Geochemistry of Chlorophylls

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Summary

In spite of intensive studies over more than 70 years, the geochemistry of tetrapyrrole pigments still presents numerous challenges. Recent studies have focused on functionalized precursors of sedimentary porphyrins, making extensive use of developments in analytical capabilities. Many new structures have been characterized, confirming the Treibs hypothesis linking chlorophyll *a* to the major sedimentary porphyrin, deoxophylloerythroetioporphyrin, and revealing a number of alternative transformation pathways. Functionalized transformation products of chlorophylls *a*, *b*, and *c* and of bacteriochlorophylls *c* and *d* have all been recognized indicating that all primary producer communities are represented in the sedimentary record. It is evident that redox status, grazing pressure and secondary reactions can all influence the fate of chlorophyll, and that the variety of transformation products present in natural sedimentary environments contains a wealth of information pertaining to the conditions present at the time of sediment deposition. Several entirely novel pigment transformation products have been identified and others represent viable precursors for sedimentary porphyrin structures previously of uncertain origin. As a direct result of the advances in analytical methods it is now possible to perform on-line analysis at very high stratigraphic resolution. At cm and mm scale resolution pigment profiles can show a significant degree of variation, indicating that the pigments are highly sensitive markers of environmental change. Thus, in addition to providing information on the source organisms present in past environments, chlorophyll derivatives have great potential for use in a range of paleoenvironmental proxies.

I. Introduction

During the mid 1930s the German chemist Alfred Treibs presented two seminal papers in which he reported the presence of the alkyl metallo-porphyrins deoxophylloerythroetioporphyrin (DPEP; **1a**) and etioporphyrin III (**2a**) and their carboxylic acid counterparts (**1b, 2b**) in sediments (Treibs 1934, a,b). Treibs isolated the components by partitioning according to acid number and assigned structures, making extensive use of ultraviolet-visible (UV/Vis) spectroscopy. The structural similarity of DPEP to chlorophyll (Chl) *a* (**3**) and of etioporphyrin III to heme (**4**) led Treibs to suggest that these components represented precursor-product pairs (Treibs, 1936). Later, Corwin (1960) noted that sedimentary porphyrin distributions were more complex than Treibs' initial work had suggested and argued, on the basis

of mass balance of the precursors, that the majority of porphyrins were derivatives of Chl. Within a few years, the application of mass spectrometry in the analysis of sedimentary porphyrins provided very clearly evidence that they are complex distributions of homologous components dominated by cycloalkanoporphyrins (CAPs) such as DPEP and etioporphyrins (Baker, 1966) such as etioporphyrin III. More recently, structural evidence for the origins of etioporphyrins from both heme (Bonnett et al., 1983) and from Chl (Fookes, 1985) has been reinforced by stable isotope analysis ($\delta^{13}C$ measurement) of sedimentary porphyrins (Boreham et al., 1989; Ocampo et al., 1989). The major pathway leading to etioporphyrins is likely to occur via oxidative cleavage of ring E of Chl as suggested by Baker et al. (1983, 1986).

Treibs' suggestion of a precursor-product relationship between biological tetrapyrroles and the ancient porphyrins present in sediments led to the development of the field of Organic Geochemistry. The precursor product relationship has since been extended to numerous classes of natural product and significant advances have been made in understanding the nature of, and controls on, the transformation reactions of natural products in the natural environment. A major part of this understanding can be linked directly to the pioneering work of Treibs in which the series of transformation reactions required to effect conversion of Chl to DPEP was proposed (Treibs, 1936). The viability of the Treibs hypothesis has been confirmed by the characterization, in three

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Abbreviations: APCI – atmospheric pressure chemical ionization; BChl – bacteriochlorophyll; biCAP – bicycloalkanoporphyrin; BPheide – bacteriopheophorbide; BPyPhe –bacteriopyropheophytin; BVir – bacterioviridin; CAP – cycloalkanoporphyrin; CCE – chlorin carotenyl ester; Chl – chlorophyll; Chlide –chlorophyllide; Chlone –chlorophyllone; COSY – correlation spectroscopy; CSE – chlorin steryl ester; CyPheide – cyclopheophorbide; DPEP – deoxophylloerythroetioporphyrin; LC-MS – liquid chromatography mass spectrometry; MS – mass spectrometry; MS/MS – tandem mass spectrometry; MS^n – multistage mass spectrometry; NMR – nuclear magnetic resonance; NP-HPLC – normal-phase high performance liquid chromatography; Phe –pheophytin; Pheide – pheophorbide; Py – pyro; PyPhe – pyropheophytin; PyPheide – pyropheophorbide; RP-HPLC – reversed-phase high performance liquid chromatography
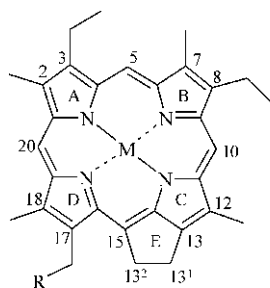
1a R=Me, M = Metal/2H
b R=CO$_2$H, M = Metal/2H
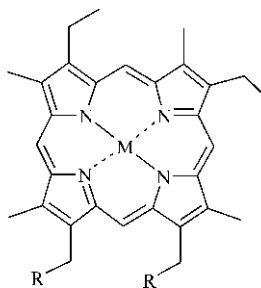
2a R=Me, M = Metal/2H
b R=CO$_2$H, M = Metal/2H
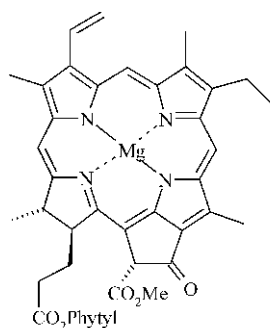
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4

sediments, of the minimum number of intermediates required to complete the conversion of Chl *a* to DPEP (Keely et al., 1990). Thus, the Treibs Scheme (Fig. 1) provides an essential framework for understanding and interpreting the significance of sedimentary tetrapyrrole distributions.

A. Porphyrins in the Geological Record

Over the years, a number of comprehensive reviews have been published detailing the variety of porphyrin structures identifi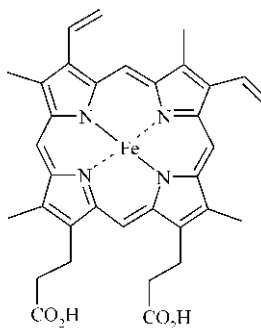ed in sediments and oils (Baker and Palmer, 1978; Baker and Louda, 1986; Louda and Baker, 1986; Chicarelli et al., 1987; Ocampo et al., 1987; Callot et al., 1990; Callot, 1991; Callot and Ocampo, 2000). Accordingly, only the key features of sedimentary porphyrin geochemistry are presented here, sufficient to provide the essential context within which functionalized sedimentary tetrapyrrole structures must be viewed. Specifically, the issues discussed relate to the ability, or otherwise, to designate a particular biological source to individual sedimentary porphyrin structures,

and the nature of the transformation pathways by which particular classes of sedimentary porphyrin are suggested to arise. Representative examples of porphyrin structures are included in this section: no attempt has been made to provide a comprehensive review. Sedimentary porphyrins occur mainly as Ni and VO complexes, though a variety of other metals have been observed in sediments, coals and oil. For detailed coverage, including the structures of the many homologues and isomers of the various classes of porphyrin discussed, the reader is directed to three excellent review articles (Chicarelli et al., 1987; Callot, 1991; Callot and Ocampo, 2000). Thorough and detailed discussion of the nature and distributions of metals in sedimentary porphyrins can be found in Filby and van Berkel (1987).

Sedimentary porphyrins identified to-date include many structures that could originate from two or more precursor Chls. Interestingly, although Chl *a* is almost certainly the major precursor of sedimentary porphyrins, no known sedimentary porphyrin structure can be linked exclusively to this precursor. Some porphyrins, however, do possess unique structural
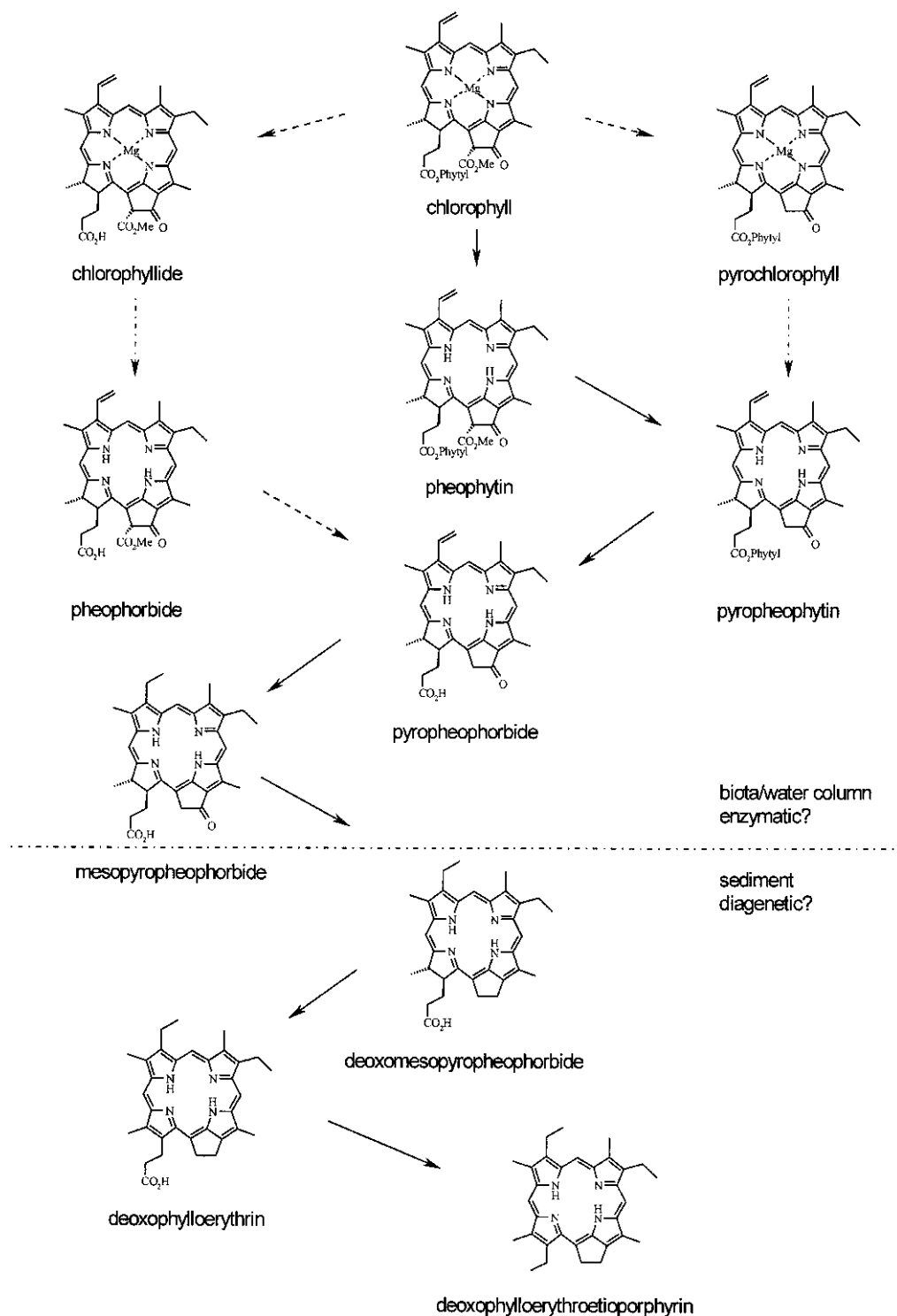
Fig. 1. Representation of the Treibs Scheme leading to cycloalkanoporphyrins, formulated for Chl *a*. The minimum number of interme-
diates required to effect conversion of Chl *a* to DPEP are connected by solid arrows, dotted arrows indicate variations in reaction order
that are known to occur — many others are possible. Structures above the broken line can be formed within the water column or surface
sediment, the timing of formation of those below the dotted line is uncertain (adapted from Keely et al., 1990).

