Protein Subunits of Bacteriochlorophylls B 802 and B 855 of the Light-Harvesting Complex II of *Rhodopseudomonas capsulata*

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Z. Naturforsch. **34 c**, 196–199 (1979); received December 4, 1978

Bacteriochlorophyll-Protein Complexes *Rhodopseudomonas capsulata*, Trypsin Degradation, IR-Absorption Spectra

The light-harvesting antenna complex II of *Rhodopseudomonas capsulata* is characterized by two prominent IR-absorption maxima at 802 and 855 nm and three polypeptides of 14,000, 10,000 and 8,000 molecular weight (SDS-polyacrylamide gel electrophoresis). Bacteriochlorophyll (Bchl) is associated with the two lower molecular weight polypeptides. The membranes of the mutant Y 5, which lacks reaction center and light harvesting complex I (B 875), were treated with trypsin. The 14,000 polypeptide was rapidly digested by trypsin, but the absorption spectrum of the membrane and the activity of the cytochrome c oxidase were not altered. Subsequently the 8,000 polypeptide was degraded. The digestion of the 8,000 polypeptide was concomitant with and proportional to the loss of absorbance at 802 nm. The absorption peak at 855 nm and the content of the 10,000 molecular weight polypeptide were, in contrast, stable for a longer time, but were also lost simultaneously.

These results, in combination with the recent publications of Sauer and Austin (Biochemistry **17**, 2011, 1978), and Cogdell and Crofts (Biochim. Biophys. Acta **502**, 409, 1978) support the idea that the two molecules of Bchl associated with the 10,000 polypeptide are responsible for the 855 nm peak while the one or two mol of Bchl associated with 8,000 polypeptide result in the 802 nm absorption maximum.

Rhodopseudomonas® *capsulata*, *R. palustris*, *R. sphaeroides* and other members of Rhodospirillaceae contain two light-harvesting (LH) antenna bacteriochlorophyll (Bchl) complexes [1–6]. The LH Bchl I of *R. capsulata*, characterized by one distinct IR absorption maximum at 870–875 nm (B 870), is associated with one polypeptide (MW 12,000 in SDS polyacrylamide gel electrophoresis) in the molar ratio 1 : 1 [6].

LH Bchl II of *R. capsulata* has two pronounced IR absorption maxima at 802 and 855 nm. The ratio of the two peaks is relatively constant under different culture conditions and their appearance in photosynthetically induced cells concomitant with the synthesis of polypeptides having molecular weights of 10,000 and 8,000 [5–7]. The isolated LH II complex contains three polypeptides of 14,000, 10,000, and 8,000 (SDS polyacrylamide gel electrophoresis) [6]. The concentrations of Bchl and carotenoids in the isolated complex are close to 3 molecules Bchl and 1 molecule carotenoid per 2 polypeptides. The smaller polypeptides, 10,000 and 8,000 are associated with Bchl and carotenoids, the large polypeptide is free of pigments [6]. The light energy absorbed by LH Bchl II seems to be transferred to the RC via LH Bchl I [8, 9].

The organization and localization of membrane components such as the reaction center, coupling factor, succinic dehydrogenase, light harvesting pigment complexes and cytochrome c_2 have been previously studied [7, 10]. We have also undertaken membrane topographic studies using *R. capsulata* with a special interest in the organization of the light-harvesting complex. In this communication we have investigated whether the Bchl species B 800 and B 855 result from association with both the 8,000 and the 10,000 polypeptide or from binding to single proteins.

Materials and Methods

Organisms: The photochemically inactive mutant Y 5 of *Rhodopseudomonas capsulata*, having LH Bchl II as the only Bchl species (a gift of Barry Marrs, St. Louis, Missouri), was cultivated under semiaerobic conditions in the dark [11].

Isolation of membranes. Twenty mM glycylglycine buffer (pH 7.8) as used in all isolation and experimental procedures. The preparation of crude chromatophores has been previously described [12].

Abbreviations: Bchl, bacteriochlorophyll; LH, light harvesting; IR, infrared; *R.*, *Rhodopseudomonas;* RC, reaction center; SDS, sodium dodecylsulfate.

