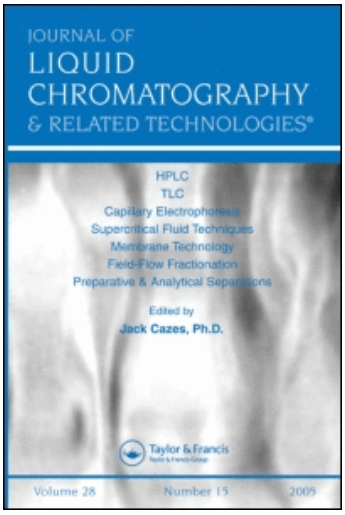


## Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Dissociation and Reconstitution Studies by High Performance Liquid Chromatography of the Light Harvesting Complex of *Rhodospirillum Vrubrum*

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**To cite this Article** Berger, G. , Andrianambinintsoa, S. , Kleo, J. , Grison, S. , Dejonghe, D. and Breton, J.(1992) 'Dissociation and Reconstitution Studies by High Performance Liquid Chromatography of the Light Harvesting Complex of *Rhodospirillum Vrubrum*', Journal of Liquid Chromatography & Related Technologies, 15: 4, 585 — 602
**To link to this Article: DOI:** 10.1080/10826079208018818
**URL:** http://dx.doi.org/10.1080/10826079208018818

JOURNAL OF LIQUID CHROMATOGRAPHY, 15(4), 585-602 (1992)

# DISSOCIATION AND RECONSTITUTION STUDIES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF THE LIGHT HARVESTING COMPLEX OF *RHODOSPIRILLUM RUBRUM*

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## ABSTRACT

Dissociation and reconstitution of light harvesting complex (LHC) of Rhodospirillum rubrum have been performed with dimethyl sulfoxide. Reconstituted LHC in the presence of five fold excess of free bacteriochlorophyll a (BChl a) or analogues were separated by gel filtration high performance liquid chromatography. Reconstitution was followed by measuring the respective quantities of bound pigments, peak amplitudes of the near infrared band, ratios of monomer to dimer bands in the presence or absence of competing BChl a. Most of the tested derivatives (BChl $a_p$ esterified with phytol, pyro BChl a, $13^2$ hydroxy BChl a, Bchl b, Chl a, Chl b) bind to LHC proteins, but only some of them (BChl $a_p$, BChl b) are maintained in such a geometry that they can dimerize, giving red-shifted absorption bands.

## INTRODUCTION

Light harvesting complexes (LHC) of photosynthetic bacteria are specific assemblies of proteins, pigments and phospholipids which absorb light and transmit their excitation energy to reaction centers where this energy is converted to chemical potential. In Rhodospirillum rubrum wild type, the LH complex is constituted by large aggregates of elementary subunits. There are 12 subunits per reaction center (1). Each subunit contains one polypeptide $\alpha$ and one polypeptide $\beta$, binding two molecules of bacteriochlorophyll a (BChl a), one carotenoid (spirilloxanthin) and two

molecules of phospholipids (2,3). The near infrared absorption maximum (BChl a Qy) is at 881 nm, or 873 nm when the carotenoid is removed or is absent, as in the case of the G 9 mutant. Using detergent (n octyl BD glucopyranoside, OGP), Parkes–Loach et al. (4,5) and Ghosh et al. (6), have dissociated BChl a from the protein and have reassociated it, starting from the dissociated mixture or from separated polypeptides and BChl a. Absorbance and circular dichroism spectra in the visible–near infrared were consistent with a reassociation process. The experimental conditions, such as OGP concentration, depended on the concentration of chromatophores and on the state of the preparation (7). In order to determine the structural features of BChl a required for binding to the light harvesting proteins, Parkes–Loach and al. (5) have tested several BChl a analogues, among which only BChl a esterified with different alcohols and BChl b led to successful reconstitutions.

We have followed an analogous approach, but with another method of dissociation (treatment with dimethyl sulfoxide, DMSO) and by using high performance liquid chromatography (HPLC) as a criterion of reassociation.



## MATERIALS AND METHODS

### Preparation of LH complex

R. rubrum wild type, strain S1, was grown in modified Hutner's medium (8), cells were harvested, centrifuged, resuspended in 50 mM Tris pH8 with DNAse and disrupted in a French press. The chromatophores were separated from the cell debris and from unbroken whole cells by centrifugation. LH complex was prepared according to a modification of Picorel's method (3) : chromatophores (O.D 881 nm = 40) were incubated one hour at 4°C with 0.5 % lauryl dimethyl amine N oxide (LDAO) and centrifuged during one hour at 45,000 RPM. The supernatant containing reaction centers was discarded and the pellet, resuspended in Tris 50 mM pH 8 (O.D. 881 nm = 25), was incubated 15 min. at room temperature with 0.3 % LDAO and centrifuged in the same conditions. The supernatant contained mostly pure LH complex.

