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Cross-species investigation of the functions of the *Rhodobacter* PufX polypeptide and the composition of the RC–LH1 core complex

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ABSTRACT

In well-characterised species of the *Rhodobacter* (*Rba.*) genus of purple photosynthetic bacteria it is known that the photochemical reaction centre (RC) is intimately-associated with an encircling LH1 antenna pigment protein, and this LH1 antenna is prevented from completely surrounding the RC by a single copy of the PufX protein. In *Rba. veldkampii* only monomeric RC–LH1 complexes are assembled in the photosynthetic membrane, whereas in *Rba. sphaeroides* and *Rba. blasticus* a dimeric form is also assembled in which two RCs are surrounded by an S-shaped LH1 antenna. The present work established that dimeric RC–LH1 complexes can also be isolated from *Rba. azotoformans* and *Rba. changlensis*, but not from *Rba. capsulatus* or *Rba. vinaykumarii*. The compositions of the monomers and dimers isolated from these four species of *Rhodobacter* were similar to those of the well-characterised RC–LH1 complexes present in *Rba. sphaeroides*. Pigment proteins were also isolated from strains of *Rba. sphaeroides* expressing chimeric RC–LH1 complexes. Replacement of either the *Rba. sphaeroides* LH1 antenna or PufX with its counterpart from *Rba. capsulatus* led to a loss of the dimeric form of the RC–LH1 complex, but the monomeric form had a largely unaltered composition, even in strains in which the expression level of LH1 relative to the RC was reduced. The chimeric RC–LH1 complexes were also functional, supporting bacterial growth under photosynthetic conditions. The findings help to tease apart the different functions of PufX in different species of *Rhodobacter*, and a specific protein structural arrangement that allows PufX to fulfil these three functions is proposed.

1. Introduction

The purple photosynthetic bacterium *Rhodobacter (Rba.) sphaeroides* has provided many insights into the molecular mechanisms underlying photosynthetic energy transduction. Light-driven charge separation is catalysed in a reaction centre (RC) that is surrounded by a light-harvesting 1 (LH1) pigment protein. This LH1 antenna is made up from multiple copies of single membrane-spanning α- and β-polypeptides that encase bacteriochlorophyll (BChl) and carotenoid pigments [1–10]. These so-called RC–LH1 core complexes are associated with a peripheral antenna formed from light-harvesting 2 (LH2) pigment proteins [11,12]. The RC–LH1 complex assembles in the photosynthetic membrane in two forms, a monomeric version in which the RC is surrounded by a C-shaped LH1 aggregate (when viewed perpendicular to the membrane) and a dimeric version displaying two-fold symmetry where two RCs are surrounded by an S-shaped LH1 aggregate [13]. To date, dimeric complexes have been documented in *Rba. sphaeroides* by electron microscopy (EM) of isolated proteins [13–15] and atomic force microscopy (AFM) of membranes [16,17] and in a second species, *Rba. blasticus*, by AFM of membranes [18]. In contrast, the RC–LH1 complex has been reported to be exclusively monomeric in *Rba. veldkampii*, as judged by AFM of intact membranes and cryo-EM of isolated complexes [19,20].

An additional component of the *Rba. sphaeroides* RC–LH1 complex is a polypeptide termed PufX which is present at a stoichiometry of one per RC–LH1 monomer (see [21] for a review). This protein has a single membrane-spanning α-helix and appears to prevent the LH1 antenna from completely encircling the RC [14,22], which is the case in some other species of purple bacteria where there is no evidence of a PufX or equivalent polypeptide [23–27]. Removal of the gene encoding PufX from the *Rba. sphaeroides* genome results in the assembly of exclusively monomeric RC–LH1 complexes in which the RC is completely surrounded by a closed ring of LH1 pigment protein [14,22], and loss of the ability of the organism to grow under standard photosynthetic conditions [28–30]. In experiments with *Rba. sphaeroides* it has been found that the truncation of the N-terminus of PufX leads to a loss of the dimeric form of the RC–LH1 complex [31,32] and there is a general consensus that PufX is a major factor dictating whether dimeric RC–LH1 complexes are assembled in this species. However the RC–LH1 complex of *Rba. veldkampii* is exclusively monomeric despite the fact that this

