Resonance Raman Spectroscopy of the Photosystem II Light-Harvesting Complex of Green Plants: A Comparison of Trimeric and Aggregated States

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ABSTRACT: Resonance Raman spectroscopy was performed on photosystem II light-harvesting complex (LHCII) in trimeric and oligomeric states with various excitation conditions. From these studies, it can be concluded that the structure of LHCII is altered during the trimer/oligomer transition. These structural changes affect the conformation of a population of carotenoid within the complex, which becomes twisted in the oligomeric form. Moreover, the interactions assumed by a chlorophyll *a* and a chlorophyll *b* are modified during the oligomerization process. This induces the formation of an H-bond to a formyl group of a chlorophyll *b* molecule and to a keto group of a chlorophyll *a* molecule. The extent to which these H-bonds to chlorophyll relate to the formation of the quencher cannot yet be precisely established. However, the structural changes they evidence may play a role in the control of the energy flux by LHCII complexes.

Harvesting of sunlight by green plants requires two photosystems, both of which comprise a reaction center and an antenna system. The antenna system of photosystem II contains approximately 200 chlorophyll (Chl)[1] molecules per reaction center. It is complex and consists of at least six different chlorophyll−protein complexes. The core antenna system of photosystem II is composed of the CP47 and CP43 subunits, whereas the chlorophyll *a*/*b* binding proteins (LHCII) form the peripheral light-harvesting system. LHCII is composed of four different complexes referred to as LHCIIa, LHCIIb, LHCIIc, and LHCIId (Peter & Thornber, 1991). The main complex, LHCIIb, binds 60% of PSII chlorophyll and is the mostly widely studied pigment−protein complex in green plants. This complex is organized into trimers, which are mixtures of three polypeptides of molecular weight of ca. 27, 27, and 25 kDa coded by Lhcb1, Lhcb2, and Lhcb3, respectively (Jansson, 1994). Its structure, determined to 3.4 Å resolution by electron diffraction of two-dimensional crystals, shows the presence of 12 chlorophyll molecules per monomer bound to a scaffold provided by three membrane-spanning α-helices, the orientation and proximity of these chlorophylls explaining the efficient energy transfer in LHCII (Kühlbrandt & Wang, 1991; Kühlbrandt et al., 1994). LHCII is associated with a class of xanthophylls, mostly lutein (60% bound carotenoid), and neaxanthin (30%), but with 10% being violaxanthin (Peter & Thornber, 1991; Bassi et al., 1993; Ruban et al., 1994a). It has been proposed that two lutein molecules are found within the LHCII structure lying parallel to the B and A α-helices, where they are in van der Waals contact with chlorophyll molecules (Kühlbrandt et al., 1994). The positions of the other carotenoids have not yet been determined.

LHCIIb can be induced to form large semicrystalline aggregates in which the spectroscopic properties of the bound pigments are drastically modified (Ruban & Horton, 1992). In particular, the fluorescence main lifetime decreases from 4.3 ns in the trimer to 110 ps in the aggregates (Mullineaux et al., 1993), and the fluorescence yield is quenched by up to 90% in the aggregates. Similarly, the emission peak shifts from 680 nm in the trimers to 700 nm in the aggregates (Ruban & Horton, 1992). Changes in the absorption spectrum in the Soret and Q_y transitions also occur upon aggregation. These changes strongly resemble those obtained when aggregates of isolated pigments (chlorophyll and xanthophyll) are formed, suggesting that upon LHCIIb aggregation significant changes in pigment−pigment and/or pigment−protein interactions occur (Ruban & Horton, 1992; Ruban et al., 1993, 1994b; Horton & Ruban, 1994). Understanding the molecular changes which accompany LHCIIb aggregation is important not only in determining the basic structure and function of this complex in light-harvesting but also in terms of understanding its dynamic aspects. There is strong evidence that alteration in LHCII structure/organization provides the mechanism by which plants dissipate excess absorbed energy as a photoprotective response to high light, the nonphotochemical quenching of chlorophyll fluorescence (Horton et al., 1991; Horton & Ruban, 1992, 1994). The changes in absorption and fluorescence spectra found in LHCIIb aggregation also occur when nonphotochemical quenching is induced in isolated chloroplasts and leaves (Ruban et al., 1991, 1992, 1993a; Ruban & Horton, 1994).

