Does the B820 Subcomplex of the B880 Complex Retain Carotenoids?

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A study is reported on the modification of the B880-RC assembly of *Chromatium minutissimum* during octyl-β-D-glucopyranoside/dodecyl-β-D-maltoside/Deriphat polyacrylamide gel electrophoresis followed by electroelution with dodecyl-β-D-maltoside and high performance liquid chromatography with octyl-β-D-glucopyranoside according to the method developed by Kerfeld *et al.* (Biochim. Biophys. Acta **1185,** 193–202 [1994a]) for isolation of the B820 subcomplexes of *Chromatium purpuratum*. The B880-RC assembly of *Chromatium minutissimum* isolated by electrophoresis was contaminated by the B800–850 complex. It was further separated into four components, three of which were in agreement with the cited work: (i) colorless contaminations, (ii) the B880-RC assembly, (iii) the B800–850 complex. In contrast with Kerfeld *et al.* (1994a), the fourth band was a band of free pigments (Bchl or Bchl+carotenoids) which had the same molecular mass as the B820 subcomplex of *Chromatium purpuratum*.

For comparison, the B880-RC enriched fraction of *Rhodospirillum rubrum* modified by lyophilization in the presence of octyl-β-D-glucopyranoside with or without carotenoids was separated by high performance liquid chromatography with octyl-β-D-glucopyranoside. The apparent molecular mass of the B820 subcomplex was 30 kDa for the sample without carotenoids and 245 kDa for that with carotenoids.

The common principles of organization of the B880 complex, the interaction of the B800–850 complex with the B880-RC assembly, the participation of carotenoids in the stabilization of the B880 complex structure and the ability of different isolation steps to modify the structure of the B880 complex are discussed. It was concluded that there are other explanations for the presence of carotenoids in the B820 subcomplex. Hence, the question of whether the B820 subcomplex retains carotenoids remains open.

Introduction

The photosynthetic apparatus of purple bacteria consists of two types of pigment-protein complexes with two major functions. The light-harvesting complexes absorb light energy and convert it into electronic excitation energy which is trapped by the reaction center (RC) where charge separation occurs through a sequence of electron transfer processes between several pigment molecules (for review see Hunter *et al.*, 1989; van Grondelle *et al.*, 1994). The light-harvesting complexes, designed according to their NIR-absorption maxima, were divided into the peripheral antenna complex (B800–850, B800–820 or LH2) and the core antenna complex (B880 or LH1). The latter surrounds the RC, forming the B880-RC assembly (Moskalenko and Erokhin, 1974a and 1974b; Drews, 1985; Nozawa *et al.*, 1987). The RC isolated from the membranes of non-sulfur bacteria is composed of the H, M and L protein subunits and there is a fourth subunit, a tetrahaem cytochrome, in those of different species of the Chromatiaceae and *Rhodopseudomonas viridis* (Erokhin and Moskalenko, 1973; Lin and Thonber, 1975; Komiya *et al.*, 1988; Deisenhofer and Michel, 1989). The B880 complex is characterized by a single NIR-absorption band near 880 nm and is present in a fixed stoichiometry to the RC in the B880-RC assembly. The light-harvesting B800–850 complex and the B880-RC assembly are located in the pigment-containing membranes or chromatophores (Brunisholz *et al.*,1986; Hunter *et al.*, 1989).

The B880 complex is composed of two small polypeptides (α and β) associated in a 1:1 molar

Abbreviations: Bchl, bacteriochlorophyll; DM, dodecyl-β-D-maltoside; HPLC, high performance liquid chromatography; LH1 and LH2, light-harvesting complexes 1 and 2; NIR, near infrared; OG, octyl-β-D-glucopyranoside; PAGE, polyacrylamide gel electrophoresis; RC, reaction center; *C., Chromatium*; *Rs., Rhodospirillum*; Au, absorbance unit.