### Dissociation and reconstitution

Dissociation of LH complex was performed at different temperatures, from 20°C to 40°C in Tris 0.02 M pH 8, LDAO 0.3 %, DMSO 20 to 30 %. DMSO is a mild dissociating agent which allows enzymatic activities to proceed, with concentrations as high as 40 %, as in the case of ATPase (9). It has no absorption band between 1 000 and 260 nm. Dissociation of LHC was followed by change in the absorption spectrum.

Reconstitution was carried out by adding concentrated BChl a or analogue in DMSO (about 5 fold excess with respect to the initial BChl a content of the LH complex). The solutions were kept at 4°C during one hour, then diluted 10 times with NaCl 1 M, Tris 0.02 M pH 8 and absorption spectra were recorded after one hour at 4°C. Separation of the reconstituted LH complexes from BChl a in excess was performed by gel filtration HPLC on TSK G 2000 SW (2 columns 0.75 x 30 cm) or on TSK

G 4000 SW (2 columns 0.75 x 30 cm) with Tris 0.02 M pH 8, NaCl 1 M, cholate (1 to 5 %) as eluent, at the rate of 0.7 to 1 ml/min. The HPLC apparatus was a Waters liquid chromatograph (M 510 pump, $U_6K$ injector, 490 E detector). Optical density was monitored at 280 and 580 nm.

**Preparation of BChl a and BChl b analogues** (10)

BChl a esterified with geranyl geranyl alcohol (BChl a $_{gg}$) and BChl a esterified with phytol (BChl a $_{p}$) were extracted respectively from Rhodospirillum rubrum G9 and from Rhodobacter sphaeroides R26, by stirring cells with methanol (3 times). A prepurification step was performed on C18 Sep Pak cartridges (Waters). The water content of the methanolic solution was brought to 30 %. In these conditions, BChl a was fixed on the resin. The cartridge was washed with this solvent in order to eliminate polar impurities, then BChl a was eluted by a minimum of pure ethanol. The purification was achieved by HPLC on Partisil 10 ODS 2 column (2 cm x 50 cm, Whatman), with ethanol 95 % - water 5 % as eluent. BChl b was extracted from Rhodopseudomonas viridis with a similar procedure : the water content of the methanolic solution was brought to 20 % for fixation on C18 Sep Pak cartridges and the eluent used in the purification step was ethanol 98 % - water 2 %. Chlorophylls a and b were extracted from spinach leaves by grinding in a Waring blendor with methanol, and the solution was treated as above. Pyro derivatives were obtained by heating the pigment dissolved in pyridin solutions in an evacuated sealed vial for 15 h at 100°C. The solution was then evaporated under vacuum and purification was carried out as for the corresponding chlorophyll. $13^2$ hydroxy BChl a $_{gg}$ was prepared by action of light (100 watts), air and LiCl (10 mg/ml) for 2 hours on BChl a $_{gg}$ in solution in ethanol, at room temperature. The extent of reaction progress was monitored by analytical HPLC ($\mu$ Bondapak C18 from Waters, ethanol 85 % water 15 % as eluent). The oxidation product was then separated from residual BChl a and other impurities, on preparative column (ethanol 91 % - water 9 %). 3 ($\alpha$ hydroxy ethyl) BChl a $_{gg}$ was obtained by action of potassium borohydride : 0.4 ml of 10 % $KBH_4$ in 0.1 M Tris buffer pH 8 for 2 ml BChl a in ethanol. The reaction was followed by analytical chromatography and the purification was carried out as for the $13^2$ OH BChl a.

The identification of the chlorophylls and of their derivatives used here was performed by UV-visible absorption and, in some cases, infrared and NMR spectroscopy. UV and visible absorption spectra were recorded on a Shimadzu UV 160 A spectrophotometer. The percentage of tetrapyrolic impurities was measured by analytical HPLC on a $\mu$ Bondapak C18 column (0.4 cm x 30 cm) (Waters), with different ethanol-water mixtures as solvent.