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0341-0382 / 79 / 0300-0196 $ 01.00/0

The membrane fraction was sedimented at 144,000 × *g* for 90 min. After washing once with buffer, the crude chromatophores were either used without further treatment or they were further purified on a linear sucrose gradient (15 – 60%, w/v) spun at 150,000 × *g* for 150 min. The intense magenta-coloured fraction in the 25% sucrose region of the gradient, which contained the intracytoplasmic membranes was collected and concentrated by sedimentation. The LH-Bchl II complex was isolated from membranes according to Feick and Drews [6].

Protease treatment. For proteolytic digestion, chromatophores were treated with trypsin (specific activity 33 units/mg enzyme; Boehringer, Mannheim) in glycylglycine buffer (pH 7.8) at 28 °C. Control samples without trypsin at 0 °C and 28 °C, respectively, were ran in parallel.

Digestion was terminated by addition of an equal volume of a termination (T) buffer containing 20 mM glycylglycine, 6.5% ethanol, a two-fold concentration of specific soybean trypsin inhibitor (w/w trypsin), 100 – 250-fold excess (mol/mol trypsin) of phenylmethyl sulfonyl fluoride (PMSF) and 4 mg bovine serum albumin/ml.

After addition of the T-buffer, the treated membranes were sedimented at 200,000 × *g* for 150 min, washed once with T-buffer and then twice with glycylglycine buffer (pH 7.0).

Polyacrylamide gel electrophoresis was performed as decribed previously [5, 6]; a 11.5 – 16.5% continuous gradient of acrylamide was used. Densitometric measurements of coomassie brilliant blue stained gels were taken on a modified Eppendorf photometer at 546 nm. Absorption spectra were measured with a Cary R 14 spectrophotometer.

Bchl was measured after extraction with acetone-methanol (7 : 2 v/v) using the molar extinction coefficient of 76 $mM^{-1}\,cm^{-1}$ [13].

Results and Discussion

Treatment of membranes from *Rhodopseudomonas (R.) capsulata*, strain Y 5 with 50 μg trypsin per mg membrane protein for 30 min resulted in degradation of the heavy polypeptide (14,000 MW) of LH complex II. Cytochrome c-oxidase activity, the *in vivo* absorption spectrum and the other LH protein bands were not affected (data not shown here).

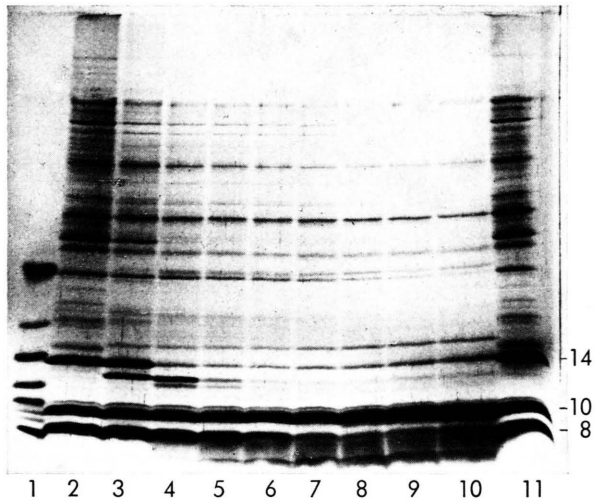

Fig. 1. Protein patterns of membranes treated with trypsin, and analyzed by SDS polacylamide slab gel electrophoresis. 1: trypsin, 2 and 11: control without trypsin incubated at 0 °C (2) and 28 °C (11), respectively, for 200 min, 3: trypsin and trypsin inhibitor were added simultaneously, 4: 3 min, 5: 6 min, 6: 15 min, 7: 30 min, 8: 70 min, 9: 130 min, 10: 200 min incubation with trypsin. The 14,000 band is at first degraded to a smaller product (3, 4) and finally disappeared. The very thin band at the same position is due to albumin in the incubation mixture. The 8,000 band is digested completely. The diffuse band at this position in 7 to 10 is from degradation products and pigments. 14, 10 and 8 indicate the positions of the three proteins of the LH Bchl complex II with MW of 14,000, 10,000 and 8,000.