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ratio. Zuber and Brunisholz (1991) and Brunisholz *et al.* (1981; 1984) sequenced both polypeptides from different complexes and bacteria. For different strains of *Rhodospirillum (Rs.) rubrum* it was established that the α polypeptide contains 52 amino acid residues, while the β polypeptide contains 54; the apparent molecular weights are 6079 Da and 6101 Da, respectively. Hydropathy profiles of the amino-acid sequence show that the N-terminal and C-terminal regions are polar and are exposed on the opposite surfaces of the membrane, while the central region of 20–23 residues is strongly hydrophobic and is assumed to be folded into an α-helix. In this pair of polypeptides there are 3 conservative histidine residues, two near the periplasmic side of the membrane and the third near the opposite side, but only two of them are ligands of the magnesium of the bacteriochlorophyll (Bchl) molecules. Thus, the pigments are located near the periplasmic side forming the Bchl dimer (Brunisholz *et al.*, 1986). The Bchl and carotenoids are present in a fixed ratio (2:1) in this type of complex (Evans *et al.*, 1988). Surface-enhanced resonance Ramman scattering spectroscopy indicated an asymmetric distribution of spirilloxanthin; one end of carotenoid must be very close to the cytoplasmic surface whereas the other is located in the vicinity of the Bchl dimer (Picorel *et al.*, 1988). Zurdo *et al.* (1991) suggested the dimeric organization of the carotenoid pigments in the B880 complex of *Rhodobacter capsulata* and *Rs. rubrum*. The purified B880 complex can easily be solubilized from the membrane of nonsulfur bacteria, such as *Rs. rubrum*, by using mild detergent treatment (Evans *et al.*, 1988; Ghosh *et al.*, 1988; Gimenez-Gallego *et al.*, 1986;).

Miller *et al.* (1987) showed that the B880 complex in the carotenoid-containing membranes of wild type *Rs. rubrum* was highly resistant towards a high concentration of octyl-β-D-glucopyranoside (OG). By contrast, when this complex is depleted of carotenoids by extraction with benzene or mutation (strain G9) it was easily dissociated into subcomplexes (subunits) during titration with increasing concentrations of OG. This dissociation was accompanied by a shift of the main NIR absorption band from 882 nm to 820 nm. These observations initiated extensive work in the isolation of the B820 type subcomplexes from carotenoid depleted membranes of non-sulfur bacteria (Chang *et al.*, 1990b; Heller and Loach, 1990; Jirsakova and Reiss-Husson, 1993). The spectral and biochemical properties of the B820 subcomplexes are well documented (Chang *et al.*, 1990a; Meckenstock *et al.*, 1992a; Meckenstock *et al.*, 1992b; Miller *et al.*, 1987; Parkes-Loach *et al.*, 1988; Visschers *et al.*, 1991; Visschers *et al.*, 1993;). The Bchl dimer is linked to the α/β heterodimer and the complex consists of several pairs of these heterodimers, i.e. they are (α/β/Bchl2) subcomplexes, where n may vary from 1 to 4 (Jirsakova and Reiss-Husson, 1993; Miller *et al.*, 1987). A recent investigation supports the idea that the B820 subcomplex consists of 4 polypeptides (Meckenstock *et al.*, 1992a; Sturgis and Robert, 1994).

Visschers and co-workers (1992) succeeded in preparing the B820 subcomplex of *Rs. rubrum* without preliminary extraction of carotenoids, by using a new detergent *n*-octyl-rac-2,3-dioxypropylsulfoxide. Later, we found the conditions for the dissociation of the B880 complex of *Rs. rubrum* containing carotenoids, in the presence of OG (Moskalenko *et al.*, 1995b). In both cited works carotenoids were lost from the B820 complex during the process of the B880 → B820 transformation. Thus, the loss of carotenoid molecules before or during solubilization with detergent shows that their absence is necessary for dissociation of the B880 complex from nonsulfur bacteria. Only Jirsakova and Reiss-Husson (1993) found carotenoids in the B820 subcomplex from *Rhodocyclus gelatinosus*. The carotenoid content was 7 times lower than in the B875 complex. It may be assumed that in the given case carotenoids unspesificallly bound with the B820 subcomplexes because of the reconstitution of the B875 complex was achieved only in the presence additional amount of hydroxyspheroidene (Jirsakova and Reiss-Husson, 1994).

