Structure of *Rhodopseudomonas sphaeroides* R-26 reaction center

C.-H. Chang, D. Tiede*, J. Tang*, U. Smith*, J. Norris*† and M. Schiffer

*Division of Biological and Medical Research, *Chemistry Division, Argonne National Laboratory, Argonne, IL 60439* and †*Department of Chemistry, University of Chicago, Chicago, IL 60637, USA*

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The molecular replacement method has been succesfully used to provide a structure for the photosynthetic reaction center of *Rhodopseudomonas sphaeroides* at 3.7 Å resolution. Atomic coordinates derived from the *R. viridis* reaction center were used in the search structure. The crystallographic *R*-factor is 0.39 for reflections between 8 and 3.7 Å. Validity of the resulting model is further suggested by the visualization of amino acid side chains not included in the *R. viridis* search structure, and by the arrangements of the reaction centers in the unit cell. In the initial calculations quinones or pigments were not included; nevertheless, in the resulting electron density map, electron density for both quinones Q_A and Q_B appears along with the bacteriochlorophylls and bacteriopheophytins. Kinetic analysis of the charge recombination shows that the secondary quinone is fully functional in the *R. sphaeroides* crystal.

Photosynthesis (Rhodopseudomonas sphaeroides) *Reaction center* *X-ray crystallography* *Molecular replacement*

1. INTRODUCTION

The photosynthetic reaction center is a complex of intrinsic membrane proteins that carries out the initial steps of photosynthesis which effect the separation of electrostatic charges. In general, the reaction centers of the purple photosynthetic bacteria are composed of three membrane-bound protein subunits, designated, L, M and H, with an aggregate molecular mass of 10^5 Da [1]. The complex contains four bacteriochlorophylls, two bacteriopheophytins, two quinones, and one non-heme iron [1]. The subunits L and M show significant amino acid sequence homologies with subunits obtained from the photosystem II of green plants [2]. In addition to the L, M and H subunits, reaction centers from *Rhodopseudomonas viridis,* and several other species of photosynthetic bacteria have an additional cytochrome subunit [3]. The structure of the reaction center from *R. viridis* has recently been determined to 3 Å resolution [4,5]. However, the structure of the reaction center from *R. sphaeroides* is of special interest since it has been extensively examined through both spectroscopic and biochemical methods. Mutants of this species are being developed as models for understanding the mechanism of herbicide inhibition in plants [6–8]. The reaction center from *R. sphaeroides* is one of only very few membrane proteins for which single crystals have been obtained that diffract to high resolution. We have previously described the crystallization of this reaction center [9,10]. We now report the structure of the complex at 3.7 Å resolution as determined by the molecular replacement method [11,12].

2. EXPERIMENTAL

2.1. *Data collection*

The crystals of the *R. sphaeroides* reaction center, which were grown from polyethylene glycol

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[10], are orthorhombic ($P2_12_12_1$) and have unit cell dimensions $a = 142.2$ Å, $b = 139.6$ Å and $c = 78.7$ Å. Diffraction data to 3.7 Å resolution were collected at room temperature by oscillation photography from a single 2-mm-long crystal. The films were scanned with an Optronics P1000 microdensitometer and processed by the method of Rossmann [13]. Scaling of intensity data (I) was performed by the Fourier-Bessel method [14] to give an $R_{sym} = 8.3\%$, where $R_{sym} = \Sigma \mid <I> - I \mid / \Sigma I$; 85% of the reflections in the 3.7 Å shell had measured amplitudes greater than three-times standard deviation.

