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Biomimetic model of a plant photosystem consisting of a recombinant light-harvesting complex and an artificial dye energy trap

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Introduction

The light-harvesting chlorophyll (Chl) *a*/*b* antenna in higher plants contributes to photosynthesis by absorbing light energy and funneling it into the photosynthetic reaction centers where the conversion into an electrochemical potential takes place. The components of this photosynthetic antenna, the light-harvesting Chl-*a/b* complexes (LHC) fulfill this task with the help of numerous protein-bound pigments, carotenoids and Chl *a* and *b* that exchange absorbed energy rapidly and at high efficiencies, within single light-harvesting complexes and then between such complexes until the energy reaches the core complexes of the photosystems containing the reaction centers (van Amerongen and van Grondelle, 2001).

The most abundant Chl-*a/b* complex is the major LHC of photosystem II, LHCIIb, comprising roughly 50 % of the total chlorophyll in higher plants. This complex can be reconstituted *in vitro* from its protein and pigment components, using either denatured thylakoid proteins (Plumley and Schmidt, 1987) or recombinant LHCIIb apoprotein, Lhcb1 (Paulsen *et al.*, 1990). Recombinant LHCIIb was shown to exhibit biochemical and spectroscopic properties very similar to those of native LHCIIb (Cammarata *et al.*, 1990; Paulsen *et al.*, 1990) and, upon 2D crystallization, yielded electron diffraction patterns closely resembling those of crystals from native LHCIIb (Hobe *et al.*, 1994).

The *in-vitro* reconstitution of recombinant LHCIIb opens up the possibility to introduce useful modifications into the structure by altering the amino acid sequence. Thus, anchors have been generated for immobilizing the complex (Rogl *et al.*, 1998; Kosemund *et al.*, 2000) or for site-specific fluorescence labeling (Paulsen *et al.*, 1993). In this paper we show that recombinant LHCIIb can be coupled to an artificial energy trap, a fluorophore that collects, via efficient energy transfer, a large part of light energy absorbed by the LHCIIb pigments. Such constructs may turn out to be useful for studying the light-harvesting function of LHCIIb or for designing biomimetic models of a photosystem, converting absorbed light energy into charge separation.

Materials and methods

Fluorescence-labeled recombinant Lhcb1
Recombinant versions of LHCIIb were used in this work (Paulsen *et al.*, 1990). Two mutants were utilized, one with the only Cys in position 79 exchanged with Ser (mutant C79S) and starting from that one with Ser in position 3 exchanged with Cys (mutant S3C). The numbering of amino acids in Lhcb1 is according to Kühlbrandt *et al.* (1994).

Maleimido-benzoylterrylenedicarboximide (BTI) was covalently coupled to the N-proximal cystein in Lhcb1 mutant S3C. One nmol Lhcb1 in 25 µl buffer (1 g/l SDS, 10 g/l n-octyl-β-D-glucopyranoside and 20 mM sodium phosphate pH 7.0) was reduced for 15 min at 37°C with 2 mM Tris-(-2-cyanoethyl)-phosphine and then mixed with 12.5 nmol BTI, solubilized in 2 µl tetrahydrofuran. After 2 h incubation at 37°C Lhcb1 was precipitated with 80% (v/v) acetone and 10 mM acetic acid. The protein pellet was dissolved in 20 µl 20 g/l SDS, 100 mM Tris/HCl pH 9.0 and boiled for 2 min in order to dissolve protein-dye aggregates and then centrifuged (5 min at 16,000 g) in order to remove dye aggregates.

Labeled Lhcb1 was purified by preparative SDS gel electrophoresis (Mini Prep Cell, Bio-Rad Laboratories GmbH, München, Germany). The purified Lhcb1-BTI fractions were concentrated by ultrafiltration (Filtron, 30 kDa, Filtron GmbH, Karlstein, Germany).

Reconstitution of recombinant LHCIIb
Reconstitution of Lhcb1-BTI followed the procedure given in Paulsen *et al.* (1993). Reconstituted LHCIIb-BTI was purified by sucrose density centrifugation for 16 h at 345,000 g using gradients of 0 M to 1 M sucrose with 1 g/l n-dodecyl-β-D-maltoside and 10 mM Tricine/NaOH pH 7.8.