The subcomplexes from sulfur bacteria have not been studied so thoroughly. Moskalenko *et al.* (1995a), using the extraction of carotenoids from the membranes of *Chromatium (C.) minutissimum* followed by electrophoresis with OG, isolated the B812 subcomplex. Kerfeld *et al.* (1994a, 1994b) developed the procedure of electroelution of the B880-RC assembly followed by gel penetration chromatography with OG for isolation of the B820 subcomplex of *C. purpuratum*. This subcomplex

the carotenoid molecules and the Bchl-carotenoid ratio varies from 1:1 to 1:2 depending on the composition of the electroelution buffer.

In this work we demonstrate that the procedure developed by Kerfeld *et al.* (1994a) is not suitable for dissociation of the B880-RC assembly of *C. minutissimum*. By using this method we isolated the fraction of free pigments (Bchl+carotenoids or Bchl) having similar apparent molecular weight to the B820 subcomplex of *C. purpuratum*. For comparison the dissociation of the B880-RC assembly of *Rs. rubrum* with or without carotenoids was studied.

Materials and Methods

Cells of *C. minutissimum* strain MSU and *Rs. rubrum* strain S1 were cultured and and chromatophores isolated as described elsewhere (Moskalenko and Erokhin, 1974a; 1978). For isolation of the B880-RC assembly of *C. minutissimum* the procedure suggested by Kerfeld *et al.* (1994a) was followed. Chromatophores were diluted to an absorbance at 850 nm of 140 cm^{-1} and solubilized with 1.5% OG (Boehringer Mannheim Gmbh, Germany) and 1.5% dodecyl-β-D-maltoside (DM, Calbiochem, Switzerland) for 2h at room temperature. The mixture was centrifuged for 1 min at 12000×*g* to pellet debris. Non-denaturing PAGE with Deriphat 160 (N-Lauryl-β-iminodipropionic acid Na-salt, Henkel KGaA., Dusseldorf, Germany) was carried out according to Fergusson *et al.* (1991) with a home-built apparatus for PAGE in glass tubes with an inner diameter of 11–12 mm. The band of B880-RC assembly was excised from the PAG and electroeluted in a home-built apparatus. The Deriphat-PAGE reservoir buffer with 0.03% DM was used as the electroelution buffer according to (Kerfeld *et al.*, 1994a). The eluted material was collected and concentrated in an Amicon cell with PM-30 membrane.

For isolation of the fraction enriched in the B880-RC assembly of *Rs. rubrum* the chromatophores (absorbance at 880 nm of 26 cm^{-1}) in 50 mM phosphate buffer (pH 7.0) were treated with 2% OG (20 mg OG per 1 ml of chromatophores) followed by centrifugation for 20 min at 20000×*g* to pellet insoluble material. The supernatant representing the B880-RC assembly was lyophilized and stored at -20 °C. The B880-RC assembly was extracted with benzene according to Miller *et al.* (1987). The benzene-extracted material was dried under a stream of nitrogen, dissolved in 50 mM Tris-buffer and titrated with OG until the 880 nm absorption band was shifted to 820 nm. The concentration of OG usually required for the sample was 0.8%.

A TSK-G3000SW column (7.5x600 mm; Pharmacia-LKB, Uppsala, Sweden) was used to separate pigment-protein complexes by HPLC. The column was equilibrated according to Kerfeld *et al.* (1994a) with 100 mM sodium sulphate, 25 mM sodium phosphate (pH 7.0), 0.8% OG and 0.02% sodium azide. The flow rate was 0.7 ml/min and the eluate was monitored at 375 nm (the Soret band of Bchl). The column was calibrated with ferritin (monomer and subunits) and cytochrome (monomer and dimer).

The absorption spectra of all isolated fractions were registered on a Shimadzu UV-160 spectrophotometer immediately after separation.

Results and Discussion

Three pigmented bands were found as a result of non-denaturing PAGE with Deriphat of the chromatophores of *C. minutissimum* treated with DM/OG mixture. According to their absorption spectra (not shown) the upper band corresponds to the B880-RC assembly (RC-LH1 complex; Fergusson *et al.*, 1991; Kerfeld *et al.*, 1994a), the middle one – to the B800–850 complex and the lower weak band – to detergent-pigment (Bchl/carotenoid) complex with two main absorption bands located near 490 nm (carotenoid) and 767 nm (Bchl). The absorption spectrum of the electroeluted assembly shown in Fig. 1 looks as usual, but for a significant amount of the B800–850 complex that is present in the sample (the shoulder near 850 nm). According to Kerfeld *et al.* (1994a) the electroelution is one of the main steps for isolation of the B820 subcomplex and it is impossible to dissociate the assembly of *C. purpuratum* to the subcomplexes using other procedures for isolation. Kerfeld *et al.* (1994a) obtained the B870-RC assembly with the normal absorbance spectrum after electroelution and did not find the B800–850 complex contaminated by absorption spectroscopy, nevertheless it was revealed by HPLC with OG (Kerfeld, personal communication). Our HPLC