2.2. *Application of molecular replacement*

The structures of the four protein subunits and the chromophores of the photosynthetic reaction center of *R. viridis* have been previously described [5]. Since the protein subunits of *R. viridis* and *R. sphaeroides* have homologous amino acid sequences [15–17], they are expected to have similar three-dimensional structures. We therefore applied the molecular replacement method to determine the *R. sphaeroides* structure by using the known *R. viridis* structure; the atomic coordinates of *R. viridis* were kindley provided by J. Deisenhofer and H. Michel. For the search structure, a model for the *R. sphaeroides* reaction center was constructed from *R. viridis* by excluding the cytochrome subunit and by removing the amino acid side chains of residues that differed between the two complexes. The resulting model had 60% of the side chains of the *R. viridis* L subunit, 44% of subunit M (some of the residues in *R. viridis* were not known), and no side chains of the H subunit (the amino acid sequence is not available in *R. sphaeroides*).

Orientation of the complex in the unit cell was determined with Crowther's fast rotation function [18] with 15 to 6 Å data. The highest peak observed in the rotation map, corresponding to our solution of the rotational match between *R. sphaeroides* and *R. viridis,* was 25% higher than any other peak. A separate rotation search, conducted with a program written by E.E. Lattman, produced essentially the same solution (the Eulerian orientation angles differ by only 0.9, 0.3 and 0.6°, respectively). The translation search to determine the location of the *R. sphaeroides* complex within its unit cell was carried out on a CRAY-XMP computer with data from 8 to 6 Å resolution. The translation vector was determined by maximizing the correlation factor γ as a function of three-dimensional translation:

$$\gamma = \frac{\sum_h F_o(h) \cdot F_c(h)}{[\sum_h F_o^2(h) \cdot \sum_h F_c^2(h)]^{1/2}}$$

where h is a diffraction index, and F_o and F_c are the amplitudes of the observed and calculated structure factors. This procedure, based on a least-squares principle, is more sensitive than the conventional R-factor search and requires less calculation (Tang and Chang, unpublished). The highest correlation factor corresponded to a minimum R value of 0.44, $R = \Sigma \| F_o \mid - \mid F_c \| / \mid F_o \mid$; at other translation values the average R value was 0.52. The translation search with higher resolution, 8 to 3.7 Å data, gave the same result. After adjustment of scale and temperature factors the R-factor decreased to 0.41. The position and orientation of the complex were further refined by two cycles of rigid-body refinement; this was followed by two cycles of refinement in which the three protein subunits and the chromophores were allowed to move independently. The R-factor for reflections between 8 to 6 Å decreased to 0.39. The maximum movements of the subunits were 0.2 Å translation and 1° rotation.

3. RESULTS

3.1. *Difference Fourier map: location of side chains*

To assess the results of the molecular replacement solution, a map with coefficients $(2F_o - F_c)$ was calculated using structure factors between 8 and 3.7 Å resolution. The chromophores were not included in the calculation of these structure factors. The correctness of the solution was suggested by the appearance of electron density in the map for those side chains and pigment molecules that were not included in the calculation of the map. A Fourier series calculated with coefficient $(2F_o - F_c)$ should have electron density for residues that were not included in the calculation of F_c. As an example, fig.1 shows how the difference electron density map correctly predicts the positions of the side chains for tryptophan residues 125 and 128 in the *R. sphaeroides* subunit M. This procedure was

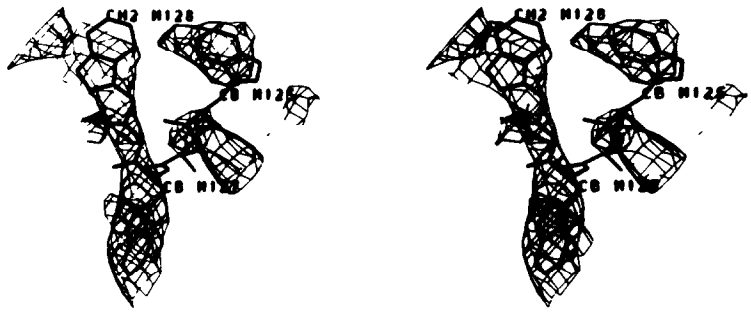

Fig.1. Electron density distribution in the initial $(2F_o - F_c)$ map for tryptophan residues 125, 127, and 128 from the M subunit. The densities for all three residues appear similar, though only M127 is a common residue for both *R. viridis* and *R. sphaeroides* and was therefore the only one included in the structure factor calculation.

used to incorporate the side chains for 180 residues of *R. sphaeroides* protein into the model, yielding an improvement in the map. The *R*-factor for reflections between 8 and 3.7 Å decreased from 0.42 to 0.39.