In this paper, we describe the first application of resonance Raman spectroscopy to investigate the changes occurring upon LHCII aggregation. It is shown that this process is accompanied by structural changes, affecting the conformation of a population of carotenoid and the H-bonding interactions of a population of Chl *a* and of a population of Chl *b*.

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[1] Abbreviations: Chl, chlorophyll; PS, photosystem; RR, resonance Raman.

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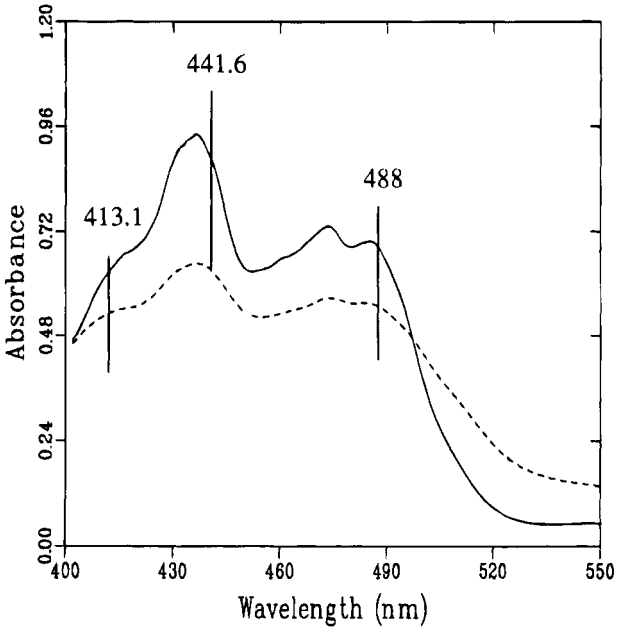

FIGURE 1: Low temperature (77 K) absorption spectra of LHCII trimers (thick line) and aggregates (dashed).

MATERIALS AND METHODS

LHCII was prepared from spinach as previously described (Ruban & Horton, 1992). Aggregates were prepared by dialysis against 10 mM tricine, pH 7.8, also as described by Ruban and Horton (1992), and trimers were prepared by the addition of 0.1% *n*-dodecyl β-D-maltoside (Sigma). In the preparations used for this work, LHCIIb was more than 90% of the total LHCII.

Raman experiments were performed with a U-1000 Jobin-Yvon Raman spectrometer equipped with a charged-coupled device detector (Spectraview 2D, Jobin Yvon). The Raman signal was recorded with a 90° geometry using grazing incidence onto the sample: 441.6 nm excitation was provided by a HeCd laser, Liconix Model 4240 PS; 413.1 nm excitation was obtained from a krypton laser (Coherent Model Innova 90); and 488 nm excitation was from an Argon laser (Coherent Model Innova 100). A detailed description of the Raman apparatus and experimental conditions has been published in Robert and Lutz (1986). To minimize Chl and carotenoid photodegradation during the Raman measurements, samples were kept at 77 K in a gas-flow cooled cryostat.

RESULTS AND DISCUSSION

Figure 1 shows the low temperature absorption spectra of aggregated and trimeric LHCII. Decreases in the oscillatory strength upon aggregation are apparent for both the Soret transition of the chlorophyll molecule and the carotenoid transitions, as reported previously for room temperature absorption spectra (Ruban & Horton, 1992; Ruban et al., 1994). More precisely, there are changes in intensity in the Soret bands with a maximum at 438 nm (chlorophyll *a*) and at 472 nm (chlorophyll *b*). The band at 485 nm, arising from the xanthophyll pigments, is also weakened upon LHCII aggregation, and an absorption increase is observed above 500 nm. In the higher wavelength region, the Q_y transitions of chlorophyll pigments appear to red-shift during the aggregation process. These data indicate that the local