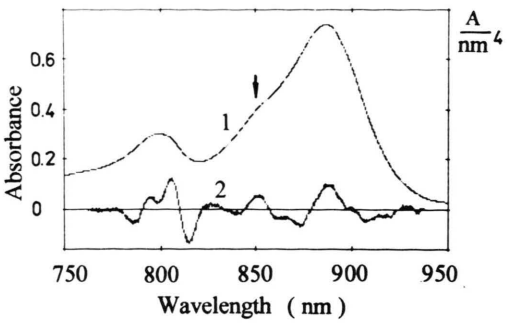

Fig. 1. Absorption spectrum (1) and its 4^{-th} derivative of the B880-RC assembly of *Chromatium minutissimum* isolated by PAGE-Deriphat followed by electroelution with DM. The arrow show a the position of the long-wavelength absorption band of the B800-850 complex.

data were similar to whose obtained by Kerfeld *et al.* (1994a): the sample was separated into four fractions (Fig. 2A). Their absorption spectra are shown in Fig. 3A. The B800–850 complex was separated from the B880-RC assembly during the HPLC with OG and its main NIR absorption maximum was located at 832 nm as compared with 850 nm in the complex after PAGE with Deriphat (Fig. 1).

In contrast with the data given by Kerfeld *et al.* (1994a) the peak which must be corresponded the B820 subcomplex (Fig. 2A, FP) resembles the band of monomer Bchl (Fig.3) which absorbs near 767 nm. Surprisingly, the apparent molecular mass of this fraction was nearly the same as that of the

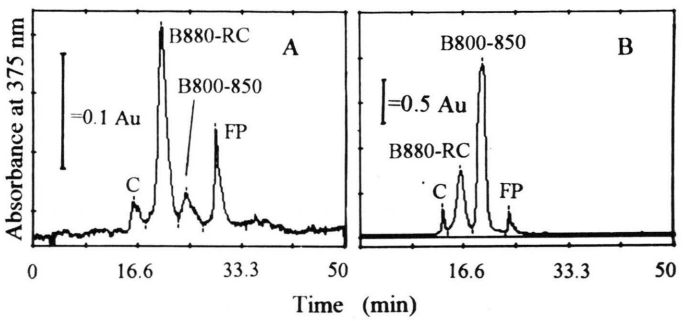

Fig. 2. HPLC elution profile of the B880-RC assembly (A) and the chromatophores (B) of *Chromatium minutissimum:* C, contaminations absorb in UV-region; FP, the detergent-pigments complex. Au, absorbance unit.

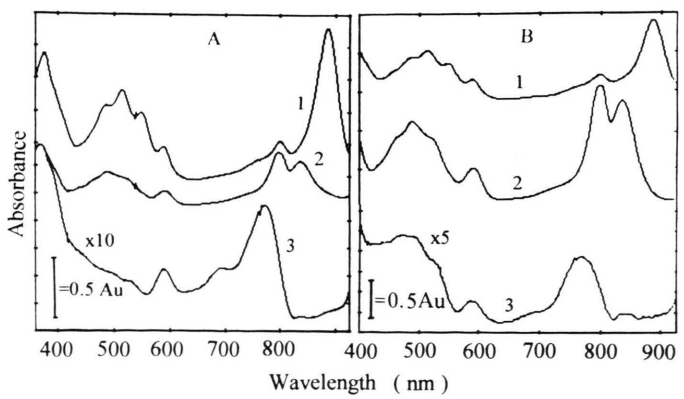

Fig. 3. Absorption spectra of the complexes isolated from the B880-RC assembly (A) and chromatophores (B) of *Chromatium minutissimum* by HPLC: 1, the B880-RC assembly; 2, the B800-850 complex; 3, detergent-pigments complex (FP, see Fig. 2).

