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Crystallization and preliminary X-ray crystallographic analysis of the B800–820 light-harvesting complex from *Rhodopseudomonas acidophila* strain 7050

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The B800–820 peripheral light-harvesting complex, an integral membrane protein from *Rhodopseudomonas acidophila* strain 7050, has been crystallized in a form suitable for X-ray diffraction analysis. The crystals belong to space group $R32$ with hexagonal cell dimensions a = 117.20, c = 295.14 Å (at 100 K). A complete 2.8 Å resolution data set has been collected and a structure solution obtained using molecular-replacement methods.

1. Introduction

Photosynthetic light-harvesting complexes are responsible for collecting the solar energy required for photosynthesis. This captured energy is subsequently transferred to the reaction centre where it is used to drive photochemical redox reactions. In purple photosynthetic bacteria the reaction centre is associated with a core light-harvesting (LH1) complex. In most species, a peripheral light-harvesting (LH2) complex is also produced to increase the light-harvesting capacity of this intramembrane system (Zuber & Brunisholz, 1991). LH2 transfers energy to the reaction centre *via* LH1.

In LH2 and LH1, the light-absorbing pigments bacteriochlorophyll *a* (Bchl *a*) and carotenoids are non-covalently bound to two low molecular weight hydrophobic apoproteins denoted α and β (Cogdell *et al.*, 1985). Within most LH2, the Bchl *a* molecules are divided into two spectrally distinct forms whose absorption maxima occur at approximately 800 and 850 nm and this is consequently known as the B800–850 complex (Fig. 1). Depending on their growth conditions, certain species and strains of bacteria are able to produce a second form of LH2 which has different spectral characteristics. This complex has Bchl *a* absorption maxima at approximately 800 and 820 nm and is hence referred to as the B800–820 light-harvesting complex (Fig. 1).

The purpose of the whole light-harvesting array is to provide a unidirectional energy gradient to the reaction centre, with maximum efficiency and minimum energy loss (van Grondelle *et al.*, 1994). The ability of the antenna apoproteins to modulate the long-wavelength absorption maxima of Bchl *a* is of fundamental importance to this process. LH2 complexes absorb at shorter wavelengths than LH1 to provide the unidirectional flow of absorbed light energy in the light-harvesting apparatus. In addition to this, the B800–820 complex is found to be a more efficient light harvester than its B800–850 counterpart as loss of energy through back transfer from LH1 to LH2 is found to be much more restricted in the presence of the B800–820 complex (Deinum *et al.*, 1991).

A comparison of the amino-acid sequences from the B800–850 and the B800–820 complexes from *Rps. acidophila* identified certain conserved residues in B800–850 complexes which are consistently different in the B800–820 complexes (Fig. 2; Brunisholtz & Zuber, 1988). Brunisholtz & Zuber proposed that the replacement of the conserved residues Tyr44 and Trp45 on the α-apoprotein was directly correlated to the spectral shift. It has since been shown (Fowler *et al.*, 1992) that a blue shift in the spectrum is

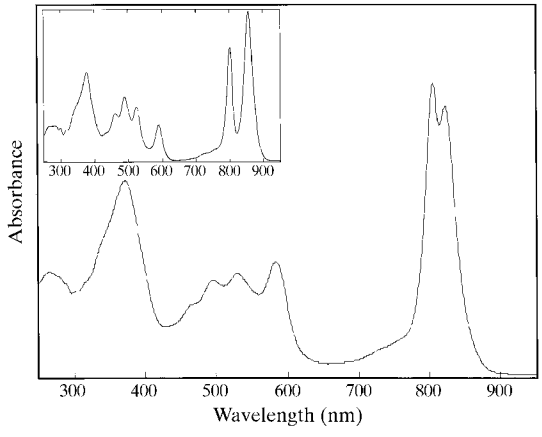

Figure 1
The main figure shows the absorption spectrum of the B800–820 complex from *Rps. acidophila* strain 7050. The amount of possible B800–820 impurity was assessed by measuring the ratio of the peak heights at 800 and 820 nm. For comparison, the insert shows the spectrum of a B800–850 complex.

induced by mutation of the residues at these positions on the α-apoprotein of the LH2 complexes from *Rhodobacter sphaeroides*. Site-directed mutagenesis was used to replace two tyrosine residues with phenylalanine and leucine (αTyr44, αTyr45$\rightarrow\alpha$Phe44, αLeu45), which are the residues present in the B800–820 complex from *Rps. acidophila* strain 7050. In this genetically modified complex the 850 nm absorption band blue-shifted to 826 nm, whereas in the B800–820 complex from *Rps. acidophila* strain 7050 this absorption peak is found at 823 nm.

