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On the structure of bacteriochlorophyll molecular aggregates in the chlorosomes of green bacteria. A molecular modelling study*

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Abstract

The supramolecular structure of methyl (3^1R)-BChlide *d* aggregation has been explored by molecular modelling in order to elucidate the unusual structure of the BChl rods in the chlorosomal antennae of green bacteria. The aggregate construction progressed from a BChlide monomer in 5c coordination which was stepwise combined to form trimeric, pentameric and decameric chlorin stacks, all incorporating Mg· · · · O-H as a basic interaction element which links two chlorins between the 3^1-hydroxyl oxygen and the Mg. Up to the level of the trimer, the structures were optimized by both a semiempirical quantum chemical method (PM3) and a force field method, while larger structures were only modelled by the force field (MM+). Strong interactions were found by extended stacking of chlorins which are in van der Waals contact. Extended hydrogen bonding networks upon stack pairing brought about by OH· · · · O = C bonds (bond length ca. 2.2 Å, angle 139–153°) between appropriately situated chlorin pairs and by electrostatic interactions lead to very large energy stabilizations. The structural features of a modelled 40mer BChl aggregate are in full accord with all spectroscopic and low-resolution structural information on the in-vitro and chlorosomal BChl aggregates. Most important, from the rotation angle between stacks of ca. 16° and the stack-to-stack distance of 7.6 Å a tubular structure can be extrapolated to form on further extension of the aggregate. It has a predicted diameter of about 5.4 nm (Mg-Mg distance), i.e. very similar to that found for the rod elements in the chlorosomes of *Chloroflexus*.

Abbreviations: BChl – bacteriochlorophyll; BChlide – bacteriochlorophyllide

Introduction

Chlorosomes, extramembraneous antenna systems of green bacteria, are organelles of about 12 nm in height, 100 nm in width and 300–400 nm in length (Olson 1980; Staehelin et al. 1978; Staehelin et al. 1988). A single chlorosome may contain up to 20.000 molecules of bacteriochlorophyll (BChl) *c*, *d* or *e* as the major pigment, surrounded by a single lipid layer (Staehelin et al. 1978). The internal space, which hosts the BChl *c* or *d*, has been found by electron microscopy to be filled mostly with rods of ca. 5–10 nm diameter (Staehelin et al. 1978, 1980). One proposal was that these rods are formed by BChl-protein complexes which, like in other chlorophyll proteins, should control the relative orientation of the BChls (Feick and Fuller 1984). An alternative model suggested that the chlorosomal BChl *c* or *d* organization was determined by pigment-pigment interaction, based on the optical properties of isolated BChl *c* in organic solvents which were very similar to those of intact chlorosomes (Bystrova et al. 1979). It had been hypothesized that these in-vitro BChl aggregates should be good models for the in-vivo chlorosomal structures. In fact, BChl *c* and *d*, by virtue of their 3^1-hydroxy group, are ideally suited to form molecular

* Dedicated to J.J. Katz, pioneer in the study of chlorophyll aggregation.

aggregates (Smith et al. 1983b), and neutron scattering of in-vitro BChl *d* aggregates (Worcester et al. 1986) revealed indeed rod shapes with approximately the diameters found also in the chlorosomes.

