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Low-temperature spectroscopy of monomeric and trimeric forms of reconstituted light-harvesting chlorophyll *a*/*b* complex

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Abstract

Low-temperature (polarized) light spectroscopy was used to study reconstituted light-harvesting complex (LHCII) of Photosystem II, both in the monomeric and trimeric form. Monomeric LHCII was reconstituted from the apoprotein overexpressed in *Escherichia coli* and pigments were extracted from chloroplast membranes and subsequently separated from unbound pigments on an anion-exchange column. These monomers trimerize in the presence of a lipid fraction isolated from thylakoids or of pure phosphatidylglycerol. The spectroscopic properties are compared to those of monomeric and trimeric forms of native LHCII and many similarities exist. However, these reconstituted complexes seem to contain slightly fewer chlorophylls, whereas one pigment that is a chlorophyll *a* in native LHCII is replaced by a chlorophyll *b* in reconstituted LHCII.

Keywords: Light-harvesting complex II; Polarized spectroscopy

1. Introduction

The most abundant photosynthetic pigment-protein complex in higher plants is light-harvesting complex II (LHCII), which functions as an antenna to enhance the efficiency of photoynthesis. LHCII exists in a trimeric form and consists mainly of a mixture of the highly homologous forms, Lhcb1 and Lhcb2 [1]. Besides several xanthophylls, each monomeric unit contains approximately 8 Chl *a* and 6 Chl *b* molecules [1]. A 3.4 Å atomic model of LHCII was obtained from electron crystallography on two-dimensional crystals of trimeric LHCII. It is unknown whether the individual trimers contain either Lhcb1, Lhcb2 or a mixture of both [2]. Twelve chlorophylls per monomer have been resolved, but the resolution is not high enough to discriminate between Chl *a* and Chl *b*.

Recently, it was reported that Lhcb1 polypeptides that are obtained after overexpression in *Escherichia coli* can be reconstituted with pigments and lipids to form trimers which in turn can form two-dimensional crystals that resemble the crystals of native LHCII at low resolution [3]. These crystals contain only one type of LHCII, which may eventually lead to an atomic model at higher resolution. A promising way of identifying the nature of the various chlorophylls might be the combination of spectroscopic and biochemical studies of reconstituted LHCII for which the presumed chlorophyll binding sites have been altered. Detailed studies are needed to investigate the structural similarity between native and reconstituted LHCII. It was shown in [3] that reconstituted monomers and trimers present CD spectra at room temperature which are largely similar to those of the native forms and this indicates a high degree of structural similarity.

A detailed comparison has been made between the polarized spectroscopic properties of trimeric LHCII and monomeric forms that were obtained in three different ways [4]. So far, eleven absorbance bands have been

Abbreviations: Chl, chlorophyll; CD, circular dichroism; fwhm, full width at half maximum; LD, linear dichroism; PG, phosphatidylglycerol; PlA_2, phospholipase A_2; PlA_2 monomers, LHCII monomers that are obtained after treating trimeric or aggregated LHCII with phospholipase A_2.

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identified for trimers in the Q_y region (roughly between 640 and 680 nm) at cryogenic temperatures, whereas significant changes were observed after monomerization. The large number of discernible spectral features allows a detailed comparison of reconstituted and native LHCII.

In [5] it was demonstrated that lipids (in particular phosphatidyl glycerol (PG)) are essential for trimer formation of LHCII and treatment of trimeric LHCII with phospholipase A_2 (plA_2) leads to monomerization. The plA_2-induced monomerization leads to the release of several pigments and the disappearance of some spectral features, especially in the Chl *a* absorption region, although some Chl *b* is also released [4]. Similar spectral changes are observed after removal of the N-terminal domain of 49 amino acids, which is essential for PG binding [5]. On the other hand, monomerization that is induced by high concentrations of non-ionic detergents does not lead to the release of chlorophylls [4].

To further characterize monomeric and trimeric forms of reconstituted LHCII we have applied various low-temperature spectroscopic techniques.

2. Materials and methods

Preparations of monomeric and trimeric reconstituted LHCII were obtained as described in [8]. For the 77 K spectroscopic experiments the reconstituted LHCII complexes were dissolved in a buffer containing about 60% (v/v) glycerol, 20 mM Hepes at pH 7.5 and 0.03% n-dodecyl β,D-maltoside. Measurements were performed as described in [4].

