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Characterization of the core complex of *Rubrivivax gelatinosus* in a mutant devoid of the LH2 antenna

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Abstract

The core complex of purple bacteria is a supramolecular assembly consisting of an array of light-harvesting LH1 antenna organized around the reaction center. It has been isolated and characterized in this work using a *Rubrivivax gelatinosus* mutant lacking the peripheral LH2 antenna. The purification did not modify the organization of the complex as shown by comparison with the intact membranes of the mutant. The protein components consisted exclusively of the reaction center, the associated tetraheme cyt c and the LH1 αβ subunits; no other protein which could play the role of pufX could be detected. The complex migrated as a single band in a sucrose gradient, and as a monomer in a native Blue gel electrophoresis. Comparison of its absorbance spectrum with those of the isolated RC and of the LH1 antenna as well as measurements of the bacteriochlorophyll/tetraheme cyt c ratio indicated that the mean number of LH1 subunits per RC-cyt c is near 16. The polypeptides of the LH1 antenna were shown to present several modifications. The α one was formylated at its N-terminal residue and the N-terminal methionine of β was cleaved, as already observed for other *Rubrivivax gelatinosus* strains. Both modifications occurred possibly by post-translational processing. Furthermore the α polypeptides were heterogeneous, some of them having lost the 15 last residues of their C-terminus. This truncation of the hydrophobic C-terminal extension is similar to that observed previously for the α polypeptide of the *Rubrivivax gelatinosus* LH2 antenna and is probably due to proteolysis or to instability of this extension.

Keywords: Light-harvesting antenna; Reaction center-LH1 core complex; Purple bacteria; *Rubrivivax gelatinosus*

1. Introduction

In all photosynthetic organisms from bacteria to plants, specialized pigment–protein complexes called light-harvesting antenna (LH) harvest the light energy and transfer it efficiently to the reaction centers (RCs) where it is converted into charge separation, initiating electron transfer. In most purple photosynthetic bacteria two types of LH can be distinguished from their in vivo localisation and their spectral properties: LH1 which are tightly associated with the RC in the so-called (LH1–RC) core complex, and LH2 which are more loosely interacting with the core complex and which are present in variable amounts with the growth conditions. In the near infrared the LH1 bacteriochlorophyll (Bchl) absorption occurs at about 870 nm, and the LH2 one at about 850 nm. Our knowledge about the energy transfers between these complexes in the photosynthetic membranes has greatly progressed (see [1,2]), owing to extensive biophysical and spectroscopic studies, and to the description at atomic resolution of the structure of the RC [3–5] and of LH2 [6,7] from several bacterial species. Lower resolution models are also available for LH1s [8] as well as for LH1–RC core complexes [9–11]. LH1 and LH2 are oligomeric integral membrane proteins, built from two hydrophobic polypeptides α and β, each containing a single transmembrane α helix. Each αβ pair is associated with pigments (bacteriochlorophyll (Bchl) and carotenoids). When isolated in detergent solution, or reconstituted in lipid bilayers, the antenna adopt a ring-like structure formed by association of 8–9 αβ pairs for LH2, and 15–16 αβ pairs for LH1. The reversible dissociation of the isolated LH1 antenna into αβ

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subunits has been demonstrated for several bacteria [12–15] and used extensively for evaluating the forces stabilizing the LH1 structure [16]. In the LH2 structures the α polypeptides form the inner part of the ring and the β ones the outer part. In the core complex, the central cavity of the LH1 ring is occupied by the RC. Recent studies performed on native membranes reveal that these oligomeric structures are also present in vivo [17–23]. However there is still a debate about the core complex structure, as to whether the LH1 ring around the RC is complete or not in some bacteria. The arguments against the formation of a closed ring of LH1 rest on functional and structural observations. It has been stressed that a closed ring would hinder the diffusion of quinone molecules exchanging between the membrane quinone pool and the RC-bound secondary quinone Q_B. In *Rhodobacter* (*Rb.*) *sphaeroides* a small hydrophobic protein called PufX is associated with the core complex, and plays a role in facilitating this exchange [24,25]; it is encoded by a gene located in the *puf* operon coding for the LH1 subunits and for two of the subunits of the RC, L and M [26]. In strains expressing PufX the LH1 ring is incomplete and adopts a S-shape around two RCs [11,17,18]; whereas in PufX$^-$ strains the ring is closed and the LH1–RC complex is monomeric [18,27,28]. A S-shape dimeric assembly has also been observed for *Rb. blasticus* [21], another bacterium with a *pufX* gene located in the *puf* operon [29]. On the other hand, several reports concluded to the presence of a closed LH1 ring around one RC in various species such as *Blastochloris* (*Bl.*) *viridis* [19], *Rhodospirillum* (*Rs.*) *rubrum* [7,11,30] and *photometricum* [20]; in the two former species the *puf* operon has been sequenced and does not contain *pufX*. Based on these observations, it would be tempting to conclude that the absence of PufX correlates with a closed, monomeric core complex structure. But it is not consistent with the structure of the core complex isolated from *Rhodopseudomonas* (*Rps.*) *palustris*, another bacterium without PufX. In this structure [10] the LH1 ring around the RC is interrupted by a single trans-membrane helix replacing one of the LH1 αβ pairs. This helix could belong to a 15 kDa polypeptide detected in the core complex; this polypeptide would thus be functionally equivalent to PufX.