Subsequent spectroscopic studies of in-vitro BChl *c* and *d* aggregation revealed, among other structural details of the BChl–BChl interaction, the essential element Mg····OH····O = C(13^1) (Hildebrandt et al. 1994; Olson and Pedersen 1988, 1990; Uehara et al. 1991; Brune et al. 1987; Chiefari et al. 1995). There is now general agreement that aggregation both in chlorosomes and in large in-vitro aggregates involves the interaction of the 3^1-OH oxygen of one BChl *c* or *d* with the Mg of a second BChl (Hildebrandt et al. 1991, 1994; Brune et al. 1987), and that the OH hydrogen should be hydrogen-bonded to the 13^1-keto group of a third BChl (Hildebrandt et al. 1991, 1994; Chiefari et al. 1995; Olson et al. 1990; Uehara et al. 1991). Such an arrangement explains all data both from vibrational spectroscopy and chemical modification studies of BChl *c* and *d* (Hildebrandt et al. 1991, 1994; Chiefari et al. 1995; Olson et al. 1990; Uehara et al. 1991; Tamiaki et al. 1992, 1994a,b; Smith et al. 1983b). Furthermore, strong excitonic coupling, which shifts the absorption to around 740 nm, has been attributed to a close interaction between different BChl rings (Olson 1980; Olson et al. 1985). Despite the large body of spectroscopic information on the in-vitro BChl aggregates and the rod shape of the aggregates, the BChl-protein complex model for intact chlorosomes (Wechsler et al. 1985) persisted until Griebenow and Holzwarth prepared chlorosomes – the so-called GEF chlorosomes – lacking any significant amount of proteins but nevertheless retaining the spectroscopic properties of intact chlorosomes (Griebenow and Holzwarth 1989). Subsequent spectroscopic studies revealed that the GEF chlorosomes actually possess the same structural BChl *c* organization as intact protein-containing chlorosomes (Holzwarth et al. 1990; Griebenow et al. 1991; Hildebrandt et al. 1991), in accord with the concept of self-organized molecular BChl *c* (or *d*) aggregates forming rod elements and maintaining their structure without any essential interaction with proteins (Holzwarth et al. 1990, 1992; Hildebrandt et al. 1991). However, there exists as yet no structure determination of such aggregates in intact chlorosomes, neither by any high-resolution scattering method (in the absence of any highly structured aggregate or crystal of BChl *c* or *d*) nor solid-state NMR (Nozawa et al. 1990).

We have now chosen to supplement the spectroscopic and low-resolution structural data by a force-field molecular modelling study (Jörgensen 1989; Dearing 1988) of the chlorosome structure problem. In particular, it will be shown that methyl BChlide *d* aggregation (according to the free energy calculations) can form highly stable tubular structures and that all of their predicted properties are in excellent agreement with the data on BChl *c* and *d* in chlorosomes and in-vitro aggregates.

Methods

The program package 'HYPERCHEM version 3.0' running on an IBM-compatible personal computer (Intel Pentium 586 processor) has been used for all the molecular modelling calculations. Semiempirical quantum-chemical calculations have been performed by the PM3 method (Stewart 1990a, b), which is a reparametrization of the AM1 method (Dewar et al. 1985). For the force field calculations the MM+ force field in HYPERCHEM was used, which is based on the MM2 force field by Allinger (1977) (using 1991 parameters). Since the original MM2 force field contains no parameters for metals, HYPERCHEM 3.0 uses Mg parameters from a supplemental generic force field similar to the one described by Mayo et al. (1990) in accord with the general MM2 structure (for further details see 'HyperChem Computational Chemistry, Part 2: Theory and Methods', Autodesk, Inc., Publication 100198–02). In all cases the original parameters provided in the HyperChem package were employed. In order to speed up the MM+ calculations, non-bonding interactions, in particular Coulomb interactions, were cut off by a switching function of 15 Å inner and 20 Å outer radius. Since aggregates involving a large number of atoms had to be calculated, it was essential to reduce the number of atoms as much as possible. Thus, all calculations were carried out for methyl BChlide *d* (which lacks the C(20) methyl group of BChl *c*), with methyl substituents at C(8) and C(12). Furthermore the methyl ester was chosen in the assumption that the ester side-chains were not decisive in the structural organization of the chlorins and their interactions. Apart from this all atoms, including hydrogens, were explicitly taken into account (no pseudo atom approach was used). For the energy minimization a steepest-descent method was chosen in the first steps until the largest strain had relaxed. The Newton-Raphson method was used for the main part of the