3.2. *Arrangement of the molecules in the crystal*

The packing of the *R. sphaeroides* reaction center complex in the unit cell is of special importance, first as a proof that the complex was correctly positioned, and second, because this is only the second crystal of a membrane protein for which the structure has been determined. The packing of the molecules in the unit cell is very reasonable. Only the carboxy-terminal two residues of the M subunit interpenetrated the neighboring molecule and, therefore, required adjustment. The M subunit in *R. viridis* has an additional 18 amino acids in its carboxy-end that are pointing away from the M subunit; these amino acids interact with the cytochrome [5]. The L and M surfaces of the complex that interacts with the cytochrome in the *R. viridis* form lattice contacts in *R. sphaeroides*. As was observed in the *R. viridis* structure, the interprotein lattice contacts are between polar residues that are located in part of the complex that would be located on the outside of the membrane. There is no contact between the transmembrane helixes of neighboring complexes. Fig.2 shows the arrangement of the complexes in the unit cell. Most of the contact between molecules is along the *a* axis and the least contact is along the *b* axis of the unit cell. This positioning may explain the observation that several heavy atoms introduced into the crystal change the *b* cell dimension but do not affect the other cell dimensions.

3.3. *The arrangement of the quinones in the* R. sphaeroides *reaction center*

In using the molecular replacement approach, initially we did not include the quinones or pigments in the molecular model. Nevertheless, in the resulting electron density map, electron density for both quinones Q_A and Q_B appears along with the pigments (bacteriochlorophyll and bacteriopheophytin). Fig.3 shows the electron density for the quinone rings at both Q_A and Q_B sites; density was also found for parts of their phytyl tails. The den-

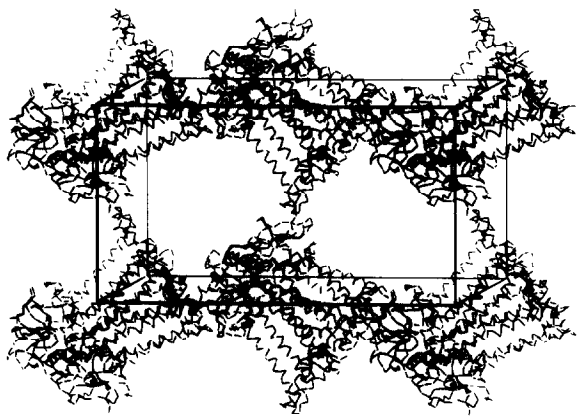

Fig.2. The arrangement of the complexes in the unit cell is shown from a perspective along the *b* axis. The contacts between neighboring complexes are formed by polar side chains. There are no contacts between the hydrophobic helices of the L, M, and H subunits of neighboring complexes.