3. Results

3.1. Absorption

The 77 K absorption spectra of reconstituted monomeric and trimeric LHCII are given in Fig. 1a and a blow-up of the Q_y region is shown in Fig. 1b. Although the spectra differ in detail, their overall appearance is rather similar and all distinguishable peaks and shoulders are present in the spectra of both preparations. The difference in the Soret region (between 400 and 500 nm) cannot be considered to be significant due to variations in scattering background.

When comparing the spectra of reconstituted and native complexes [4,7] it is most striking that the relative Chl *b* contribution is larger for reconstituted complexes as evidenced from the relatively larger contributions from the Chl *b* 474 and 650 nm bands. This agrees with the fact that the Chl *a*:Chl *b* ratio is around 1.1 for reconstituted complexes [6], whereas it is around 1.3–1.4 for native trimers [2,7]. Nevertheless, the most notable features in the Q_y absorption region of native LHCII (around 650, 661,

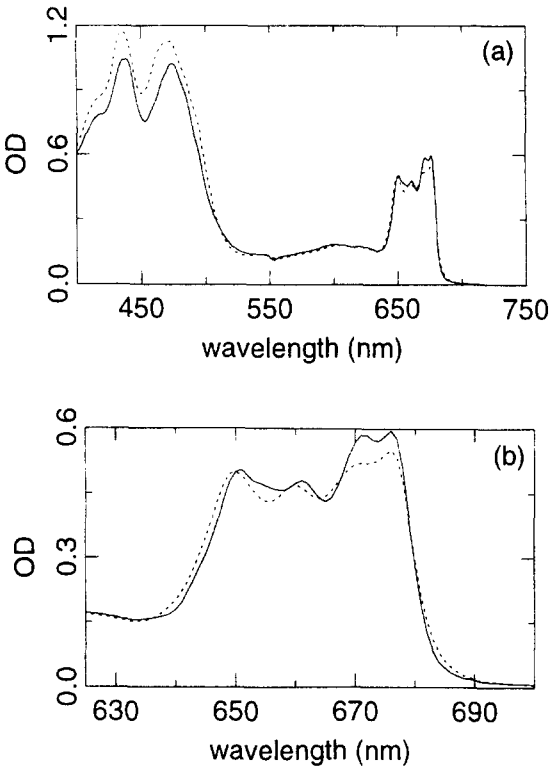

Fig. 1. (a) Absorption spectra of reconstituted trimeric LHCII (————) and monomeric LHCII (---) at 77 K. The spectra were recorded with an optical bandwidth of 1 nm and were normalized to each other at 650 nm. (b) See (a): spectra were normalized to 0.5 at 650 nm.

670 and 676 nm) are also present for reconstituted complexes, although their respective heights differ. As was already pointed out in the Introduction, some pigments are released upon treatment of native trimeric LHCII with plA_2. Since reconstituted LHCII monomers do not contain lipids, it seems most appropriate to compare their absorption spectrum to that of 'native' monomers, which have been obtained with a plA_2 treatment. Indeed, the spectrum of the latter complex resembles that of the reconstituted complexes in the sense that the intensity of the 670 and 676 nm bands is lower than for native trimers. A notable difference between the absorption spectrum of plA_2 monomers and reconstituted monomers and trimers is the presence of the 661 nm absorption band in the latter preparations. This may be partly explained by the fact that preparations of plA_2 monomers contain free Chl *a* which possesses a broad absorption band around 670 nm which could (partly) mask the 661 nm band. Future experiments will have to reveal whether the 661 nm band is still present in plA_2 monomers.