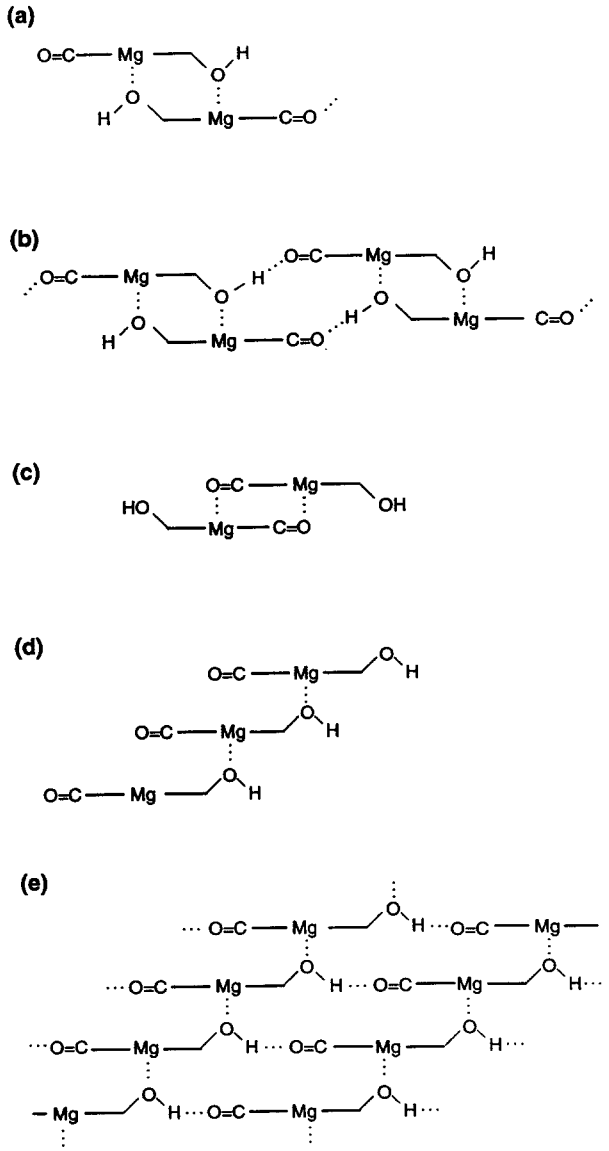

Fig. 1. Models of aggregate structures which have been tested both by semi-empirical quantum chemical (PM3) and force field (MM+) methods.

optimization. Generally a stop criterion of gradient < 0.01 or smaller was employed.

Results and discussion

A variety of aggregation and interaction models for the BChls *c* and *d* has been proposed. Most of these models, ranging from various dimer arrangements to some higher aggregates, have been tested in our approach, applying both PM3 and MM+ methods (see Fig. 1 for a list of structures). These studies will be described in detail elsewhere. We focus here only on the model(s) which have led to a stable structure with properties that are in agreement with experimental data of chlorosomes and in-vitro aggregates.

Modelling of BChlide d *monomer and small aggregates*

We started with the structure optimization of a BChlide *monomer* in a 5c coordination. The fifth ligand was provided by the oxygen of an isopropyl alcohol attached to the Mg. The most stable structure, energy-minimized by PM3, had a slightly bowl-shaped chlorin skeleton, with the Mg dislocated out-of-plane of the nitrogens by 0.25 Å (not shown) and the fifth ligand in anti configuration to the ester side-chain. These features are in good agreement with X-ray data of 5c chlorin structures (Chow et al. 1975; Kratky and Dunitz 1986). Maintaining the distribution of partial charges determined for PM3-optimized BChlide, the structure was then again energy-minimized, this time by MM+. In comparison to the PM3 structure a slight reorganization of the chlorin ring was observed, and the Mg was somewhat less out-of-plane. However, these changes were modest and affected neither the general bowl shape of the chlorin nor the out-of-plane position of Mg.

BChlide *dimers* with HO···Mg bonds were optimized next by PM3 and then used as building blocks for larger aggregates (tetramers and hexamers) employing MM+ (cf. Fig. 1a,b). While several of such higher aggregates resulted in energetically stable structures which possibly are relevant for some in-vitro aggregates, none of them showed structural features consistent with the rod-like BChl organization in chlorosomes. For this reason such aggregates are not described here in detail either.