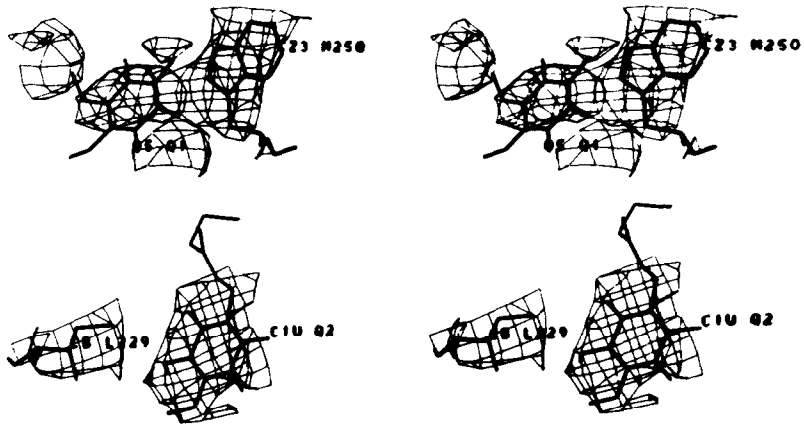

Fig.3. Stereo figures of the quinone rings and a neighboring residue in the Q_A and the Q_B sites. The backbones of the structures are superimposed on the electron density from the initial $(2F_o - F_c)$ map that was calculated without including any of the chromophores. The orientations of the quinones are approximate; their precise orientation will have to be determined after further refinement of the structure.

sity found for the quinones obeys the approximate local two fold as shown in fig.4. In the *R. viridis* crystal there is no quinone in the Q_B position [4,5,19]; the inhibitor orthophenathroline can be diffused into the crystal and was found in a position similar to the Q_B site [5].

The kinetics of charge recombination on the bacteriochlorophyll dimer, B_2, from the light-induced $B_2^+Q^-$ state show that the secondary quinone is fully functional in the crystals. The light-induced $g=2$ B_2^+ EPR signal decays with halftimes of 1 s both in single crystals and in the reaction center solution before crystallization. This rate is characteristic of charge recombination from the secondary quinone $B_2^+Q_B^- \rightarrow B_2Q_B$ [1,6]. Addition of orthophenathroline, which blocks electron transfer from Q_A to Q_B, to the reaction center solution shortens the charge recombination halftime to about 80 ms, consistent with a recombination from the $B_2^+Q_A^-$ state.

4. DISCUSSION

The success of the molecular replacement technique in correctly revealing (i) the amino acid side chains that are unique to *R. sphaeroides* and (ii) electron densities for the reaction center chromophores proves that the solution is a correct one and that the tertiary and quaternary structures of the L, M, and H subunits are essentially conserved.

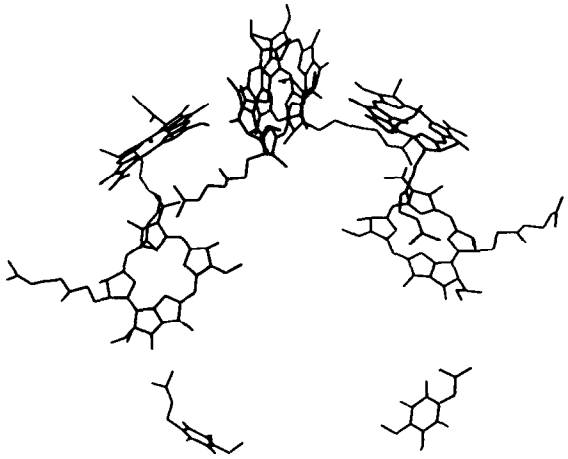

Fig.4. The position of the quinone in the Q_B site in *R. sphaeroides* relative to the other chromophores. To construct the molecular model shown here, the quinones Q_A and Q_B have been moved independently and located in accordance with the electron density map, while the bacteriochlorophyll and bacteriopheophytin positions are essentially as determined for *R. viridis* [5]. We emphasize that the electron density map at present is not sufficiently good to make statements about the precise angles and orientations between Q_A and Q_B or about the difference between the pigments in *R. sphaeroides* and *R. viridis*. This figure, therefore, must be used with caution as some significant changes will likely occur upon refinement.

This finding suggests that reaction center structures from many different organisms, including the green plant photosystem II, may be highly homologous. The current structure of the *R. sphaeroides* reaction center at 3.7 Å resolution shows that the general features of the chromophore organization are also conserved. However, a unique feature of our crystals is that they retain the secondary quinone. Further refinements will provide details of the molecular structures, which are responsible for the different functional activities of these two quinones, and details on the small variations in the organization of the other chromophores.

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