Fluorescence measurements, quantification of resonance energy transfer
Fluorescence emission spectra were measured using a Fluoromax-2 (Jobin Yvon, Grasbrunn, Germany) at 297 K and 77 K (excitation wavelength 410 nm, excitation and emission bandwidths 3 nm, integration time 0.1 s). The spectra were corrected for fluctuations in excitation light intensity and wavelength dependent changes in photomultiplier sensitivity. All LHCIIb samples had an optical density of <0.1 to minimize fluorescence reabsorption. For 77 K measurements the samples were brought to 60% (v/v) glycerol.

Energy transfer from Chl *a* to BTI was calculated from the quenching of donor (Chl *a*) emission in the presence of the acceptor. LHCIIb with and without BTI attached were brought to the same Chl-*a* absorption at 410 nm. The dye absorption was negligible at this wavelength. Energy transfer efficiency was estimated through the comparison of maximal Chl-*a* fluorescence with and without energy transfer to the dye.

$$\text{Energy transfer (\%)} = \left(1 - \frac{\text{maximum Chl - } a \text{ emission in LHCIIb - BTI}}{\text{maximum Chl - } a \text{ emission in LHCIIb}}\right) \bullet 100$$

Trypsin digestion
LHCIIb (about 3 µg/ml), purified by sucrose density centrifugation was incubated with 0.1 mg/ml trypsin in 10 mM Tricine/NaOH pH 8.0, 0.2 mM EDTA for 30 min at room temperature.

Reduction of BTI with sodium dithionite
About 1 nmol Lhcb1-BTI in 1 ml 1 g/l SDS, 50 mM Tris, 384 mM Glycin and 1 mM EDTA was reduced through the addition of a few mg of sodium dithionite. Emission spectra of Lhcb1-BTI were measured before and after dye reduction.

Results

Site-specific fluorescence labeling of recombinant LHCIIb
Recombinant LHCIIb was labeled site-specifically with BTI, a rylene dye. BTI is a fluorophore capable of accepting excitation energy from Chl *a*. BTI was functionalized with a maleimido-group and reacted with the singular mercapto group in Lhcb1 mutant S3C at Cys3. Labeling experiments with the cystein-free protein mutant C79S demonstrated site specificity of the BTI label in S3C. BTI was attached to about 50% of the protein. Labeled Lhcb1 was distinguishable from the non-labeled protein by a different migration behavior in a Laemmli polyacrylamide gel, enabling us to purify the Lhcb1-BTI coupling product by preparative gel electrophoresis (not shown).

Excitation energy transfer from Chl a to BTI
Lhcb1-BTI could be reconstituted with pigments *in vitro*, yielding fluorescently labeled recombinant LHCIIb, following the same procedures as for unlabeled protein. The complexes were found to be of similar stability e.g. in partially denaturing gel electrophoresis as those reconstituted with the wildtype protein and exhibited virtually 100% energy transfer from Chl *b* to Chl *a* (not shown).

Figure 1a shows a fluorescence emission spectrum of LHCIIb-BTI at 297 K. Excitation was at 410 nm where Chl *a* but not Chl *b* absorbed. The emission spectrum of LHCIIb-BTI exhibited a maximum at 678 nm, near the emission maximum of Chl *a*, and a small shoulder at 730 nm, near the emission maximum of BTI. Chl-*a* emission in LHCIIb-BTI was significantly reduced compared to Chl-*a* emission of the same concentration of non-labeled LHCIIb. Therefore, an apparent efficiency of energy transfer from Chl *a* to BTI was calculated from the quenching of donor (Chl *a*) fluorescence, i.e. the maximum intensity of Chl-*a* emission in the donor-acceptor construct (LHCIIb-BTI) relative to Chl-*a* emission in the absence of the acceptor (LHCIIb), and found to be 70% ± 4% at 297 K (Fig. 1a) and 85% ± 4% at 77 K (Fig. 1b). Despite the strong quenching of donor fluorescence, BTI fluorescence was low in LHCII-BTI due to its quantum yield of 3% ± 0,3%, measured at 297 K. In order to visualize acceptor dye emission in the LHCIIb-BTI construct, figures 1a and 1b also show its emission spectrum normalized to the same maximum emission as unlabelled LHCIIb.