The pigments from reconstituted LH complexes were extracted by shaking 1 volume of diethyl ether with 1 volume of solution to which a small quantity of ethanol was added. The extraction was repeated three times. In the case of BChl b, traces of dithiothreitol were added to avoid oxidation. Water was removed from the organic phase by calcium chloride. Then the solvent was evaporated by a stream of nitrogen and the pigments were resolubilized in ethanol, for later HPLC analysis, on a $\mu$ Bondapak C18 column, with ethanol-water mixtures as solvent (0 to 15 % $H_2O$)

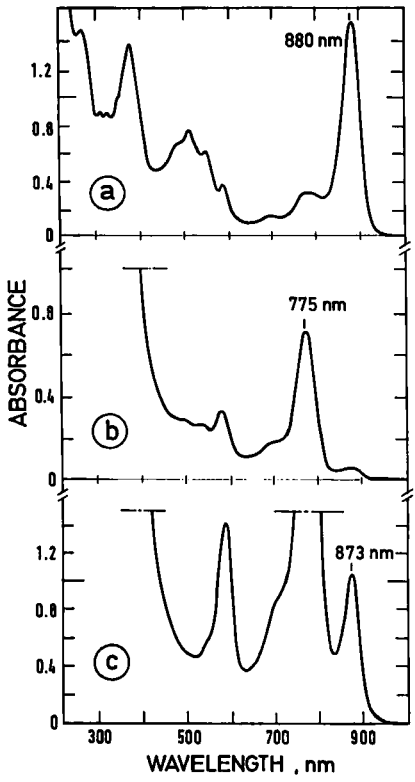


Fig. 1) Dissociation and reconstitution of LHC followed by absorption spectroscopy
a) native LHC
b) after 20 % DMSO treatment, 4 minutes at 40°C
c) after addition of excess BChl $a_{gg}$ and dilution with 1 M Tris 20 mM pH 8, 4°C

## RESULTS

### Dissociation and reconstitution with BChl $a_{gg}$

The BChl a dimer of LHC of Rhodospirillum rubrum S1 absorbs at 880 nm (Fig. 1a). A shoulder around 780 nm is observed, due to the presence of monomeric BChl a, estimated to 5–10 % of the total BChl a, and depending on the preparation and on the conditions of storage. In the presence of DMSO, the 880 nm band decreases with time and is replaced by the 775 nm band of monomeric BChl a (Fig. 1b). There is an isobestic point at 800 nm, and no intermediary band at 820 nm, as observed by Parkes-Loach et al. (4) with OGP. The rate of dissociation increases with

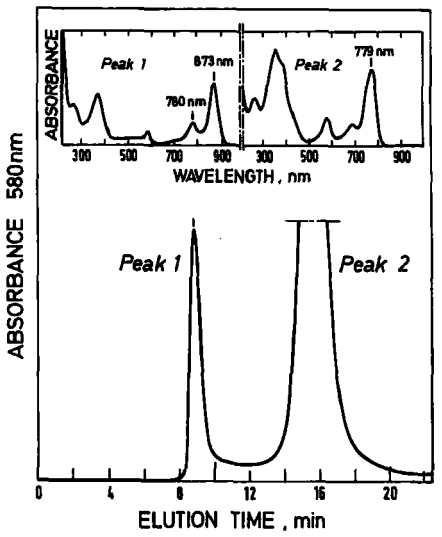


Fig. 2) Chromatography of reconstituted LHC with excess BChl a$_{QQ}$. Conditions : 2TSK G 2000 SW columns (0.75 x 30 cm), eluent : NaCl 1 M, Tris 20 mM pH 8, cholate 2 %. Insert : absorption spectra

temperature and concentration of DMSO. As an example, dissociation with 20 % DMSO was 95 % complete in 4 minutes at 40°C, whereas the same result was obtained after one hour at 20°C.

Reconstitution was achieved by adding excess BChl a (5 times the content of chlorophyll of the LHC), storing the sample one hour at 4°C and diluting it 10 times with NaCl 1M Tris 0.02 M pH 8. It was followed by changes in the absorption spectrum and measured by the amplitude of the near infrared band, at 873 nm. The ratio between the amplitude of the 873 nm band of the reconstituted BChl a dimer (Fig. 1c) and that of the 775 nm band of the dissociated monomeric BChl a is 2.0 ± 0.15. This value is close to that found by Chang et al. (11) when dissociation and reconstitution were performed with OGP (2.16). Reconstitution by our method is then as effective as by the detergent method. However, this ratio is smaller than the 880 nm to 775 nm ratio observed during the dissociation (2.85). The discrepancy is most probably due to the presence of carotenoids in the LHC of Rhodospirillum rubrum S1, while they are absent in reconstituted complexes or in the Rhodospirillum rubrum G9 mutant, which absorbs at 873 nm.

No 873 nm peak was visible with BChl a alone, at the same concentration, treated in the same conditions. Aggregation occured sometimes at higher concentrations, resulting in a broad peak around 850 nm.

