Crystal Structure of the RC-LH1 Core Complex from *Rhodopseudomonas palustris*

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Supporting Online Material

Materials and Methods

Protein preparation

The RC-LH1 core complex was isolated and purified from photosynthetic membranes of the purple bacterium *Rhodopseudomonas palustris* strain 2.1.6. The membranes were adjusted to an OD_{850} of 50 in 20 mM Tris HCl pH 8.0 and solubilised by the addition of LDAO to a final concentration of 1% v/v. The RC–LH1 core complex was initially separated from the LH2 complexes by sucrose density centrifugation as previously described (*S1*). The RC–LH1 band was removed from the gradient and the core complex was further purified by a combination of ion exchange chromatography on a BioCAD/Sprint using a HQ20 column and molecular sieve chromatography with Sephadex S200. An IR-CD spectrum (Fig. S1) and absorption spectrum (Fig. S2) of the purified complex solubilised in 20mM Tris HCL, pH 8.0, 0.1% (v/v) LDAO indicate an intact, functional assembly. Typically, samples with purity ratios of OD_{875}:OD_{280} of > 2.2:1 were used for crystallisations.

Crystal growth

Just prior to crystallisation the core complex was exchanged into 1% (w/v) sucrose monocholate using centrifugal ultrafiltration. Crystals were grown by vapour diffusion at

10-16°C using the 'sitting drop' method. The 'well' contained RC-LH1 complex (OD 875 nm of 60), 0.5% (w/v) sucrose monocholate, 1% (w/v) spermidine, 10 mM $MgCl_2$, 100mM Tris HCl pH 8.5 and 8% (w/v) MMePEG2000. The reservoir contained 16-25% (w/v) MMePEG2000 and 20 mM $MgCl_2$ in 100mM Tris HCl pH 8.5. The crystals grew in about 7 days. 35% (w/v) MMePEG2000-containing mother liquor was used for cryo-protection. Diffraction was observed to a resolution of 4.4 Å but most crystals showed very serious non-merohedral twinning.

Crystallographic analysis

Diffraction data were measured at station 14.1 of the SRS at Daresbury, UK, with the ADSC Quantum4 CCD-detector at the cryogenic temperature of 100K. Indexing, integration and scaling were performed for data to a resolution of 4.8 Å using the DENZO and SCALEPACK programs (*S2*). The crystals belong to the space group *P*1 with cell dimensions of a = 76.04 Å, b = 119.02 Å, c = 130.43 Å, α = 69.3°, β = 72.7° and γ = 66.5°. Data reduction and subsequent calculations used programs from the CCP4 suite (*S3*). Initial phases were obtained by molecular replacement with the program AmoRe (*S4*) using the structure of the RC from *Rb. sphaeroides* as a model (*S5*). The best and the second best MR solutions had correlation coefficients of 37.5% and 29.7%, and *R* factors of 60.6% and 63.3%, respectively. The best AMoRe solution was refined by rigid-body refinement with the program REFMAC (*S6*) using data between 60 and 4.8 Å (R = 51.5%), and a SigmaA weighted (*S7*) $2mF_o$-DF_c electron density map calculated. Models built using the program QUANTA (Molecular Simulations Inc.) underwent subsequent rigid-body refinement with REFMAC. The LH1-complex was modelled using the α-helices (in poly-Ala form) and the pigments (as bacteriochlorin macrocycles)

from the structure of the LH2-complex from *Rps. acidophila* (*S8*). The crystallographic factors R and R_{free} (for 5% reflections excluded from the refinement) were reduced to 0.463 and 0.484, respectively.

Purification of protein W

RC-LH1 complexes were extracted into an organic solvent mixture (1:1 chloroform:methanol, 0.1M ammonium acetate, 10% (v/v) acetic acid) and passed through a Sephadex LH-60 molecular sieve column at a flow rate of 0.5 mL/min. Fractions were collected in volumes of 3.5mL. The elution profile is shown in Fig. S3. A silver stained SDS-PAGE gel of the putative protein W purified on the LH-60 column showed a single protein band with a mass of about 11KDa (Fig. S4).