features that permit an origin from a particular Chl to be recognized. Thus, as a result of the restricted occurrences of individual Chls among photoautotrophs, porphyrins can provide direct evidence for contributions to the sedimentary organic matter pool from specific groups of organisms.

1. Source Specific Porphyrins

The presence of a β-hydrogen at C-7 (see structure **1** for numering) was initially suggested to indicate that porphyrin (**5**) was a specific transformation product of Chl *b* (**6**; Chicarelli and Maxwell, 1984), implying an origin from Chlorophyta or from type II cyanobacteria, though the former are a more likely source based on relative abundance in nature. Subsequently, however, the discovery of Chl c_3 (**7c**) in a coccolithophorid alga (Fookes and Jeffrey, 1989) provided an alternative source. Consequently, there are no porphyrin structures known that can be assigned

an origin exclusively from either Chl *a* or Chl *b*. The best candidates for source-specific porphyrins of algal origin are CAP structures (**8**; Ocampo et al., 1984; Verne-Mismer et al., 1988, 1990) derived from Chls *c* (**7**). These compounds can be recognized as Chl *c* derivatives by the unusual structural motif whereby a methyl- or ethanoic acid- substituted five-membered exocyclic ring exists between C-15 and C-17 and not between C-13 and C-15 as is usual in CAPs (cf **1**). The ring system originates from an acid catalyzed Michael-type addition reaction involving the acrylic acid side chain of the Chls *c* and the nucleophilic C-13^2 carbon atom of the ring-E ketoester system, with loss of the C-13^1 and C-13^3 carbon atoms by decarboxylation and decarbomethoxylation. The C_{31} derivative (**8a**) could arise from Chls c_1, c_2 or c_3 (**7a-c**) whereas the C_{30} derivative (**8b**) could only originate by C-7 decarbomethoxylation of Chl c_3 (**7c**).
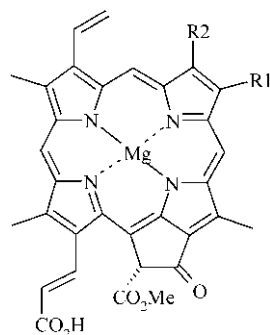
Bacteriochlorophylls (BChls) *a* (**9**) and *b* of the purple bacteria also can not be linked to unique alkyl
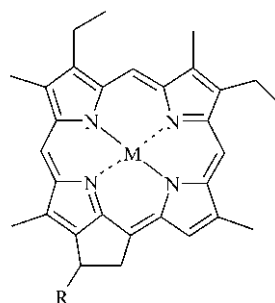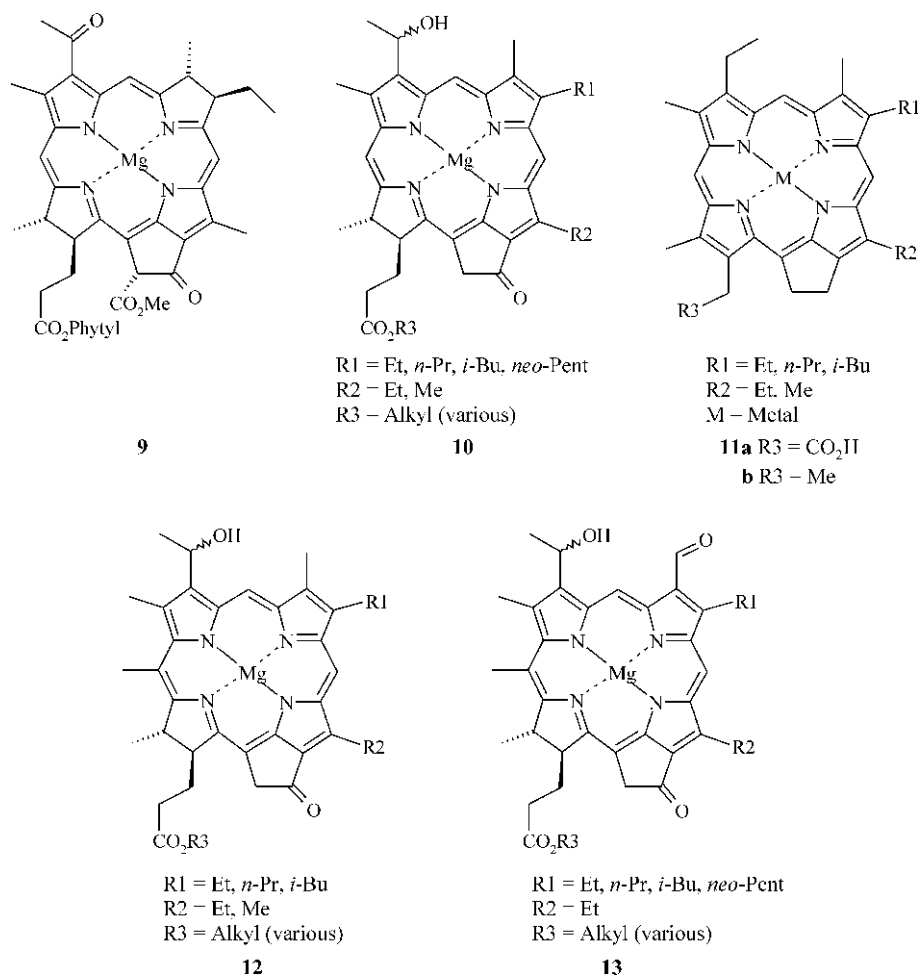

5 M = Metal

6
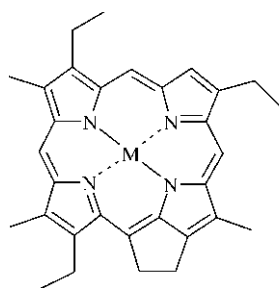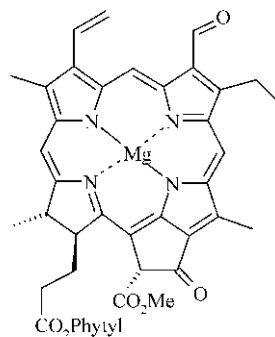
7a R1 = Et, R2 = Me
  b R1 = Vinyl, R2 = Me
  c R1 = Vinyl, R2 = CO_2Me

8a R = Me, M = Metal
  b R = CH_2CO_2H, M = Metal

R1 = Et, *n*-Pr, *i*-Bu, *neo*-Pent
R2 = Et, Me
R3 = Alkyl (various)

10

R1 = Et, *n*-Pr, *i*-Bu
R2 = Et, Me
M = Metal

11a R3 = CO$_2$H
  b R3 = Me

9

R1 = Et, *n*-Pr, *i*-Bu
R2 = Et, Me
R3 = Alkyl (various)

12

R1 = Et, *n*-Pr, *i*-Bu, *neo*-Pent
R2 = Et
R3 = Alkyl (various)

13

porphyrin structures: reduction of the C-3 methyl ketone and oxidation of the bond between C-7 and C-8 would lead to structures that could equally well arise from any of the Chls *a*, *b* and *c* or from the BChl *d* (**10**) homologues possessing the [C-8, C-12] alkylation pattern [Et, Me]. By contrast, BChl *d* from green sulfur bacteria can be recognized as the source of the porphyrin carboxylic acids (**11a**) and alkyl-porphyrins (**11b**) with the unique structural feature of extended alkylation at C-12 and/or C-8 (Ocampo et al., 1985; Keely and Maxwell, 1993; Gibbison et al., 1995). Similarly, partial structure analysis of a sedimentary porphyrin from the Oulad Abdoun oil shale of Morocco suggested the presence of a C-20 Me substituent, leading Callot et al., (1990) to suggest an origin from either BChl *c* (**12**) or *e* (**13**) of the green bacteria.