Table I. The apparent molecular masses of three pigment-protein complexes isolated by HPLC (in kDa).

	Bacterial species and samples used for isolated of the complexes							
	Chromatium minutissimum				*Chromatium purpuratum*[a]		*Rhodospirillum rubrum*	
Complex	B880-RC re-HPLC	K_n[b]	Membrane	K_n	B880-RC	K_n[c]	B880-RC Car$^-$	B880-RC Car$^+$
B880-RC	346	1	569	1	470	1	390	>650
B820	–		–		70.5	1.4	33.1	245
B770	81.3	1.41	186	1.47	–		–	–

[a] Data of Kerfeld *et al.* (1994a); [b] K_n – normalized retention time (volume) calculated as a ratio $V_t/V_{B800\text{-}RC}$ where V_t is the retention time of the corresponding complex; [c] was calculated on data obtained by Kerfeld *et al.* (1994a).

B820 subcomplex of *C. purpuratum* and both of them had the same K_n (Table I).

Similar results were obtained after separation of the chromatophores of *C. minutissimum* treated with OG/DM mixture by HPLC (Figs 2B and 3B). It is necessary to mention that: (i) the B880-RC assembly was isolated without contamination of the B800–850 complex since a significant amount of OG was present in the eluted buffer; (ii) the fraction of free pigments (Fig. 2B, FP) consists of the monomer Bchl (767 nm) and carotenoids (490 nm), and its apparent molecular mass was 2.3 times higher than that of the fraction obtained by PAGE-HPLC. However, their K_n were almost the same (Table 1).

Thus, the detergent-pigment complex (FP) has an unexpectedly large apparent molecular mass. To check these data we carried out HPLC of the B880-RC assembly of *Rs. rubrum* with extracted carotenoids titrated with OG (Figs 4A and 5A). This procedure is widely used for isolation of the B820 subcomplexes from different types of purple bacteria (Heller and Loach, 1990; Meckenstock *et al.*, 1992a; Miller *et al.*, 1987). In this case, the B820 subcomplex has higher retention time than the detergent-pigment complexes obtained in the presence of different amounts of DM and, consequently, smaller apparent molecular mass (Table I). This value agrees with the results of other authors (Meckenstock *et al.*, 1992a).

For comparison we separated the B880-RC assembly of *Rs. rubrum* containing carotenoid by HPLC. Its structure was modified by lyophilization in the presence of the detergent. Only a part of the B880-RC assembly dissociated into the RC and the B820 subcomplexes (Figs 4B and 5B). A part of the B880 complex was a partially dissociated complex retaining nearly 50% of carotenoids, where both types of the complexes (B880 and B820) were present (curve 1, Fig. 5B). The large

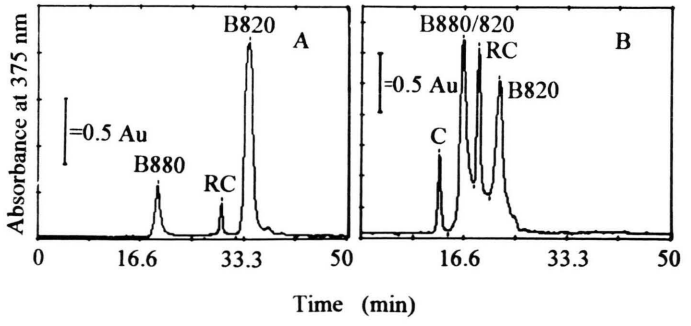

Fig. 4. HPLC elution profile of the B880-RC assembly of *Rhodospirillum rubrum* with extracted carotenoids (A) and the B880-RC assembly containing carotenoids (B): C, contaminations absorb in UV-region.