Mass spectroscopic analyses

The MALDI-TOF spectrum (Fig. S5) was obtained with the whole RC-LH1 complex, with the MALDI conditions optimized for the 9–12 KDa region. The peaks were compared with the m/1e$^-$ masses of known protein components, LH1α, β and the L, M and H subunits of the reaction center. The only peak which could not be explained in terms of the primary mass/ne$^-$, or by the LH1 apoproteins dimerising and having a higher charge per dimer was the peak at 10707.95. We tentatively assigned this peak to protein W.

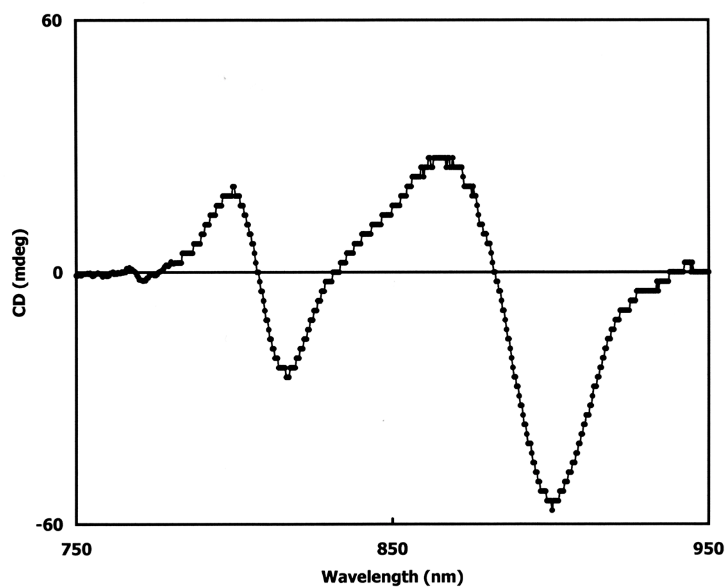

Fig. S1. Room temperature near IR-CD spectrum of *Rps. palustris* RC-LH1 complex solubilised in 20mM Tris HCL, pH 8.0, 0.1% (v/v) LDAO.

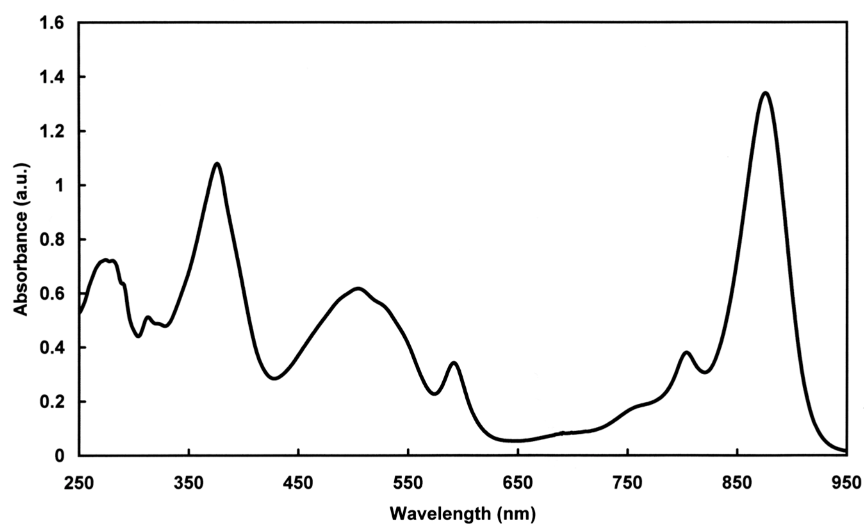

Fig. S2. Absorption spectrum of solubilised RC-LH1 core complex.

