Chapter 26

Reconstitution and Pigment Exchange

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Summary

Reconstitution and pigment exchange are two experimental techniques that have proven extremely useful to elucidate structure-function relationships in chlorophyll (Chl)-protein complexes. In reconstitution experiments the Chl-binding apoproteins, usually in their recombinant form, are folded in the presence of pigments to form pigment-protein complexes that are often virtually indistinguishable from their native counterparts. Since both the protein and the pigment building blocks in such an assembly kit can easily be modified, this approach serves to elucidate the functional significance of the structural elements modified. Pigment exchange can be viewed as a partial reconstitution: rather than completely taking a Chl-protein complex apart and then reconstituting it, only a limited number of pigments is dissociated and then restored. This, too, allows alteration of the pigments bound to specific positions and, thus, to learn more about the functional contribution of these particular pigment binding sites. Reconstitution and pigment exchange are complementary techniques in that some complexes are accessible to pigment exchange that cannot (yet) be reconstituted in vitro.

This chapter lists a selection of recent examples where the reconstitution in vitro of light-harvesting complexes from purple bacteria and light-harvesting Chl-*a/b* complexes from higher plants has been instrumental in assessing their function and where the reconstitution of other Chl-protein complexes has opened up new possibilities for their analysis. Very different kinds of light-harvesting complexes all exhibit an astonishing capability of self-organization during in vitro reconstitution. It is proposed that, if this also reflects any significant in vivo feature, then this ability to self-organize may help to regulate the light-harvesting capacity by rapid dis- and re-assembly of the light-harvesting complexes.

An exciting new possibility, opened up by the pigment-exchange approach, is to place the Ni derivative of (B)Chl in well-defined binding sites. This pigment dissipates excitation energy extremely rapidly and, therefore, will help to elucidate pathways of excitation energy migration in photosynthetic complexes.

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I. Introduction

A number of chlorophyll- (Chl-) protein complexes can be reconstituted in vitro from their apoprotein and pigment components. The term reconstitution potentially leads to confusion because it often describes the integration of proteins into native or synthetic liposomes. In the context of this chapter it denotes the formation of Chl-protein complexes that are similar to or virtually identical with their native counterparts in the same environment. These complexes are not necessarily reconstituted in lipid vesicles during this process and several Chl-protein complexes can be reconstituted, i.e. formed, in the complete absence of lipids. Typically, the components to be reconstituted are mixed in detergent solution and brought under conditions promoting self-assembly, resulting in the formation of pigment-protein complexes. The Chl-protein complexes amenable to this technique include all Chl *a/b* complexes of higher plants and some of their relatives as well as the inner and outer light-harvesting complexes, LH1 and LH2, respectively, of purple bacteria. The advantage of assembling these complexes in vitro is the possibility of introducing modifications into the components. Thus, recombinant apoproteins or, in the case of the shorter LH1 and LH2 proteins, even synthetic peptides have been employed. Studies of the minor Chl *a/b* complexes CP29, CP26, and CP24 (Jansson et al., 1992) that are difficult to isolate in substantial amounts, have been greatly facilitated by their reconstitution with apoprotein overexpressed by bacteria whereby large amounts of the protein became easily accessible. More importantly, the use of recombinant or synthetic apoprotein makes it easy to introduce alterations into the amino acid sequence to perform mutational analyses of protein function. Likewise, in Chl complexes assembled outside the organism, or with its biosynthetic capacity altered, the pigment composition of the complex can easily be varied to yield information about the specificity of pigment binding by these apoproteins.

Pigment exchange can be viewed as a partial reconstitution of Chl-protein complexes. The complexes are not completely dissociated into pigments and proteins; instead one or more pigments are removed and then re-assembled. Pigment exchange has been established in several pigment-protein complexes that cannot be reconstituted in vitro such as the RCs in some purple bacteria, or allows for site-selective replacements. The pigment binding sites that are susceptible to pigment exchange reactions can be analyzed to determine the structural requirements and functional properties of the pigments bound.

