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Isolation and characterization of a light harvesting complex II lacking the γ-polypeptide from *Rhodobacter capsulatus*

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Extraction of membranes isolated from the wild-type strain 37b4 of *Rhodobacter capsulatus* with Na_2CO_3 removed quantitatively the 14000 M_r γ-polypeptide from the light-harvesting complex II (LHC II). The same procedure was used to extract the γ-polypeptide from the isolated LHC II. The LHC II free of the γ-polypeptide (M_r 14000) and containing the pigment-binding polypeptides α (M_r 10000) and β (M_r 8000) showed the same absorption spectrum as LHC II in the presence of the γ-polypeptide. Reaction center (RC) bleaching of the extracted and untreated membranes showed the same kinetics. Furthermore, kinetics of RC bleaching at different light intensities were identical in extracted and unextracted preparations, indicating that the LHC II lacking γ-polypeptide was able to transfer excitation energy efficiently to the LHC I-RC complexes. This efficient coupling was also confirmed by low temperature (77 K) fluorescence spectroscopy. The results indicate that the γ-polypeptide is not required for the function of the LHC II.

Introduction

The photosynthetic apparatus of wild type *Rhodobacter capsulatus* strain 37b4 contains three different Bchl-protein complexes: RC and two light harvesting complexes known as LHC I and LHC II [1]. Fluorescence measurements have shown that the LHC I component is able to interconnect several RC complexes whereas LHC II connects many photosynthetic units [2].

The level of the LHC II is variable and depends on the external oxygen tension, light intensity and other growth conditions [3–5]. It has been reported that the LHC II of *Rb. capsulatus* consists of three polypeptides with apparent M_r 8000 (β); 10000 (α); and 14000 (γ) evealed by SDS-PAGE [6–8].

The only polypeptides of the LHC II which bind pigments noncovalently are α and β [9]. The N-termini of the α- and β-polypeptides are exposed on the cytoplasmic surface whilst the C-termini are exposed on or directed to the periplasmic side with the hydrophobic core containing the α-helical stretch transversing the cytoplasmic membrane [10]. The γ-polypeptide is exposed on the cytoplasmic side of the membrane [10].

In addition to the α- and β-polypeptides (coded by *pucAB*), the formation of the LHC II also requires the synthesis of Bchl, carotenoids and the expression of genes localized downstream of the *pucAB* operon. Three open reading frames have been observed in the downstream region (puc C, D and E) [11]. *pucE* codes for a 14000 subunit of the LHC II and may represent the γ-polypeptide species.

The function of the γ-subunit in the LHC II is largely unknown. We describe here a modified method for the isolation of the native LHC II from *Rb. capsulatus* free of the γ-polypeptide and examine its properties. We show that the γ-subunit is not necessary for the function of the LHC II.

Materials and Methods

Organisms and culture conditions

Rhodobacter capsulatus strain 37b4 (DSM 938) was cultivated in RÄH medium [5]. The average light intensity behind the culture flasks was 2000 W/m^2 and the growth temperature 30 °C.

Abbreviations: LHC, light harvesting complex; RC, reaction center; Bchl, bacteriochlorophyll; LDAO, lauryldimethylamine oxide.

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Chemicals and analytical measurements

DE 52 was obtained from Whatmann (U.K.), LDAO from Fluka (F.R.G.). Bchl concentrations were calculated from absorption measurements in acetone/methanol (7 : 2, v/v) extracts at 770 nm by using an extinction coefficient of 76 mM · cm^{-1} [12]. Membrane proteins were separated by SDS-PAGE on 1.0 mm slab gels [7] with an 11.5–16.6% continuous gradient of acrylamide. Gels were stained with Coomassie brilliant blue. Absorption spectra were recorded on a Kontron UV 860 spectrophotometer.