In this context we present a biochemical characterization of the core complex of *Rubrivivax* (*R.*) *gelatinosus*. This purple bacterium belongs to the β group of proteobacteria and the organisation of its *puf* operon is known to be different from those of the α group such as *Rb. sphaeroides* and *Rb. capsulatus* [31,32]. Notably it does not contain a gene analogue to *pufX*; the *pufC* gene coding for the tetraheme cyt c bound to the RC is found instead. Furthermore the genes *pufB* and *pufA* coding respectively for the β and α polypeptides of LH1 are preceded by *orf1* (coding for a putative soluble protein) and separated by *orf2* (coding for a putative transmembrane protein); the function of these two orfs is unknown. The photosynthetic apparatus of *R. gelatinosus* is located in the cytoplasmic membrane which is devoid of extensive invaginations and which presents at most a few short tubules [33]. Whereas the nonameric structure of *R. gelatinosus* LH2 has been recently demonstrated [34,35], the structure of its core complex has not been studied so far. Isolation of the core complex from wild type strains was already reported [36,37] but in our hands the yield was quite low. As a prerequisite for structural studies we have now isolated in good yield and in a native state the core complex of a *R. gelatinosus* mutant lacking the LH2 antenna. We paid particular attention to the composition of the complex, in order to determine if it contained another component in addition to the RC, to the tetraheme cyt c and to the LH1s. No such component was found. On the other hand we observed that the LH1 subunits of the isolated core complex presented several modifications. In particular, the α polypeptide was heterogeneous, consisting of a mixture of the full-length polypeptide and of a truncated one lacking the last 15 residues of its C-terminus. Only the truncated polypeptide was detected previously by direct sequencing of the protein [38], and it was proposed later to result from a post-translational processing [31]. In fact this truncation is similar to that observed for the α polypeptide of the isolated LH2 antenna [34] and probably occurs by the same mechanisms.

2. Materials and methods

2.1. Materials

The following detergents were used: *n*-nonyl β-D-glucopyranoside (C9G, Bachem), 3-((3-cholamidopropyl)dimethylammonio)-1-propanesulfonate (CHAPS, Fluka) and sodium cholate (Serva).

2.2. Bacterial growth conditions

Wild type *R. gelatinosus* (strain S1) and the PUCAΩ strain (containing a *pucA* gene interrupted by a Ω cartridge, therefore devoid of LH2) were grown at 30 °C anaerobically in the light in completely filled 2 L bottles in malate medium [39], with streptomycin (25 μg/ml) and spectinomycin (25 μg/ml) added to the growth medium for the mutant.

2.3. Purification of membranes

Cells were suspended in 50 mM Tris–HCl buffer, pH 8, containing 5 mM EDTA and 1 mM PMSF and broken in a French press. Crude membranes were isolated by differential centrifugation, washed in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8) and suspended in TE buffer containing 5% (w/w) sucrose. They were purified on a discontinuous sucrose gradient, formed by 4 layers containing respectively 15%, 30%, 40% and 50% (w:w) sucrose in TE buffer; after 2 h centrifugation at 200,000 g the coloured bands were recovered, diluted with TE buffer and spun down (200,000 g, 45 min). In a few experiments a continuous 15–50% sucrose gradient and overnight centrifugation (200,000 g) were used.

2.4. Isolation of photosynthetic complexes

R. gelatinosus strain S1 was used for isolating the RC and the LH1 antenna following published procedures [15,37]. The mutant PUCAΩ (lacking LH2) was used for the isolation of the core complex. Crude membranes were isolated as above and washed in TE buffer containing 1 M NaCl. The pellet was suspended in TE buffer to an OD_{877nm} of 40 and solubilised at 5 °C by the addition of C9G and CHAPS at final concentrations of 1% and 0.5% respectively. After 30 min incubation and centrifugation (1 hr, 200,000 g) the crude core complex was recovered in the supernatant. It was purified by a combination of ion exchange chromatography on DEAE-Sepharose Fast Flow (Amersham) with gradient elution (0 to 0.4 M NaCl in 10 mM Tris–HCl buffer, 1 mM EDTA, 0.2% C9G, 0.2% CHAPS, pH 8), followed by molecular sieve chromatography on Sepharose CL-6B (Amersham) in 50 mM Tris–HCl