In this chapter, the reconstitution and pigment-exchange studies reviewed will be grouped by the Chl proteins studied. No experimental procedures will be presented. For these, the reader is referred to the original literature cited or, in the case of Chl-*a/b* complexes, to a review focusing on various reconstitution techniques for these complexes (Paulsen and Schmid, 2001).

Abbreviations: CP24, CP26, CP29 – minor subunits of LHCII with apoproteins Lhcb6, Lhcb5, and Lhcb4, respectively; LH1, LH2 – Core and peripheral light-harvesting complexes of purple bacteria, respectively; LHCI, LHCII – light-harvesting Chl *a/b* complex of PS I and PS II, respectively; LHCI-730, -680 – subunits of LHCI with apoproteins Lhca1,4 and Lhca2,3, respectively; LHCIIb – major subunit of LHCII with apoproteins Lhcb1-3; PS I, PS II – photosystems I and II, respectively

II. Reconstitution

The Chl-protein complexes whose reconstitution has been described so far are listed in Table 1. In the following, a few selected examples are given of the kinds of questions that have been addressed and answered by reconstituting these complexes.

A. Light-harvesting Complexes of Purple Bacteria

Based on the dissociation experiments of R. Ghosh and, in particular of P. A. Loach, the latter group devised a simple procedure to reconstitute the core light-harvesting complex LH1 of purple bacteria: The α and β subunits plus BChl are mixed in detergent and then assembled by diluting the detergent below the critical micellar concentration (Parkes-Loach et al., 1988). Not only does this establish pigment binding to the protein but also the oligomeric complex absorbing at 873 nm is formed. Reconstitution studies of LH1 allowed a detailed analysis of protein segments involved in assembly (Meadows et al., 1995, 1998; Davis et al., 1997; Kehoe et al., 1998) as well as a description of the structural features in BChl that are essential for binding (Parkes-Loach et al., 1990; Davis et al., 1996).

The core light-harvesting complex LH1 from several strains of purple bacteria has been successfully reconstituted, whereas among the peripheral

Table 1. Reconstituted Chl-protein complexes

Complex	Reconstituted protein[a]	References[b]
LH1	n.p. from purple bacteria	(Parkes-Loach et al., 1988, 2001)
	r.p. from purple bacteria	(Davis et al., 1997)
	s.p. from purple bacteria	(Kehoe et al., 1998)
LH2	n.p. and r.p. from purple bacteria	(Todd et al., 1998)
LHCI-730	r.p. from tomato	(Schmid et al., 1997; Rupprecht et al., 2000)
LHCI-680	r.p. from tomato	(V. H. R. Schmid, personal communication)
LHCIIb	n.p. from pea	(Plumley and Schmidt, 1987)
	r.p. from pea	(Paulsen et al., 1990; Reinsberg et al., 2001; Rogl et al., 2002)
	r.p. from maize	(Remelli et al., 1999)
CP29	r.p. from maize	(Giuffra et al., 1996; Pascal et al., 2001)
CP26	r.p. from maize	(Ros et al., 1998; Frank et al., 2001)
CP24	r.p. from maize	(Pagano et al., 1998)
Chl-*a/b* LHC	n.p. from *Chlorella fusca*	(Meyer and Wilhelm, 1993)
Chl-*a/b/c* LHC	n.p. from *Mantoniella squamata*	(Meyer and Wilhelm, 1993)
LhaR1	r.p. from red algae	(Grabowski et al., 2000)

[a] n.p., native protein; r.p., recombinant protein; s.p., synthetic polypeptide; [b] The list of references is far from being complete and meant to provide a starting point for surveys of the literature.