2. Unusual Porphyrin Structures

A number of sedimentary porphyrins possess ring structures that can only be rationalized as arising through modification of precursor Chls. In addition to the Chl *c*-derived porphyrins discussed above, these components include methylcyclohexano- (**14**; Chicarelli et al., 1984) and cycloheptano- (**15**; Fookes, 1983; Wolff et al., 1983) porphyrins, bicyclo-alkanoporphyrins (bi-CAPs, e.g., **16**; Prowse et al., 1987) and a 13^1-methyl-substituted bicycloalkeno-porphyrin (**17**; Chicarelli and Maxwell, 1986), tetrahydrobenzo- (**18**; Verne-Mismer et al., 1987) and benzoporphyrins (**19**; Kaur et al., 1986). Plausible mechanistic pathways to the cyclohexanoporphyrin and bi-CAP structures have been suggested, involving condensation reactions between the C-17 propionic acid substituent and the C-13^2 carbon atom. Despite attempts to identify likely precursors, the origins of
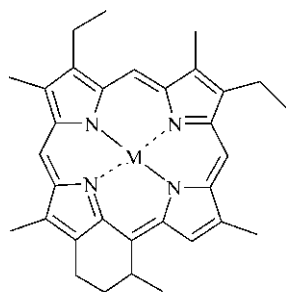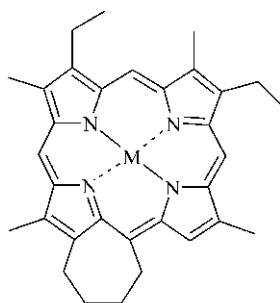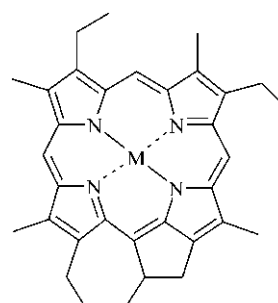
14 M = Metal   **15** M = Metal   **16** M = Metal / 2H
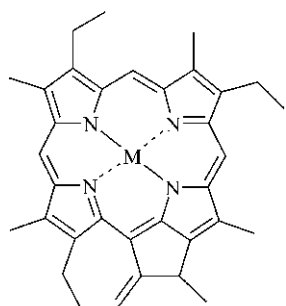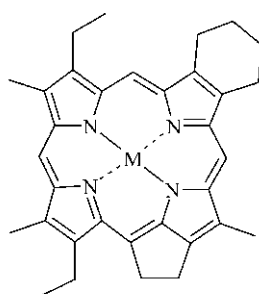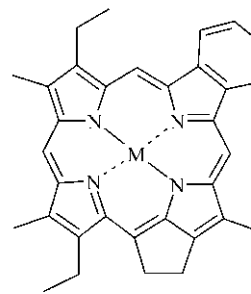
17 M = Metal   **18** M = Metal   **19** M = Metal

the 13^1-methyl-substituted component (**17**), tetra-hydrobenzo- (**18**) and benzoporphyrins (**19**) remain uncertain, though a Diels-Alder reaction involving [8-vinyl]-Chl *a* (present in some type II cyanobacteria; Chapter 1, Scheer) has been suggested for the last two (May and Lash, 1992).

The brief review above illustrates the wide range of structural variations within natural porphyrin distributions, alludes to their complexity and shows that questions as to the origins of some structures remain to be answered. In order to provide insights into the origins of sedimentary porphyrins, recent work has largely focused on identification of functionalized derivatives in Recent sediments and on characterizing distributional differences within, and between, a range of depositional settings. These studies owe much to the early work of Baker and co-workers who identified functionalized tetrapyrroles in cores recovered under the Deep-Sea Drilling Project (for reviews see Baker and Louda, 1983, 1986; Louda and Baker, 1986). Owing to the limited capabilities of analytical instrumentation at that time, those studies were restricted in the extent to which structures could be confirmed. Over the last ten years, the considerable

advances in development of analytical techniques and their application to identification of the functionalized precursor chlorins have allowed greater certainty in structure assignments and have revealed a number of novel components. During that time research activity in this area has been substantially greater than in the analysis of the corresponding sedimentary alkyl porphyrins. Major targets in the studies of functionalized precursors have been the identification of transformation products and the pathways by which they are formed, and the recognition of environmental- and source-specific markers. Accordingly, the main focus of this article concerns the functionalized precursors of sedimentary porphyrins, representing the earliest stages of transformation. Derivatives of all of the major Chls are included, encompassing dihydroporphyrins (chlorins, e.g., Chl *a*; **3**), tetrahydroporphyrins (bacteriochlorins, e.g., BChl *a*; **9**) and porphyrins (e.g., Chl *c*; **7**). A brief resume of the most widely used methods of analysis is presented, followed by discussion of the main transformation pathways, transformation products and uses in environmental assessment.

B. Analytical Methods

Sedimentary tetrapyrroles occur as complex distributions, necessitating chromatographic separation prior to analysis. The main spectroscopic methods employed in characterization are UV/Vis, mass spectrometry (MS) and nuclear magnetic resonance (NMR). Other techniques, including Raman spectroscopy and stable isotope ratio analysis, have also been applied but will not be considered further here. Owing to the chemical reactivity of many of the functionalized tetrapyrroles there is considerable opportunity for the formation of artifacts during extraction and workup. Hence, the methods used to extract and analyze the tetrapyrroles must be sufficiently mild and rapid so as to avoid reactions leading to such products.

1. Extraction

The sensitivity of Chl derivatives to oxidation reactions in alcoholic solutions was recognized around 100 years ago (Willstätter and Stoll, 1913). More recently, it has been established that these are facile autoxidation reactions that are specific to components possessing a carbomethoxy substituent at position C-13^2: they depend on the activation of the C-13^2 hydrogen atom provided by the β-ketoester system (Hynninen, 1991; Woolley et al., 1998). Accordingly, extraction of tetrapyrroles possessing this structural feature requires the avoidance of alcohols in the extraction medium. Acetone is the preferred extraction solvent and, as a precaution to avoid photo-oxidation, solutions are protected from exposure to strong light. In order to prevent autoxidation, which occurs only very slowly in acetone, rapid extraction methods such as sonication are employed (Keely et al., 1990; Airs et al., 2001a).

2. Reversed-phase High Performance Liquid Chromatography (RP-HPLC) Analysis

Several RP-HPLC methods have been developed for the analysis of functionalized tetrapyrroles (for examples, see the review by Roy, 1987). To be of maximum value in geochemical analysis the analytical methods need to give good resolution over a wide polarity range, encompassing highly polar derivatives such as Chls *c* through to highly apolar derivatives including chlorin steryl esters (CSEs; see later). The availability of photodiode array detectors for HPLC

places an additional constraint on the mobile phase composition. In order to obtain spectral information of Chl derivatives and co-occurring carotenoids over a wide range, UV absorption by the mobile phase should be as low as possible. Thus, replacement of acetone (Eckardt et al., 1991; Verzegnassi et al., 1999; Zapata et al., 2000) by ethyl acetate (Wright et al., 1991) is favored. To achieve the combined goals of excellent chromatographic resolution, UV-transparency and compatibility with liquid chromatography mass spectrometry (LC-MS), a method was developed for the analysis of sedimentary tetrapyrroles (Method A; Airs et al., 2001a). Variants of the method were also developed, allowing flexibility according to the requirements of the analysis and the availability of instrumentation and preparative apparatus. Methylation of carboxylic acids by treatment with diazomethane is recommended in order to obtain the best chromatographic peak shape possible and to permit long term storage of extracts (Airs et al., 2001a and references therein).

3. Normal-phase High Performance Liquid Chromatography (NP-HPLC) Analysis

Chlorophyll autoxidation reactions lead to the formation of a range of products, some of which are not well resolved under RP-HPLC conditions. Better chromatographic resolution of Chl oxidation products can be achieved using NP-HPLC (Kuronen et al., 1993) and the compatibility of this approach with LC-MS analysis has been demonstrated for mixtures of Chl oxidation products formed in the laboratory (Jie et al., 2002).

4. Liquid Chromatography-Mass Spectrometry Analysis

Broad distinctions between different classes of Chl derivative can be made according to chromatographic elution order and on-line UV/Vis spectra, and further refinement is possible from co-elution with authentic standards. For rigorous assignment, however, it is necessary to obtain molecular mass and structural information by MS. The value of tandem MS (MS/MS) in assignment of functionalized sedimentary tetrapyrrole structures was demonstrated using fractions isolated by preparative HPLC and ionized using fast atom bombardment (Keely and Maxwell, 1990a). Fragment ions yielding structure-specific information were readily obtained, but the off-line analytical ap-

proach is laborious and places severe constraint on the number of samples that can be processed. For this reason, LC-MS, which enables on-line analysis, is now the method of choice for MS analysis of sedimentary tetrapyrrole distributions. Significant improvements in the ease and rapidity of MS/MS analysis result from use of the atmospheric pressure ionization techniques electrospray (Chillier et al., 1994) and atmospheric pressure chemical ionization (APCI; Airs et al., 2001a; Airs and Keely, 2002). APCI has been shown to give excellent response for production of radical anions (negative ion-mode) and of protonated molecules (positive ion mode) from functionalized free-base tetrapyrroles (Verzegnassi et al., 1999; Airs et al., 2001a). Combined with post-column addition of acid, to effect on-line demetallation of the Mg-containing derivatives (Airs and Keely, 2000), the technique also provides excellent response for the Mg-containing species (Airs et al., 2001a; Airs and Keely, 2002). The application of ion-trap mass spectrometry, with its unique multistage (sequential or MS^n) approach to MS/MS, has been of particular benefit in distinguishing between structurally similar Chl derivatives (Airs et al., 2001a; Airs and Keely, 2002; Squier et al., 2002; Wilson et al., 2003, 2004a). In APCI ion trap MS of Chl derivatives the precursor ion, the protonated molecule, is isolated within the trap and is subjected to resonance excitation, forming product ions by fragmentation induced by collision with the He bath gas in the trap. The product ion spectrum (MS^2) is generally dominated by one fragment ion. The process of isolation and fragmentation can be repeated on one of the MS^2 product ions and subsequently up to at least MS^7 (Airs and Keely, 2002; Wilson et al., 2003, 2004a). In this way, the detailed fragmentation behavior of a significant number of Chls and their derivatives has been determined and it is apparent that the derivatives follow systematic fragmentation pathways (Jie et al., 2002; Airs and Keely, 2002; Walker et al., 2003; Wilson et al., 2003, 2004a).