In a next step various *trimers* were generated, with the aim to model a BChlide aggregate capable of winding up in a circular or helical fashion. For this reason the BChlides were attached to each other via the 3^1-OH oxygen coordinating to Mg of the next BChlide (see Fig. 1d). In such a structure the BChlides can be attached to each other with the Mg···OH bonding either syn or anti to the ester side-chain. A further differentiation – by the stereochemistry at C(3^1) – has not yet been introduced. Rather, the calculations were carried out for the (3^1R) configuration only, which corresponds to the more abundant diastereoisomer of BChl *c* in *Chloroflexus* under our conditions (Fages et al. 1990). Several cycles of PM3 followed by MM+ opti-

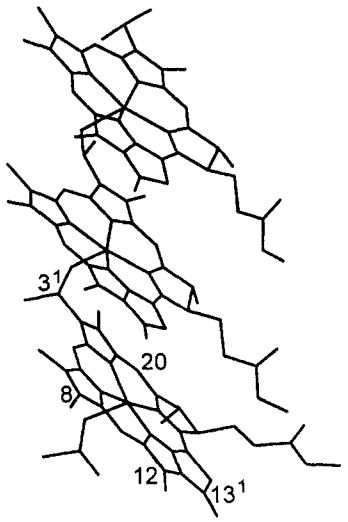

Fig. 2. Trimeric methyl BChlide *d* stack. The magnesium of the terminal (bottom) chlorin is capped with an isopropyl alcohol molecule. The structure was optimized consecutively by PM3 and MM+. The viewing angle is selected for showing as much detail as possible. For the purpose of comparison with the pentameric stacks shown in Fig. 3, this trimer should be rotated around its long axis. *Note:* In this and the following figures hydrogens are omitted from the structures, except for those hydrogens involved in a hydrogen bond (indicated by dashed lines).

mizations (always preserving the partial charges from the PM3 calculation) were carried out on such possible arrangements of BChlide trimers from a variety of starting structures.

The results can be summarized as follows: The Mg····OH coordination anti to the ester side-chain was energetically favored substantially (about 40 kJ/chlorin) over the syn arrangement in all cases, in accord with the study of the 5c BChlide monomer. Furthermore, all trimers tended to form planar stacks with the chlorin planes tilted by about 34° with respect to the connection axis of the BChlides. In these stacks the two 5c chlorins assumed equal conformations, whereas the one of the terminal 4c chlorin deviated substantially (not shown). This irregularity was avoided by capping the terminal chlorin with methyl or isopropyl alcohol in order to compensate for the missing hydroxyethyl ligand from the hypothetical next BChlide in a longer chain. The most stable trimeric structure consecutively optimized by PM3 and MM+ is shown in Fig. 2. The chlorins are in van der Waals contact and assume an energetically relaxed conformation similar to that of the optimized 5c monomer. The interactions between the chlorins are quite favorable. In addition to the O····Mg coordination, electrostatic interactions between the chlorins contribute strongly to the stacking. For example, the negatively charged 13^1-keto oxygen of one chlorin is close to the positively charged Mg of the adjacent chlorin.

The trimers optimized by PM3 alone and by PM3 followed by MM+ are quite similar. This is encouraging for the modelling of larger aggregates which cannot be treated anymore by PM3 but only by force-field methods.

The proper charge distribution in the chlorins, as calculated by PM3, is essential for achieving this stacking. When all partial charges on the atoms of the chlorin rings were set to zero and the standard bond dipoles were used in the FF calculations instead, such a stack did not form. Rather, a fairly irregular structure with a flat potential energy surface results. The decisive contribution of the electrostatic interaction to the stabilization is reflected in its contribution to the total energy (Table 1). This finding is in line with recent reports attributing the π-π-interaction mainly to electrostatic interaction (Hunter and Sanders 1990).