At a higher concentration of trypsin (1 mg per mg membrane protein) the 14,000 polypeptide of the LH proteins disappeared very fast from the SDS polyacrylamide pattern of the chromatophore membranes while the 8,000-polypeptide was slowly degraded (Fig. 1). The absorption spectra of the membrane preparations after different times of treatment with trypsin showed a stepwise and selective reduction of the 802 nm peak relative to the 855 nm peak (Fig. 2 and Table I). During the first 70 – 90 min of trypsin treatment the absolute heights of the Bchl absorption maxima, at 855 and 375 nm, do not change. The relative absorption at 800 nm was compared to the content of the 8,000 band in treated membranes as determined from densitometric tracings of coomassie brilliant blue stained gels (Fig. 3). A linear proportionality between the decrease of the 800 nm absorption peak and the degradation of the LH polypeptide of 8,000 MW was observed (Fig. 3). Since the mutant Y 5 does not contain reaction

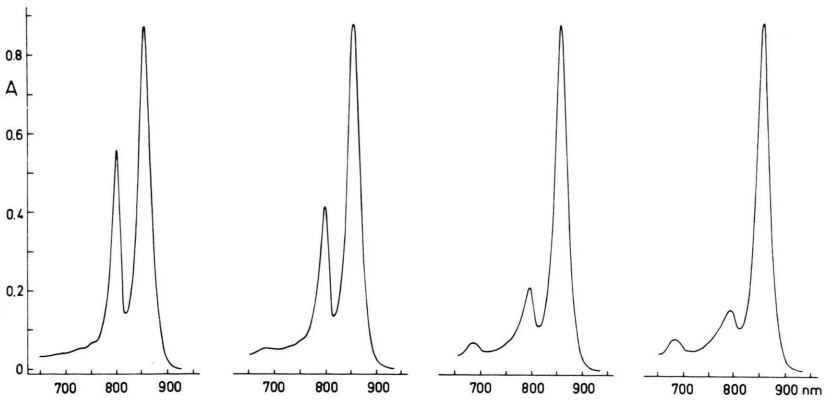

Fig. 2. Infrared absorption spectra of membranes from *R. capsulata* strain Y 5. From left to right: untreated control, 10 min, 200 min and 270 min incubation of membranes with trypsin (0.8 mg trypsin per mg protein).

Table I. Absorption changes in membrane preparations incubated at 28 °C with 1 mg trypsin per mg membrane protein. The digestion was stopped with T. buffer (see Methods).

Incubation time [min]	Treatment	Absorption of the incubation mixture ($d=1.0$ cm)			Absorption of the washed membrane		nmol bacteriopheophytin per absorption $1.0_{855\,nm}$ in washed membranes
		855 nm	855/800 nm	855/375 nm	855/800 nm	855/375 nm	
200	control, without trypsin, 0 °C	0.99	1.55	1.74	1.55	1.70	14.0
200	control, without trypsin, 28 °C	1.00	1.59	1.72	—	—	14.6
0		0.99	1.55	1.71	1.63	1.73	13.3
3	trypsin	0.99	1.72	1.71	2.46	1.74	13.2
6	treated	1.00	1.90	1.73	3.24	1.75	13.3
15	samples	1.00	2.00	1.73	3.68	1.72	13.0
30		0.98	2.23	1.70	4.15	1.68	13.6
70		0.96	2.52	1.62	4.32	1.54	14.3
130		0.79	3.46	1.31	4.62	1.53	16.7
200		0.62	4.30	1.07	4.96	1.04	20.8

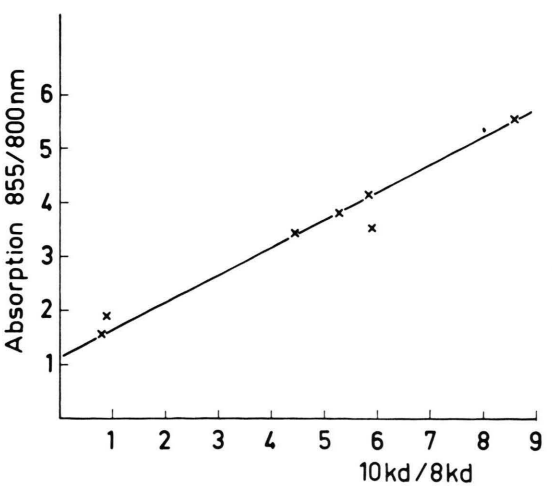

Fig. 3. Proportionality between absorption ratio 855/800 nm of membranes and the absorption ratio of densiometric scanning of coomassie blue stained 10,000 and 8,000 MW protein bands from samples treated for different periods with trypsin (1 mg/mg membrane protein; 15 min to 270 min of incubation).