A study of BChls from green and brown bacteria exemplifies the need for the combined approach offered by LC-MS analysis. Bacteriochlorophylls from cultured organisms reveal highly complex distributions in which variations are evident both in the extent of alkylation at C-8 and/or C-12 and in the nature of the esterifying alcohol (Airs et al., 2001b). Within each of the BChl series (*c, d* or *e*), which can be assigned from the on-line UV/Vis spectra, the *m/z* value of the protonated molecule provides very

limited structural information as a result of the large number of structural isomers possible for each molecular mass. The application of tandem MS allows designation of the esterifying alcohol to a restricted range of possibilities (Airs et al., 2001b; the exact structure of each can be determined by gas chromatography-mass spectrometry of the free alcohols after hydrolysis) and the total extent of alkylation at [C-8, C-12]. Detailed studies of the MS^n spectra of the (demetallated) Chls (Airs and Keely, 2002) or their corresponding pheophorbides (Pheides; Wilson et al., 2003, 2004a) allow assignment of the alkyl substitution patterns at each of the two positions. Notably, the use of MS^n analysis allowed contributions from co-eluting isobaric bacteriopheophorbides (BPheides) to be detected and the overlapping signals to be deconvoluted (Wilson et al., 2003, 2005), highlighting the necessity for mass spectral analysis as an essential tool in the determination of complex sedimentary pigment distributions.

Normal-phase LC-MS/MS has also been employed in the analysis of sedimentary alkyl porphyrins (Rosell-Melé et al., 1996, 1999; Mawson et al., 2004). In the later of these studies the complexity of the distributions, and lack of chromatographic resolution as a result of the preponderance of isomeric structures, precluded the isolation of individual components for NMR analysis. The application of LC-MS^n allowed assignment and quantification of many porphyrins, including structures exhibiting only partial chromatographic resolution (Mawson et al., 2004).

5. Nuclear Magnetic Resonance Analysis

Proton NMR analysis is one of the most rigorous methods for characterizing sedimentary tetrapyrroles: analysis of spin-spin interactions and through space connectivities determined from nuclear Overhauser enhancement allows full assignment of structures of individual components isolated from sediments and oils (Chicarelli et al., 1987; Callot et al., 1990; Callot and Ocampo, 2000). The application of two dimensional techniques (e.g., correlation spectroscopy, COSY; Keely and Maxwell, 1990b and COSY and rotating-frame Overhauser enhancement spectroscopy; Ocampo and Repeta, 1999) can lead to greater structural specificity by avoiding effects due to irradiation spill, and simplifies the analysis by alleviating the need to select individual irradiation positions in setting up the experiment. In the case of porphyrins, where the number of isomeric structures

is greatest, NMR is still required to discriminate some porphyrin structures and is the method of choice to confirm novel structures. By contrast, the majority of the more functionalized derivatives can be fully assigned using LC-MS/MS (e.g., Airs et al., 2001a; Squier et al., 2002; Wilson et al., 2004a), alleviating the need for isolation and purification for NMR analysis.

II. Chlorophyll Transformations

Geochemical interest in Chl derivatives relates both to their potential as sources of the porphyrin derivatives that occur in sediments and oils and their potential as indicators of specific environmental conditions and processes. Since the latter depends on an understanding of their origins, the following discussion considers Chl transformation products in the context of common transformation pathways. Transformation reactions of Chls that leave the macrocycle intact have been termed type I transformation reactions and ones that lead to cleavage of the macrocycle are termed type II (Hendry et al., 1987). In situations where the environment is oxygen-limited or oxygen-deficient (termed reductive below) type I reactions dominate. In environments where oxygen is readily available (termed oxidizing below) both type I and type II reactions can occur.

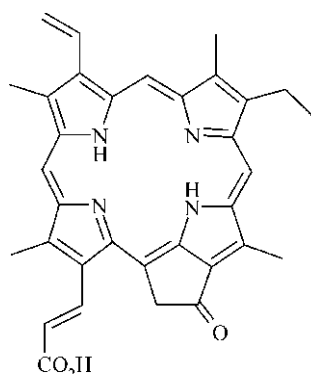### A. Regular Reductive Transformation Products

1. Products of Simple Type I Defunctionalization Reactions

The characteristic structural feature that identifies the products of reductive transformation reactions of Chls is the intact carbon skeleton of ring E. Structures including pheophytins (Phes), Pheides and their pyro- (Py) derivatives (Fig. 1) are formed by simple defunctionalization reactions that involve modifications of functional groups around the periphery of the macrocycle. Pheophytins, Pheides and their Py-counterparts are among the earliest derivatives produced in natural environments, being formed either through the processes of senescence (Schoch et al., 1981; Spooner et al., 1994a; Chapter 17, Kräutler and Hörtensteiner) or herbivory (Shuman and Lorenzen, 1975). Typically, they are among the major products in contemporary and Recent sediments (Keely et al., 1990; Villanueva et al., 1994a; Airs et al., 2001a; Squier et al., 2002; Airs and Keely, 2003; Squier et

al., 2004a; Wilson et al., 2004a) and Py-pheoporphyrins (**20, 21**) derived from Chls c_1 and c_2 have also been detected (Harradine and Maxwell, 1998). Similar transformation products of BChl a have been identified (Villanueva et al., 1994b), indicating that the same transformation mechanisms affect the prokaryote and eukaryote communities. In the case of BChls c, d and e, the main derivatives identified in Recent sediments are the Phes (Squier et al., 2002, 2004a; Airs and Keely, 2003; Wilson et al., 2004a); the absence of a C-13^2 carbomethoxyl group in these BChls limits the variety of structures possible. Thus, the bacteriopheophytins (BPhes) c, d and e are structurally analogous to the Py-derivatives of Chls a, b, c and BChl a, but require one fewer reaction step in their formation. The presence of Phe derivatives of BChls and of bacteriopyropheophytin a (BPyPhe a) in sediments provides evidence that senescence is important in their formation. The major BChl-producing organisms are confined to dysoxic and anoxic regions of the water column (van Gemerden, 1983), hence grazing organisms can play only a minor role in the transformation reactions leading to their sedimentary derivatives.

Phes and PyPhes are also commonly found within fecal material (Burkill et al., 1987; Talbot et al., 1999a,b) where they are considered to be products of the grazing process. Clearly, however, the process of grazing itself can induce senescence reactions and the distinction between endogenous algal and exogenous (e.g., grazer) enzyme effects is unclear. For example, Spooner et al. (1994b) incubated lysed diatom cells in the dark and found that the rate of formation of chlorophyllide (Chlide), Pheides, Phe and PyPhe, produced by the actions of the algal enzymes, matched zooplankton gut clearance rates. High abundances of Pheides in marine environments have been suggested to be indicative of grazing (Shuman and Lorenzen, 1975). Although Pheides occur in high relative abundance in the fecal material of some grazers, they can also be produced by demetallation of Chlide formed by the actions of chlorophyllase, an active enzyme in certain diatoms (Jeffrey and Hallegraeff, 1987) and various other algae. Accordingly, caution must be exercised in the interpretation of Pheides in sediments. The occurrence of Pheides in a submerged microbial mat system from which grazers were absent (Villanueva et al., 1994a) attests to formation resulting from chlorophyllase activity. In that case, the enzyme appeared to have its origins in diatoms co-existing with the cyanophyta that comprised the main photoautotrophs in the mat.

Other Chl derivatives formed through reductive

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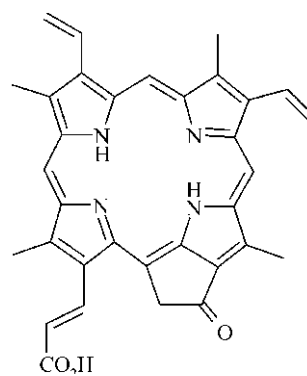
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transformation pathways include the products of C-3 vinyl and C-13[1] ketone reduction, aromatization and decarboxylation (Keely et al., 1990, 1994). Notably, none of the derivatives formed by these reactions have been identified in the water column, though they have been identified in unconsolidated and thermally immature sediments from Maraú (Keely et al., 1990), Willershausen (Keely et al., 1994), Messel oil shale (Ocampo et al., 1992) and marls of the Vena del Gesso evaporitic sequence (Keely et al., 1995). Precedent for the C-3 vinyl reduction depending on microbial activity comes from the formation of mesoPyPheide *a* (Fig. 1) in laboratory microcosms in which algal cells were incubated in the presence of sulfate reducing bacteria (Spooner et al., 1995). The mechanisms leading to C-13[1] ketone reduction, aromatization and decarboxylation remain to be established, though it is evident, by the occurrence of derivatives in immature sediments, that the reactions are not exclusively thermal.

The occurrence, as the predominant BChl derivative in an Antarctic sediment, of BPhe a_{gg} in which the esterifying alcohol is geranygeraniol signifies an input from the aerobic purple bacteria, which are heterotrophs (Squier et al., 2004b). The recognition that such aerobic bacterial inputs can impart a dominant signature on the sedimentary pigment profiles indicates the caution that must be exercised in interpreting the presence of de-esterified counterparts of BChl *a*.

