A potential role of chlorophylls *b* and *c* in assembly of light-harvesting complexes

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Abstract Chlorophyll (Chl)-containing light-harvesting complexes (LHCs) in chloroplasts of plant and algal cells usually include an oxidized Chl (Chl *b* or *c*) in addition to Chl *a*. Oxidation of peripheral groups on the tetrapyrrole structure increases the Lewis acid strength of the central Mg atom. We propose that the resulting stronger coordination bonds between oxidized Chls and ligands in LHC apoproteins (LHCPs) stabilize the initial intermediates and thus promote assembly of LHCs within the chloroplast envelope.

Key words: Light-harvesting complex; Chlorophyll *b*; Chlorophyll *c*; Chloroplast; Photosynthesis

1. Introduction

Core complexes of photosystems I and II contain little, if any, Chl *b* [1], although, as demonstrated with reconstitution of LHCII, a number of Chl-binding sites on proteins can not distinguish between Chl *a* and Chl *b* [2,3]. Nevertheless, the major peripheral LHCs contain nearly equal numbers of Chl *a* and Chl *b*, which are held in highly specific positions. Several of these complexes, in particular those with Lhca4, Lhcb1 and Lhcb6 as apoproteins, do not accumulate in chlorotic, Chl *b*-less mutants of plants [4,5]. The regular structure of LHCII and the requirement of Chl *b* for accumulation have led to the consensus that the complex is assembled via a defined pathway and the final product is stabilized by Chl *b*.

Chl *b* is possibly confined to LHCs by its site of synthesis, or assembly of the complexes may require a unique interaction with LHCP. The chloroplast envelope is a site of synthesis of Chlide *a* [6]. Chl *b* is synthesized from Chlide *a* by Chlide *a* oxidase [7], and subsequent esterification, but does not accumulate when synthesis of LHCPs in the cytosol is inhibited [8]. Although newly synthesized LHCPs were detected in Chl *b*-less mutants of Arabidopsis and barley, these proteins were not recovered in chloroplasts purified from these plants [9,10]. Furthermore, when LHCPs were synthesized in the absence of Chl synthesis in *Chlamydomonas reinhardtii* cells, import into the chloroplast was aborted and the proteins were shunted to vacuoles [11]. These results suggest that the specific properties of Chl *b* are important not only for assembly of stable LHCs but perhaps also for import of the apoprotein into the plastid. Thus it seems possible that stability of Lhcb1 is a consequence of its interaction with Chl *b* within the chloroplast envelope, as import is initiated, which prevents retrograde transfer to vacuoles for degradation.

2. Role of retention motifs

We proposed [12] that assembly of LHCs, and retention of Lhcb1 in the chloroplast, requires initial binding of two molecules of Chl to a highly conserved sequence that we designated a 'retention motif' (Fig. 1). We envisaged interaction of Chl with this motif to be *necessary* to hold the protein in the envelope inner membrane, during its import into the chloroplast, long enough for additional Chl and xanthophyll molecules to bind and complete assembly of the complex. However, binding of Chl *a* is apparently not *sufficient* within the residence time of the protein under autotrophic growth conditions. A means to stabilize the initial association of Chl with Lhcb1 would increase the probability that assembly continues to completion, particularly when the rate of Chl synthesis is low. An approximately two-fold greater strength of binding of Chl *b*, as compared with Chl *a*, to Lhcb1 was measured during detergent-induced dissociation of LHCII [13]. Thus Chl *b* possibly binds the retention motif more strongly than Chl *a* and *prolongs* residence of the protein in the envelope.

Direct assays [14] indicated that two molecules of Chl *a* bind to a retention motif (RT). Then, RT+Chl⇋[RT·Chl]; RT·Chl+Chl⇋[RT·2Chl]; or, RT+2Chl⇋[RT·2Chl]. K_D = [RT][Chl]^2/[RT·2Chl].

The equilibrium positions of these reactions can be driven toward complex formation by increasing either the concentration of Chl or the stability of the complex. In support of the former possibility, enhancing the rate of Chl *a* synthesis in Chl *b*-less *C. reinhardtii* cells allowed accumulation of a full complement of LHCPs [11,15]. A two-fold increase in the strength of the coordination bond with the Mg in Chl *b* over that with Chl *a* would increase stability of the complex and reduce the dissociation constant, K_D, by a factor of approximately 4. Thus, at lower rates of Chl synthesis, as would occur during autotrophic growth, an initial intermediate formed with Chl *b*

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Abbreviations: Chl, chlorophyll; LHC, light-harvesting complex; LHCII, LHC associated with photosystem II; LHCP, LHC apoprotein; Lhca and Lhcb, apoproteins of LHCI and LHCII, respectively; Pchlide, protochlorophyllide

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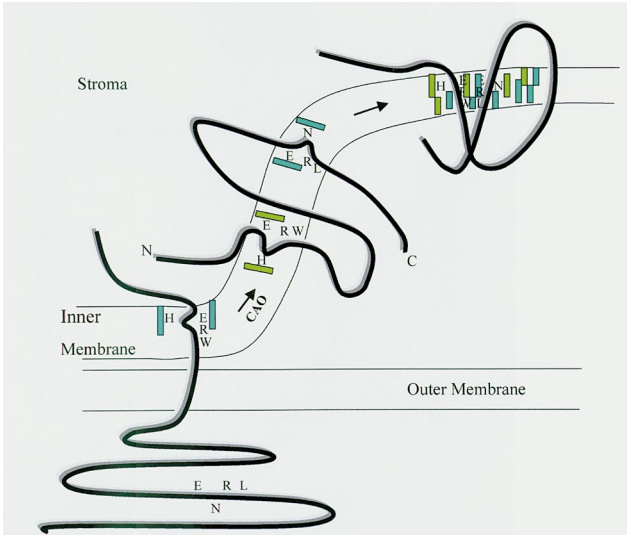