Abbreviations: AFM, atomic force microscopy; A_{LH1}/A_{RC}, ratio of LH1 absorbance to RC absorbance; BChl, bacteriochlorophyll; DM, decyl β-D-maltoside; DDM, n-dodecyl-β-D-maltoside; DHPC, 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine; EM, electron microscopy; LDAO, N,N-dimethyldodecylamine N-oxide; LH1, light-harvesting 1 pigment protein; LH2, light-harvesting 2 pigment protein; NMR, nuclear magnetic resonance; OG, octyl β-D-glucoside; PDB, Protein Data Bank; PMS, phenazine methosulphate; RC, reaction centre; *Rba*, *Rhodobacter*; *Rps*, *Rhodopseudomonas*

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species also possesses a PufX [19,20], and it would therefore appear that the assembly of dimeric RC–LH1 complexes is not simply dictated by the presence of PufX *per se*, but is dependent on other factors.

It is not yet clear why some *Rhodobacter* species assemble dimeric RC–LH1 core complexes while others do not, and what advantage (s) the dimeric architecture confers. Monomeric RC–LH1 complexes are visible in AFM images of membranes of *Rba. sphaeroides*, forming a minor population alongside the major population of dimeric RC–LH1 complexes [16,33]. A systematic, quantitative analysis of the relative amounts of monomer and dimer in AFM images of *Rba. sphaeroides* membranes has not been carried out to date. In large membrane patches from *Rba. blasticus* formed by fusion of smaller fragments in two freeze–thaw cycles, 75% of the observed RC–LH1 complexes were dimeric and the remainder monomeric [18], but it was not possible to determine whether the monomers were produced from the dimers during sample preparation, or whether the two forms existed in equilibrium in intact bacterial cells. It is clear that PufX determines the open architecture of the *Rba. sphaeroides* RC–LH1 complex, enlarged and closed rings of LH1 being seen for PufX-deficient RC–LH1 complexes in AFM topographs and in EM data [14,22]. Such PufX-deficient complexes are exclusively monomeric, the open architecture seen in PufX-containing RC–LH1 complexes being required for dimerisation. Studies of the impact of these different architectures for the RC–LH1 complex on cyclic electron transfer in the photosynthetic membrane have focussed on the effects of removal of PufX and the resulting closed structure for the LH1 antenna [34–36], and to date there has been no attempt to determine whether the monomeric and dimeric forms of the RC–LH1 complex result in differences in the characteristics of cyclic electron transfer. A complication is uncertainty over the relative populations of RC–LH1 monomers and dimers in a particular type of strain grown under particular conditions, available data being limited to a handful of studies employing either sucrose gradient fractionation of detergent-isolated complexes [17,32,37] or analysis of patches of intact membrane by AFM or EM [14–16,31].

The finding that the RC–LH1 complex from *Rba. sphaeroides* assembles in a dimeric form whereas that from *Rba. veldkampii* is exclusively monomeric has raised the question of which component(s) of the complex are responsible for this difference. Particular attention has been focussed on PufX, as a feature of this polypeptide is the relatively low degree of sequence identity displayed across the *Rhodobacter* genus [38]. Alignment of the five available PufX sequences shows only eight absolutely conserved residues in a protein of between 75 and 83 amino acids (see [21] for a discussion). The PufX from *Rba. sphaeroides* shows a high degree of identity with that from *Rba. azotoformans* (89% — see Table 1), but much lower identity with the PufX proteins from *Rba. blasticus* (26%), *Rba. veldkampii* (23%) and *Rba. capsulatus* (23%). The latter three percentage identities are much lower than those for the RC and LH1 polypeptides that make up the remainder of the corresponding RC–LH1 complex (Table 1).

Table 1
Identity between the sequences of the component polypeptides of the RC–LH1 core complex from *Rba. sphaeroides* and other *Rhodobacter* species where the sequence of PufX is known.[a].

