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Chemical crosslinking studies of the isolated light-harvesting B800-850 complex of *Chromatium minutissimum*

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The spatial relationship of polypeptides comprising the light-harvesting B800-850 complex of *Chromatium minutissimum* has been studied by means of chemical crosslinking of the isolated complex with cleavable, 1.2 nm-long dithiobis(succinimidyl propionate). The samples were analyzed by different types of electrophoresis and spectrophotometrically. No difference was shown between crosslinking of the B800-850 complex either solubilized or incorporated into proteoliposomes. It was found that two main polypeptides form only one type of heterodimer. The crosslinked complex was more thermostable. This crosslinkage restricted the conformational transitions causing the shift of the long wavelength band in the near infrared region. A structure of the complex is discussed.

Light-harvesting complex; Crosslinker; Conformational change; Thermostability; Bacteria

1. INTRODUCTION

The intracytoplasmic membranes (chromatophores) of most purple sulfur and nonsulfur bacteria contain the reaction center complex and three light-harvesting complexes B800-820, B800-850 and B890(870) designated on the basis of their near-IR absorption maxima. A detailed understanding of the structure and function of the complexes requires the investigation of either isolated complexes or mutants lacking the different types of the complexes [1–3].

The light-harvesting complexes of the bacteria are conservative structures which consist of α and β low molecular weight polypeptides with the central hydrophobic region being a transmembrane α-helix. Conserved histidine residues are present in this region and are supposed to bind Bchl molecules in the complexes [3]. The isolated B800-850 complex contains α and β polypeptides in equimolar amount. Each $\alpha\beta$ polypeptide pair binds 2–4 Bchl molecules and 1–2 carotenoids [3]. The B800-850 has been supposed to be made of six $\alpha\beta$ pairs (subcomplexes) on the basis of biochemical and biophysical characteristics [4–6].

Reversible chemical crosslinking procedures are an accepted method for probing relationships between the pigment–protein complexes in the chromatophores of sulfur and nonsulfur bacteria [7–10]. The lateral organization of the B800-850 complex and spatial organization of constituent polypeptides have been studied using membrane preparations isolated from the cells of *Rhodobacter capsulatus* phototrophic negative mutant strain Y5 ($B875^{-}RC^{-}$ $B800\text{-}850^{+}$) or *Rhodopseudomonas viridis* [7,9]. Here we present a study on the interaction of the α and β polypeptides in the isolated B800-850 complex from *Chromatium minutissimum* using cleavable bifunctional crosslinking reagent DSP and its influence on the structure of the complex.

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Abbreviations: Bchl, bacteriochlorophyll; DSP, dithiobis(succinimidyl propionate); SDS, sodium dodecylsulfate.

2. MATERIALS AND METHODS

Cells of *Chromatium minutissimum* strain MSU were grown anaerobically in an inorganic Larsen's medium as described [10]. The cells were sonicated and pigmented membranes were purified on a sucrose gradient, then sedimented and resuspended in 50 mM Tris-HCl buffer to 50 units of optical density in the main maximum in the near IR-region. The B800-850 complex was isolated by Triton X-100 electrophoresis in a 7% polyacrylamide gel according to [10]. The complex was concentrated to a protein concentration of 3 mg/ml followed by the substitution of the buffer with 5 mM BICIN-NaOH (pH 8.0). The proteoliposomes with the B800-850 complexes were prepared in the same buffer as described [11] except that dithiothreitol was omitted as we used a crosslinker cleavable by S-S reagents.

For crosslinking the B800-850 complex or the one incorporated into proteoliposomes and resuspended in BICIN-NaOH buffer were treated with freshly prepared DSP solution in dimethylsulfoxide. The final concentration of DSP was 10 mM and of dimethylsulfoxide 5%, respectively. The reaction was stopped by adding the 1 M Tris to the final concentration of 50 mM. The electrophoresis with Triton X-100 or SDS was carried out according to [10,11]. The absorption spectra were measured on Shimadzu UV-160 spectrophotometer.