The stacking of the trimer in Fig. 2 can evidently be extended in both directions along the axis. The OH hydrogens point all in one direction of the stacks, and the 13^1-keto groups in the opposite direction. This suggests that two or more of such (extended) stacks in parallel might possibly implement the OH····O = C hydrogen bonding network, required by the spectroscopic data, into a stable two- or three-dimensional aggregate structure.

Building of longer stacks

Higher aggregates formed from two and three of the trimeric stacks were placed next to each other and were optimized by MM+. These arrangements, forming the above-mentioned hydrogen bonding network, indeed resulted in a substantial stabilization of the total energy as compared to the energies of the individual trimer stacks. However, the cut-off effects in the interactions at the border of such small stacks are unfavorable towards a completely regular structure. In order to obtain longer stacks, the central chlorin of the PM3-optimized trimer was replaced by units of two or more of its copies, retaining the individual charge distributions of all chlorins. The resulting higher aggregates (up to decameric stacks were generated) were then energy-minimized by MM+. With respect to the relative orientation of the chlorins and their individual conformations, the energy-minimized structures were basically identical to that of the optimized single trimer

Table 1. Energies (kJ/mol) of optimized structures from force field (MM+) calculations on various BChlide *d* aggregates (for details of the calculations see text). All stacks were capped with propanol or isopropyl alcohol at the terminal BChlide *d* in order to have all Mg atoms five-coordinated

Number of BChlides *d* in stack	Number of stacks in aggregate	Total energy	Van der Waals energy	Electrostatic energy	Energy per BChl monomer
1 (Monomer with 5c Mg)[a]	1	75	29	− 258	75
3	1	− 75	− 54	− 821	− 25
5	1	− 196	− 120	− 1433	− 39
5	2	− 2888	− 317	− 5504	− 288
5, 10, 10, 10, 5[b]	5	− 21416	− 1216	− 32408	− 539

[a]BChlide *d* capped with isopropyl alcohol coordinated to Mg.

[b]Aggregate containing five stacks; the peripheral stacks have 5 BChl/stack and the inner three stacks have 10 BChl/stack.

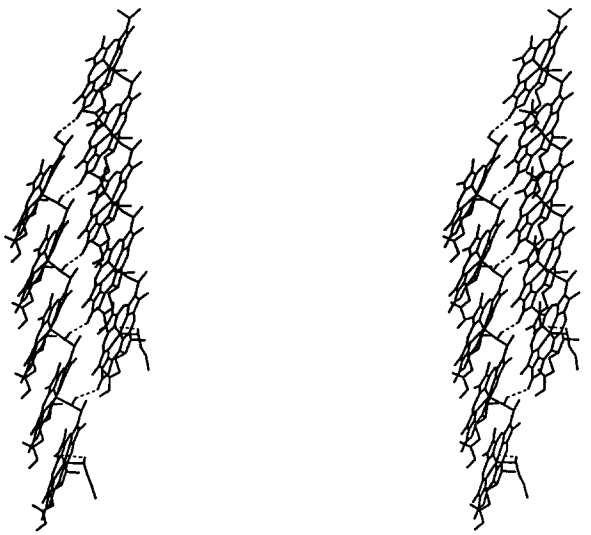

Fig. 3. Stereoview of an aggregate of two pentameric methyl BChlide *d* stacks, energy-minimized by the force-field method (MM+). The magnesium atoms of the terminal (bottom) chlorins are capped with a propanol molecule. Note the out-of-plane position of Mg and the bowl-shaped chlorins. Hydrogen bonds are shown as dashed lines. The view shows the inside of the tentative tubular structure.

(Fig. 2) and also pentamer (Fig. 3). For the contributions of the interactions to the total energy see Table 1.