complexes LH2, only that of *Rhodospirillum molischianum* has been assembled in vitro (Todd et al., 1998). When mutations are introduced into the LH2 β subunit of *Rhodobacter sphaeroides*, including the exchange of 4 C-terminal amino acids with those of the LH1 β subunit, this protein also forms monomeric pigment-protein complexes but, even in the presence of LH1 or LH2 α subunits, forms no LH1- or LH2-type oligomeric complexes (Todd et al., 1999). Consistently, the 4 C-terminal amino acids in the β subunit of *Rs. molischianum* LH2 are identical with those of the β subunit in *Rb. sphaeroides* LH1, confirming the significance of these C-terminal amino acids for the proteins' ability to bind pigments under reconstitution conditions. It is unclear why LH1 appears to reconstitute more readily than LH2 although the overall structure is thought to be similar, except for the different number of subunits in the circular arrangements. Either this is a technical problem and the optimum conditions for LH2 reconstitution have not yet been identified. Or this different behavior is based on structural differences. In an NMR structural analysis of an LH1 β subunit it was recently discovered that the trans-membrane domain of this protein contains two α-helical segments forming a kink (Conroy et al., 2000) whereas LH2 β subunits contain one consecutive trans-membrane α helix (McDermott et al., 1995; Koepke et al., 1996). Possibly the bent structure in LH1 adds some flexibility to the structure that is advantageous for assembly in vitro.

B. Chlorophyll a/b Proteins

In 1987 the group of G. W. Schmidt found that the major light-harvesting Chl *a/b* protein, LHCIIb, reconstitutes with pigments when the denatured apoprotein is mixed with pigments in sodium dodecylsulfate solution and refolded by freeze-thaw cycles (Plumley and Schmidt, 1987). This was then extended to the recombinant LHCIIb apoprotein (Cammarata et al., 1990; Paulsen et al., 1990) and later it eventuates that all known Chl *a/b* proteins of higher plants can be reconstituted following this or a similar procedure (see Table 1).

Deletion mutations in LHCI (Rupprecht et al., 2000) and LHCIIb (Cammarata and Schmidt, 1992; Paulsen and Hobe, 1992) defined the minimum protein requirements for complex formation. A major question addressed by reconstitution studies has been the assignments of Chl *a* and *b* to binding sites in LHCIIb and other Chl *a/b* complexes. The resolution of the electron-crystallographic structure analysis of LHCIIb was not sufficient to distinguish between these two pigments; however, a tentative assignment was made (Fig. 1) on the basis of theoretical considerations (Kühlbrandt et al., 1994). These assignments have been tested by mutating single Chl-binding amino acids and testing, biochemically or spectroscopically, whether Chl *a* or Chl *b* had disappeared from the complex. In these studies, most of the original assignments have been confirmed whereas for some binding sites contradic-

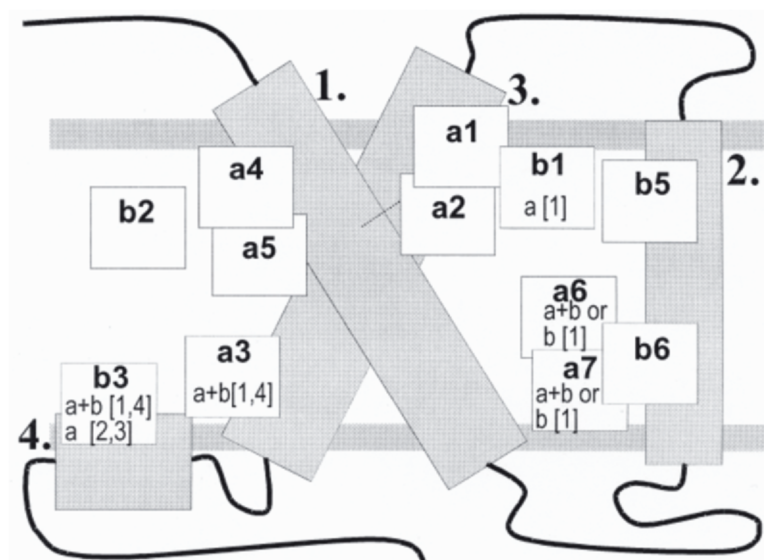

Fig. 1. Sketch of LHCIIb structure as derived from Kühlbrandt et al. (1994). The hatched boxes indicate the trans-membrane α helices numbered as counting from the N terminus, the horizontal gray bars indicate the stromal (upper) and lumenal (lower) surface of the thylakoid membrane. The squares denote Chl molecules with the assignments given by Kühlbrandt et al. (1994) in bold text. Given in plain text are the alternative assignments made on the basis of mutational analyses of reconstituted LHCIIb. [1], (Remelli et al., 1999); [2], (Rogl and Kühlbrandt, 1999); [3], (Yang et al., 1999); [4], (Rogl et al., 2002).