3.2. Fluorescence

The 77 K fluorescence spectra of reconstituted monomers and trimers are given in Fig. 2. The spectra peak at 679 and 680 nm, respectively; the widths are 9 and 11 nm fwhm. According to the small fluorescence band

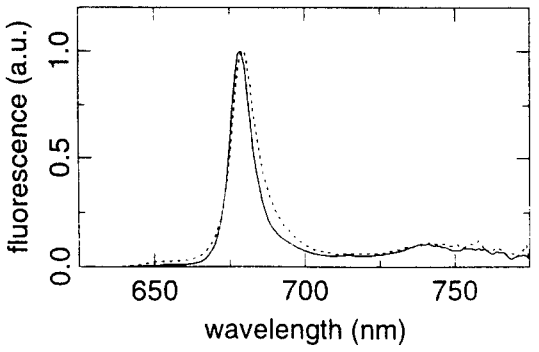

Fig. 2. Fluorescence emission spectra (optical bandwidth 3 nm) of reconstituted trimeric LHCII (————) and monomeric LHCII (---) at 77 K after excitation at 610 nm (bandwidth 12 nm). The spectra were normalized to 1 in the peak.

below 660 nm, samples of the reconstituted complexes contain some uncoupled Chl *b*. In the specific preparations of reconstituted monomers used in this experiment the amount of uncoupled Chl *b* was apparently not very high.

The spectrum of reconstituted trimers is similar to that of native trimers (peak at 680–681 nm, fwhm 9 nm) [7]. A comparison with spectra of native plA_2 monomers [4] is not appropriate, since those preparations contained significant amounts of free Chl *a*, partly indicated by the broad Chl *a* fluorescence band. The shoulder around 700 nm in the fluorescence spectrum of native trimeric LHCII (which is probably due to the presence of some LHCII aggregates [9]) is missing for the reconstituted trimers.

3.3. Circular dichroism

The room-temperature CD spectra of reconstituted monomers and trimers are given in Fig. 3. The characteristic changes upon trimerization of reconstituted complexes that were reported previously [3], notably the appearance of a shoulder in the Chl *b* Q_y band (just below 650 nm) and of the negative band near 475 nm can be observed in Fig. 3a (room temperature). In the 77 K spectra of Fig. 3b it is clear that trimerization leads to considerable fine structure in the Chl *b* region.

Although the spectra for reconstituted and native complexes [4] differ in detail, the global similarity is striking; especially the difference in the Chl *b* region between monomers and trimers is very similar for reconstituted and native complexes [4]. This similarity indicates that a considerable part of the pigment–pigment interactions present in native LHCII is also present in the reconstituted LHCII.

3.4. Linear dichroism

The 77 K LD spectra of reconstituted monomers and trimers in the Q_y region are presented in Fig. 4. In the Chl *a* region both spectra are characterized by a positive band at 676 nm and a shoulder in the 660–665 nm region. In the Chl *b* region both spectra show a minimum at 655–657

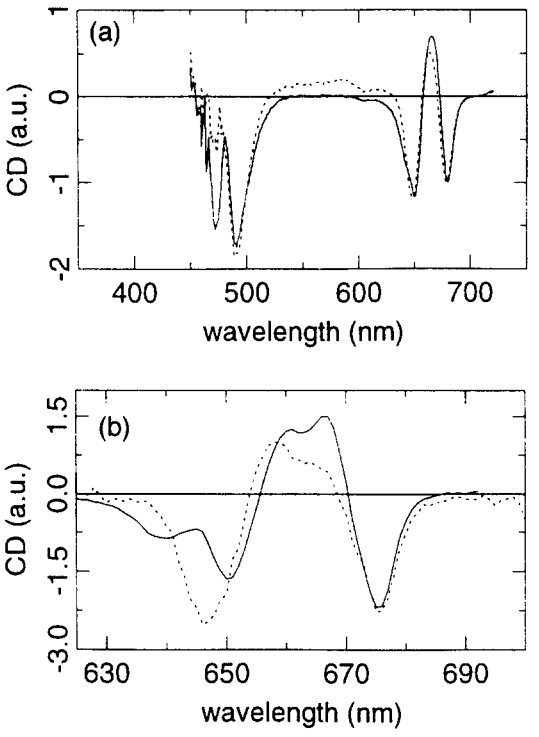

Fig. 3. Circular dichroism spectra of reconstituted trimeric LHCII (————) and monomeric LHCII (---) at room temperature (a) and at 77 K (b). All spectra were recorded with an optical bandwidth of 3 nm.

nm and a maximum (shoulder for the monomers) near 640 nm. The spectrum of monomers shows another positive band at 646 nm.