Large aggregates of pentameric and decameric stacks

Two pentameric stacks were then placed parallel next to each other enabling close hydrogen bonding interactions between the hydroxyls of one stack with the 13^1-keto groups of the other. This structure was energy-minimized by MM+. No unfavorable van der Waals interactions with or between the ester side-chains occurred after an appropriate conformational search for the ester chain. The HO···Mg bridge can assume two different configurations in a stack, i.e., the free electron pair on oxygen and the hydrogen can be interchanged. Only one of these configurations allows a favorable hydrogen bonding to the 13^1-keto group. For the (3^1R) epimer the favorable orientation corresponds to the (*R*) configuration of the oxygen. The optimized hydrogen bond distance was $\leq$ 2.5 Å. In order to speed up the calculation for the larger aggregates involving several pentameric and larger stacks, the hydrogen bond distance (OH hydrogen-to-keto oxygen) was first restrained to 2.5 Å and a series of minimization steps was carried out in order to allow the system to relax. When the gradient in the minimization algorithm had reached a value of less than about 0.3, these restraints were removed and the system was further energy-minimized in an unrestrained manner. The unrestrained system tended towards the same conformation as well, although more slowly. No other restraints were applied. In this way aggregates of two, three and five pentameric stacks were docked together.

Both the energy minimization and the computer presentation of the stacks as space-filling models indicated early that a rotation of each stack by a small angle around its long axis relative to the adjacent stack provides for the optimal hydrogen bonding interaction and avoids unfavorable van der Waals interactions. *This is important since it could eventually lead to a circular stack arrangement, i. e., to tubular structures making*

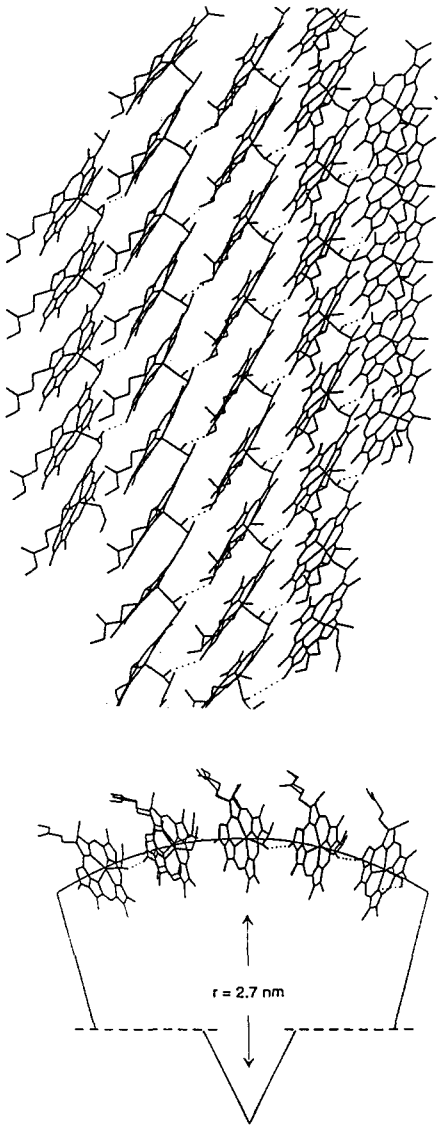

Fig. 4. Energy-minimized aggregate of 40 methyl BChlides *d* arranged in five stacks each of 5, 10, 10, 10, and 5 chlorins, in partial views from the outside of the tentative tubular structure (above) and the top (below). The bottom representation indicates the sector of the tubular structure (diameter ca. 5.4 nm for the Mg-Mg distance) which is obtained on extension of the 40mer aggregate. Hydrogen bonds are indicated by dashed lines.

up the rods in the chlorosome which we are searching for. The energy-minimized aggregate consisting of two pentameric stacks is shown in Fig. 3.

Figure 4 shows the largest energy-minimized aggregate created so far, made up of 40 methyl BChlides *d* arranged in five stacks each of 5, 10, 10, 10, and 5 chlorins. This arrangement minimizes cut-off effects and permits to determine, in the center of this aggregate, the optimal parameters for hydrogen bonding and stack rotation: the hydrogen-to-keto oxygen distance is 2.2 ± 0.1 Å, the hydrogen bond angle is 139–153° (which encompasses the values of hydrogen bonds in the aggregate center and at the periphery), and the long axis of each stack rotates by an angle of 16 ± 1° relative to the adjacent stack. The energy parameters are given in Table 1.