The reconstituted LHC was separated from the excess of BChl a by gel filtration HPLC in the presence of 2 % cholate. The elution profile shows two peaks (Fig. 2). The high molecular weight

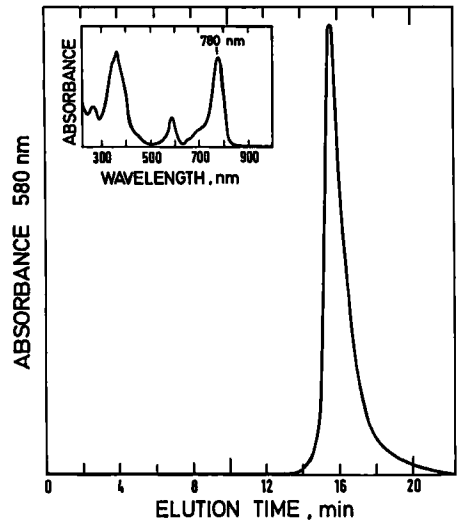


Fig. 3) Chromatography of free BChl a $_{gg}$
Same conditions as Fig. 2. Insert : absorption spectrum



fraction (Peak 1) exhibits a major absorption band at 873 nm and a smaller one at 780 nm (Fig 2 insert) while peak 2 contains essentially free BChl a..

No free BChl a was eluted at the retention volume of peak 1 in these chromatographic conditions (Fig 3). Consequently, the minor 780 nm band in peak 1 (Fig. 2) corresponds to LHC bound BChl a in the monomeric form. The percentage of this form depended on different factors : percentage of cholate, flow rate of eluent, size of the pores of the particles of the gel. After correction for the 780 nm absorption of LHC purified by HPLC, it was estimated to about 30 % of the total BChl a of the reconstituted LHC and could hardly be reduced. As reconstitution was more than 90 % quantitative before chromatography, on the basis of the dimer absorption and extinction coefficient value of Chang et al. (11), one must admit that the monomeric BChl a bound to LHC corresponds to monomerisation of about 30 % of the dimer during chromatography. The dissociation proceeds also after chromatography, at a rate depending on the temperature and the percentage of cholate.

Under the same chromatographic conditions, untreated LHC did not show such a monomerisation phenomenon. The 780 nm absorption of chromatographed LHC was comparatively less important than that of DMSO treated and reconstituted LHC : it is thus very likely that the carotenoid which shifts the absorption to 880 nm contributes to the stability of the BChl a dimer. If no excess BChl a was added after dissociation of LHC by DMSO, the extent of reconstitution with the BChl a of LHC only, by using this procedure, was very low (< 10 %). Pheophytinization of dissociated

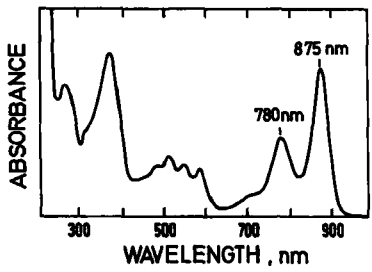


Fig. 4) Absorption spectrum of reconstituted LHC with excess BChl $a_p$
Same conditions as Fig. 2



BChl a by acidic treatment (≈ pH2), followed by neutralization, did not modify the ability of LHC to reconstitute in the presence of excess BChl a. However, running the dissociated mixture on C 18 Sep Pak cartridge, in order to remove BChl a, takes away also an essential part of the LHC, necessary for reconstitution (possibly phospholipid). Heating at 40°C during 4 minutes in 20 % DMSO of the mixture of reconstituted LHC and excess BChl a, leads to complete dissociation of the dimer.

**Reconstitution with different chlorophylls**

When BChl $a_p$ from Rhodobacter sphaeroides R 26 was used in place of BChl a from Rhodospirillum rubrum, the same spectrum of the reconstituted LHC was obtained (Fig 4). After separation from excess free BChl $a_p$, pigment from reconstituted LHC was extracted by diethyl ether and analyzed by reversed phase HPLC (Fig 5). The percentage of BChl $a_p$ is 70 to 80 % of the total BChl a, about 10 % less than in the initial mixture. Thus, we must conclude that BChl $a_{gg}$ is exchanged with BChl $a_p$ in the reconstituted LHC. Minor peaks of isomer a' and of $13^2$ OH derivative (< 5 %) indicated that BChl a underwent partial allomerization during the reconstitution or extraction processes.

BChl b from Rhodopseudomonas viridis gives, by this method, reconstituted LHC absorbing between 885 nm and 895 nm (Fig 6). The same result has been obtained by Parkes-Loach et al. (4,5), with the OGP system. The high molecular weight fraction, separated by gel filtration HPLC, contained mixture of BChl b and BChl a, as can be seen after diethyl ether extraction and RP HPLC (Fig 7). These pigments are LHC bound, since neither free BChl b nor BChl a eluate at this volume, in these conditions.

$13^2$ hydroxy (Fig. 8 and 9) and pyro BChl a (Fig. 10 and 11) bind also to dissociated LHC : they are recovered in the high molecular weight fraction (after diethyl ether extraction and RP HPLC).