tory data have been obtained (Fig. 1). One surprising observation was that some binding sites in LHCIIb appear to bind Chl *a* or Chl *b* rather non-specifically. Mutational analyses of recombinant CP29 led to the same conclusion (Giuffra et al., 1997; Bassi et al., 1999; Simonetto et al., 1999). This raises the question whether in vivo some binding sites also hold a mixed population of Chl *a* and Chl *b* or whether some selection mechanism ensures a higher specificity of these binding sites towards the two Chl species than is seen in vitro. Single-molecule studies may help to solve this question. Spectroscopic studies on individual (B)Chl-protein complexes have already been performed with LH2 (Bopp et al., 1999; Tietz et al., 1999; van Oijen et al., 1999; Köhler et al., 2001, see also Chapter 21, Köhler and Aartsma), LHCIIb (Tietz et al., 2001), and PS I (Jelezko et al., 2000).

Spectroscopy of reconstituted Chl-*a/b* complexes with a modulated Chl *a/b* population, or with individual binding sites staying empty, permitted the deduction of the spectroscopic properties of individual Chl molecules in CP29 (Giuffra et al., 1997; Bassi et al., 1999; Simonetto et al., 1999; Cinque et al., 2000; Pascal et al., 2001) and LHCIIb (Remelli et al., 1999; Rogl and Kühlbrandt, 1999; Cinque et al., 2000; Rogl et al., 2002; Zucchelli et al., 2002). The same extends to the carotenoid binding sites in Chl *a/b* complexes (Croce et al., 1999a,b, 2001; Hobe et al., 2000; Caffarri et al., 2001; Formaggio et al., 2001). Such studies led Croce et al. (1999a) to calculate even the orientation of neoxanthin, the carotenoid presumably invisible in the electron-crystallographic structure analysis of LHCIIb (Kühlbrandt et al., 1994), with regard to the rest of the complex. It will be interesting to see whether this prediction will be verified by a more refined structure determination.

Time-resolved measurements of LHCIIb reconstitution (Booth and Paulsen, 1996) revealed some molecular details of the assembly process. The concentrations of all components, protein, Chl and carotenoids, are rate-limiting, indicating that at least some of each molecule species is bound into the complex during the rate-limiting step(s) (Reinsberg et al., 2000, 2001). Moreover, the formation of the protein α helix in the reconstitution process occurs during the same kinetic step(s) as does the assembly of pigments; so, protein folding and pigment binding are apparently closely coupled processes (Horn and Paulsen, 2002).

Reconstituted LHCIIb forms trimers in the presence of phosphatidyl glycerol (Hobe et al., 1994). Mutational analyses revealed a ‘trimerization motif’

near the N-terminus of the protein (Hobe et al., 1995), and a C-proximal tryptophan residue (Kuttkat et al., 1996) which when deleted or involved in a non-conservative amino acid exchange abolishes trimer formation. Similarly, mutant versions of recombinant LHCI-730 apoproteins were used to identify the protein segments essential for formation of heterodimeric LHCI-730 (Schmid et al., 2002)

C. Other Light-harvesting Proteins

Some light-harvesting proteins from marine algae, showing sequence homology to the Chl *a/b* proteins of higher plants and green algae, have also been reconstituted in vitro. These are the Chl *a/b/c* protein of the unicellular prasinophyte *Mantoniella squamata* (Meyer and Wilhelm, 1993; Meyer et al., 1996) and the LHCI protein, LhcaRI, from the red alga *Porphyridium cruentum* (Grabowski et al., 2000). Interestingly, both of these recombinant algal proteins exhibit astonishing flexibility in their pigment binding. The Chl *a/b/c* protein, from *M. squamat* can be reconstituted with pigment extracts from the green alga *Chlorella vulgaris* (Meyer and Wilhelm, 1993; Meyer et al., 1996), and the Chl *a*-zeaxanthin protein from *P. cruentum* forms complexes with pigments extracted from a higher plant (Chl *a/b*, lutein, neoxanthin, violaxanthin, β-carotene), a diatom (Chl *a/c*, fucoxanthin, diadinoxanthin) and a dinoflagellate (Chl *a/c*, peridinin) (Grabowski et al., 2001). This finding confirms the relationship between Chl *a/b*-like proteins and suggest that the flexibility of apoproteins in binding various Chls and carotenoids has helped in the evolution of different types of light-harvesting units in various branches of plant evolution.