Bleaching and recovery of the RCs in chromatophores were measured with a kinetic photometer. Using a Silicon diode detector flash-induced formation of the Bchl cation radical was measured at 1000 nm. The xenon flash (20 μs duration) used for activation was filtered using interference filters at 857 nm (Balzers, Lichtenstein) and alternated to give a flash energy of 7 mJ at the sample position. The optical absorbance of all samples was 0.4 at 860 nm, optical path 10 mm, for both membrane preparations. When necessary, light energy from the flash was reduced by means of neutral density filters. Alternatively, light saturation of the RC bleaching was measured under continuous actinic illumination. In this case, the same filter combination was used as for the flash experiments and the actinic light source originated from a 50 W halogen lamp. Maximum light intensity on the surface of the sample cuvette was 25 W/m^2. LDAO was used at 1% final concentration to interrupt energy flux from the LHC II to the LHC I-RC complex as revealed by an increase in the fluorescence yield at 880 nm originating from the LHC II.

Isolation and purification of LHC II

The LHC II was isolated as in Ref. 8 with the following modifications: cells in the exponential growth phase were harvested, washed two times with 10 mM Tris-HCl (pH 7.6) and broken in a French pressure cell in the presence of DNase and phenylmethylsulfonyl fluoride. The preparation was centrifuged for 20 min at 15000 rpm in a Sorvall SS 34 rotor at 4°C. The supernatant was further centrifuged for 60 min at 45000 rpm in a Ti 60 rotor of a Beckman ultracentrifuge. This last pellet was washed with 10 mM Tris-HCl buffer (pH 7.6). The membranes were resuspended in 10 mM Tris-HCl buffer (pH 7.6) also containing 5 mM EDTA and sonicated twice for 30 s at 0°C under an atmosphere of nitrogen. The sonicated membranes were finally pelleted by ultracentrifugation in the Ti 60 rotor at 4°C.

The membranes of *Rb. capsulatus*, resuspended in Tris-HCl buffer to a concentration of 400 μg Bchl/ml were further diluted 1 : 1 with 10 mM Tris-HCl, buffer (pH 7.6) containing 1% LDAO. After solubilization by stirring at room temperature in the dark for 30 min the suspension was diluted with an equal volume of 10 mM Tris-HCl (pH 7.6) and layered on top of a sucrose step gradient (0.3/0.6/1.2 M sucrose) in 10 mM Tris-HCl (pH 7.6) buffer also containing 0.5% LDAO.

After centrifugation (38000 rpm in a Ti 60 rotor for 18 h) the three pigmented bands were collected. The uppermost band containing LHC II (B800–850) was dialyzed for 48 h against 10 mM Tris-HCl buffer (pH 7.6) containing 0.08% LDAO. The center and the lower fractions from the sucrose gradient contained mainly LHC I and RC in different proportions.

The complex from the upper band (B800–850) was applied to a DE52 column and eluted with 10 mM Tris-HCl buffer (pH 7.6) containing 0.5% LDAO and 0.09 mM NaCl. The eluate was dialysed against 10 mM Tris-HCl buffer (pH 7.6) containing 0.08% LDAO for 48 h. The sample was further concentrated with sucrose. The γ-polypeptide (M_r) was extracted from the LHC II by the following procedure. A 5 ml sample having an absorption of 40 at 260 nm (1 cm optical path) was treated with 150 ml of 200 mM Na_2CO_3 for 30 min at 4°C in the dark under gentle stirring. The extracted LHC II was washed twice with Tris-HCl buffer (pH 7.6) and centrifuged 2 × 1 h at 38000 rpm, Ti 60 rotor, 4°C.