### B. Oxidative Transformation Products

1. Products of Type II Reactions

Oxidation reactions of Chls follow two distinct mechanistic pathways. Photo-oxidation involves reaction with singlet oxygen and follows a pathway in which the initial attack of oxygen is at position C-5, generating products in which the macrocycle is cleaved (Mühlecker et al., 1993). Reactions involving cleavage of the Chl macrocycle are designated type II (Hendry et al., 1987) and produce linear tetrapyrrole products that are colorless or weakly-colored, and which are subsequently degraded to maleimides (monopyrrolic derivatives). Maleimides have been identified in sediments of the Kupfershiefer and the Serpiano shale, where the alkylation patterns of individual structures (e.g., **22, 23**) could be assigned an origin from BChls *c*, *d* or *e* (Grice et al., 1996). Isotopic signatures enriched in ^{13}C lend support to the proposed partial origin of **22** and origin of **23** from Chlorobiaceae (Grice et al., 1997). The characterization of maleimides, both as free components and following chromic acid oxidation of bound residues, has since been applied to marine sediments of Neogene age from the Shinjo basin (Kozono et al., 2001) and Cretaceous-Tertiary boundary sediments from Kawaruppu (Shimoyama et al., 2001). In those settings, 2-methyl-3-ethylmaleimide was the dominant component, occurring with lesser amounts of 2-methyl-3-*n*-alkyl derivatives (methyl to pentyl at C-3 in Shinzo and methyl to butyl in Kawaruppu). The extended *n*-alkylation up to C-5 at C-3 does not suggest a specific origin from pigments of green bacteria. Rather, it can only be rationalized as resulting from retention of carbon atoms C-13 to C-17, most likely from derivatives in which the C-13[1] ketone had been reduced. Lesser amounts of 2-methylmaleimide, benzomaleimide (**24**) and methylated homologues, and tetrahydrobenzomaleimide were also present. Marked differences in the distributions of the free and bound maleimides were attributed to the former being products of photo-oxidation of Chls and the latter being formed from bound porphyrins. Evidence for the formation of maleimides by oxidative trans-
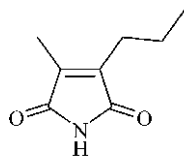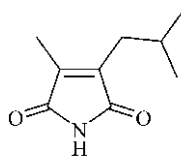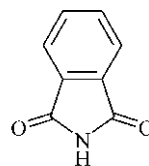
22   **23**   **24**

formation in the water column of a contemporary environment was provided by their recognition in the surface sediments of Tokyo Bay (Kozono et al., 2002). The benzo-derivatives in the ancient sediments were suggested to be products of oxidation of benzo- and tetrahydrobenzoporphyrins. Notably, evidence of maleimides with branched side chains was lacking (Kozono et al., 2001; Shimoyama et al., 2001), implying the absence of Chlorobiaceae at the time of sediment deposition. By contrast, clear evidence of photic zone euxinia associated with the major oceanic anoxic event of the Permian (Zechstein basin) was provided by recognition of maleimides with branched C-3 alkyl groups (Pancost et al., 2002), and later in sediments of Cenomanian-Turonian (North Atlantic), early Toarcian and early Aptian (Marche-Umbria, Italy) age, times also associated with oceanic anoxic events (Pancost et al., 2004).

2. Products of Regular (Type I) Reactions

Chlorophyll reacts with molecular oxygen (triplet oxygen) via type I reactions, leading to oxygen incorporation centered on ring E. A range of oxidation products form in alcoholic solution, including hydroxyl derivatives, lactones and ring-opened derivatives (Hynninen, 1991; Woolley et al., 1998). A number of related structures occur in natural sedimentary environments. The C-13^2 hydroxyl derivatives of Phe a (**25**) were identified in Loch Ness and Lake Baikal (Airs et al., 2000) and in Kirisjes pond (Walker et al., 2002), and corresponding C-13^2 hydroxyl derivatives of BPhe a and BPheide a in a coastal salt pond (Salt Pond) from the USA (Ocampo and Repeta, 2002, 2004). The ring-opened derivative purpurin-7 phytyl ester (**26**) was identified (as the dimethyl ester) in the sediments of Priest Pot, Loch Ness and Lake Baikal (Airs et al., 2000) and the anhydride derivatives purpurin-18 (**27a**) and its phytyl ester counterpart (**27b**) in Loch Ness and Lake Baikal (Naylor and Keely, 1998). The former anhydride was also found to occur in surface sediments of the Peru margin

(Ocampo and Repeta, 1999). Derivatives formed by disruption of ring E with incorporation of oxygen are likely precursors of sedimentary etioporphyrins (Naylor et al., 1998; Airs et al., 2000). The presence of the hydroxyl and purpurin-18 derivatives in senescent algae (Louda et al., 1998, 2002) indicates viable mechanisms for formation within the water column. Clearly, therefore, parallels exist between laboratory oxidation products and the products of natural oxidation reactions. Although the main oxidation products found in the natural environment can be explained according to the autoxidation mechanism proposed by Hynninen (1991), a full treatment of the reaction mechanism remains to be performed.

Products of BChl a in which the 3-acetyl-tetrahydroporphyrin structure has been converted to a dihydroporphyrin (the so-called bacterioviridins, BVirs) have recently been identified in sediments where exposure to oxygen occurred. Specifically, the BVirs (**28a, b**) derived from BPhe a and BPheide a were identified (Wilson et al., 2004b), indicating breakdown of the dysoxic conditions required by the bacteria, leading to incursion of oxygen. It was not possible to determine if the ingress of oxygen occurred before, during or after deposition. Conversely, some environments containing BChl a derivatives do not contain BVirs (Villanueva et al., 1994b), consistent with their formation being dependent on the redox status of the environment. The BVir derivatives also have parallels in the laboratory oxidation of BChls (Woolley et al., 1998; Walker et al., 2003), indicating that oxygen penetration into the anoxic zone can impact on the bacterial community and leave a signature in the biological marker record.

Although it is not conclusive, evidence that water column redox status controls the presence and relative abundances of Chl oxidation products comes from the relative abundances of oxidation products in sediments. Thus, the highest levels of oxidation products have been observed in environments with long and more highly oxygenated waters (Naylor and Keely, 1998; Airs et al., 2000). Clear support
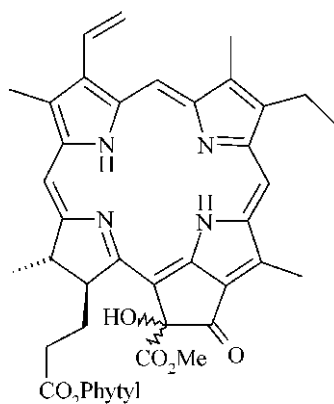
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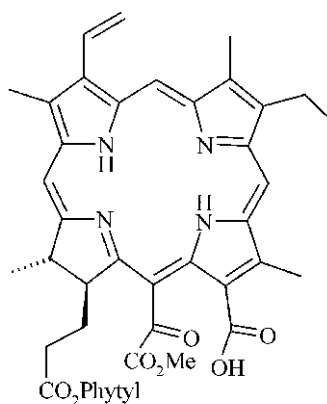
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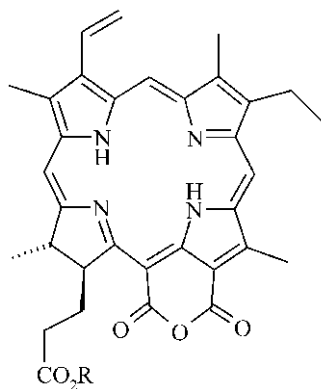
27a R = H
  b R = Phytyl
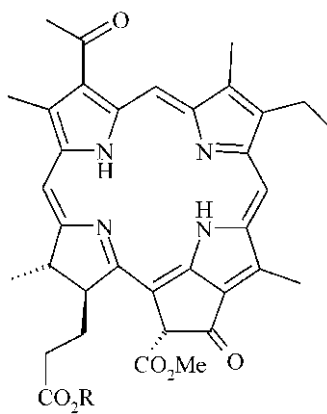
28a R = Phytyl
  b R = H

for this hypothesis comes from the presence, in an Antarctic lake sediment, of Chl oxidation products during periods where algae were the main primary producers, and absence in regions of the sediment core deposited when anoxygenic bacteria dominated the primary producer community, but where algae were still present (Walker et al., 2002). Furthermore, a later study, examining distributions of Chls and their oxidation products during and following the Spring bloom in the Celtic Sea, found higher relative abundances of oxidation products during the later stages of the bloom (Walker and Keely, 2004). The timing of their formation indicates that these components are produced during overturn of the bloom, probably following exposure of the Chls as a result of cellular disruption associated with senescence. The presence of substantial amounts of Chl oxidation products in

algal cultures subjected to senescence under oxidizing conditions (Louda et al., 1998, 2002) attests to their origins through autoxidation mechanisms, perhaps influenced also by the presence of peroxides released by the senescent algae (Merzlyak et al., 1993). Given that either route involves and depends on the availability of molecular oxygen, it is evident that Chl oxidation products have potential for use in paleoenvironmental proxies for the availability of molecular oxygen at the time of sediment deposition (see later). In order for this to be extended beyond a relative measure, the exact mechanism of oxidation needs to be determined, the role of peroxyl species defined, and the dependence on algal type examined.

C. Non-Regular Transformation Products

A number of non-regular Chl transformation products have been identified in which the normal functional group modifications are supplemented by reactions involving the coupling of non-Chl derived substituents or forming non-biosynthetic exocyclic ring structures. The departure from the typical degradative transformation reactions of Chls means that these components hold great potential as informative biological markers for specific processes affecting the primary producer community.