Fig. 1. Model of LHCII assembly in the chloroplast envelope and the proposed role of Chl *b* and Chlide *a* oxidase (CAO). Several proposed intermediates are shown in sequence, *left* to *right*. After synthesis in the cytosol, an LHCP precursor is imported sufficiently for the first retention motif to engage the inner membrane. Removal of the transit sequence occurs soon after the N-terminal portion of the protein enters the stroma. Chl(ide) *a* binds to ligands in the motif provided by an ion pair between the side chains of Glu and Arg and the His residues. In conjunction with the Trp residue next to the motif, Chlide *a* oxidase catalyzes the oxidation of bound Chlide *a* (blue-green) to Chlide *b* (olive-green), and esterification anchors the protein in the membrane. The xanthophyll lutein is possibly inserted at this stage. Subsequent folding of the protein pulls the second motif into the membrane, which binds Chl *a* but contains a Leu residue next to the motif. Addition of more Chl and replacement of the *intrahelix* Glu–Arg ion pairs with *interhelix* ion pairs completes assembly of the complex. The approximate positions of the conserved amino acids in retention motifs at each stage of folding of the protein are indicated. When Chlide *a* oxidase activity is absent, cytosolic chaperones apparently cause the proteins to slip back into the cytosol or be transferred to vacuoles for degradation.

may be sufficiently stable to allow continuation of assembly of LHCII.

A Trp residue is next to Arg in retention motifs (-E*xx*H*x*RW-) in the first membrane-spanning region of all LHCPs that form complexes containing Chl *b* [16] and may play a role in its synthesis. Support for this possibility was the synthesis of Chlide *b* in *C. reinhardtii* cells treated with phenanthroline derivatives [17], which are analogues of the phenylindole portion of Trp. The motif in the third membrane-spanning region of Lhcb1 is followed by Leu instead of Trp (-E*xx*N*x*RL-) and would not be expected to promote Chl *b* synthesis. This proposal is consistent with models [18] based on excitation dynamics of native LHCII [19], which indicated that two of the four Chl molecules bound by the two interacting membrane-spanning helices of LHCP, and proximal to the central luteins [20], are Chl *b* molecules. The remaining five Chl *a* and three Chl *b* molecules are distributed throughout the complex [21,22].

3. Modulation of the Lewis acid strength of Mg^{2+}

The chemistry of chlorins provides an explanation for the increased stability of Chl *b*–protein complexes. The final stage of Chl synthesis from protoporphyrin IX includes, in sequence, insertion of Mg^{2+}, formation of the isocyclic ring, reduction of ring D of protochlorophyllide (Pchlide) to produce Chlide *a*, oxidation of Chlide *a* to Chlide *b* and finally esterification to the final Chls [23]. Each modification of the tetrapyrrole structure results in withdrawal of electrons from, and thereby a weakening of the basicity of, the nitrogen atoms [24]. Most strikingly, introduction of the electronegative formyl group on a chlorin, such as in Chl *b*, reduces the p*K* values of the pyrrole nitrogens by 2 pH units [25]. This change in basicity of the nitrogens is reflected in the well-known demetallation of Chls in slightly acidic solutions, whereas much stronger acids are required to remove the Mg atom from Pchlide [26]. Of importance for assembly of LHCs, the lessened basicity of the pyrrole nitrogens increases the strength of the Mg as a Lewis acid, and interaction of the metal atom with a protein ligand, through the fifth coordination bond of its square pyramidal structure, is enhanced. Surprisingly, experiments to test this proposal directly indicated that Chl *b* binds less readily than Chl *a* to the retention motif (L.L. Eggink and J.K. Hoober, unpublished results). Binding of Chl *b* is possibly inhibited by the expected increase in the strength of the coordination bond of Mg with water molecules [27]. This interpretation suggests that Chlide *a* binds first to the retention motif in LHCP and then is converted to Chlide *b*. The protein thus serves as an 'effector' in this reaction, which would explain the requirement of LHCP for Chl *b* synthesis.

In those species that are products of a secondary endosymbiotic event and thus have additional membranes surrounding the chloroplast, Chl *b* is usually replaced with Chl *c*. Chl *c* is synthesized by introduction of a double bond in the propionate side chain on ring D of Pchlide, to produce a *trans*-acrylate group [28]. This extension of conjugation transmits electronegativity of the carboxyl group, which remains unesterified, to the ring π system and reduces basicity of the pyrrole nitrogens in a manner similar to that caused by the formyl group on Chl *b*. LHC apoproteins in chromophytic algae contain the same conserved amino acids in the retention motif sequence as those in chlorophytic organisms [16,29], which suggest that Chl *c* should also form strong coordination bonds with these ligands. The absence of Chl *b* correlates with replacement of Trp with Ile, Leu, or Ala next to the motif in these proteins. Confirmation [30] that some species of red algae contain Chl *d*, an analog of Chl *b* except that the vinyl group of ring A rather than the methyl group on ring B is oxidized to a formyl group [31], reflects an additional mechanism that organisms have found to oxidize the Chl molecule.

LHCPs do not accumulate in plants unable to make Chl in the dark, which suggests that Pchlide does not form sufficiently strong coordination bonds with protein-bound ligands to form stable complexes. Thus, we propose that modifications of the Chl molecule functionally influence equilibria of Chl–protein associations. Chl *a* is sufficient for complex formation with proteins synthesized by chloroplast ribosomes and co-translationally inserted into thylakoid membranes, whereas the stronger interaction of Chl *b* is required for LHC assembly with some of the LHCPs imported post-translationally into the chloroplast.

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