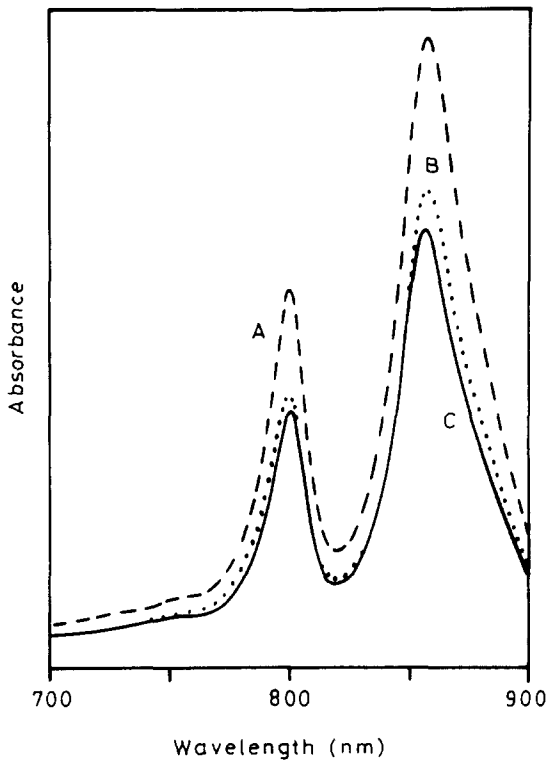

Fig. 1. Absorption spectra of Na_2CO_3-treated as well as untreated ICM isolated from *Rb. capsulatus* wild type strain 37b4. (A) LHC II isolated from intact membranes. (B) LHC II isolated from intact membranes and further extracted with Na_2CO_3. (C) LHC II isolated from membranes that were previously extracted with Na_2CO_3 to remove quantitatively its γ-polypeptide.

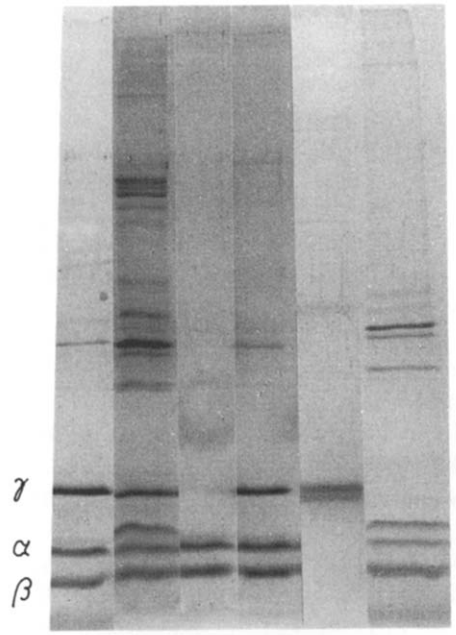

Fig. 2. SDS-polyacrylamide gel electrophoresis. Lane 1, LHC II isolated from the photosynthetic negative mutant of *Rb. capsulatus* Y 5; lane 2, purified ICM from *Rb. capsulatus* wild type strain 37b4; lane 3, LHC II complex isolated from previously Na_2CO_3 extracted ICM from *Rb. capsulatus* 37b4; lane 4, LHC II isolated from intact ICM from *Rb. capsulatus* 37b4; lane 5, Na_2CO_3 extracts from ICM from *Rb. capsulatus* 37b4 and lane 6, polypeptide composition of the *Rb. capsulatus* ICM after extraction with Na_2CO_3. On the left margin of this figure the position of the α, β and γ-polypeptides of *Rb. capsulatus* phototrophic negative mutant Y 5 are indicated.

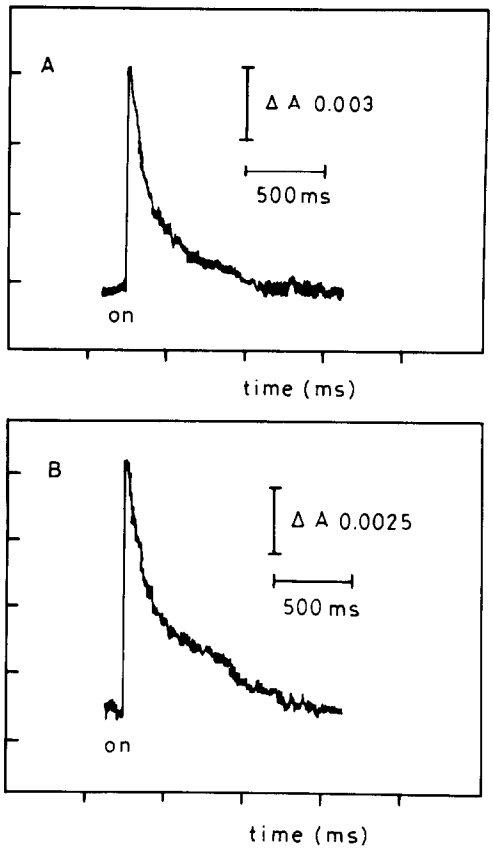

Fig. 3. Kinetic of the flash-induced bleaching of RC in untreated (A) and Na_2CO_3-treated membranes (B). In these cases the appearance of the Bchl radical was monitored at 1000 nm and a downwards deflection indicated an increase in the absorption at this wavelength. The flash had a duration of 20 μs. The absorption of the samples at 850 nm was identical at 0.4 absorption units. The intensity of the flash was 7 mJ measured at the position of the sample.