Surprisingly, another member of the extended Chl *a/b* protein family, the PS II protein PsbS, has resisted attempts to reconstitute it with pigments in vitro (Dominici et al., 2002). Sequence analysis of this protein suggests 4 trans-membrane domains of which the first and third show extensive similarity with Chl *a/b* light-harvesting proteins (Kim et al., 1992; Wedel et al., 1992). PsbS has been isolated in a pigmented form (Funk et al., 1995b) but, in contrast to Chl *a/b* proteins, is also stable without bound pigments (Funk et al., 1995a). Of course, a negative result in reconstitution experiments does not prove that PsbS in fact is unable to bind pigments in vitro; possibly the correct conditions for this protein simply have not yet been found. Thus, it remains to be seen whether or not PsbS can be reconstituted with pigments.

A recombinant water-soluble light-harvesting peridinin-Chl protein (PCP) from a diatom can be reconstituted with pigments in vitro (R. G. Hiller, personal communication). PCP bears no resemblance with Chl-*a/b* proteins since it contains a 30-kDa protein wrapped around eight peridinin and two Chl *a* molecules (Hofmann et al., 1996).

D. Water-soluble Chlorophyll Protein

A recombinant fusion of a water-soluble Chl protein (WSCP) from cauliflower with maltose-binding protein was reconstituted by simply adding it to a thylakoid suspension; the protein apparently extracts Chl molecules from the membrane and binds them tightly (Nishio and Satoh, 1997). A number of water soluble Chl proteins have been isolated from higher plants: WSCP from *Chenopodium album* (Yakushiji et al., 1963) changes its absorption spectrum upon illumination whereas WSCPs from *Brassicaceae* such as cauliflower (Murata et al., 1971), cress (Murata and Ishikawa, 1981) and Japanese radish (Shinashi et al., 2000) do not. The physiological function of WSCPs is unknown: those from *Brassicaceae* share extensive sequence homology with a drought-induced protein, Bnd22, from rapeseed and, therefore, are thought to be involved in scavenging Chls during (stress-induced) senescence. Nothing is known yet about how the Chls are bound. WSCP fused to maltose-binding protein appeared to oligomerize upon Chl binding (Nishio and Satoh, 1997). It is to be expected that a closer examination of this reconstitution in vitro will yield information about Chl organization and possible functions of this Chl-protein complex.

III. Pigment Exchange

A. LH1 and LH2

Instead of fully reconstituting *Rs. rubrum* LH1 from its apoprotein and pigments (see above), the complex can also be partially dissociated by detergents at an elevated temperature and then be re-associated, by detergent removal, to contain various amounts of exogenously added pigment derivatives such as the Zn analogue of BChl *a*. This approach allowed the titration of different BChl *a* derivatives competing for their binding into the B873 sites. An interesting observation in these experiments was that there are at least two biochemically distinguishable B873 binding sites in LH1, possibly due to their localization either

Table 2. Chl exchange in Chl-protein complexes

Complex	Pigment(s) exchanged	References[a]
LH1	B873 → [Zn]-BChl *a* etc.	(Lapouge et al., 2000)
	B873 → [Ni]-BChl *a*	(Fiedor et al., 2000; Fiedor et al., 2001)[b]
LH2	B800 → [3^1-OH]-BChl etc.	(Bandilla et al., 1998)
	B800 → BChl *a*	(Fraser et al., 1999)
	B800 → [3-acetyl]-Chl *a*	(Herek et al., 2000)
RC[c]	B_A, B_B → [3-vinyl] BChl *a*	(Hartwich et al., 1995)
	B_A, B_B → [13^2-OH]-BChl *a*	(Storch et al., 1996)
	H_A, H_B → Phe *a*	(Meyer and Scheer, 1995; Franken et al., 1997)
PS II RC	Chl *a* → [3-acetyl]-Chl *a*	(Scheer und Hartwich, 1995; Gall et al., 1998)

[a] Not a complete list of references; [b] This work actually describes complex reconstitution but is listed here because its main point is partial pigment exchange; [c] RC of purple bacteria

on the α or the β subunits (Lapouge et al., 2000).