Rba. sphaeroides protein	Encoding gene	Percentage identity			
		Rba. azotoformans	*Rba. blasticus*	*Rba. veldkampii*	*Rba. capsulatus*
LH1 β (PufB)	*pufB*	–	–	–	79
LH1 α (PufA)	*pufA*	–	–	–	77
RC L (PufL)	*pufL*	95	80	70	78
RC M (PufM)	*pufM*	96	77	74	76
RC H (PuhA)	*puhA*	–	–	–	61
PufX	*pufX*	89	26	23	23

[a] Determined using Swiss-Prot [74] to search for relevant sequences and ClustalW [75] to align and calculate percentage identity. No entry indicates relevant gene sequence was not available.

Taken together with the loss of dimeric RC–LH1 complexes that accompanies the deletion of PufX in *Rba. sphaeroides*, the finding that some species of *Rhodobacter* assemble dimers but others do not has led to attempts to use PufX sequence alignments to identify a "dimerisation motif". The low identity between the five available PufX sequences makes it challenging to identify specific residues that could dictate whether the RC–LH1 complex will be dimeric or monomeric, but specific proposals have been made and these are detailed in the Discussion. Some of the logic underlying these proposals is based on the premise that the RC–LH1 complex from both *Rba. capsulatus* and *Rba. azotoformans* assembles in the dimeric form, an assumption that has not been tested experimentally. One aim of the present study was therefore to look for evidence of dimeric RC–LH1 complexes in these two species, and also address the wider question of how common the dimeric variant of the RC–LH1 complex is within the *Rhodobacter* genus by examining the pigment-protein content of two newly characterised species, *Rba. changlenesis* [39] and *Rba. vinaykumarii* [40].

The second aim of the present study was to further investigate the factors that dictate whether the RC–LH1 complex assembles in a dimeric form by utilising strains of *Rba. sphaeroides* that contain chimeric pigment-protein complexes. In previous work [41] it was reported that photosynthetic growth was retained in a *Rba. sphaeroides* strain where the native PufX had been replaced with the PufX from *Rba. capsulatus* (a strain termed RCLH1sXc), despite the limited sequence identity between the two (Table 1). The spectral characteristics of the membrane-embedded RC–LH1 complex from the RCLH1sXc strain were similar to those of the native complex and the strain was also able to grow photosynthetically, which implied that some of the roles of the native PufX could be fulfilled by the *Rba. capsulatus* variant [41]. Also produced in this work were strains heterologously expressing the *Rba. capsulatus* LH1 antenna alongside either the *Rba. sphaeroides* PufX, the *Rba. capsulatus* PufX or no PufX [41]. The present work examines whether dimeric RC–LH1 core complexes are assembled in these strains of *Rba. sphaeroides* expressing non-native LH1 and/or PufX proteins, and studies in more detail the composition and functionality of their chimeric RC–LH1 complexes.

2. Materials and methods

2.1. Sources and growth of native bacterial strains

Wild-type strains *Rba. sphaeroides* NCIB8253 and *Rba. capsulatus* Kb-1 were revived from laboratory stocks. *Rba. azotoformans* (JCM number 9340) was obtained from the Japan Collection of Microorganisms (RIKEN Bioresource Centre). Native strains of *Rba. changlensis* and *Rba. vinaykumarii* were kindly donated by Dr. Sasikala from the Jawaharlal Nehru Technological University in Hyderabad, India. The LH2-deficient *Rba. sphaeroides* strain DBCΩ was also used [42], which is strain NCIB8253 with the LH2-encoding *puc* operon replaced by a cassette conferring resistance to streptomycin.

Rba. sphaeroides NCIB8253 and *Rba. changlensis* were grown in M22 + medium [43] and *Rba. capsulatus* Kb-1 was grown in RCV-PY medium [44]. *Rba. azotoformans* was grown in MMYS medium (Japanese Collection of Microorganisms website (http://www.jcm.riken.jp/)) and *Rba. vinaykumarii* was also grown in this medium supplemented with 68 mM sodium glutamate and 137 mM glycerol. *Rba. sphaeroides* was grown at 34 °C and the remaining species were grown at 30 °C.

For growth under dark/semi-aerobic conditions a 10 ml aliquot of the relevant medium in a 30 ml universal bottle was inoculated with cells from a freeze-dried stock, glycerol stock or agar plate and placed in an orbital incubator at 180 rpm for 24 h. These 10 ml starter cultures were then used to inoculate 70 ml of medium in a 100 ml Erlenmeyer