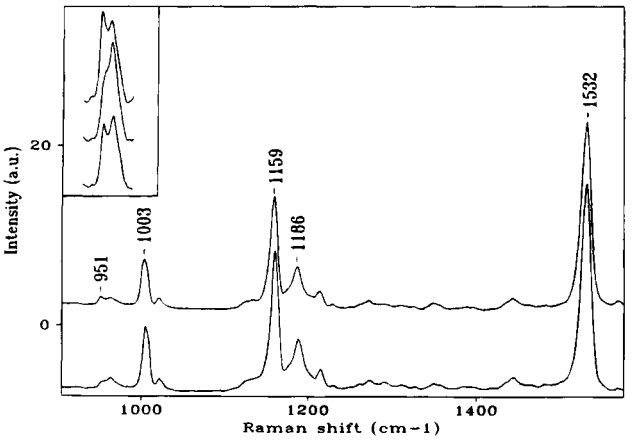

FIGURE 2: Resonance Raman spectra of LHCII trimers (bottom) and aggregates (top) (900−1600 cm^{-1}). Excitation conditions: 488.0 nm; temperature, 77 K. Inset: 900−980 cm^{-1} region, 8× expanded. Top: LHCII aggregates; center: LHCII trimers; bottom: thylakoids.

environment and/or the interactions of some of these pigments are altered in aggregated LHCII as compared to LHCII trimers.

Resonance Raman spectra of aggregated and trimer LHCII were measured with various visible excitation wavelengths between 413.1 and 488 nm. As reported before (Lutz, 1977), in such preparations, carotenoid contributions should predominate the Raman signal detected for all these excitation wavelengths. In particular with wavelengths longer than about 460 nm, only very small contributions from the other types of absorbing molecules (mainly Chl *b*) are observable in the RR spectra. The 488-nm line is thus expected to give rise to a signal from the carotenoid and coincides with an absorbance difference between aggregated and trimeric LHCII. At 441.6 nm, contributions of Chl *a* and Chl *b* pigment are present together with those of carotenoid, and at 413.1 nm, mostly Chl *a* and carotenoid contributions are present in the spectra.

RR Studies on Carotenoids in LHCII According to Aggregation States. Figure 2 displays RR spectra of aggregated (top) and trimer (bottom) LHCII at 488-nm excitation. These spectra are composed of three groups of bands at ca 1530, 1160−1230, and 1000 cm^{-1}, which can be used to diagnose the molecular configurations (Saito & Tasumi, 1983; Koyama et al., 1988). All these bands have identical frequencies within the accuracy of our measurements, and they indicate that most (if not all) of the carotenoid contributing at this excitation wavelength are in the *all-trans* configuration (Saito & Tasumi, 1983). However, in the 940−970-cm^{-1} region, aggregation of the LHCII induces a change in the intensity ratio between the 951- and the 963-cm^{-1} bands, the 951-cm^{-1} band being more intense in oligomers than in trimer RR spectra (Figure 2, inset). These bands have been ascribed to out-of-plane wagging modes of the chain hydrogens bound to the C_7/C_8 and to the C_{11}/C_{12}, respectively, coupled to the torsion of the corresponding C=C bond (Saito & Tasumi, 1983; Koyama et al., 1988). They are not totally symmetric in the C_{2h} group and are very weak in resonance with the $^1B \leftarrow {}^1A$ transition. They are known to gain intensity when the molecule assumes a lower molecular symmetry, and they are affected by changes of the configuration of the carotenoid molecules (Koyama et al., 1988). However, in this latter case, shifts of the 1530-cm^{-1} band are generally observed

(Koyama et al., 1988). One may thus conclude that, upon oligomer formation, a torsion of the polyene chain occurs for at least a population of carotenoid molecules, around the C(12)−C(13) bond (Saito & Tasumi, 1983; Koyama et al., 1988). A restricted population of carotenoid molecules only is affected by the aggregation of the LHCII; indeed, if all carotenoids were similarly affected by this process, one should expect to observe similar modifications in the RR spectra independent of the excitation wavelength used for enhancing the contributions of the carotenoid molecules. This is not the case, and the changes described above are not observed under 441.6- or 514.5-nm resonance conditions. In these conditions of excitations, RR spectra are extremely similar between aggregated and trimer LHCII (data not shown). In RR spectra of thylakoids and even whole leaves, this 951-cm^{-1} band is clearly more intense than in LHCII trimers, (Figure 2, inset), although less intense than in aggregated LHCII.