A similar approach can be extended to LH2 and RCs from purple bacteria and to PS II from higher plants (Table 2).

LH2 of *Rhodopseudomonas acidophila* was completely stripped of its B800 pigments by low pH/detergent treatment; subsequently, up to 80% of B800 binding sites could be re-filled with BChl *a* or derivatives such as [13^2-OH]-, [3-vinyl] BChl *a* or [3-acetyl-Chl] *a*. The energy transfer of these pigments to B850 was significantly higher than predicted by the Förster theory, indicating that B800 and B850 interact more closely than merely by resonance of their transition dipoles (Herek et al., 2000)

B. Reaction Centers

Pigment exchange experiments with bacterial reaction centers have been reviewed by Scheer and Hartwich (1995). The BChl *a* molecules in the monomer binding sites(B_A, B_B) of both the A and B branches in *Rb. sphaeroides* RCs were selectively exchanged with a BChl derivative, e.g. with [3-vinyl]-BChl. The experimental procedure took advantage of the fact that the carotenoid spheroiden(on)e present in these complexes selectively protects the B_B site. Thus, B_A was selectively exchanged in these complexes. In RCs from a carotenoid-deficient mutant, both monomeric BChl molecules were exchanged at the same time. If subsequently the carotenoids were reconstituted into the complex, the [3-vinyl]-BChl in the B_A site could then be re-exchanged with non-modified BChl, yielding a RC specifically modified in the B_B binding site. This allowed the individual analysis B_A and B_B with regard to their spectroscopic properties and BChl-carotenoid interaction. (Hartwich et al., 1995). The binding of BChls into B_A and B_B is selective for the configuration of $C13^2$ in the isocyclic ring. If BChls are hydroxylated at $C13^2$, which blocks epimerization and fixes BChls in either the natural or the unnatural configuration, only the natural epimer binds into B_A and B_B (Storch et al., 1996).

The pigment exchange reaction established for RCs of purple bacteria can be extended to PS II RCs of higher plants. Several Chl molecules can be exchanged with derivatives such as the Zn-containing analogue or the [3-acetyl] derivative (Gall et al., 1998). First steps towards assigning the sites of pigment exchange have been taken (Zehetner et al., 2002). Also the Phe molecules in PS II RC have been successfully exchanged (Germano et al., 2000).

C. [Ni]-BChl a, a 'Black-hole Pigment'

A particularly interesting (B)Chl *a* derivative to be exchanged or reconstituted into pigment-protein complexes is the Ni analogue as it dissipates excitation energy very efficiently and thus acts as an excitation trap. [Ni]-BChl *a* has a much shorter excited state lifetime than BChl *a*, due to extremely rapid internal conversion from the electronically excited to the ground state (Musewald et al., 1999; Noy et al., 2000). When [Ni]-BChl *a* is introduced into LH1 from *Rb. sphaeroides*, it dissipates BChl excitation energy within 60 fs, even when only one Ni-derivative is bound per LH1 holocomplex. This is much faster than the time required for Förster resonance energy transfer between pigments. Therefore, all the pigments in the LH1 ring must be excitonically coupled to each other (and the Ni-derivative). This excludes alternative models of LH1 being composed of excitonically isolated clusters of BChl *a* that exchange energy only by the Förster transfer mechanism (Fiedor et al., 2000, 2001). Such an ap-

proach, in combination with the reconstitution or pigment exchange technique, clearly offers a great advantage in elucidating excitation energy pathways in photosynthetic complexes by placing excitation energy traps in defined positions.

