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BACTERIOPHEOPHYTIN *c* IN REACTION CENTER COMPLEXES OF GREEN PHOTOSYNTHETIC BACTERIA

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Two reaction center complexes prepared from cytoplasmic membranes of *Chlorobium limicola* f. *thiosulfatophilum* were compared by absorption and CD spectrophotometry. Bacteriopheophytin *c* (670 nm), which is optically active in one complex but not in the other, may serve as a secondary electron acceptor in the reaction center.

Purified cytoplasmic membrane fractions from *Prosthecochloris aestuarii* [1,2] and *Chlorobium limicola* f. *thiosulfatophilum* [2–4] show a relatively sharp absorption peak at 672–674 nm, which has been ascribed mainly to monomeric BChl *c* by Fowler et al. [2] and to BPh *c* by Olson et al. [3,4]. The ratio of 674 nm material to BChl *a* has been observed to be highly variable in different membrane preparations [3,4], but in the 600 kdalton complex from Triton-treated membranes of *P. aestuarii* the peak, now at 670 nm, is always found in the same ratio to the 840 nm absorbance change due to the reaction center chlorophyll [5]. Excitation of this complex with light absorbed mainly by the 670 nm pigment can cause preferential fluorescence emission at 835 nm, presumably from the reaction center chlorophyll [6]. Upon illumination by light absorbed by BChl *a*, there is apparently an electrochromic red shift from 660 to 678 nm of a pigment included in the 670 nm band. Thus, the 670 nm pigment(s) may be closely associated with the reaction center in the Triton complex. When this complex is treated with guanidine hydrochloride about half the BChl *a* is removed in the form of two BChl *a* proteins, and a 350 kdalton complex remains [5]. Although the new complex contains about the same amount of 670 nm pigment as does the Triton complex, its photochemical activity is greatly impaired. The quantum yield of charge separation is about 0.5 in the Triton complex; it is about zero in the guanidine hydrochloride complex, the main product being a BChl *a* triplet state [7]. The present work was designed to see whether the 670 nm pigment in membranes and complexes is aggregated, and to see whether there is a correlation between the state of aggregation and photochemical activity.

Unit-membrane vesicles (complex I) were prepared from *C. limicola* f. *thiosulfatophilum* 6230 (strain Tassajara) according to the procedure of Olson and Thornber [8] and dissolved in 10 mM phosphate buffer (pH 7.4) and 10 mM sodium ascorbate. Triton and guanidine hydrochloride complexes were prepared from vesicles according to the procedures of Swarthoff and Amesz [5]. In the preparation of guanidine hydrochloride complexes, the step-gradient layers were 25% sucrose, 25% sucrose plus 1 M guanidine hydrochloride, 40% sucrose, and 50% sucrose.

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; I, primary electron acceptor.