The LD spectra of reconstituted LHCII show significant differences from the spectra of native LHCII. Similar is the (positive) LD for monomers and trimers of native and reconstituted complexes in the Chl *a* region, pointing to a comparable average Chl *a* orientation. Other similarities include the negative band around 655–657 nm and the positive band (shoulder) near 640 nm. Notably, the latter is absent for plA_2 monomers, but present for trimers and for monomers obtained in high detergent concentrations [4]. There are, however, significant differences in the Chl *b*

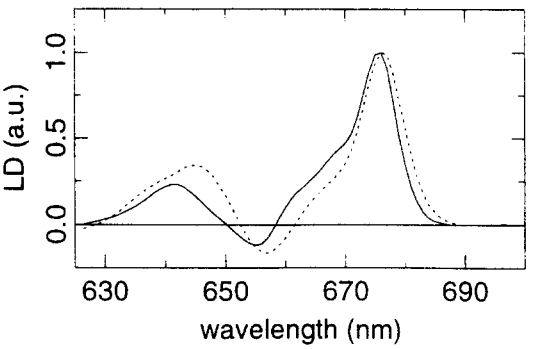

Fig. 4. Linear dichroism spectra of reconstituted trimeric LHCII (————) and monomeric LHCII (---) at 77 K. The spectra were recorded with an optical bandwidth of 3 nm and were normalized to 1 in the peak.

region. Native monomers contain a positive band around 649 nm whereas this band is much more pronounced for reconstituted monomers and has a maximum near 646 nm. An important difference between the LD spectra of native monomers and trimers is the presence of the fine structure for trimers with negative/positive bands at 647 and 652 nm, respectively. These bands correspond to the bands that can also be observed in the CD spectrum at the same wavelengths [4]. Although reconstituted trimers also contain the fine structure in the CD spectrum, no fine structure can be observed in the LD spectrum. This can easily be explained if a negative LD band at 646 nm is compensated by a positive band at the same wavelength. Indeed, the reconstituted monomers exhibit significantly more positive LD at 646 nm than the native monomers and it is very likely that the same band contributes to the LD of the trimer.

4. Discussion

It was demonstrated earlier that, at room temperature, reconstituted monomers and trimers have absorption and CD spectra that strongly resemble those of native complexes [3,6]. In the present study we performed measurements at 77 K and also used different techniques in order to compare the spectral properties of native and reconstituted complexes in more detail. A comparison with the spectra of native complexes [4,7] is allowed, since the medium composition is very similar. No essential spectral differences exist between LHCII with n-dodecyl β,D-maltoside ([7], the reconstituted complexes) or n-octyl β,D-glucopyranoside [4]. Even at low temperatures, the native and reconstituted complexes (both monomers and trimers) have many spectral features in common [4,8], which points to a similar structural organization. However, some specific differences exist, most notably the relative increase of the Chl *b* absorption. The differences may be due to a lower lipid content in the LHCII monomers used in this study as compared to native LHCII. Biochemical analysis of lipid content in these LHCII preparations is underway. Whereas PG is essential for trimerization and is also required for the formation of reconstituted trimers, it is also essential for the binding of some chlorophylls to LHCII [4]. Monomers that have been obtained from native trimers after treatment with plA_2 give rise to an absorption spectrum that resembles those of reconstituted complexes especially in the Chl *a* region. It was shown in [10] that pigments are essential for the folding of the LHCII polypeptide into its native conformation. Despite the fact that some chlorophylls are absent in the reconstituted complexes, trimerization is still possible. Besides this difference in pigment content, it seems likely that one pigment that is a Chl *a* in the native complex is 'replaced' by a Chl *b* in the reconstituted complex. The reconstituted trimers exhibit a positive LD band in the Chl *b* region, which is absent for native trimers. On the other hand, the integrated area of the positive Chl *a* around 676 nm is larger for the native trimers than for the reconstituted trimers when compared to the intensity of the shoulder between 660 and 665 nm. This is most easily explained by the 'replacement' of a Chl *a* by a Chl *b* molecule with a similar orientation. Recently, it has become possible to reconstitute LHCII with pigment compositions that vary to a large extent. Hopefully, this will allow us to gain a more detailed understanding of the spectroscopy of native LHCII.

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