3. RESULTS AND DISCUSSION

DSP is a bifunctional crosslinking reagent which reacts preferentially with amino groups. Its span is 1.2 nm long. Two pigmented bands were formed (not shown)

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as the result of electrophoresis with Triton X-100 of the B800-850 complex treated with DSP. The upper small band corresponds to the dimer and the lower one to the monomer of this complex. Earlier we have shown that a lot of B890-RC/B800-850 associates and a small amount of B800-850 dimers are revealed in the membrane of *Chromatium minutissimum* treated with *N*-hydroxysuccinimide esters [10]. Thus the distance between amino groups in the different B800-850 complexes was more than 1.2 nm and the crosslinking bridges appeared to be basically within one B800-850 complex. There is good indirect evidence for this assumption: the increase in electrophoretic mobility of the monomer complex after treatment with DSP. It is known that *N*-hydroxysuccinimide esters react with positively charged amino groups, essentially with ε-amino groups of lysine. Indeed the positive charge of DSP-treated B800-850 complex and respectively the impeding force under electrophoresis were decreased resulting in the enhancement of the complex mobility. The identical effects have been found during Triton X-100 electrophoresis of chromatophores or the isolated B890-RC assembly treated with bifunctional crosslinkers [10,12]. Thus this effect is usable as a probe of crosslinkers interaction with bacterial pigment–protein complexes.

No difference in the electrophoretic behaviour of the B800-850 complex treated with DSP in buffer or in proteoliposomes was observed. Therefore the monomer B800-850 complex treated with DSP in buffer was studied later on. It was isolated by Triton X-100 electrophoresis, eluted from the gel and concentrated. The treatment with the crosslinker of the B800-850 complex did not change its absorption spectrum (Fig. 1). Anionic detergent SDS caused the red shift from 837 to 853 nm of the long wavelength band B850 in the control (Fig. 1A) and practically did not influence the position of the B850 band in the DSP-treated complex (Fig. 1B). Nonionic detergent Triton X-100 on the contrary did not influence the control B800-850 complex but in DSP-treated complex after addition of the detergent the B850 band was shifted from 835–837 nm to 825 nm (Fig. 1D). We designated the state of the B800-850 complex with the position of the long wavelength band at 853–855 nm, 835–837 nm and 825 nm as conformation 1, 2 and 3, respectively. The conformation 1 corresponds to the native state of the complex. After the treatment of *Ch. minutissimum* chromatophores with Triton X-100 followed by electrophoresis, elution and concentration, the B800-850 complex was isolated in conformation 2. It is clear that conformational transition 2 → 1 was prohibited and 2 → 3 was stimulated in the B800-850 complex treated with DSP (Fig. 1). As the shift of the B850 band reflects the specific changes in the structure of the complexes, consequently some of these changes were limited in the DSP-treated complex. These data are in good agreement with results on the destruction of the B800-850 complex by heating: after crosslinking its thermostability markedly increased (Fig. 2). It is known that crosslinking bridges in proteins augment their thermostability. For example human hemoglobin A crosslinked by diaspirin between two Lys β82's or Lys α99's had a markedly higher T_m of 57°C, while the T_m of control protein was 41°C [13].

The investigated B800-850 complex consists of two main (α and β) and one minor polypeptide (Fig. 3). The latter is not necessary for the native structure of the complex. All bands of these polypeptides disappeared in the complex treated with DSP and three new bands were found. The main band belongs to the αβ heterodimer, and two other bands are crosslinking products between the α polypeptide or αβ heterodimer and additional polypeptide, respectively. These results disagree

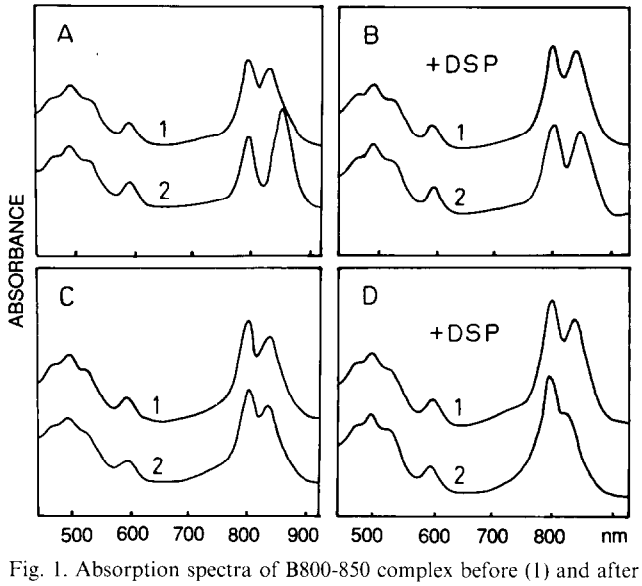

Fig. 1. Absorption spectra of B800-850 complex before (1) and after (2) addition of SDS (A,B) or Triton X-100 (C,D). Complex treated with DSP (B,D).