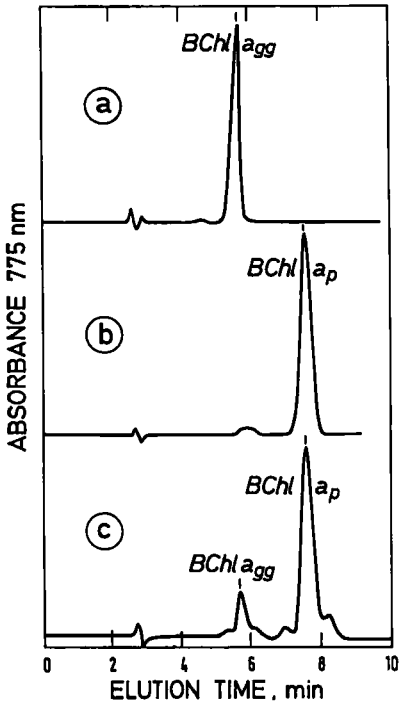


Fig. 5) Chromatography of BChl a extracted from reconstituted LHC with BChl $a_p$ (from Rb sphaeroides)
Conditions : $\mu$ Bondapak C18 column (0.4 x 30 cm), eluent : ethanol with 10 % H2O, 1 ml/min
a) : BChl $a_{gg}$, b) : BChl $a_p$, c) : ethyl ether extract, detection 775 nm

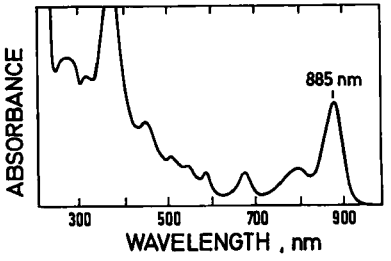


Fig. 6) Absorption spectrum of reconstituted LHC with excess BChl b
Conditions : 2 TSK G 4000 SW Columns (0.75 x 30 cm), eluent : NaCl 1 M, Tris 20 mM pH 8, cholate 5 %

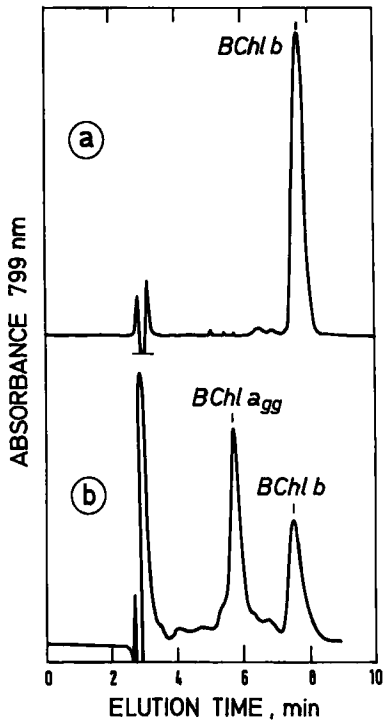


Fig. 7) Chromatography of chlorophylls extracted from reconstituted LHC with BChl b
Same conditions as Fig. 5
a) : BChl b, b) : ethyl ether extract, detection 799 nm

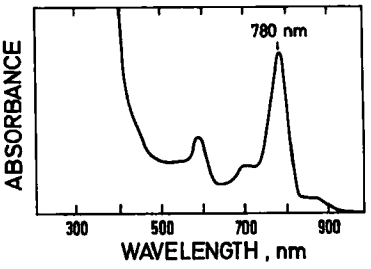


Fig. 8) Absorption spectrum of reconstituted LHC with excess $13^2$ OH BChl a
Conditions : 2 TSK G 2000 SW Columns (0.75 x 30 cm), eluent : NaCl 1 M, Tris 20 mM pH 8, cholate 5 %

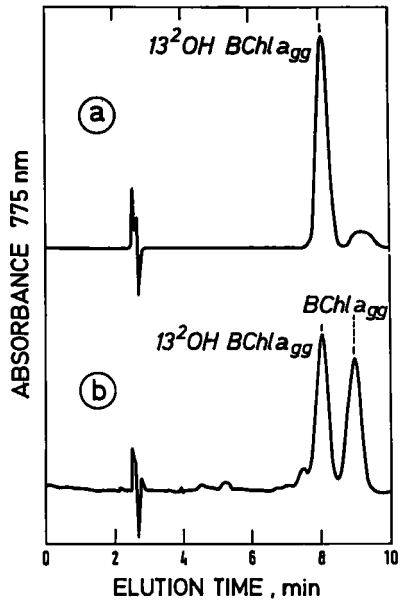


Fig. 9) Chromatography of chlorophylls extracted from reconstituted LHC with $13^2$OH BChl a $_{gg}$
Conditions : $\mu$ Bondapak C18 column (0.4 x 30 cm), eluent : ethanol with 15 % H2O, 1 ml/min
a) : $13^2$ OH BChl a $_{gg}$ b) : ethyl ether extract, detection 775 nm