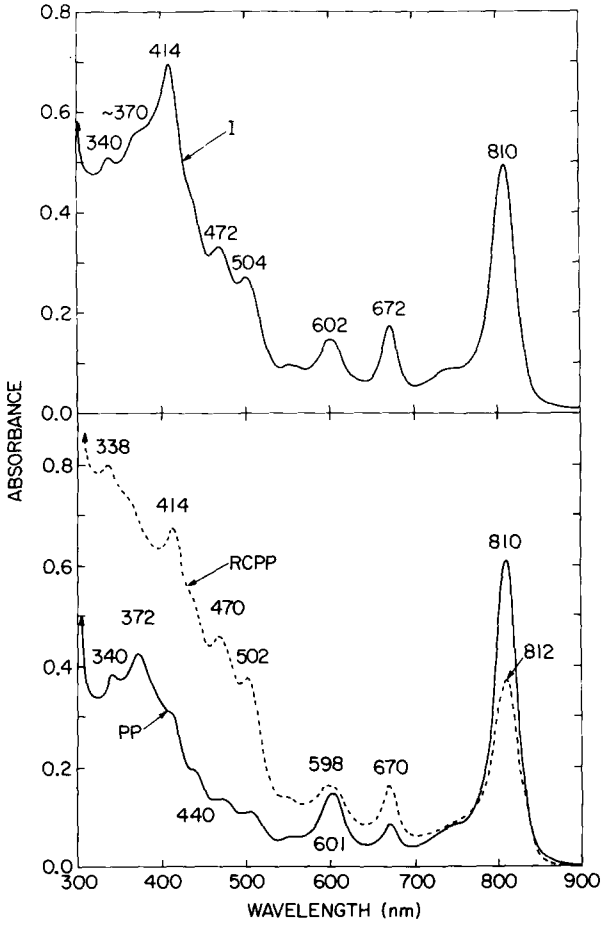

Fig. 1. Absorption spectra of unit-membrane vesicles (complex I) and of Triton (PP) and guanidine hydrochloride (RCPP) complexes. Curve I, complex I in 10 mM phosphate buffer (pH 7.4). Curve PP, Triton complex in buffer and 0.1% Triton X-100. The reaction center concentrations in these three samples are not necessarily the same.

Absorption spectra were measured with Cary 14R and 118C spectrophotometers. CD spectra were measured with a dichrometer described by Sutherland et al. [9]. The dichrometer and the Cary 118C spectrophotometer were operated as described by Sutherland and Olson [10]. Light-induced oxidation of cytochrome *c*-553 was assayed at 553 nm with saturating far-red actinic light (projector + 2 cm H_2O + Corning 2-58 filter + Wratten 88A filter) and the Cary 14R spectrophotometer. Light-induced oxidation of *P*-840 was assayed at 842 nm, with near-saturating 605 nm actinic light (Bausch & Lomb interference filter + Corning 3-67 filter + glass heat filter).

The absorption spectrum of monomeric BChl *c* in ether solution has its major peaks at 432 and 660 nm, whereas the spectrum of BPh *c* has its major peaks at 408 and 660 nm [11]. As shown in Fig. 1, the absorption spectrum of unit-membrane vesicles (complex I) has a major peak at 414 nm associated with 672 nm pigment; the spectrum of the Triton complex has a shoulder at about 410 nm; and the guanidine hydrochloride complex has a major peak at 414 nm associated with 670 nm pigment. It seems unlikely that the 432 nm peak of BChl *c* in ether would shift to 414 nm in the complexes at the same time that the 660 nm peak shifts to 670 nm. We suggest that the 408 nm peak of BPh *c* in ether would shift to 414 nm in the complexes, and that BPh *c* is responsible for