center and LH Bchl I (B 875), the absorption maximum at 802 nm represents exclusively the spectral form B 800 of the LH-Bchl II complex. The selective disappearance of this spectral form and the quantitatively coordinated degradation of the 8,000 protein suggested that Bchl is associated with the polypeptide of 8,000 MW to form the B 800 moiety of the LH-Bchl II complex. A selective irreversible bleaching of the 800 nm absorption band of the LH-complex from *R. sphaeroides* was described recently [14].

After 100 min of incubation, the 855 nm peak and 10,000 polypeptide were also observed to decrease concomitantly. When the isolated LH II complex was treated with trypsin, the sequence and kinetics of polypeptide digestion and diminution of absorption maxima were different from those obtained from the membrane studies. However, the results still supported the idea that Bchl B 800 is associated with the 8,000 polypeptide whereas Bchl 850 is associated with a 10,000 polypeptide.

The fourth derivative of the infrared absorption spectrum of the LH Bchl II complex of *R. sphaeroides* indicated that the 850 nm band contained a pair of exciton-coupled Bchl's [15]. The circular dichroism spectra indicated that the complex is present in whole membranes of *R. sphaeroides* in large aggregates [14], and that the Bchl-protein association in the isolated complex is similar to that in membranes [15].

A molecular weight of approx-95,000 was found for the native complex of *R. capsulata* [6] indicating an octamer of polypeptides. We were not able to separate B 800 and B 855 while conserving the *in vivo* absorption spectra. At present we are studying the smallest size of the native complex and its localization in the membrane.

The results presented here, together with the previously published date [14, 15] suggest that the LH Bchl II complex consists of two Bchl-polypeptide subunits, one mole Bchl B 800 associated with a polypeptide of 8,000 MW and two mole Bchl B 855 associated with a polypeptide of 10,000 MW. The location of the one mole carotenoid associated with the complex is not known.

This work was supported by grants of the Deutsche Forschungsgemeinschaft. The authors thank Dr. Judy Shiozawa for discussions and reading the manuscript.

[1] J. Aagaard and W. R. Sistrom, Photochem. Photobiol. **15,** 209 (1972).
[2] S. Lien and H. Gest, Arch. Biochem. Biophys. **159,** 730 (1973).
[3] R. A. Niederman, D. E. Mallon, and J. J. Langan, Biochim. Biophys. Acta **440,** 429 (1976).
[4] N. N. Firsow and G. Drews, Arch. Microbiol. **115,** 299 (1977).
[5] A. Schumacher and G. Drews, Biochim. Biophys. Acta **501,** 193 (1978).
[6] R. Feick and G. Drews, Biochim. Biophys. Acta **501,** 499 (1978).
[7] G. Drews, Curr. Topics Bioenerg. **8b,** 161 (1978).
[8] K. L. Zankel and R. K. Clayton, Photochem. Photobiol. **9,** 7 (1967).
[9] T. G. Monger and W. W. Parson, Biochim. Biophys. Acta **460,** 393 (1977).
[10] G. Drews, R. Feick, A. Schumacher, and N. N. Firsow, Proc. Fourth Int. Congr. Photosynthesis, p. 83, Reading 1977.
[11] K. F. Nieth and G. Drews, Arch. Microbiol. **96,** 161 (1974).
[12] R. Dierstein and G. Drews, Arch. Microbiol. **106,** 227 (1975).
[13] R. V. Clayton, Photochem. Photobiol. **5,** 669 (1966).
[14] K. Sauer and L. A. Austin, Biochemistry **17,** 2011 (1978).
[15] R. J. Cogdell and A. R. Crofts, Biochim. Biophys. Acta **502,** 409 (1978).