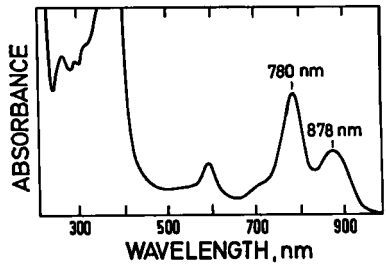


Fig.10) Absorption spectrum of reconstituted LHC with excess pyro BChl a $_{gg}$
Same conditions as Fig. 8

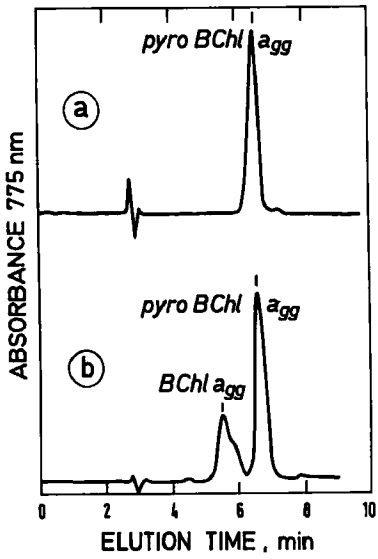


Fig.11) Chromatography of chlorophylls extracted from reconstituted LHC with pyro BChl $a_{gg}$
Same conditions as Fig. 5
a) : pyro BChl $a_{gg}$, b) : ethyl ether extract, detection 775 nm



However, beside the monomeric pigment absorption peak, practically no red-shifted peak was visible when reconstitution was performed with $13^2$ hydroxy BChl a (Fig. 8) and only a minor broad one with pyro BChl a (Fig. 10). Nevertheless, these pigments are bound to LHC proteins, as the free pigments eluted much later in the conditions of chromatography used here.

3 ($\alpha$ hydroxy ethyl) BChl $a_{gg}$ seems to have no affinity for LHC proteins and could not be detected in the high molecular weight fraction.

Chl a (Fig 12 and 13) and Chl b (Fig 14 and 15) can be reassociated to LHC with the technique described here, without shift of their maximum of absorption.

Table 1 shows the different chlorophyll analogues to BChl a ratios found in the high molecular weight fraction, when reconstitution was performed with a five fold excess of pigments, and gives an estimate of the relative affinities of the pigments for the LHC proteins.

**Competitive binding experiments**

In order to determine whether the different chlorophylls compete with BChl $a_{gg}$ for the same sites, the extent of reassociation obtained with a five fold excess of BChl $a_{gg}$ was compared to that obtained with, in addition, the same excess of analogue. As can be seen in table 2, BChl $a_{p}$ from

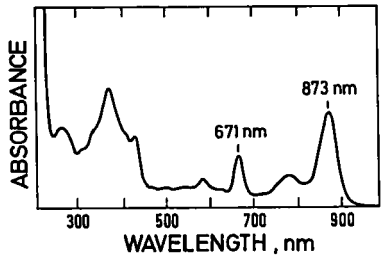


Fig.12) Absorption spectrum of reconstituted LHC with excess Chl a
Same conditions as Fig. 2



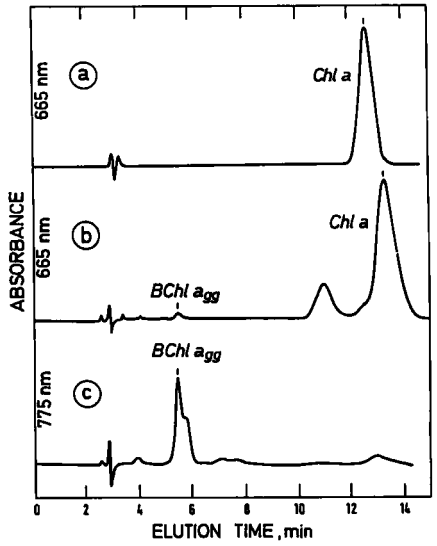


Fig.13) Chromatography of chlorophylls extracted from reconstituted LHC with Chl a
Same conditions as Fig. 5
a) : Chl a, b) : ethyl ether extract, detection 665 nm, c) : ethyl ether extract, detection 775 nm

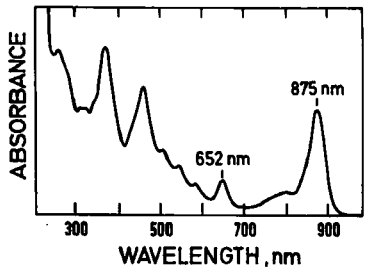


Fig.14) Absorption spectrum of reconstituted LHC with excess Chl b
Same conditions as Fig. 2

