Erkut İnan İseri · Demet Gülen

Chlorophyll transition dipole moment orientations and pathways for flow of excitation energy among the chlorophylls of the major plant antenna, LHCII

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Abstract We have attempted in this work an assignment of the Q transition dipole moment orientations for all the chlorophylls in the major plant antenna, light-harvesting complex II (LHCII). Information that has recently become available through a structural model of the LHCII, site-directed mutagenesis, and spectroscopy of both LHCII and CP29 has been evaluated to model the electronic excited state structure in the presence of chlorophyll-chlorophyll and chlorophyll-protein interactions. An assignment has been obtained which satisfactorily reproduces the polarized linear absorption characteristics. The assignment proposed has also been found to be adequate in reproducing the time scales of the energy transfer processes. The pathways for the flow of excitation energy among the chlorophylls of the complex have been suggested in the context of identity and orientation assignments.

Keywords Light-harvesting complex II · Electronic excited states · Energy transfer · Photosynthesis

Abbreviations ABS: absorption · CD: circular dichroism · Chl: chlorophyll · LD: linear dichroism · LHC: light-harvesting complex · PSII: photosystem II · 3PEPS: three-pulse photon echo peak shift

Introduction

Photosynthetic organisms contain light-harvesting pigment-protein complexes (LHCs) that absorb light and transfer energy efficiently to other pigment-protein complexes called reaction centers to initiate the photochemistry of photosynthesis. The efficiency of the light-harvesting processes depends on the ultrafast energy transfer processes in the LHCs (antenna) of green plants, bacteria, and algae, which are mainly governed by the pigment-pigment and the pigment-protein interactions. For a comprehensive understanding of the ultrafast energy transfer processes on the molecular level, one of the important tasks is the determination of the electronic excited states of the LHCs (van Grondelle et al. 1994).

Light-harvesting in photosystem II (PSII) of green plants is performed by a collection of LHCs buried in the photosynthetic membrane (thylakoids). The LHC associated with the PSII consists of two parts: an outer antenna binding chlorophyll a (Chl a), Chl b, and carotenoids as pigments and an inner antenna binding Chl a and carotenoids. The proteins forming the outer antenna are usually called as the Lhcb proteins. The major Lhcb protein, LHCII, binds almost 65% of the PSII chlorophyll (accounting for 50% of the total Chls in the thylakoids), and three of the minor Lhcb proteins homologous to LHCII (usually called as CP24, CP26, and CP29) bind altogether 15% of the PSII chlorophyll (Boekema et al. 1999).

Currently, LHCII is the only Lhcb protein which is structurally resolved. A large part of the structure of the LHCII is modeled to a resolution of 3.4 Å (Kühlbrandt et al. 1994). The LHCII pigments identified per monomeric subunit of the C3 symmetric trimer of the current model are 12 Chl molecules surrounding two central carotenoid molecules (see Fig. 1).

The current LHCII model does not allow a direct access to several parameters that are essential for understanding the light-harvesting function. At 3.4 Å resolution the Chls could only be modeled as naked tetrapyrole rings. Therefore, the model does not provide any distinction between the Chls a and b (the identity problem) and no distinction could be made between the molecular x- and y-axes of the Chl molecules (the orientation problem).

However, most of the chlorophyll binding sites in LHCII are disclosed by the structural model, which also
led to the identification of the probable binding sites in highly homologous CP29 (Bassi et al. 1999). Site-directed mutagenesis of chlorophyll binding residues has allowed construction of several mutant proteins lacking individual Chl molecules. Biochemical and spectral characterization of these mutants has recently been used for determination of the Chl identities in both CP29 and LHCII (Bassi et al. 1999; Remelli et al. 1999; Rogl and Kühlbrandt 1999; Simonetto et al. 1999). Bassi and co-workers have reported that in both complexes the evolutionarily conserved core (Green and Kühlbrandt 1995), consisting of the binding sites A1, A2, A4, and A5, is found to be occupied by the Chls a. In CP29 the remaining four binding sites (A3, B3, B5, and B6) are found to have mixed Chl a/b occupancies, with the respective Chl a binding probabilities of 70%, 30%, 60%, and 40%. However, in reconstituted monomeric LHCII, A3 and B3 are found to be mixed sites with equal Chl a/b binding affinities and B5 and B6 are found to be pure Chl b sites. Three of the remaining LHCII sites are suggested to bind either Chl b (A7 and B2) or Chl a (B1), and A6 is proposed to have equal affinity for Chl a and b. On the other hand, Rogl and Kühlbrandt (1999) have suggested that A1, A2, A3, and B3 are pure Chl a sites and B5 and B6 are pure Chl b sites in reconstituted trimers.

There is no doubt that determination of the Chl identities is a major step in correlating the spectra with the structure. However, understanding of the structure-function relationship is still hampered since the orientations of the molecular y-axis of the Chls are not unambiguously determined.

The Lhcb proteins are spectroscopically complex objects, exhibiting considerably heterogeneous spectra in the spectral range of 630–685 nm (Qy band) and many spectral forms are commonly observed in all the Lhcb proteins in this absorption region. The number of spectral bands decomposed in the Qy band almost matches the number of bound Chls in all these complexes (Zucchelli et al. 1994). Owing to a fewer number of Chls, the minor antennae show a lower degree of complexity and their spectral data may prove more easily interpretable than that of the LHCII. It has long been noted that the spectral bands resolved in the Chl a absorption region of the LHCII and CP29 proteins are essentially identical, suggesting an identical organization for the Chls a common to both complexes (Zucchelli et al. 1994; Giuffra et al. 1997). In the recent site-directed mutagenesis work of Remelli et al. (1999), it has been furthermore confirmed that not only the site selectivity is largely conserved between the two complexes but also the distribution of the absorption forms among different protein domains. In addition, the energy equilibration in CP29 is observed to be very similar to part of the energy equilibration in LHCII, both in terms of temporal and spectral characteristics (Gradinaru et al. 1999). It has therefore been expected that a better interpretation of the light-harvesting process in the major plant antenna, LHCII, can be obtained by understanding the structurally and electronically less complicated CP29.

Recently, we have suggested an electronic excited state structure for the CP29 complex (Mørk et al. 2000), by assuming a structure common with the relevant part of the LHCII and using the Chl identities reported by the mutational analysis of Bassi et al. (1999). We have determined the orientations of the Qy transition dipole moments of all the Chls of CP29 by a simultaneous simulation of the key features of the low-temperature absorption (ABS) and linear dichroism (LD) spectra (Pascal et al. 1999). We have also discussed that the model we proposed can explain the general character of the energy equilibration in CP29 (Gradinaru et al. 1999) on the basis of our preliminary energy transfer rate estimates.

In the present study we have attempted to assign the orientations of all the LHCII chlorophylls using our