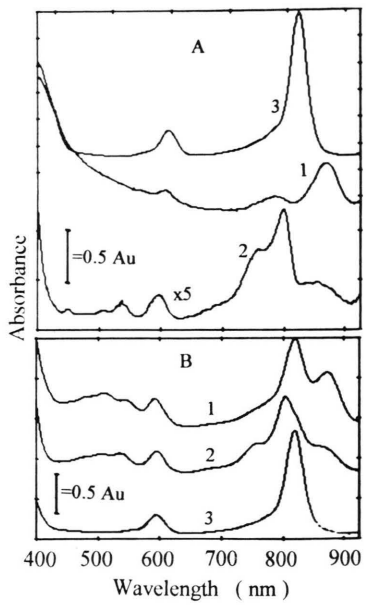

Fig. 5. Absorption spectra of the complexes isolated by HPLC from the B880-RC assembly of *Rhodospirillum rubrum*: (A), with extracted carotenoids: 1, the B880 complex; 2, RC; 3, the B820-subcomplex; (B), containing carotenoids: 1, B880/820 complex; 2, RC; 3, the B820 (sub)complex.

apparent molecular mass of the B820 (sub)complex of *Rs. rubrum* shows that the process of the B880 complex dissociation in the presence of carotenoids differs from that in the sample with extracted carotenoids.

Previously we observed that PAGE with Triton X-100 separated the B880-RC assembly and the B800–850 complex of *C. minutissimum*. The B800–850 complex was in conformation when its main absorption band in the NIR-region was located at 830 nm and there was no contamination about the B800–850 complex in the B880-RC assembly (Moskalenko and Erokhin, 1974a). In contrast with these results, the B880-RC assembly with the admixture of the B800–850 complex was isolated through the non-denaturing Deriphat-PAGE of chromatophores of *C. minutissimum* (Fig. 1) and *C. purpuratum* (Kerfeld *et al.*, 1994a). The blue shift of the main absorption band of the B800–850 complex reflects the conformational changes between the C-terminal of α and β polypeptides within the complex which influences the exitonic interaction in Bchl dimer by modifying the mutual arrangement of Bchl molecules (Moskalenko *et al.*, 1996). A correlation was observed between the location of the NIR absorption band of the B800–850 complex of *Rhodobacter sphaeroides* and site mutation in the C-terminal region of the α subunits of the complex. The alterations αTyr-44 → Phe and αTyr-45 → Phe or Leu caused the hypsochromic shift of this absorption band to 11 nm and 24 nm (77 °K), respectively (Fowler *et al.*, 1992; Fowler *et al.*, 1994). The conformational changes in the native B800–850 complex could be limited by cross-linking bridges (Moskalenko and Toropygina, 1993). Thus, the detergent caused changes in the shape of the B800–850 complex. The absence of these changes in a small part of the B800–850 complex hinders the dissociation of the B800–850/B880-RC associated and explains the presence of the B800–850 complex in the band of the B880-RC assembly after PAGE. During the second step of purification (HPLC with OG) the B800–850 complex changes its conformation and separates from the B880-RC assembly (Figs 2A and 3A). These results are in good agreement with the following data: (i) a small part of the B800–850 complex is firmly bound with the RC as established while using bifunctional cross-linking reagents (Moskalenko and Toropygina, 1988); (ii) it was impossible to reconstitute the B880–850/B880-RC native interaction if the conformation of the B800–850 complexes differed from the native one (Moskalenko *et al.*, 1992).

Given below is a more detailed consideration of the procedure of the B820 subcomplexes isolation according to Kerfeld *et al.* (1994a). It consists of three steps: 1) non-denaturing Deriphat-PAGE; 2) electroelution of the sample from the PAG in the presence of Deriphat or DM and its concentration; 3) HPLC with OG. Before PAGE with Deriphat these authors (Kerfeld *et al.*, 1994a) treated the membranes of *C. purpuratum* with DM/OG mixture. The position of the NIR absorption band of the B800–850 complex of *C. minutissimum* is a very convenient probe for the presence of the bound OG in the sample. In the control complex it is located near 850–855 nm and is shifted to 830 nm under OG (or Triton X-100) treatment. This process was completely reversible and the removal of the detergent caused the bathochromic shift of the corresponding band. OG has a high CCM and can be easily removed by dialysis. Therefore, one

could assume that OG was removed from the complexes during their penetration in the gel in the first step of OG/DM-PAGE according to Kerfeld *et al.* (1994a) since OG was not included into the gel: the NIR absorption band of the B800–850 complex is located near 850 nm (Fig. 1). This assumption was also supported by our observation concerning the dissociation-reassociation process in the B880 complex *Rs. rubrum* during PAGE: in the presence of a high concentration of OG in the PAG the dissociation of the complex was achieved, while the decrease in the detergent concentration in the PAG stimulated the back process (Moskalenko *et al.*, 1995b). Thus, the B880-RC assembly after such PAGE bound mainly DM and some amount of Deriphat. DM was shown to stabilize the structure of the different pigment-protein complexes (Moskalenko, 1990; Moskalenko and Kuznetsova, 1992; Moskalenko and Kuznetsova, 1993; Seibert *et al.*, 1988). Therefore it is clear that any possible modification of the structure of the complexes in the presence of these detergents during PAGE is doubtful.