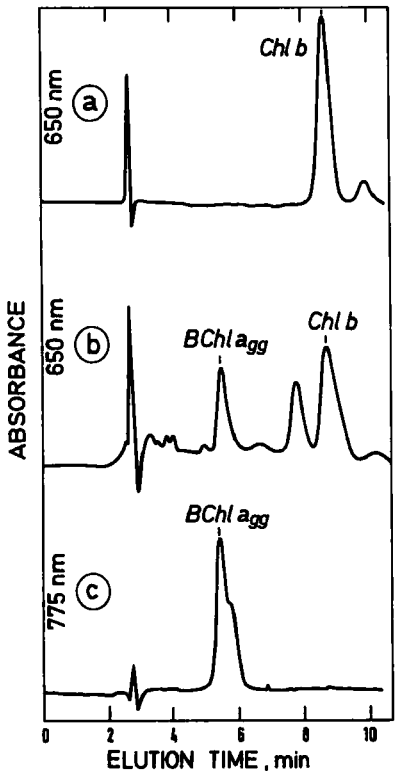


Fig.15) Chromatography of chlorophylls extracted from reconstituted LHC with Chl b
Same conditions as Fig. 5
a) Chl b, b) : ethyl ether extract, detection 650 nm, c) : ethyl ether extract, detection 775 nm

TABLE 1

*Chlorophyll analogue to residual Bchl a $_{gg}$ ratios in the high molecular weight reconstituted complex, when reconstitution was performed with a five fold excess of chlorophyll analogue*

| Pyro BChl $a_{gg}$ / BChl $a_{gg}$ | BChl $a_p$ / BChl $a_{gg}$ | $13^2$ OH BChl $a_{gg}$ / BChl $a_{gg}$ | BChl b / BChl $a_{gg}$ | Chl a / BChl $a_{gg}$ | Chl b / BChl $a_{gg}$ | 3αhydroxy ethyl BChl $a_{gg}$ / BChl $a_{gg}$ |
|---|---|---|---|---|---|---|
| 6.5 | 2.4 | 1.4 | 0.61 | <0.58 | 0.13 | 0 |

TABLE 2

*Competitive binding experiments with five fold excess of BChla $_{gg}$ and five fold excess of chlorophyll analogue*

| | BChl $a_{gg}$ | Pyro BChl $a_{gg}$ | BChl $a_p$ | $13^2$OH BChl $a_{gg}$ | BChl b | Chl a | Chl b |
|---|---|---|---|---|---|---|---|
| Relative amplitude of the 873 nm band | 1 | 1.05 | 0.96 | 0.68 | – | 1.09 | 1.33 |
| 873/780 bands ratio | 2.1 | 0.79 | 1.19 | 0.74 | – | 1.82 | 2 |
| Chlorophyll analogue/ BChl a $_{gg}$ ratio | 1 | 1.02 | 0.58 | 0.4 | 0.12 | 0.05 | 0.05 |

Rhodobacter sphaeroides R26 competes with BChl a $_{gg}$, but is less fitted to LHC proteins from Rhodospirillum rubrum : the ratio of the dimer to monomeric bands in the high molecular weight fraction is smaller with BChl a $_{p}$ than with BChl a $_{gg}$. The ratio of BChl a $_{p}$ to BChl a $_{gg}$ in that fraction, measured by RP HPLC is less than 1 (0.58) when reconstitution was performed with equal concentrations of the two types of BChl a. The same results were obtained with $13^2$ OH BChl a $_{gg}$, but with a smaller affinity for LHC proteins. In each case, the derivatives are bound to the proteins, at the same site as normal BChl a $_{gg}$, since they decrease the near infrared absorption peak of the dimer when set in competition. This is not due to aggregation, since no free chlorophyll derivatives were eluted at the retention volume of LHC in the conditions used for chromatography.

Pyro BChl a $_{gg}$ binds efficiently to LHC proteins, with an affinity comparable to that of Bchl a $_{gg}$. However, the 873 to 780 nm bands ratio is significantly lower, indicating that pyro BChl a $_{gg}$ replaced BChl a $_{gg}$ at its site but does not form a dimer. This will be discussed later.

On the other hand, Chl a and Chl b did not modify significantly the recombination of BChl a $_{gg}$ when they were in competition with it, as shown by the 873 nm band amplitude and the ratio of the 873 to the 780 nm bands of the separated high molecular weight fractions. The extent of binding of these two chlorophylls is relatively low, which could explain the lack of competition.