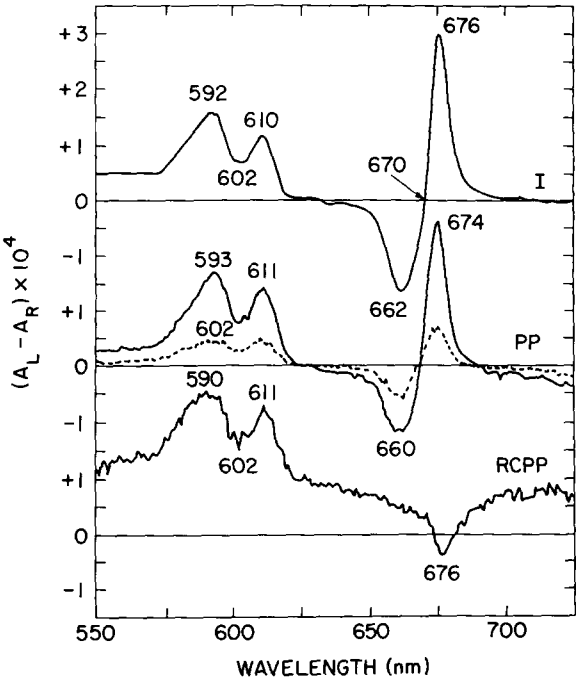

Fig. 2. CD spectra of membranes and reaction-center complexes. Curve I, membranes (complex I) in 10 mM phosphate buffer (pH 7.4), $A_{602} - A_{645} = 0.49$, $A_{671} - A_{645} = 0.44$. Curve PP (solid line), Triton complex in 0.1% Triton X-100 and buffer, $A_{602} - A_{645} = 0.74$, $A_{671} - A_{645} = 0.26$. Curve PP (dashed line), Triton complex in 1.6% Triton and buffer, $A_{601} - A_{645} = 0.53$, $A_{670} - A_{645} = 0.18$. Curve RCPP, guanidine hydrochloride complex in 0.2% Triton X-100 and buffer, $A_{598} - A_{645} = 0.92$, $A_{670} - A_{645} = 0.69$.

TABLE I

COMPARISON OF MEMBRANES, TRITON COMPLEX AND GUANIDINE HYDROCHLORIDE COMPLEX

BChl *a* concentrations are normalized to 10 μM. For BPh *c* $\epsilon_{670} - \epsilon_{645} \approx 50\ mM^{-1} \cdot cm^{-1}$ [11]. *P*-840 was estimated from BChl *a*/*P*-840 = 80 in membrane vesicles and the Triton complex and 35 in the guanidine hydrochloride complex [5]. Reduced cytochrome *c* was estimated from $\epsilon_{553} - \epsilon_{540} \approx 20\ mM^{-1} \cdot cm^{-1}$ based on assumed similarities to cytochromes c_2 (*Rhodospirillum rubrum*) and *c*-553 (*Chromatium vinosum*) [14]. Light-induced *P*-840 oxidation was estimated on the assumption $\Delta\epsilon_{842} \approx 100\ mM^{-1} \cdot cm^{-1}$. Light-induced cytochrome *c*-553 oxidation was estimated from $\Delta\epsilon_{553} \approx 20\ mM^{-1} \cdot cm^{-1}$ based on assumed similarities to cytochromes c_2 (*R. rubrum*) and *c*-553 (*C. vinosum*). All reactions were in the presence of 10 mM ascorbate.

Property/reaction	Membrane (complex I)	Triton complex	Guanidine hydrochloride complex
Bacteriopheophytin *c* (μM)	5.0	2.1	5.4
P-840 (μM)	0.1	0.1	0.3
Cytochrome *c*-553 (μM)	1.3	0.4	0.4
Light-induced oxidations			
P-840 (μM)	0.11	0.09	≈0.01
Cytochrome *c*-553 (μM)	0.57	0.30	0.13
Dark reductions			
P-840 half-time (s)	3	1–2	1–2
Cytochrome *c*-553 lag (s)	2–3	2–3	≈1
Half-time (s)	15–18	6–8	5–6

the peaks at 414 and 670 nm. Some monomeric BChl *c* in the membranes might account for some of the absorbance at 440 nm and peak positions at about 416 and 674 nm [8].

In the absorption spectrum of complex I (Fig. 1) the peaks assigned to BChl *a* are at 340, 370, 602 and 810 nm. Those assigned to mainly BPh *c* with perhaps a little BChl *c* are at 414 and 672 nm, and those assigned to carotenoids are at approx. 440, 472 and 504 nm. The small peak at 552 nm is probably due to reduced cytochrome *c*-553. For BChl *c* and BPh *c* in ether, $\epsilon_{660} \approx 100$ and $60\ mM^{-1} \cdot cm^{-1}$, respectively [11]. In cell-free extracts, monomeric BChl *c* appears to have a peak near 680 nm [12]. We estimated $\epsilon_{680} \approx 60\ mM^{-1} \cdot cm^{-1}$ and $\epsilon_{670} - \epsilon_{645} \approx 50\ mM^{-1} \cdot cm^{-1}$ for monomeric BChl *c* in the various complexes. For BPh *c* in these complexes, we estimated $\epsilon_{670} - \epsilon_{645} \approx 50\ mM^{-1} \cdot cm^{-1}$. For BChl *a* protein from *P. aestuarii*, $\epsilon_{601\pm2} - \epsilon_{645} \approx 27\ mM^{-1} \cdot cm^{-1}$ [13] which we assumed for BChl *a* in the various complexes. The ratio of (BChl *c* + BPh *c*) to BChl *a*, estimated from the ratio $(A_{670} - A_{645})/(A_{602} - A_{645})$, was $0.92 \times 27/50 = 0.50$ (see also Table I). Data on light-induced oxidation of *P*-840 and cytochrome *c*-553 are also given in Table I.