Is this structure reasonable for the BChl aggregates in chlorosomes?

This question can be answered positively in every respect. From the extensive conformational search for the monomer, trimer and pentamer we are confident that these arrangements correspond to global minima within the force field used. The conformation of each chlorin aggregate is close to that of the monomeric 5c BChlide and the intra-stack interactions are favorable (Table 1). Furthermore, the relative orientation of adjacent stacks in the aggregates shown in Figs. 3 and 4 with respect to the stack rotation corresponds to the global minimum, since the optimization had been started from several different rotation angles and had always ended in the same minimum. The energy stabilization due to stack pairing is very large (Table 1). It is determined by electrostatic interactions and hydrogen bonding. *Evidently, the MM+ modelling is internally consistent and the proposed large aggregate structure represents a highly stable conformation at or close to the global minimum within the MM+ limitations.*

Is this structure also reasonable from a chemical point of view? Intuitively one would expect chlorins to orient themselves sandwich-like. Both $\pi\pi$ interactions as well as electrostatic interactions have been discussed in the literature (Abraham and Smith 1983; Smith et al. 1983b). Given the restrictions imposed by the hydroxyethyl···Mg bridge, two adjacent BChlides in a stack orient themselves favorably with regard to the partial charges on nearby atoms of different chlorins. Several close-lying pairs of opposite charges exist. The most important one is the keto oxygen of one chlorin and the Mg of the adjacent chlorin. It is of particular interest that the energy-minimized chlorin conformation with an out-of-plane Mg and an anti configuration of the fifth ligand are in agreement with the X-ray structure of ethyl Chlide *a*, which is a close analog to BChlide *d* and which is present in a 5c coordination in the crystal (Kratky and Dunitz 1977; Strouse 1974; Chow et al. 1975). The Mg-N distances in the structures

of Figs. 3 and 4 (2.02–2.06 Å) are in good agreement with the BChlide *a* data (2.02–2.16 Å). Furthermore the optimal relative arrangement within a stack is very similar to the packing in the 'one-dimensional polymer' found in crystalline ethyl Chlide *a* dihydrate (Kratky and Dunitz 1977; Strouse 1974) and in pheophytin *a*, and it is close to the principal structure suggested by Smith et al. (1983b) for BChl *c* and *d* oligomers.

In the ethyl Chlide *a* crystal the hydrogen bond bridging is provided by a water molecule. In the BChl *c* and *d* aggregates this function is taken over by the hydroxyethyl group. The respective bonding requirements of the hydroxyethyl group put more strict limitations on the relative orientations of chlorins than in the ethyl-Chlide a dihydrate. All modelling runs suggest that the keto group as an additional ligand to Mg, as proposed earlier (Bystrova et al. 1979; Lutz and Van Brakel 1988) and spatially favorable, would lead to an energetically highly strained conformation and can thus be ruled out. Such a coordination is in fact not necessary since the equally strong electrostatic interaction exerts fewer conformational restrictions than an actual coordination. The ester side-chains, even with a long alkoxyl rather than the methoxyl used in the simulations, are directed away from the stack and do not sterically hinder the stack orientation.

An inter-stack hydrogen bond is readily formed between each pair of chlorins, without introducing steric hindrance and with hydrogen bond lengths and angles indicating strong bonding. This and further electrostatic interactions make up for the large stabilization energy of the stack-stack interaction (Table 1). Here the trends only are essential since they are independent of the method of calculation employed, whereas the actual numbers may depend on details of the method.