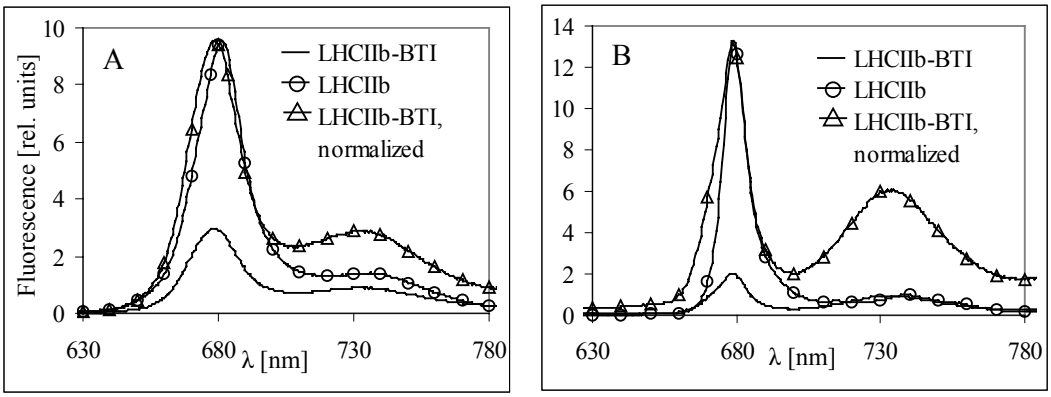

Figure 1: Fluorescence emission of LHCIIb and LHCIIb-BTI at 297 K (A) and 77 K (B). Purified LHCIIb and LHCIIb-BTI were brought to exactly (±1%) the same absorption at 410 nm (predominantly Chl-*a* absorption). Fluorescence emission spectra (excitation at 410 nm) were measured of these solutions. In order to visualize the BTI contribution (emission maximum at about 735 nm) to the LHCIIb-BTI emission spectrum, the latter is also shown normalized to the maximum signal of LHCIIb emission.

In order to test whether the apparent quenching of Chl-*a* fluorescence in LHCIIb-BTI was due to energy transfer from Chl *a* to the dye, we measured the fluorescence emission of the trypsin-digested LHCIIb-BTI. Only the N-terminal 35 amino acids in LHCII are cleaved off by trypsin (Paulsen *et al.*, 1993). Trypsin was therefore expected to separate donor and acceptor and, thus, to abolish energy transfer. Treatment of non-labeled LHCIIb with trypsin decreased Chl-*a* emission which was due in part to diluting the LHCIIb solution (Fig. 2). By contrast, when LHCIIb-BTI was treated with trypsin, Chl-*a* emission rose to the same level as in trypsin-treated LHCIIb. This observation strongly suggests that Chl-*a* emission in LHCIIb-BTI was quenched by efficient energy transfer to the acceptor dye.

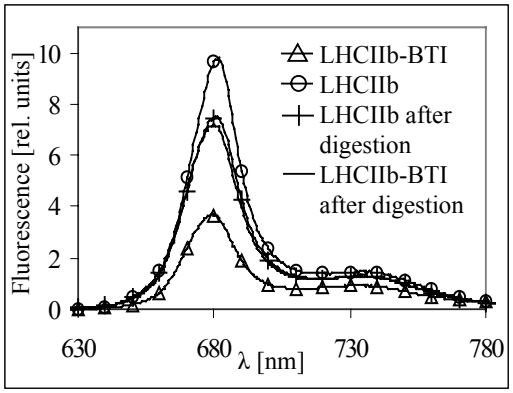

Figure 2: Fluorescence emission of LHCIIb and LHCIIb-BTI befor and after protein digestion with trypsin. Purified LHCIIb and LHIIb-BTI were brought to the same Chl-*a* absorption at 410 nm and treated with trypsin. Emission spectra (excitation at 410 nm) were taken before and after trypsin digestion.

In order to exclude that charge transfer from Chl *a* to BTI was the reason for the quenching of Chl-*a* emission in Lhcb1-BTI, we tested the spectral effect of reducing the protein-bound dye by sodium dithionite. We observed a 13 nm bathochromic shift that was not detectable upon illuminating LHCIIb-BTI at 410 nm (not shown), indicating that no photoreduction of BTI occurred.

Discussion

Resonance energy transfer from Chl a to BTI

In the LHCII-BTI constructs strong quenching of Chl-*a* fluorescence appeared in the presence of the potential energy acceptor BTI. This reduction of the Chl-*a* quantum yield was not due to environmental effects, as Chl *b* kept transferring its excitation energy to Chl *a* in the construct and therefore could not suffer drastic changes in its molecular surrounding due to the dye coupled to LHCIIb. Moreover the reduction of the Chl-*a* quantum yield was reversible as the trypsin digestion experiment proved. When the energy accepting dye was separated from LHCIIb Chl-*a* fluorescence rose to the same extent as in unlabeled LHCIIb complexes.