The second step – electroelution – is the main step for the isolation of the B820 subcomplexes from carotenoid containing *C. purpuratum* membranes. It was shown that the B870-RC assembly did not dissociate into subcomplexes if this step was excluded (Kerfeld *et al.*, 1994a). Obviously during electroelution the modification of the B870-RC assembly's structure took place. Principally, it could be expected that modification of the structure resulting from electroelution should be accompanied by the protein-protein or protein-pigment interaction and should be recorded as the blue shift of the main absorption band as shown for the B880-RC assembly of *Rs. rubrum* (Fig. 5). There were no changes in the structure of the B880-RC assembly of *C. minutissimum* as measured by spectroscopic characteristics (Figs 1 and Fig 3A).

The last step for isolation of the B820 subcomplexes of *C. purpuratum* is the routine technique which is generally accepted for isolation of such complexes (Heller and Loach, 1990; Meckenstock *et al.*, 1992a; Miller *et al.*, 1987). Only Moskalenko *et al.* (1995a; 1995b) used PAGE with OG for isolation of the B812 subcomplexes from the extracted membrane of *C. minutissimum* and the carotenoid containing membranes of *Rs. rubrum*. According to the submitted data, we did not achieve using gelpenetration chromatography the complete dissociation of the modified B880-RC assembly of *Rs. rubrum* which was easily dissociated into the B820 subcomplexes during PAGE with OG. By these means we cannot achieve the dissociation of the B880-RC assembly of *C. minutissimum* into the B820 subcomplexes and the RC using the procedure developed by Kerfeld *et al.* (1994a) for *C. purpuratum* because of this step provides very mild condition.

It is necessary to emphasize that the apparent molecular masses and, especially, K_n of the B820 subcomplex of *C. purpuratum* as HPLC chromatogram are similar to the band of free pigments and the chromatogram of *C. minutissimum* (Table I). Since water soluble proteins were generally used for calibration, this technique gives a significant deviation in the apparent molecular mass values of the complexes measured by different authors (Jirsakova and Reiss-Husson, 1993; Miller *et al.*, 1987). Normalization of the retention time (volume) eliminates this discrepancy (Table I). The other factor influencing the molecular mass values is the presence of detergents and lipids in the sample. A decrease in the DM concentration in the B880-RC assembly as compared with that in the membrane of *C. minutissimum* caused a decrease in the molecular mass of the B880-RC assembly by 1.6 times and that of the detergen-pigments complex (FP) by 2.3 times, while K_n did not change (Table I). Obviously, the discrepancies in the apparent molecular masses of the detergent-pigments complex (FP), as well as the complexes, are associated with the presence of DM, which is a larger surfactant (molecular weight = 511) than Deriphat or OG. However, at least two possibilities cannot be excluded in the case of *C. purpuratum*. The first one is that the band of free pigments comigrates with the B820 subcomplex. This assumption is supported by the same K_n of this band (Table I) and the B820 subcomplex (Kerfeld *et al.*, 1994a). Kerfeld *et al.* (1994a) also noticed that the B820 subcomplex lost Bchl selectively but the authors did not recover the band of free Bchl on the chromatograms. The second is that the presence of carotenoids in the B820 subcomplex could result in the reassociation of the free pigments and the subcomplexes since the B820 subcomplex has inverted circular dichroism lobes as

compared with that of the B870-RC assembly (Kerfeld *et al.*, 1994a).