1. Chlorin Steryl and Triterpenoid Esters

Chlorin steryl esters (e.g., **29**), unusual components that were first identified in sediments (King and Repeta, 1991; Prowse and Maxwell, 1991), result from an esterification or transesterification reaction between a Chl derivative and a sterol. The major components found in sediments are derivatives of PyPheide *a* (Pearce et al., 1993), and there is much variety in the nature of the esterifying sterols (Harris et al., 1995a; Pearce et al., 1998). Lesser amounts of derivatives of PyPheide *b* and Pheide *a* have also been reported (Talbot et al., 1999b; Riffé-Challard et al., 2000). The sterols identified to-date represent typical algal sterols, though methyl sterols are under-represented compared with the free sterol distributions (Talbot et al., 2000). A significant overprint of the C_{27} sterol cholesterol from the grazing organism is apparent, and alterations to the sterol distribution can occur if the diet of the grazer is limited in cholesterol, in which case phytosterols are metabolized. The formation of CSEs has been observed during experiments in which diatoms were grazed by copepods (Harradine et al., 1996a; Talbot et al., 1999a). Their presence in high relative abundance in sediment traps (King and Repeta, 1994) and in the gut contents of marine salps, but absence in suspended particulate matter (King and Wakeham, 1996), has led to wide acceptance that CSEs are markers of herbivory. Similar derivatives, containing tetrahymanyl (**30**) or a hopanyl (**31**) in place of the steryl moiety, were identified in the sediments of Lake Valencia, Venezuela (Harradine et al., 1996b). The incorporation of tetrahymanol, which occurs in ciliates when dietary levels of sterols are low, together with hopanols was attributed either to zooplankton grazing a mixed diet comprising cyanophyta and ciliates or a direct effect of ciliates grazing cyanophyta (Harridine et al., 1996b).

Although high levels of CSEs in sediments have been attributed to their greater stability compared with free pigments, sediments of Lake Valencia and the Black Sea contain amounts of CSEs up to *c.* 20% of the pigment preserved in the sediment (Eckardt et al., 1992). Both are highly productive environments and grazing activity in the latter is confined to the surface waters (ca. 80 m), the water column being predominantly anoxic. Hence, the conditions are favorable to pigment preservation. Clearly, it is still necessary to reconcile the levels observed in natural environments with the lower levels observed in grazing experiments. Possible causes of these abundance differences include algal specificity and/or different extents of conversion by different grazers. Thus, for the CSEs to be employed as a quantitative measure, broader ranges of algae and grazing organisms need to be examined.

2. Chlorin Carotenyl Esters

Structures resulting from the esterification/transesterification of Chl derivatives with carotenol derivatives of fucoxanthin (e.g., **32**) were identified in sediments from the Southern California Bight, San Pedro and Santa Barbara Basins, Monterey Bay Canyon and Oman and Peru margins (Goericke et al., 1999). The assignments were made on the basis of HPLC retention times, on-line UV/Vis spectra and LC-MS[n] analysis, and selected derivatives were prepared for comparison. Esters of both PyPheide *a* and Pheide *a* were detected and presumed to form in a manner analogous to CSEs. The apparent restriction of the carotenol source to fucoxanthin derivatives was suggested to reflect a specific grazing activity involving copepods and other crustaceans feeding on diatoms (Goericke et al., 1999). Studies of sediments from the Louisiana continental shelf also revealed CCEs to be restricted to fucoxanthinol derivatives, including novel structures in which dehydration of the carotenol had not occurred (Chen et al., 2003). In that study CCEs were also identified in fecal pellets collected from zooplankton trawled from surface waters (Chen et al., 2003).

3. Chlorophyllones and Cyclopheophorbide Enols

Chlorophyllones (Chlones, **33**) have been reported to occur in a wide range of sediments (Chillier et al., 1993; Harris et al., 1995b) and the structurally related

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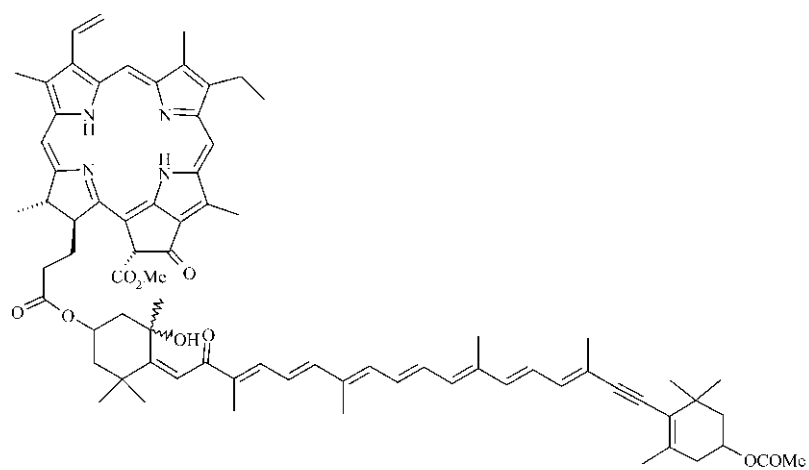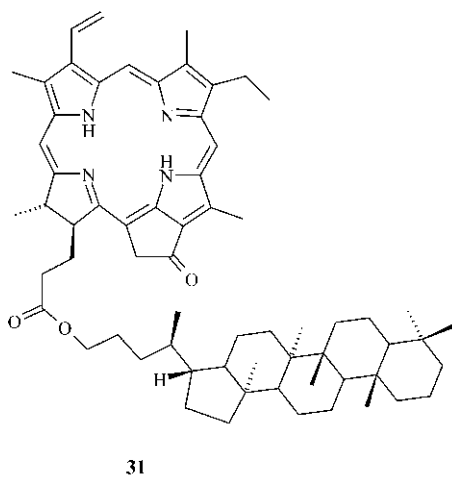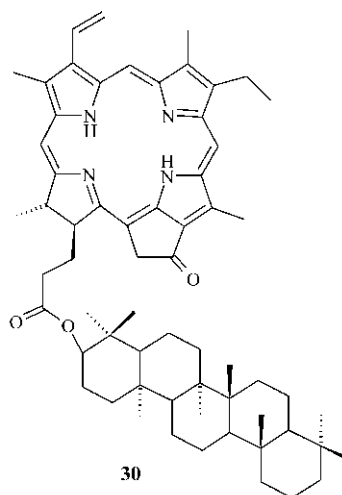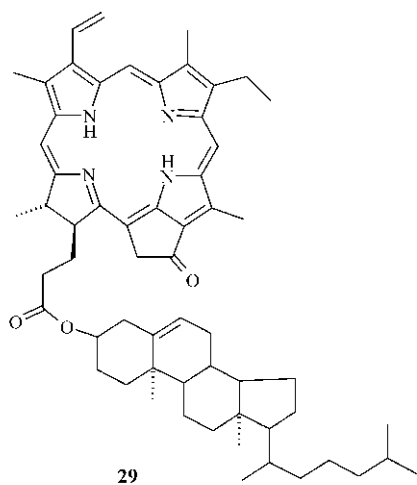
cyclopheophorbide (CyPheide) enols (**34**) have been identified in sediments (Ocampo et al., 1999; Louda et al., 2000) and in water column particulates (King and Wakeham, 1996; Ocampo et al., 1999). Both structural types exhibit features analogous to the bi-CAP porphyrins (e.g., **16**) and chlorin (**35**) found in older sediments. The enols were first identified in marine organisms including a sponge (Karuso et al., 1986), clams (Sakata et al., 1990; Yamamoto et al., 1992; Watanabe et al., 1993), and in the diatoms that comprised the diet of the latter (Watanabe et al., 1993). It was noted that the enols were unstable during chromatography (Ocampo et al., 1999) and in solvent in the presence of oxygen (Goericke et al., 2000), producing approximately 1:1 mixtures of the Chlone 15^1 (*R:S*) diastereomers and minor amounts of [13^2-oxo]-PyPheide *a* (**36**; Ocampo et al., 1999). It has been suggested that Chlone in sediments formed as a product of transformation of the enols during chromatography on silica (Louda et al., 2000). Recently, however, it was noted that Chlone in natural environments often occurs with the 15^1S diastereomer in vast excess and it was confirmed that alteration during chromatography leads to 1:1 mixtures of the

two diastereomers (Aydin et al., 2003).

The Chlone and CyPheide enol structures represent Chl *a* derivatives that can be rationalized to form by a condensation reaction between the C-13^2 position in ring E and the C-17 propionic acid substituent, in a manner analogous to the Dieckmann condensation or Claisen condensation. Synthetic derivatives have been prepared by these methods (Falk et al., 1975; Ma and Dolphin, 1996, respectively), in both cases involving generation of a carbanion from the demethoxycarbonyl derivatives using a strong base. It is uncertain if the natural precursor requires the presence of a labile hydrogen at position C-13^2, *i.e.,* if the β-ketoester functionality that is present in the Chls *a*, *b*, and *c* and the BChls *a* and *b* is a pre-requisite for reaction. Consideration of the environments in which such condensation products have been identified, either as the functionalized derivatives or as the alkyl porphyrins, suggests that basic conditions may favor their formation, consistent with the mechanism involving deprotonation of a β-ketoester intermediate. Issues remaining to be addressed include: determination of a mechanistic basis for the formation of Chlones and structurally related species
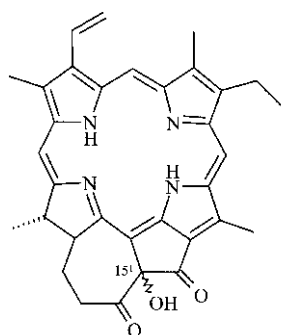
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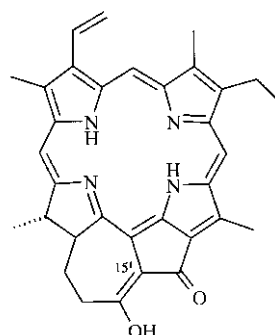
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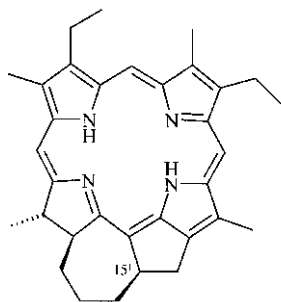
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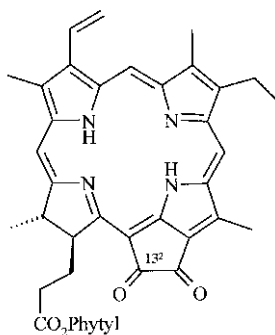
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in nature; determining if the reactions are chemically- or biologically-mediated; determination of the quantitative significance of the derivatives in the sedimentary record; determination of the potential for formation from Chls other than Chl *a* that possess the β-ketoester functionality.