Another scenario would be that BTI was engaged in charge transfer since it is a fairly good electron acceptor (BTI^-/BTI = - 0.83 V, Quante, 1995). However there were no indications for electron transfer from Chl *a* to BTI. The emission spectrum of BTI exhibited marked alterations when the dye was reduced with the strong reductant sodium dithionite, and we found none of these changes in the emission of LHCIIb-BTI when Chl *a* was excited. Therefore we are left with the conclusion that Chl-*a* emission is quenched in LHCIIb-BTI because of resonance energy transfer to BTI.

Maximum distance between emitting Chl a and BTI in LHCIIb-BTI

Since the N-terminal part of LHCIIb could not be resolved in crystallographic measurements (Kühlbrandt *et al.*, 1994) there is no information on the molecular distance between the donor (Chl *a*) and the acceptor (BTI) in LHCIIb-BTI. Furthermore, the relative orientations of donor and acceptor and, consequently, the orientation factor κ^2 are unknown. The maximum possible value of κ^2 is 4 which makes the critical Foerster distance R_0 83 Å (details of R_0 calculation to be published elsewhere). From this maximum R_0 a maximum distance between the emitting Chl-*a* molecule(s) in LHCIIb and BTI was calculated to be 72 Å. If the hydrophilic N-terminal domain would be fully extended away from the pigment-binding α helices the estimated distance
between Chls and N terminus is more than 200 Å. Even if the N-terminal domain was organized into an α helix which, according to the crystal structure, only part of it is, the distance would be about 95 Å. We conclude that the N-terminal domain in solubilized LHCIIb is not fully extended.

Biomimetic model of a plant photosystem

The apparent energy transfer between Chl-*a* molecules and BTI of 70% at 297 K and about 85% at 77 K is rather efficient. Most likely these values are still underestimated. Since we calculated transfer efficiencies from the quenching of donor fluorescence, all Chl-*a* molecules in the recombinant LHCIIb that were not coupled to BTI, either directly or via other Chl-*a* molecules, emitted to the same extent as in the absence of the energy accepting dye and thus lowered the apparent energy transfer efficiency. In LHCIIb-BTI constructs we saw virtually complete energy transfer from Chl *b* to Chl *a* but we cannot exclude partial uncoupling between Chl-*a* molecules.

Moreover, there may be significant back transfer of energy from BTI to Chl *a*. The critical Foerster distance is at least 1.5 times higher for the energy transfer from Chl *a* to BTI but this leaves R_0 for the back transfer of energy (calculated maximum of 54 Å)

well within the range of possible donor-acceptor distances. If significant energy back transfer in fact occurs then the apparent energy transfer efficiency from Chl *a* to BTI could be further raised by decreasing the fluorescence lifetime of the acceptor, e.g. by engaging the acceptor dye in a fast photochemical reaction.

A large number of constructs modeling photosystems have been described. Mostly those models are multichromophoric systems with highly ordered pigment arrays. Such arrays were achieved by covalently coupling synthetic porphyrins funneling excitation energy toward one specific porphyrin (Lin *et al.*, 1994; Wagner and Lindsey, 1994). Self assembling models of photosystems were designed by Haycock *et al.* (2000) and Tamiaki *et al.* (1996). Haycock *et al.* (2000) obtained a spontaneous aggregation of synthetic porphyrins in organic solvent funneling excitation energy toward one of the aggregated chromophors. Tamiaki *et al.* (1996) achieved self-organization of bacteriochlorophyll *c* and its derivatives in a detergent or lipid environment in the absence of protein, thus modeling a chlorosome with an energy acceptor. A model
including the charge separation function (Kuciauskas *et al.*, 1999) consists of a porphyrin system as antenna and a free-base porphyrin-fullerene dyade acting as a reaction center.

To our knowledge the LHCIIb-BTI construct is the first self-assembling biomimetic model of a plant photosystem that contains a recombinant light-harvesting complex of the photosynthetic apparatus in higher plants. It mimics the efficient transfer of absorbed light energy to an acceptor but differs from a photosystem in that light energy is not converted into charge separation. Experiments to add this feature to our model construct are underway.

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