RR Studies on Chlorophyll Pigments in LHCII in Different Aggregation States. In order to study the structural changes induced by the oligomerization of the LHCII complexes, we have recorded RR spectra of these complexes at 441.6 nm, a wavelength located on the red side of the Soret electronic transition of Chl *a* and near to the maximum of the Soret band of Chl *b*. At this wavelength, it is possible to observe RR contributions of both these types of pigments, although the carotenoid signal is quite intense [see, e.g., Lutz (1984) and Lutz and Robert (1988)]. Under these conditions of excitation, the most sensitive spectral region for diagnosing intermolecular interactions assumed by the chlorin pigments is the higher frequency region of RR spectra, where stretching modes from the conjugated carbonyl groups (namely, 9-keto and 9-keto and 3-formyl for Chl *a* and Chl *b* molecules, respectively) contribute. The frequency of these modes is extremely sensitive to the H-bonding state of these chemical groups. Bands arising from the stretching mode of the 9-keto carbonyl groups are observed at ca. 1695 cm^{-1} when these groups are free from intermolecular interactions, in an apolar medium, and their position may shift down to ca. 1655 cm^{-1} (1640 cm^{-1} in the particular case of H_2O-bridged Chl *a* polymers) when these groups are involved in intermolecular H-bonding interactions. Similarly, bands arising from the 3-formyl carbonyl group of Chl *b* are observed at 1665 cm^{-1} when these groups are free from interactions, and they may shift down to 1620 cm^{-1} upon formation of H-bonds between this group and its environment (Lutz, 1984).

Figure 3 displays the high frequency region (1550−1750 cm^{-1}) of LHCII aggregated (top) and trimers (bottom) as well as a difference spectrum computed from these spectra. In the 1620−1710-cm^{-1} region, a broad and unresolved cluster can be observed, reflecting the presence of formyl and keto carbonyl groups involved in various types of intermolecular interactions. However, there is a clear difference between these two spectra, and a 1639-cm^{-1} component present in the oligomeric LHCII is clearly missing in the RR spectra of the trimer LHCII. The only difference between these two samples is the presence of 0.1% dodecyl β-D-maltoside used for preparing trimeric LHCII. This band thus cannot arise from chemicals that would be present in the sample containing LHCII oligomers and not in that containing LHCII trimers. RR spectra of carotenoids are not expected to display a band with such an intensity in this

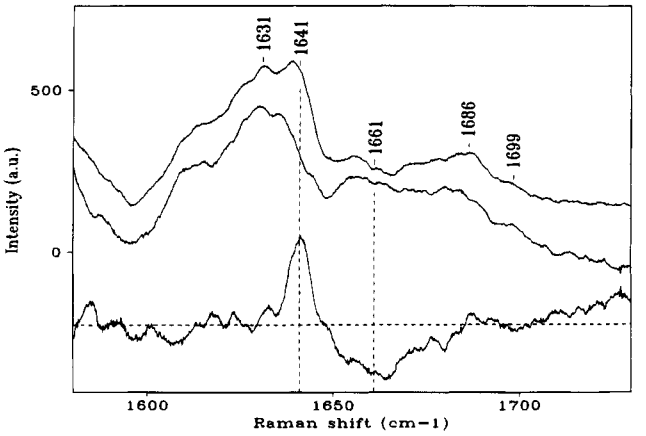

FIGURE 3: Resonance Raman spectra of LHCII trimers (medium trace) and aggregates (top) (1600−1725 cm^{-1}). Bottom: computed difference between spectra of aggregates and trimers. Excitation conditions: 441.6 nm; temperature, 77 K.