## DISCUSSION

Different chlorophyll analogues such as pyro BChl a $_{gg}$, BChl a $_{p}$, $13^2$ OH BChl a $_{gg}$, BChl b, Chl a, Chl b are able to replace BChl a $_{gg}$ in LHC from Rhodospirillum rubrum, in the order of decreasing affinity. They are effectively bound to proteins since no aggregated free chlorophyll derivative eluates at the retention volume of LHC in these conditions and they appear to compete for the same polypeptide site as BChl a $_{gg}$. However, they do not give rise in every case to near infrared absorption peak. The bathochromic shift of the Qy transition has been attributed by Scherz et al. (12–16) to pigment–pigment $\pi$–$\pi$ interactions corresponding to a decrease of energy. It has been shown to occur at 853 nm with BChl a alone, in formamide–water – Triton X 100 mixtures and we have obtained a maximum at 843 nm in isopropanol–water mixtures (table 3).

The positioning of Bchl a $_{gg}$ molecules is achieved by Rhodospirillum rubrum polypeptides so as to allow maximum dimer formation : in Bchl a $_{gg}$ reconstituted LHC, separated by HPLC, about 70 % of the bacteriochlorophyll is in the dimer form (based on the ratio of the 873 to 780 nm bands). However, when BChl a $_{p}$ replaces Bchl a $_{gg}$ in Rhodospirillum rubrum LHC, the new interactions between pigments and polypeptides do not permit such close association between two molecules of BChl a : the percentage of dimerized molecules is lower and the bound molecules which are sufficiently distant from the others, behave as monomers and absorb at 780 nm. There is no intermediate wavelength between 780 and 873 nm, as in the detergent system (4), so that it seems that dimerization is, in these conditions, an all or none phenomenon.

The near infrared band of aggregated form of pyro Bchl a $_{gg}$ is at 922 nm in water–formamide (16) and at 932 nm in water–isopropanol mixtures (table 3). This large shift shows that

TABLE 3

*Maximum wavelength of the Qy transition for several chlorophylls and derivatives in formamide–water mixtures (ref. 12,16), isopropanol and isopropanol–water–mixtures.*

| | BChl $a_{gg}$ | Pyro BChl $a_{gg}$ | $13^2$OH BChl $a_{gg}$ | BChl b | Chl a | Chl b |
|---|---|---|---|---|---|---|
| Isopropanol | 776 nm | 778 nm | 776 nm | 803 nm | 664 nm | 647 nm |
| Isopropanol–water 35/65 mixture | 843 nm | 932 nm | 806 nm | 868 nm | 670 nm | 655 nm |
| Formamide–water 3/1 TX100 mixture | 853 nm | 922 nm | 838 nm | 882 nm | | |



aggregation of pyro BChl $a_{gg}$ is accompanied by a decrease of free enthalpy. However, reconstitution of LHC with pyro BChl $a_{gg}$ gives a small and relatively broad band, between 878 and 882 nm. Moreover, the ratio of dimer to monomer bands shows that pyro BChl $a_{gg}$ is less fitted than BChl $a_{gg}$ for dimerization when set in competition in LHC of Rhodospirillum rubrum. Interactions of pyro BChl $a_{gg}$ with polypeptides prevent dimerization of the pigment, probably by maintaining the two molecules of the pair at a distance or an orientation such that association is partly hindered.

$13^2$ OH BChl $a_{gg}$ gives aggregates absorbing at 838 nm in water–formamide (16) and at 806 nm in water isopropanol (table 3). The shift is smaller than for BChl $a_{gg}$ in the same conditions, which indicates that the decrease of the free enthalpy by formation of the dimer is also smaller and that the dimerization is less probable. The same phenomenon occurs with Chl a and Chl b : aggregation in isopropanol–water mixtures induces a displacement of the Qy band of 6 and 8 nm, which explains the lack of shift and of formation of dimer after binding to LHC proteins.

On the other hand, the shift observed upon aggregation of BChl b in formamide–water or isopropanol water (respectively 79 and 65 nm) is comparable to that of BChl $a_{gg}$ (77 and 67 nm) (table 3). As we observe approximately the same shift for BChl b reinserted in LHC from Rhodospirillum rubrum (82 to 92 nm) and for BChl $a_{gg}$ (97 nm), we surmise that BChl b is organized in the same geometry as BChl $a_{gg}$ in the complex although its affinity of binding is smaller (table 2).

Therefore, two phenomena seem to occur in the reconstitution of LHC with pigments : first, the binding of the pigment, measured by the ratio of the pigment to BChl $a_{gg}$, when the two

molecules are in competition. Second, the dimerization of the pigment, which depends not only on the energy of the $\pi - \pi$ interactions (evaluated by the shift of the absorption band upon aggregation in formamide or isopropanol–water mixtures), but also on polypeptide–pigment interactions which at least permit an approximately favourable positioning of the pigments for dimerization. Further experiments are needed, especially with LHC from Rhodobacter sphaeroides and Rhodopseudomonas viridis, to test these hypotheses.

**Acknowledgements**

We wish to thank B. Fournal for careful typing of the manuscript, and Dr E. Nabedryk for encouraging and stimulating discussions.

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