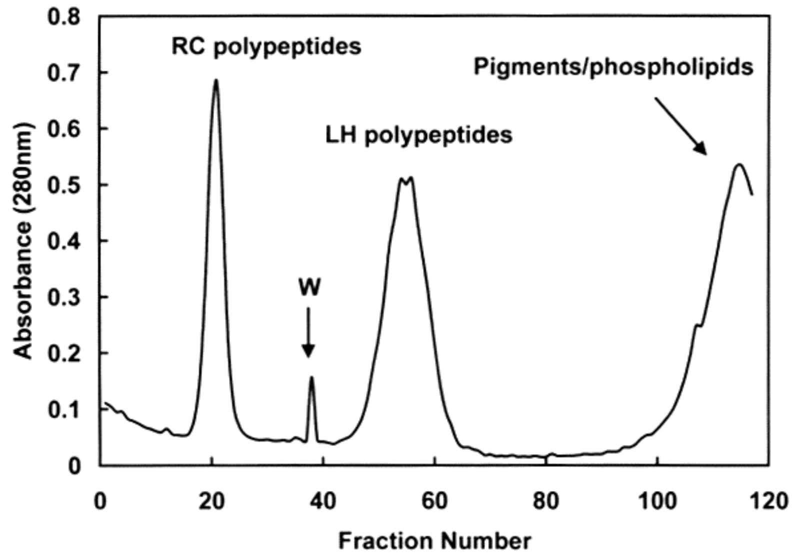

Fig. S3. Gel filtration fractionation of the RC-LH1 core complex extracted into organic solvent and passed through a Sephadex LH-60 column.

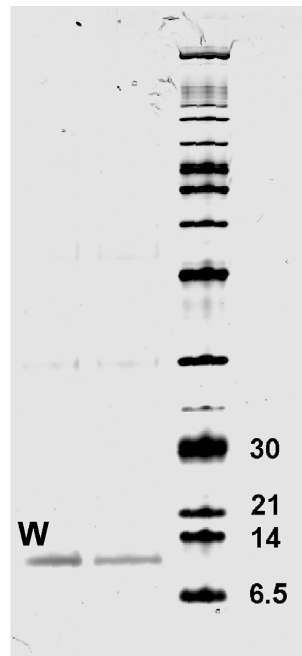

Fig. S4. Silver stained SDS-PAGE gel of protein W purified from the RC-LH1 core complex.

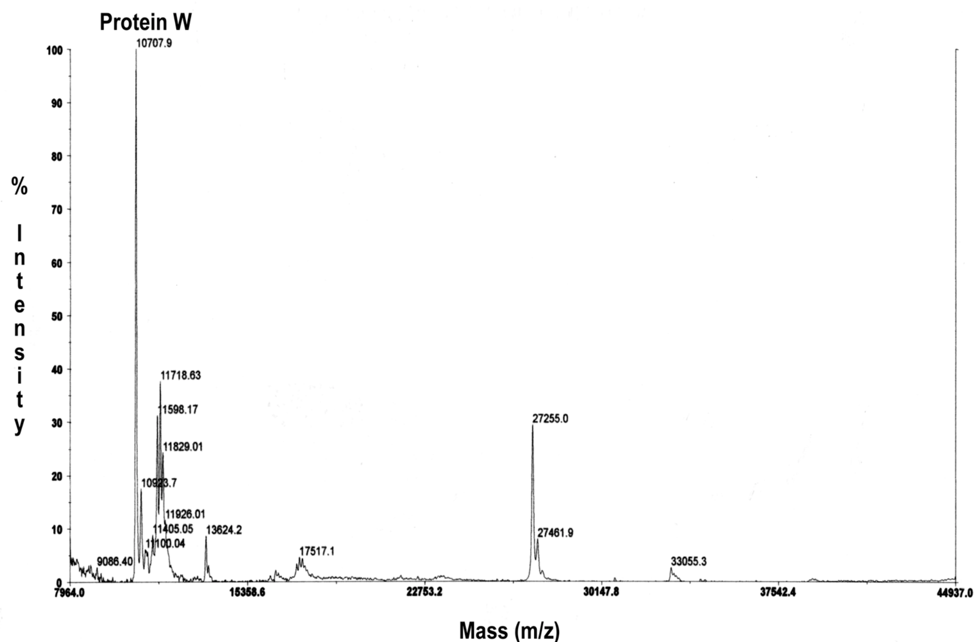

Fig. S5. Mass spectrum (MALDI-TOF) of the RC-LH1 complex.

References and notes

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