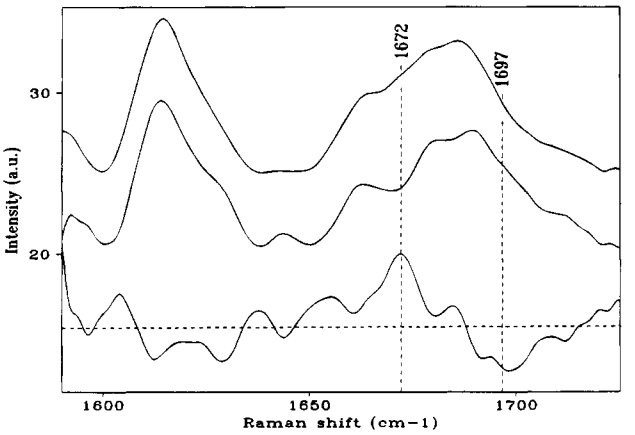

FIGURE 4: Resonance Raman spectra of LHCII trimers (medium trace) and aggregates (top) (1600−1725 cm^{-1}). Bottom: computed difference between spectra of aggregates and trimers. Excitation conditions: 413.1 nm; temperature, 77 K.

spectral range. One must thus conclude that this band arises from one population of Chl molecules and, because of its frequency, from a carbonyl stretching mode of these molecules. A change in the resonance conditions between these two samples due to perturbations of the Soret electronic transition of one or more population of Chl molecules could induce the disappearance from the RR spectra of the contribution of this population of pigments. Considering the excitation profiles of the different RR bands of chlorophyll in the 1300−1700-cm^{-1} spectral range (Lutz, 1979, 1984), it is very unlikely that such a phenomenon would not be accompanied by a decrease of the overall Chl *a* or Chl *b* contribution over the whole spectrum, and it would in particular affect the intensity of the bands arising from the keto carbonyl stretching modes. This is not observed, and the absence of this band in LHCII trimers cannot be induced by changes in resonance conditions. This spectral change thus reveals a change in the interactions assumed by one population of carbonyl groups, i.e., that one population of carbonyl groups assumes different intermolecular interactions in LHCII trimers and oligomers.

The absence of any observed change at 1639 cm^{-1} when Chl *a* pigments are excited at 413.1 nm (see Figure 4) indicates that this band arises from Chl *b* molecules. These molecules possess two carbonyl groups, namely, a formyl and a keto. A 1639-cm^{-1} frequency has been observed for

keto carbonyl stretching modes only in models involving direct Chl−Chl interactions. This type of interaction induces specific alterations in resonance Raman spectra, which have never been observed up to now in higher plant and algae complexes (Lutz, 1977). This band thus more likely arises from the stretching mode of a formyl group of a Chl *b* molecule involved in H-bonding interactions with its environment. In spectra of LHCII trimers (Figure 3), there is a weak, broad band at ca 1660 cm^{-1} that is not present in the RR spectra of the oligomers. As this is the main component selectively observed in the trimer spectra, we assign this band as being the counterpart of the 1639-cm^{-1} one observed in the oligomer spectra. According to this result, one population of formyl carbonyl, which would be free from interactions in the LHCII trimers, finds an H-bonding partner during LHCII oligomerization. It is worth noting that the calculated area for this 1639-cm^{-1} band corresponds to ca. 1/20th of the whole intensity observed in the carbonyl stretching mode region. Considering the number of Chl *a* and Chl *b* present in the LHCII complexes and considering that each Chl *b* has two carbonyl groups and each Chl *a* has one, the population of Chl *b* concerned by the structural changes described here represent about one Chl *b* per LHCII. However, this quantitation just constitutes an estimate as many parameters may influence it, in particular differences in the resonance Raman cross-section of the different pigments at any given wavelength. In RR spectra of whole thylakoids, there is a 1639-cm^{-1} contribution under these excitation conditions, the frequency of which matches well with that of this band (data not shown).