The same procedure was also used for the isolation of the LHC II from crude membranes.

Results

The LHC II isolated from *Rhodobacter capsulatus* and purified as described under Methods (preparation A) has two absorption bands in the near-infrared centered at 802 and 855 nm and an absorbance ratio at 855/802 of 1.5 (see Fig. 1, curve A). It contains three polypeptides having M_r of approx. 8000, 10000 and 14000 (Fig. 2, lane 1).

Preparation (A) was extracted with 200 mM Na_2CO_3 for 30 min in the dark as described in Materials and Methods. This new preparation was designated as B. It has the same absorption maxima at 802 and 855 nm (Fig. 1, curve B). Analysis of this sample by SDS-PAGE showed only two protein bands having apparent M_r of 10000 and 8000 (Fig. 2, lane 3). The 14000 polypeptide was quantitatively extracted and remained soluble in the extract (Fig. 2, lane 5).

We also isolated the LHC II from the same wild-type strain but from membranes that were previously extensively treated with Na_2CO_3 in order to remove the γ-polypeptide from the membrane. This procedure resulted in the same LHC II preparation containing α and β (10000 and 8000 M_r) polypeptides. This isolated LHC II (preparation C) showed also two near-infrared

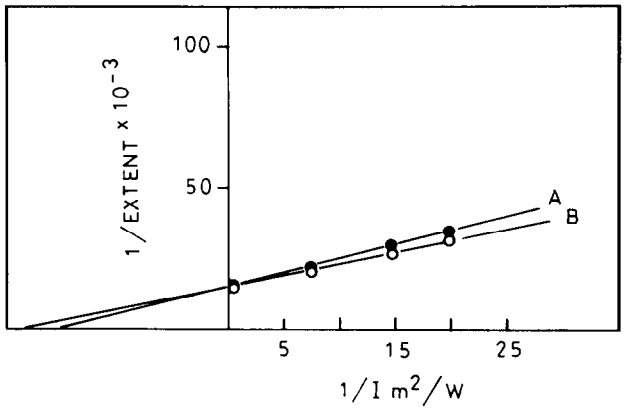

Fig. 4. Reciprocal plots of the extent of the RC bleaching under continuous illumination vs. the reciprocal of the actinic light. The actinic light originated from a 50 W tungsten halogen lamp and the excitation wavelength was selected by means of blue cut-off filters. The intensity of the actinic light was decreased, when required, by means of neutral density filters.

absorption bands, which are slightly shifted in their main peaks, those being centered at 805 and 852 nm, respectively, but otherwise identical to those shown in Fig. 1, curve A or curve B (Fig. 1, curve C).

Fig. 2, lanes 3 and 6 shows the polypeptide composition of the membranes before and after extraction with Na_2CO_3 clearly indicating the absence of the 14000 M_r polypeptide (γ) in the extracted one.

Membranes of a strain that expressed *puc B, A, C,* but not *DE* showed absorption maxima at 802 and 855 nm, but a relatively lower 802 nm peak and a protein pattern containing α and β but not γ (Tichy, H.V., personal communication).