4. Alkyl Sulfides

An entirely novel series of transformation products has been identified in which a carbon-sulfur bond has been formed through diagenetic reaction. The derivatives identified comprise C-3[1] alkyl sulfides (e.g., **37**) containing between one and five additional carbon atoms and representative structures occur for the Chl and Phe counterparts (Squier et al., 2003, 2004a). Although the mechanism of formation remains to be established, it is evident that the derivatives are formed early during diagenesis and it appears likely that their formation involves reaction of the Chl derivative with an alkylthiol or alkylsulfide (Squier et al., 2004a). Notably, the derivatives were identified in a lake sediment in which BChls form a major component of the signature from the primary producer community, indicating the environment to have been one in which organosulfur species were likely to form. One significant feature of the presence of the Chl alkysulfide derivatives is that they provide evidence on the timing of sulfur incorporation into organic matter in general, as well as providing a rationale for the occurrence of sulfur cross-linked alkyl porphyrins (**38**), albeit linked at a different position in the structure (Schaeffer et al., 1993). The recognition of the alkysulfide derivatives raises many

questions including: the nature of the mechanism of their formation; their environmental significance; their relevance as determinants of the extent of sulfur incorporation into sedimentary organic matter and the potential for tetrapyrroles to be cross-linked to macromolecular organic matter by a variety of different mechanisms.

III. Timing and Nature of Transformations

It is apparent that many of the transformation reactions of Chls in the natural environment take place during the very earliest stages of diagenesis. The majority of the early stage reductive transformation reactions can be linked to biological activity associated with senescence and/or herbivory. Others, such as oxidation and sulfur incorporation, most likely relate to secondary reactions that occur following release of the Chls into the environment as a result of cellular disruption. As such, it appears that reactions at least up to and including the formation of PyPheides (Fig 1) occur mainly within the water column or, particularly in the case of benthic communities, in the surface sediment. A number of the transformation steps appear to be later stage processes, possibly associated with burial of the sediments. These include reduction of the keto- groups, aromatization, decarboxylation and metallation. In spite of the long-standing interest in the geochemistry of tetrapyrroles, these later-stage reactions in the natural environment remain to be explained in full, both in terms of the reaction mechanisms and their causes and how the environmental conditions influence the product distributions.
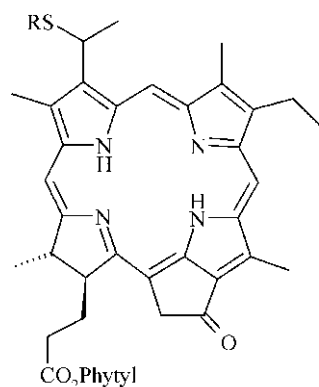
37 R = Me, Et, Pr, Bu, Pent
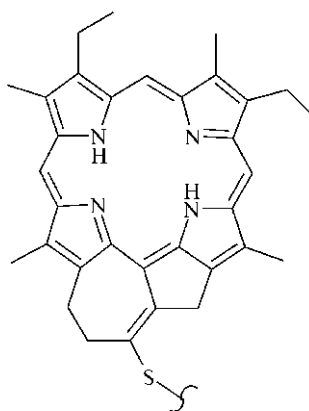
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The transformation pathways of Chls in terrestrial environments differ significantly from those that operate in aquatic environments. This is partly the result of differences in cellular structure and organization but is also a reflection of the different environmental stresses to which plants are subjected in these two distinct regimes.

A. Terrestrial

Net Chl production in terrestrial environments has been estimated at 2.92×10^8 tons per annum (Hendry et al., 1987). The vast majority of that Chl is destroyed through the natural processes involved in autumnal senescence in leaves. These reactions involve oxidative cleavage of the macrocycle to generate weakly colored and colorless derivatives (Mühlecker et al., 1993). For this reason, the amount of Chl from higher plant sources that enters the long-term sedimentary reservoir, through preservation of structures with the macrocycle intact, can be assumed to be essentially zero. Accordingly, the absolute abundances of porphyrins in coals, the main terrestrial reservoir of sedimentary organic matter, are considerably lower than in accumulations originating from aquatic environments. In addition, the depositional environments in which coals that contain porphyrins accumulate are most likely to be stagnant deltaic settings in which algae and phototrophic bacteria are present and possibly serve as the main sources of coal porphyrins.

B. Aquatic

Net Chl production in aquatic environments has been estimated at 8.63×10^8 tons per annum (Hendry et al., 1987). Chlorophyll preservation in aquatic environments is strongly influenced by the redox conditions and depth of the water column. Under strongly oxidizing conditions Chl photo-oxidation reactions have been implicated in destruction of the macrocycle, leading to removal of most of the Chl before it reaches the anoxic conditions that exist either in the lower reaches of the water column or within the first few centimeters of the sediment. Under fully oxic and intermediate redox conditions, heterotrophic processes are highly favorable and utilize the high-energy pathways associated with the use of oxygen as the oxidant. By contrast, under reducing conditions Chls can be exceptionally well preserved with the carbon skeleton being structurally conserved. Under intermediate redox conditions, or darkness, the macrocycle can remain intact but with modification at the periphery through oxidation reactions centered on ring E (Naylor and Keely, 1998; Airs et al., 2000; Walker et al., 2002). The major Chls found in aquatic sediments typically comprise algal pigments, the most important being Chl *a*. In some situations, however, the major Chls comprise bacterial pigments originating from anoxygenic purple and green photosynthetic bacteria (Villanueva et al., 1994b; Squier et al., 2002, 2004a). These organisms require dysaerobic (purple bacteria) and anoxygenic (green bacteria) conditions, respectively, and can be quantitatively important components of the primary producer community (Hurley and Watras, 1991; Vila et al., 1998; Villanueva and Hastings, 2000; Chen et al., 2001). Owing to their intolerance to oxygen, these bacteria utilize H_2S as the electron donor in photosynthesis, leading to the production of elemental sulfur rather than molecular oxygen. Thus, the presence of bacterial Chls as the major components in sediments indicates the existence of anoxic and euxinic conditions within the photic zone, and prevalence of conditions favorable to the preservation of organic matter. Such conditions are often associated with highly productive lakes, where eutrophication leads to depletion of oxygen in the water column, or very steep-sided and deep lakes where wind mixing is minimal and the waters can become stratified, but can also occur in estuarine settings. The other fundamental requirement, the presence of H_2S, demands a significant activity among the sulfate reducing bacterial community. Sulfate reducers are heterotrophs that rely on relatively low energy-yielding oxidation reactions in which sulfate is the source of the oxidizing power. Thus, the relative abundances of Chl oxidation products versus indicators of reducing conditions reflect the redox status of the depositional environment and the prevalence of different communities of heterotrophic and autotrophic bacteria.

C. Water Column Studies

Temporal studies of contemporary lake and marine waters reveal the great variability in pigment composition that accompanies species succession in natural aquatic environments (Villanueva et al., 1994b; Walker and Keely, 2004). The duration of photosynthetic activity of individual species of primary producers varies, ranging from a matter of a few days for some microalgae through to several months or even years in the case of macroalgae. The

pigments provide a good means to monitor changes in the primary producer community, and changes in the nature and abundances of transformation products reflect the processes of senescence and grazing activity on the bloom. For example, the succession of transformation products during the overturn of a bloom in the Celtic sea commenced with demetallation, de-esterification and decarbomethoxylation reactions, followed by increasing extents of oxidation reactions with increasing exposure of the pigments to the oxidizing conditions within the water column (Walker and Keely, 2004). The oxidation reactions become increasingly important with duration of exposure within the water column and so appear, to some degree, to be dependent on the particle size, which exerts a strong influence on settling rate.

D. Sediment Studies

Early studies of pigment compositions in sediments largely focused on the identification of novel structures and the development of an understanding of the transformation pathways by which the original Chl derivatives are gradually converted into alkyl porphyrins. More recently, in the light of the increased knowledge of the structures and their significance, the focus has shifted towards characterizing the variability in conditions within depositional environments through development of detailed stratigraphic profiles of pigments. For example, Ocampo et al. (1992) examined changes in the distributions of alkyl porphyrins in the Messel shale and demonstrated that the distributions differ markedly over the timescale represented by the sediment core examined, reflecting the changes in depositional environment within the anoxic basin. Similarly, Keely et al. (1995) demonstrated changing profiles of porphyrins within a narrow time zone within the Miocene Vena del Gesso evaporitic sequence. In those studies the stratigraphic resolution that could be achieved was limited by the length and complexity of preparative stages prior to analysis. The development of on-line LC-MS approaches has allowed dramatic improvements in the stratigraphic resolution that can be achieved.