Figure 4 displays the higher frequency range (carbonyl stretching frequency range) of RR spectra of LHCII oligomers (top) and trimers (bottom) at 413.1-nm excitation, in conditions where contributions of Chl *a* molecules are enhanced relative to those of Chl *b*. In this frequency range, the RR signal appears clearly dependent on the aggregation state of the LHCII. Although these clusters of bands are somewhat congested, in the trimer spectra, the 1662-cm^{-1} contribution is clearly resolved while it appears as the shoulder of a 1672-cm^{-1} band in the oligomer spectra. Beside this, no major difference is observable between these spectra, but an increase in the intensity on the higher frequency side of the C=O stretching band cluster in the trimer spectra, between 1690 and 1710 cm^{-1}, is better resolved in the difference spectrum. The integrated area corresponding to the missing RR intensity at 1672 cm^{-1} represents 1/15th of the whole area integrated over the C=O stretching frequencies. It is thus most likely that one Chl *a* pigment only per LHCII complex has its keto carbonyl group affected by the aggregation process. It should be noted that both the C=O stretching bands clusters in this figure have the same integrated area, i.e., the missing RR intensity in RR spectra of trimers is compensated by the additional RR intensity observed at ca. 1697 cm^{-1}. Since 1672 cm^{-1} is a frequency of keto carbonyl groups involved in intermolecular interactions while 1690−1710 cm^{-1} corresponds to the frequency observed for free from interaction keto carbonyls, we may conclude that the keto carbonyl group of one Chl *a* pigment per LHC monomer changes from interactions upon oligomer formation. Considering that this keto carbonyl group is vibrating at ca. 1697 cm^{-1} in LHCII trimers and at 1672 cm^{-1} in LHCII oligomers, it is possible to estimate that it enters a ca. 3.6 kcal/M H-bond during oligomer formation (Zadorozhnyi & Ishchenko, 1965).

In summary, it is clear that the structure of the LHCII trimer is altered when it forms aggregates. The conformation of one population of carotenoid molecules is changed, and there is a change in hydrogen bonding to chlorophyll *a* and chlorophyll *b*. At present, it is not possible to assign these changes to particular pigments in the LHCII structural model. However, given the twisting of the lutein polyene chain that is evident in this structure (Kühlbrandt et al., 1994), it is tempting to conclude that the 950-cm^{-1} band arises from these intrinsic carotenoids. Given their structural importance, if correct, this would imply a rather large shift in protein conformation upon LHCII aggregation. The occurrence of these structural changes shows that the LHCII is a dynamic structure in which the properties of bound pigments can be altered by the organization of the complex. This is consistent with the suggestion that changes in organization of LHCII provide a possible mechanism by which the thylakoid membrane *in vivo* can switch between an efficient light-harvesting unit under light-limiting conditions to a powerful dissipative structure when light is in excess (Horton et al., 1991; Horton & Ruban, 1992, 1994). In the first discussion about how the increase in energy quenching may arise both from LHCII *in vitro* and for qE *in vivo*, it was suggested from changes in the visible absorption spectra that new pigment interactions, involving both chlorophyll and xanthophyll were involved (Ruban & Horton, 1992; Ruban et al., 1992). The resonance data shown here provide strong support for the existence of such changes in LHCII. The extent to which the increased H-bonding to chlorophyll relates to the actual formation of the quencher can obviously not be established at the present. The extra H-bonding may restrict the position of chlorophyll molecules to give rise to close pigment distances and precise orientations that are apparent in the structural model. In this model, it indeed appears that most chlorophyll molecules are in van der Waals contact with another chlorophyll molecule, and a cluster of three pigments is even described (Kühlbrandt et al., 1994). Such close pigment−pigment interactions may be necessary for quenching. The possibility that such dynamic response of LHCII occurs *in vivo* is supported by evidence for a higher order of organization of this complex above the trimer level derived from thylakoid fractionation (Bassi & Daniese, 1992), 77 K fluorescence emission spectroscopy (Ruban & Horton, 1994), circular dichroism (Garab et al., 1988), and singlet-triplet annihilation studies (Kolubayev et al., 1986). Moreover, in the case of circular dichroism and 77 K fluorescence experiments, there is also evidence to support the occurrence of changes in LHCII structure/organization upon illumination. Features characteristic of aggregated LHCII have also been observed in resonance Raman spectra of thylakoid membranes and whole leaves (experiments in progress). Therefore, resonance Raman spectroscopy may in the future provide a way of exploring the specific molecular changes underlying the regulation of light harvesting *in vivo*.

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