The reversible RC-Bchl photooxidation was not impaired by Na_2CO_3 treatment of mebranes [Fig. 3). In order to determine whether light energy is transferred from the LHC II to LHC I-RC complex after extractions of membranes, the light saturation properties of the RC photooxidation were measured under continuous actinic light. It was expected that any decrease in the size of the antenna Bchl or change in the organization of the LHC II, as it might occur in the Na_2CO_3-treated membranes, should increase the light energy required to saturate the RC bleaching. Fig. 4 shows that the light saturation properties of both Na_2CO_3-treated and untreated membranes were almost identical with an apparent K_m for light of 1.14 and 1.32 $W \cdot m^{-2}$ respectively. Moreover, we have also determined, as control, that an increase of the K_m for light up to 5-times its value is to be expected if, as it is the case in the presence of added detergents (1% LDAO final concentration) the LHC II become uncoupled from the LHC I-RC complexes, as evidenced by an increased fluorescence yield originating from the B850 Bchl (appearance of a fluorescence band at 77 K centered at 882 nm). Such an increase, as mentioned above, was clearly not observed after extracting the chromatophores with Na_2CO_3 and the K_m for light of this preparation was even slightly lower than that of untreated membranes. These results allowed us to conclude that apparently no interruption of energy flux between the LHC II and LHC I-RC complexes occurs in the membranes after extraction of the 14000 M_r polypeptide and that the modified LHC II is, by this criterium, indistinguishable in its function from that normally present in the untreated membranes.

Additional information on the energy transfer between LHC II and the LHC I-RC complex was obtained by measuring the low temperature fluorescence (77 K) of both types of membrane. Fig. 5 shows that the low-temperature emission spectra are identical with a single emission band originating from the B870 band.

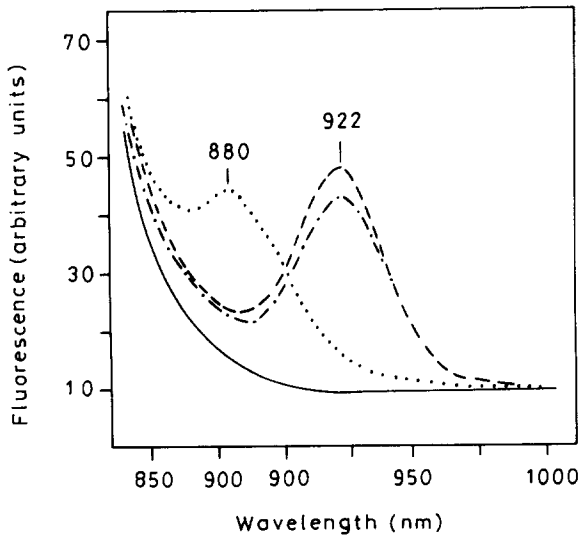

Fig. 5. Low-temperature fluorescence (77 K) of treated (– – –) and untreated membranes (·–·–·). Also shown is the fluorescence spectrum of the purified LHC II (·····). The samples were resuspended in 60% glycerol to decrease turbidity due to freezing.

Discussion

In the present paper we demonstrate that removal of the 14000 M_r polypeptide (γ) directly from the chromatophore membrane before the isolation of the LHC II has no influence on the absorption properties or the function of the LHC II. We have also observed that energy transfer from the LHC II to the RC evaluated from low temperature fluorescence spectra and from light saturation properties of the RC bleaching in either Na_2CO_3 extracted or in unextracted membranes, occurs with similar characteristics. These results indicate that the extraction of the 14000 M_r polypeptide does not affect the interaction of the α- and β-polypeptides with the Bchl molecules producing the known absorption bands at 802 and 855 nm.

So far as it has been studied, in all species of Rhodospirillaceae containing the LHC II, Bchl and carotenoids are non-covalently bound to the two low molecular weight polypeptides called β and α. In *Rb. capsulatus* the LHC II contains a third polypeptides, which does not bind Bchl or carotenoids, called γ [9].

It has been shown [11] that the formation of LHC II in *Rb. capsulatus* requires aside from the synthesis of the corresponding α- and β-polypeptides, Bchl and carotenoids, the expression of at least one gene of the open reading frames CDF localized downstream of the *pucAB* operon.

The results presented in this paper clearly shows that a functional LHC II only contains the α- and β-polypeptides. In addition, the γ-polypeptide is not required for either the absorptions properties or the function of the LHC II. However, it may play a role in the assembly or regulation or biosynthesis of the LHC II. These 12 possibilities are currently being investigated using gene disruption and deletion studies.

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