The peak at 670 nm in the absorption spectrum of the Triton complex (Fig. 1) was assigned to BPh *c* alone because of the 2 nm blue shift from the position of the corresponding peak in the absorption spectrum of the membrane (complex I). The ratio of BPh *c* to BChl *a* was 0.2, about 60% less than that estimated for the intact membrane. Light-induced *P*-840 oxidation and cytochrome oxidation (Table I) were similar to the reactions observed in complex I.

In the absorption spectrum of the guanidine hydrochloride complex (Fig. 1), the peak assigned to BPh *c* remained at 670 nm, but the peak assigned to BChl *a* was diminished relative to that assigned to BPh *c* and was shifted to 598 nm. The ratio of BPh *c* to BChl *a* was 0.5, more than double that for the Triton complex. Light-induced *P*-840 oxidation was almost imperceptible (see Table I). Since the reaction center concentration in the guanidine hydrochloride complex was estimated to be about 3-times that in the Triton complex, the light-induced oxidation of *P*-840 in the guanidine hydrochloride complex appeared to involve only about 3% of the reaction centers.

Swarthoff and Amesz [5] showed that the light-on response in the guanidine hydrochloride complex

from *P. aestuarii* was about 50-times slower than the responses in the intact membrane and the Triton complex. The very low quantum yield of charge separation and the very high yield of the BChl *a* triplet state in the guanidine hydrochloride complex suggested some damage to the electron acceptor side of the reaction center such that electron transfer from I to an iron-sulfur protein (secondary acceptor) is severely inhibited [13].

The CD spectra (Fig. 2) of the intact membrane (complex I) and the Triton complex show strong maxima (+) and minima (–) from BChl *a* at 592–593 (+), 602 (–), and 610–611 (+) nm, and even stronger signals from BPh *c* at 660–662 (–) and 674–676 (+) nm. The ratios of BPh *c* to BChl *a* in the membrane and the photosystem protein complex are 0.5 and 0.2, respectively, yet the ratio of BPh *c* CD signal to BChl *a* CD signal is about the same in both. This indicates that only the BPh *c* remaining in the Triton complex is optically active in the membrane. Most of the BPh *c* in the membrane gives no CD signals. The simple shape of the CD signals in question is consistent with the type of induced optical activity produced by a dimer. However, since there are still about 16 BPh *c* molecules per reaction center in the Triton complex, some higher aggregate of BPh *c* might be suggested to account for the optical activity.

In the CD spectrum (Fig. 2) of the guanidine hydrochloride complex, one BChl *a* maximum has shifted from 592–593 nm to 590 nm; the CD signal due to BPh *c* has completely changed with a minimum at 676 nm replacing the minimum at 660–662 nm and the maximum at 675–676 nm. The ratio of BPh *c* to BChl *a* is 0.5 in the guanidine hydrochloride complex, so there is more than twice as much BPh *c* relative to BChl *a* as in the Triton complex. The aggregated state of BPh *c* characteristic of the membrane and the Triton complex has been destroyed by the procedures used to prepare the guanidine hydrochloride complex. Although the correlation between the aggregated state of BPh *c* and the photochemical activity may be coincidental, I suggest that aggregated BPh *c* may function on the acceptor side of the reaction center. Fajer (personal communication) has suggested that BChl *a* may serve as I; BPh *c* might function as an intermediary electron carrier between I and the iron-sulfur protein [7]. The evidence indicates that if BPh *c* does serve as an electron carrier, it must be a dimer or higher aggregate.

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