In an attempt to examine the extent to which pigment variations can be monitored over very short timescales, sediments have been examined at cm and sub-cm scale resolutions. The record of sedimentary pigments from an Antarctic lake was shown to exhibit dramatic shifts between a lacustrine and a marine influenced environment, which was evident with sampling at cm scale resolution (Squier et al., 2002;

Walker et al., 2002). Furthermore, the profile within the marine section reveals subtle variations in pigment structure that probably relate to population shifts within the anoxygenic bacterial community (Wilson et al., 2004a). The studies demonstrate clearly that much detail on population changes, which are intrinsically linked to environmental conditions, is contained within the sedimentary pigment record. Through further examination of high-resolution stratigraphic records of pigments from appropriate sedimentary records (i.e., those in which bioturbation is absent), the significance of changes in pigment compositions in relation to variability within different depositional environments will become clear.

Ultra-high resolution studies of pigments in sediments were carried out in a microbial mat (Airs and Keely, 2003). Sampling at intervals of 2 mm demonstrated the feasibility of conducting pigment analysis at this scale and revealed that substantial changes in the pigment profiles are apparent at this stratigraphic resolution. Further ultra-high resolution studies of sediments from a range of depositional environments are warranted in order to determine the extent to which pigment profiles record short term variations in environmental conditions and the types of depositional environments in which variations are most marked.

IV. Transformation Scheme

It is evident that a much greater variety of reactions affect Chls than those recognized in the Treibs Scheme. In an attempt to reflect the number and nature of the reactions to which Chls are subjected a revised transformation scheme has been developed (Fig. 2). Although the scheme goes some way to addressing the complexity of processes in the natural environment, it is evident that there are still gaps in understanding. For example, it is not yet possible to include transformation reactions that would lead to the cleavage of the C-3 substituent to generate precursors for the C-3 Me or C-3 H porphyrins, structures that are evident in ancient sediments. Clearly, therefore, it must be recognized that the scheme is incomplete. It is hoped that it might serve as a stimulus to direct efforts towards identifying the missing links: increased understanding of the structures and the mechanistic basis by which sedimentary tetrapyrroles are formed is still needed in order to liberate the full potential of the pigment geochemical record.

Fig. 2. Representation of various transformation reactions to show the expansion of the Treibs scheme as illustrated for Chl *a*. For simplicity, only one functionalized derivative and one potential product is shown in each case. The defunctionalization reactions leading to the alkylporphyrin products can be assumed to follow pathways such as those outlined in Fig. 1.

V. Applications

Development of applications to which the analysis of sedimentary pigment distributions is beneficial continues to be an active area. One of the most compelling reasons for an interest in the geochemistry of these derivatives is their origin in the primary producer community. Thus, their signatures provide informa-

tion about the most fundamental component of the food web, on which the vast majority of other forms of life depend. Some applications have already been realized and others have the potential for development in the future.

A. Realized

Specific Chl pigments act as biological markers for the presence of selected broad groups of organisms. Thus, Chl *b* indicates the presence of green alga (Chlorophyta) and Chl *c* indicates a contribution from one or more of a restricted range of algae including diatoms, dinoflagellates, and cryptophytes. The BChls are more specific: BChl *a* indicates an input from purple bacteria, the BChls *c* and *d* an input from Chloroflexaceae or from green strains of Chlorobiaceae, and BChl *e* indicates contributions from brown strains of Chlorobiaceae. Similarly, the absence of an accessory Chl pigment accompanying Chl *a* is suggestive of contributions from cyanobacteria or some cryptophytes. In addition to revealing contributions from specific primary producers, the algal pigments indicate regions of oxygenated waters whereas the bacterial pigments (with few exceptions) indicate photic zone anoxia and euxinia (see above). Where algal and bacterial pigments both occur, it is likely to suggest either stratified waters, or redox conditions alternating at a frequency higher than that represented by the stratigraphic resolution achieved by the sampling. The other main applications suggested for pigment geochemistry at the present time are the assessment of past productivity (Harris et al., 1996), the impact of grazers on the algal community from examination of CSE distributions and amounts (Dahl et al., 2004), and the indication of different relative extents of oxygen availability from the relative abundances of Chl oxidation products versus unaltered derivatives (Walker et al., 2002).

B. Outstanding Issues and Potential Developments

Further advances are required in order to develop the uses of pigments as rigorous proxies for environmental parameters. The potential applications include quantitative estimates of: grazing pressure; extent of senescence; oxygen availability; sulfur incorporation into sedimentary organic matter and photic zone anoxia and euxinia. It is evident that pigment abundances in sediments are influenced

both by the productivity and by the extent of preservation: decoupling these two factors is an issue of importance to the assessment of carbon cycling as well as being of specific interest in the area of pigment geochemistry. In general, approaches required to develop pigment-based proxies will involve evaluation and validation of the proposed proxy against the parameter of interest both in controlled laboratory trials and in field trials. For BChls, where the distributions exhibit marked variation in response to different environmental conditions, studies need to be conducted in conjunction with controlled manipulation of bacterial cultures. In addition, the stabilities of components included in the proxies need to be established over the longer term.

One important issue that remains to be addressed fully is the determination of the controls on the metallation of sedimentary porphyrins. While it is clear that metallation is related to the availability of various metals in the depositional environment and their relative stabilities in the porphyrin macrocycle, the connection between metal availability and speciation remains to be translated from a theoretical consideration based on E_H pH stability fields (Lewan and Maynard, 1982, Lewan, 1984) to one based firmly on environmental measurements. The difficulty here is that transmetallated functionalized tetrapyrroles have not been observed in natural aquatic systems prior to deposition in sediments, hence it appears that sediments at the appropriate stage of diagenesis need to be examined.

C. Nature of Transformation Reactions

The exact nature of the reactions leading to a number of specific transformation products in the natural environment remain to be established. These include the reactions that yield the Chlone and CyPheide enol structures, reactions leading to the sulfur-containing Chl derivatives, reactions leading to CSEs and CCEs and the precise mechanisms leading to the formation of Chl oxidation products.

D. Quantifying Possible Links with Climate (Environmental Variability)

A major goal over the next few years is likely to be the establishment of links between the pigment distributions preserved in sediments and factors controlling climate. The primary producer community is undoubtedly strongly influenced by climatic changes

and it is highly likely that these influences are recorded within the pigment record either through changes in species composition (though this would be difficult to decouple from other forcing factors) or through changes in the specific nature and the abundances of various transformation products. Such influences on the pigment compositions are most likely to be revealed by studies in which other biological markers are evaluated in conjunction with the pigments, for example, carotenoids, UV screening pigments such as scytonemin (Squier et al., 2004c) and lipids are all possible candidates.

VI. Concluding Remarks

Since its development nearly 100 years ago, pigment geochemistry has evolved from a focus on the characterization and identification of individual compounds as biological markers to a field focused more on the examination of complex pigment distributions for the evaluation of past environments. This development reflects that the field is developing towards, but has not yet achieved, a mature status. The developments over the next few years are likely to arise from studies that involve combinations of laboratory and field studies aimed at revealing the factors that are of greatest importance in controlling pigment compositions and transformation reactions. It is likely that a variety of new analytical approaches will be developed and, through the use of rigorous methods to separate and identify components in complex mixtures, that further novel Chl transformation products will be identified. The ultimate goal might be to construct three dimensional maps from temporal and spatial distributions of pigments and to translate these to reconstruction of environmentally important parameters, for example intensity of primary productivity, heterotrophy and water column redox potential and the prevalence of euxinia in times past.

Note Added in Proof

The following notes summarize developments in the field that have been published since the writing of this chapter.

Grazing experiments with freshwater crustaceans examined the formation of CSEs from mixed phytoplankton sources, confirming a significant level of incorporation of cholesterol from the grazer into the CSEs along with the sterols of the dietary phytoplankton. Grazing of mixtures of algae containing sterols ranging from C_{27} to C_{29} revealed a bias towards incorporation of C_{29} sterols within the CSEs, attributed to easier metabolism of the lower carbon numbered species. The potential for under-representation of contributions from specific algal sources was noted (Soma et al., 2005).

Comparison of pigment decay rates in oxic and oxygen depleted sites in the Mississippi Delta led Chen et al. (2005) to conclude that sedimentation rate and lability of organic matter were more important than bottom water hypoxia in controlling the preservation of pigments, and organic matter in general, in sediments. Reuss et al. (2005) showed clear differences in pigment abundances and distributions between sites that exhibited significant differences in the environmental conditions and sedimentary regime and concluded that more work is required in order to assess the full potential of pigments as biomarkers in marine and estuarine environments.

Studies of older sediment sequences have examined variations in pigments over glacial-interglacial periods. Nara et al. (2005) showed distinct changes in algal-sourced pigments over glacial-interglacial cycles and suggested that changes in productivity had occurred as a result of increased fertilisation due to higher precipitation during the warmer climatic periods. Squier et al. (2005) similarly observed marked changes in pigment profiles and also detected periods of water column euxinia from the presence of BPhys *e,* markers of brown strains of green sulfur bacteria. Both studies demonstrate that extreme climate events can exert major influences on aquatic, resulting in clear signatures in the sedimentary pigment record. Another interglacial sediment also yielded BChl *e* derivatives indicative of photic zone euxinia (Mallorqui et al., 2005). The recognition of derivatives of BChl *e* in sediments means that the preservation of each of the BChls of green sulfur bacteria has now been demonstrated in the sedimentary record, further supporting the view that all of these BChls are possible precursors of sedimentary porphyrins.

It is also noteworthy that a marine cyanobacterium in which the major pigment has been shown to be Chl *d*, the 3-desvinyl-3-formyl derivative of Chl *a,* has been identified as a free-living and widespread organism that inhabits environments restricted in visible radiation but enriched in near infrared (Larkum and Kühl, 2005). The significance of this

observation lies in the potential for Chl *d* to be a major source of 3-methyl and 3-H porphyrins in the geological record. Thus, these porphyrins may have value as indicators of defined biological inputs and of specific environmental conditions. It will be of particular interest to try to relate environments that the contemporary Chl *d*-containing organisms inhabit with paleoenvironments in which the 3-methyl and 3-H porphyrins are prevalent.

Acknowledgments

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