The data present in Figs 4B and 5B proved that the process of the B880 → B820 transformation was accompanied by loss of carotenoids. According to Miller *et al.* (1987) the apparent molecular mass of the B820 subcomplex seemed to be within the range of 42–65 kDa. We received a lower value (30 kDa) which is in good agreement with the data obtained for other species (Meckenstock *et al.*, 1992a). As the ratio α:β:Bchl is 1:1:2 in the B820 subcomplex, the sum of the molecular masses of α and β polypeptides (6079 and 6101 Da) and two Bchl molecules (911,5.2 Da) results in a total molecular mass of 14003 Da. Thus, our data support the idea that the B820 subcomplex of *Rs. rubrum* consists of two (α/β/Bchl2) subunits (Meckenstock *et al.*, 1992a; Sturgis and Robert, 1994). The apparent molecular mass of the B820 subcomplex isolated from the carotenoid containing B880-RC assembly indicates that it is present in the aggregate form (6–8 the B820 subcomplexes) in agreement with the model B880-RC assembly suggested by Karrash *et al.* (1995). According this model a small part of the B880 complex (two α/β–heterodimer) is tightly bound with RC and the main part (two [α/β]7 domens) don't interact with it. The second represents the part of the B880 complex don't interacting with RC and probably transformed into the B820 form during OG treatment and separation. Thus, if our data is true, in the course of dissociation of the carotenoid containing B880 complex during HPLC with OG only a partial transformation B880 → B820 takes place without the dissociation of the complex to the subcomplexes. The absorption spectra of these two samples are identical (curve 3, Figs 5A and B).

In conclusion we should mention that the method described by Kerfeld *et al.* (1994a) could not be used to isolate the B820 subcomplex from the B880-RC assembly of *C. minutissimum*. Our failure to isolate the B820 subcomplex from the B880-RC assembly of *C. minutissimum* could depend on the different carotenoid compositions of the B880-RC assemblies of *C. minutissimum* (main carotenoid is spirilloxanthin) and *C. purpuratum* (okenone). The structures of these carotenoids are shown in Fig. 6. Both have the methoxy group in C1 position, while okenone has the ketogroup in C4 position and the aromatic ring at the opposite side of the molecule instead of the methoxy group in spirilloxanthin. Thus, they differ in substitute groups and the length of molecules. The carotenoids have been shown to influence the absorbance of Bchl and the interaction between the N-terminal of polypeptides in the B880 complex (Brunisholz *et al.*, 1986). One could speculate that a shorter okenone molecule has a lower influence on the complex structure, that is why Kerfeld *et al.* (1994a) managed to isolate the B820 subcomplex

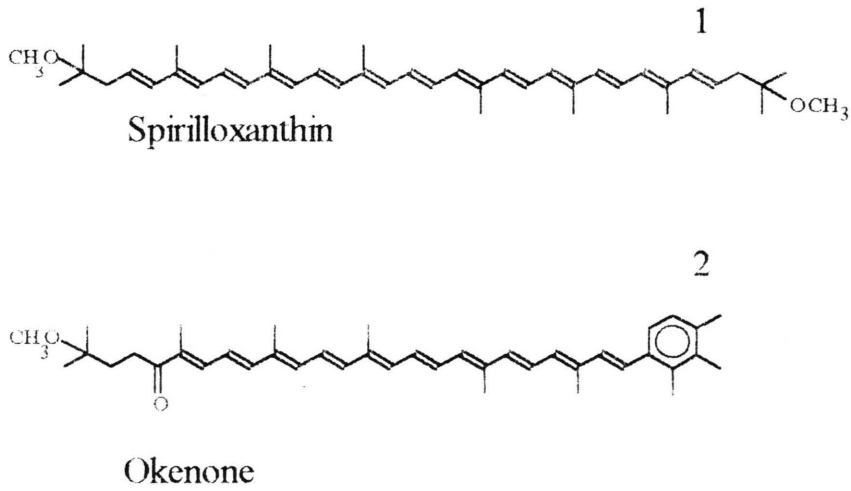

Fig. 6. The structure of the main carotenoid of the B880-RC assembly of *Chromatium minutissimum* (1) and *Chromatium pirpuratum* (2).

from the modified B870-RC assembly of *C. purpuratum*. For isolation of the B820 subcomplexes from the membranes or the B880-RC assemblies of spirilloxanthin type containing carotenoids species (Chromatiaceae and others) preliminary elimination of carotenoids or essential modification in the C-terminal region are required. Hence the question if the B820 subcomplex retains the carotenoids remains open. At present time we have not firm evidence for binding carotenoids by the B820 subcomplexes.

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