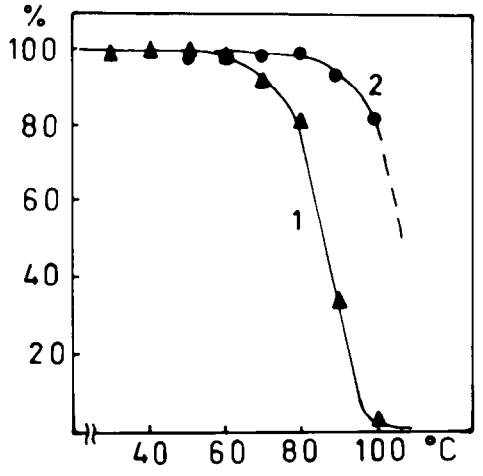

Fig. 2. Destruction of the long wavelength band B850 in the B800-850 complex under heating (10 min): 1, control; 2, treated with DSP.

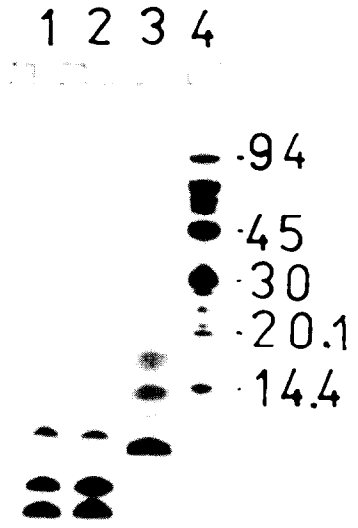

Fig. 3. SDS-electrophoresis of B800-850 complex incorporated into liposomes (1) or in buffer (2) and treated with DSP (3); protein markers (4).

with those of the previous workers. Peters and Drews [7] studied the B800-850 complex in the membranes of *Rhodobacter capsulatus* strain Y5 (RC^-, $B875^-$, $B800\text{-}850^+$) and obtained different crosslinking products. These were dimers and trimers of α and β polypeptides, $\alpha\beta$ heterodimers in a different stoichiometric ratio. The spots of α and β monomeric polypeptides have also been revealed after two-dimensional electrophoresis of membranes treated with DSP. Ludwig and Jay found crosslinkage between $\alpha\alpha$ and $\beta\beta$ as well as $\alpha\beta$ polypeptides of the light-harvesting complex in the membranes of Bchl b containing bacterium *Rhodopseudomonas viridis* [15]. In the cited works the membranes were analysed, therefore it was not possible to separate the crosslinkage between the complexes from the one within the complexes. The discrepancy between the results can be also related to different organization of the light-harvesting complexes from different bacterial species, or with different distance between reacting amino groups in the complexes. Thus the main crosslinking product in the monomer of the B800-850 complex treated with DSP was $\alpha\beta$ heterodimer and this crosslinkage limited the conformational transition in the complex and increased its thermostability.

The isolated B800-850 complex shows the spectral shift of the long wavelength band B850 in the near infrared region. It is probably connected with the change in the excitonic interaction between two molecules of Bchl. According to structural models the interacting Bchl molecules (dimer Bchl) are bound to α and β polypeptides by histidine residues in such a manner that one Bchl molecule is bound with α-polypeptide and the other with β-polypeptide [2–4]. Therefore, conformational changes of α and β polypeptides could modify the excitonic interactions in the Bchl dimer by changing the mutual arrangement of Bchl molecules. The crosslinking bridges limited both the changes of interaction between α and β polypeptides, and between two molecules of Bchl.

The B800-850 complex consists of 6 pairs of α and β polypeptides. It is not possible to dissociate it into smaller subcomplexes as it has been shown for the B880 complex with extracted carotenoids [14,15]. The interaction between subcomplexes in the B800-850 complex is stronger in comparison with the same one in the B880 complex. The question arises whether the crosslinking bridge binds α and β polypeptides from the same or different smaller subcomplexes. There is no direct evidence to answer this question. Probably α and β polypeptides from different subcomplexes interact with each other by their N- or C-terminal domains to form the spatial structure of the B800-850 complex providing both conformational transition in the complex and the increase in its stability. Obviously α and β polypeptides from different subcomplexes as well as within a single subcomplex could be crosslinked. It is clear that only one type of crosslinkage is permitted, otherwise several different products such as $\alpha\alpha$, $\beta\beta$, etc. would be present.

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