IV. Concluding Remarks

The number of self-organizing membrane proteins that can be refolded in vitro is still rather small. It is actually quite astonishing that light-harvesting Chl-binding proteins of both plants and bacteria belong into this group. All of these proteins are capable of binding quite a number of different pigments more or less specifically. The contribution of these pigments to the total mass of the complexes is significant (roughly one third in LHCIIb and somewhat less in LH1 and LH2). In terms of pigment-protein ratios, the chlorosome of green sulfur bacteria goes to the extreme: the arrays of BChl *c* in these complexes can self-organize in the absence of any protein (Chapter 20, DeBoer and DeGroot). This raises the possibility that also in the other pigment-protein complexes, the self-organizing capacity is partly, or even mostly, due to pigment-pigment interactions. However, the observation that very limited alterations in the LHCIIb protein structure, such as single amino-acid exchanges in a loop domain, can totally destroy the stability of the complex (Heinemann and Paulsen, 1999) proves that proteins, even protein domains not directly involved in pigment binding, make important contributions to the cooperative stabilization of Chl-protein complexes. Moreover, the experimental procedure of re-folding Chl *a/b* proteins is very similar to the procedure used for re-folding bacterioopsin (London and Khorana, 1982; Booth, 2000) This membrane protein binds one chromophore, retinal, to form bacteriorhodopsin but virtually completely folds in vitro even in the absence of retinal (Riley et al., 1997). Although Chl *a/b* proteins, by contrast, need pigment binding as a trigger for re-folding (see below), the example of bacteriorhodopsin demonstrates that a membrane protein can principally re-fold in vitro without the help of any pigments or other co-factors.

No successful reconstitution of any Chl-binding RC protein nor of the Chl-*a* binding inner antennae CP43 and CP47 has yet been reported. Why is it that among the pigment-protein complexes of the photosynthetic apparatus only the more peripheral ones, involved in light harvesting (Chl *a/b* complexes and their relatives, LH1 and LH2, and PCP), seem to be able to spontaneously organize? Although the possibility to reconstitute these complexes is relevant only in vitro, it is tempting to speculate that it reflects a property that plays some role also in vivo; possibly during the biogenesis and degradation of the various photosynthetic units. Light harvesting is a process regulated, depending on light intensity, in all photosynthesizing organisms. A minimum light-harvesting capacity is necessary to render photosynthesis efficient enough to meet and compete for the energy needs of the organism, whereas over-energizing of the photosynthetic apparatus must be avoided as it is potentially very harmful. Possibly the self-organizing power of light-harvesting units helps to accelerate the assembly and disassembly of light-harvesting units in vivo, thus facilitating the regulation of the light-harvesting capacity.

A somewhat different prerequisite applies to Chl complexes for individual pigments to be exchanged. These complexes must stay sufficiently stable with the corresponding Chl binding sites at least transiently empty. In fact, for LH2 from purple bacteria it has been shown that the B800 binding sites can be completely stripped and then re-filled (Fraser et al., 1999). An NMR study of LH1 from *Rb. sphaeroides* showed that this protein folds into its apparently native structure even in the absence of pigments or lipids (Kikuchi et al., 1999). By contrast, the apoprotein of LHCIIb needs pigment binding to refold in vitro (Reinsberg et al., 2000, 2001; Horn and Paulsen, 2002). On the other hand, mutation analyses of Chl binding sites in LHCIIb and CP29 show that stable complexes can be formed lacking one or few Chl molecule(s). Therefore, it seems likely that the pigment exchange technique should also be amenable for the Chl molecules in Chl *a/b* complexes.

Note Added in Proof

This chapter has been written in mid-2002 and reflects the knowledge available at that point. It is not possible here to give a full update—my apologies to all colleagues whose new and important contributions have not been included.

It should be noted, however, that Fig. 1 is thoroughly outdated. Two X-ray crystallographic structures have been published of LHCIIb from spinach (Liu et al., 2004) and pea (Standfuss et al., 2005) at 2.72 Å and 2.5 Å resolution, respectively. Both groups find eight

Chl-*a* and six Chl-*b* molecules in LHCIIb, residing in non-mixed binding sites. Thus, the binding sites accommodating both Chl *a* and Chl *b* (described in Section II.B of this review) appear to be a reconstitution artifact.

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