From the mutagenesis, it was suggested that the loss of two hydrogen bonds from αTyr44 and αTyr45 to an acetyl carbonyl O atom on the 850 nm absorbing Bchl *a* (B850) and the strengthening of another to a B850 keto carbonyl group were responsible for a blue shift in the absorption spectrum (Fowler *et al.*, 1994). However, in the structure of the B800–850 complex from *Rps. acidophila* strain 10050 (McDermott *et al.*, 1995), it was found that while the residues αTyr44 and αTrp45 form hydrogen bonds to the acetyl O atoms, there is no hydrogen bonding to keto carbonyl groups on any of the B850 molecules (Prince *et al.*, 1997).

2. Experimental details

Purification and crystallization protocols were based upon the procedures used to obtain crystals of the B800–850 complex from *Rps. acidophila* strain 10050 (Papiz *et al.*, 1989; McDermott, 1997). The optimized purification protocol differed from that of McDermott mainly in the substitution of gel-filtration chromatography for anion-exchange chromatography. This was necessary as in all cases the isolated LH2 was found to contain a mixture of both B800–820 and B800–850 complexes.

All cultures of *Rps. acidophila* strain 7050 were grown anaerobically on Pfennig's media (Pfennig, 1969) at 303 K. Initially, cells were grown to a volume of 500 ml, using incandescent light of intensity 10 W cm^{-2}, producing a B800–850 peripheral light-harvesting complex. Subsequent inoculated cultures were grown with light of intensity 0.2 W cm^{-2} to induce the synthesis of a B800–820 complex. Harvested cells were mechanically disrupted by two passages through a French pressure cell at a pressure of 154 MPa and the membranes were adjusted to a concentration which gave an optical density at 820 nm (OD_{820}) of 40 cm^{-1} using 20 m*M* Tris–HCl buffer at pH 8.00. Solubilization of the complex was achieved adding lauryl dimethylamine *N*-oxide (LDAO) to a concentration of 2%(*v*/*v*) and incubating at 277 K for 3 h. The solution was then centrifuged at 15000*g* for 20 min and the supernatant containing the solubilized complexes collected.

The LH2 complex was separated from LH1/RC complexes by discontinuous sucrose density gradients. On removal from the gradients, 5 ml of the solubilized complex ($OD_{820} \simeq 20$ cm^{-1}) was loaded onto an 1 ml RESOURCE Q column (Pharmacia Biotech, Uppsala, Sweden) which had been equilibrated with 10 column volumes of buffer. Elution was achieved by a salt gradient of 0–200 m*M* NaCl in buffer, 0.1% LDAO. A small amount of the B800–850 complex was eluted initially, followed by fractions containing a mixture of B800–850 and B800–820 complexes in varying amounts, until at higher salt concentrations pure B800–820 complex was obtained.

Throughout the preparation, and in particular on removal from the anion-exchange column, the purity of the B800–820 complex was monitored by measuring the ratio of the absorption peaks at 820 and 280 nm (McDermott, 1993) and the amount of B800–850 complex monitored by measuring the ratio of the absorbancies at 800 and 820 nm. The presence of a B800–850 'impurity' enhances the 800 nm absorption peak relative to the 820 nm peak. Samples with 820:800 nm ratios greater than 0.93 were collected for crystallization trials. Prior to crystallization, centrifugation dialysis was used to exchange the purification detergent for 1% β-octyl glucopyranoside (β-OG) which contained 0.35 *M* NaCl in buffer. The concentration of the resulting protein solution was adjusted to give an OD_{820} of 90 cm^{-1}.

3. Results and discussion

Crystals were grown using the sitting-drop vapour-diffusion method. The 15 µl drop contained three parts protein solution to one part 4.0 *M* K_2HPO_4 (pH $\simeq$ 9.2) and 2.5%(*w*/*v*) benzamidine hydrochloride as the small amphiphile. This was equilibrated against a well solution of 1 ml 2.3 *M* $(NH_4)_2SO_4$ pH 9.7 at 291 K. Tabular crystals were obtained after three to four weeks, growing to a maximum size of 0.6 × 0.6 × 0.2 mm.