The factors discussed so far are necessary but insufficient requirements for accepting the structures as a model for the BChl organization in chlorosomes. The most important structural element that has come forth from vibrational spectroscopy is the Mg····OH····O = C interaction. It is fully incorporated in the models of Figs. 3 and 4. Also, the close Mg-Mg distance of 6.8 ± 0.2 Å within a stack is consistent with the strong exciton interaction in chlorosomes and BChl aggregates. However, other arrangements, e.g. those using dimers as building blocks (Fig. 1b) also possess several of these structural elements and could insofar also qualify as chlorosome models on this basis alone. The decisive discrimination between the different models is the ability to adopt a rod-like structure. Inspection of Fig. 4b suggests that a tubular aggregate structure, hence a rod shape, is likely to form if this arrangement, and none of the others we have modelled, is properly extended. The rotation angle between stacks is ca. 16°, which would result in 22 ± 2 stacks per closed ring. Taking this rotation angle and a stack-to-stack distance of 7.6 Å, diameters of either 5.4 nm (based on the Mg-Mg distance) or 6.0 nm (based on the chlorin edge-to-edge distance) can be calculated for the anticipated tubular structure. This would be consonant with the rod diameter of 5.2 nm found by electron microscopy for the chlorosomes of *Chloroflexus*. Preliminary modelling runs suggest that such a tubular structure can indeed be formed without any difficulty. All ester side-chains would point towards the outside of the rod and the aliphatic C(8) and C(12) substituents to the inside. The core of the rod is a large hole of about 5 nm diameter. The interactions between adjacent BChls on the inside of the 40mer tube indicate that a smaller bending than that found for the stack of Fig. 4 should result when the substituents at C(8) and C(12) are larger (note that these substituents in Fig. 4 are methyls and that substituents of up to neopentyl occur at C(8) of BChl *d* (Smith et al. 1983a)). Thus, tubes with larger diameters than extrapolated above may readily be formed as well (note that rod diameters of up to 10 nm have been found in *Chlorobium* chlorosomes and in artificial aggregates from *Chlorobium* BChl *d* (Worcester et al. 1986; Staehelin et al. 1980).

Finally, taking into account that the Q_y transition moment of a single chlorin being directed from nitrogen III to nitrogen I, the main optical transition in the proposed tubular structure will be oriented along the long axis. This is in accord with the polarization of the long-wavelength transition found in intact and GEF chlorosomes (Griebenow et al. 1991; Van Dorssen et al. 1986) – another criterion for evaluating the structure developed in this work.

The overall arrangement of the computer-modelled tubular structure, with the ester side-chains pointing all to the outside, is in general agreement with that of the tubular micelle proposed by Worcester et al. (1986). However, the arrangement of the chlorins in the tube is quite different, determined by the hydroxyethyl ···· Mg coordination. Modelling shows that the bending of the chlorin plane as such is not responsible for the curvature (Fig. 4b) leading to the tubular structure as proposed by Worcester et al. (1986) (note that the chlorins are parallel to the rod surface in the Worcester model). Rather, the curvature of the tube results from the relative orientation in the chlorins of

the 3^1-hydroxyethyl and the 13^1-keto groups, which form the hydrogen bonding network.

Conclusion

The tubular structure expected to result from an extension of the computer-modelled 40mer, appears to represent an excellent model for the organization of BChl chlorosomes and aggregates. Nevertheless, an experimental verification of this structure is still desirable, although the basic features of the chlorin-chlorin arrangement and bonding should remain correct irrespective of possible insufficiencies in the force field which might cause some deviations from the actual structure.

We should finally like to point out here that in the computer-modelled structure all primary potential binding sites of the Chlides with proteins, i.e. the Mg atom and the keto group, are occupied by other BChlides already. This leaves no obvious favorable interaction sites with proteins, barring some sites of secondary importance such as the end of a rod. Also, the large stabilization energy of the large aggregates suggests that such structures can form without supporting interaction with a protein. This confirms our previously expressed view that the rods in chlorosomes arise from self-organization of the BChls in an unpolar environment and that they are maintained without requiring any major interaction with a protein (Holzwarth et al. 1990; Hildebrandt et al. 1991, 1992).

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