The critical step in obtaining X-ray quality crystals of the complex was to employ procedures which allowed the complex to be sufficiently purified and for the degree of purity to be monitored throughout the preparation. The work reported here emphasizes the importance of an adequate purification protocol when working with membrane proteins, thus allowing the crystallization to be left to simple screening procedures.

The X-ray diffraction data were collected on beamline 9.6 at the Daresbury Laboratory Synchrotron Radiation Source using a wavelength of 0.87 Å and a MAR Research 345 imaging-plate system. A cryoprotectant containing 50% saturated sucrose and artificial mother liquor (1.5 *M* K_2HPO_4, 0.5% β-OG in buffer) was introduced to the crystal by dialysis and loop-mounted crystals were flash-cooled to 100 K with an Oxford Cryosystems Cryostream. A native data set to 2.8 Å resolution was collected using 0.5° oscillations (plate diameter 30 cm) and a low-resolution data set was also collected from the same crystal to 4.4 Å using 2.0° oscillation frames (plate diameter 18 cm).

Data were processed with *DENZO* and scaled with *SCALEPACK* (Otwinowski, 1993). The crystal is of a rhombohedral space group (*R*32), with hexagonal cell dimensions a = 117.20, c = 295.14 Å. The data set is 99.5% complete in the resolution range 50–2.8 Å and 99.2% complete in the highest resolution shell (2.85–2.8 Å). The outer shell has $\langle I\rangle/\sigma\langle I\rangle$ of 1.3 and 67.6% of the overall data have $\langle I\rangle/\sigma\langle I\rangle > 3$. Data processing gave an overall $R_{\rm merge}$ [$R_{\rm merge} = \sum_h \sum_j |\bar{I}_{(h)} - I_{(h)j}| / \sum_h \sum_j I_{(h)j}$, where $\bar{I}_{(h)}$ is the mean intensity] of 6.5% (57.5% in the highest resolution shell) and an average multiplicity of 3.7. The data are somewhat anisotropic which results in the high *R* factor in the highest resolution shell.

		31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
10050	B800-850	H	L	A	I	L	S	H	T	T	W	F	P	A	Y	W	Q	G	G	V	K	K	A	A
7050	**B800-820**	H	A	A	V	L	T	H	T	T	W	Y	A	A	F	L	Q	G	G	V	K	K	A	A
7050	B800-850	H	A	A	V	L	S	H	T	T	W	F	P	A	Y	W	Q	G	G	L	K	K	A	A
7750	B800-850	H	L	A	I	L	S	H	T	T	W	F	P	A	Y	W	Q	G	G	V	K	K	A	A
7750	**B800-820**	H	L	A	V	L	T	H	T	T	W	F	P	A	F	T	Q	G	G	L	K	K	A	A

Figure 2
Primary sequence of the C-termini of the α-apoproteins from *Rps. acidophila*, shown from the conserved Bchl *a* binding histidine residue at position 31. The highlighted section shows the residues conserved in the B800–850 complexes and differing in the B800–820 complexes, which Brunisholtz & Zuber (1988) proposed to be associated with the spectral shift.

A Patterson map calculated with the diffraction data was compared to a Patterson map calculated with the corresponding data from the B800–850 complex from *Rps. acidophila* strain 10050 (McDermott *et al.*, 1995) (computations using programs from the Collaborative Computational Project, Number 4, 1994). This comparison gave an overall correlation coefficient of 77%, rising to 95% at the Harker section $w = 0$, indicating that the two complexes are approximately isostructural.

The program *AMoRe* (Navaza, 1994; Collaborative Computational Project, Number 4, 1994) was used for a molecular-replacement solution. The search model consisted of the apoproteins of the asymmetric unit of the B800–850 complex from *Rps. acidophila* strain 10050 (McDermott *et al.*, 1995), using data within the resolution limits of 10–4 Å. The solution corresponded to zero rotation and a translation (δx, δy, δz) of −0.31, −0.05, −0.38 Å with respect to the orthonormal cell axes. This solution had favourable crystal contacts and a correlation coefficient of 50.4% after rigid-body refinement.

The refinement of the structure of the B800–820 light-harvesting complex from *Rps. acidophila* strain 7050 is in progress. The elucidation of this structure will allow the role which the protein plays in modulating the long-wavelength absorption band of Bchl *a* to be determined.

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