Structural similarity of cytochrome $c_2$ from *Rhodopseudomonas viridis* to mitochondrial cytochromes $c$ revealed by its crystal structure at 2.7 Å resolution

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Abstract

The crystal structure of cytochrome $c_2$ from *Rhodopseudomonas viridis* has been refined using molecular dynamics and restrained least-squares methods to a crystallographic R-factor of 0.216 at 2.7 Å resolution. A structural comparison between *Rps. viridis* cytochrome $c_2$ and the other bacterial cytochromes $c_2$ or mitochondrial cytochromes $c$ indicates that the overall protein foldings are very similar to each other with the exception of the surface loop and terminal region of the polypeptide chain. However, the position and hydrogen-bond pattern of the evolutionarily conserved water molecule buried within the heme binding pocket in *Rps. viridis* cytochrome $c_2$ are common to those in the mitochondrial cytochromes $c$. This fact indicates that *Rps. viridis* cytochrome $c_2$ is structurally more similar to mitochondrial cytochromes $c$ than to the other bacterial cytochromes $c_2$.

**Key words:** Cytochrome $c_2$, Crystal structure; X-ray crystallography; *Rhodopseudomonas viridis*

1. Introduction

In photosynthetic bacteria, light energy is converted into chemical energy by a series of electron transfer reactions. The light-driven cyclic electron transfer process in the non-sulphur purple bacterium, *Rhodopseudomonas viridis*, involves two membrane proteins, the photosynthetic reaction center and the cytochrome $bc_1$ complex, and a water-soluble cytochrome $c_2$ [1]. The primary charge separation and subsequent light-driven electron transport from the bacteriochlorophyll dimer to quinone occur in the reaction center. The determination of the three-dimensional structure of the reaction center from *Rps. viridis* provided information for the advancement for detailed analysis of this electron transfer process [2-4]. On the cytoplasmic side of membranes, the ubiquinone molecules transfer electrons from the reaction center to the cytochrome $bc_1$ complex. Electrons are then transferred to cytochrome $c_2$ from the cytochrome $bc_1$ complex, which is accompanied by proton transfer across the membrane. On the periplasmic side of the membrane, cytochrome $c_1$ re-reduces the photo-oxidized bacteriochlorophyll dimer through a tightly bound cytochrome subunit in the reaction center.

Amino acid sequences have been determined for a variety of bacterial cytochromes [5,6]. Among the bacterial cytochromes $c_2$, that from *Rps. viridis* is closer to the mitochondrial cytochromes $c$ than to the other bacterial cytochromes $c_2$ in terms of homology of the amino acid sequences. The molecular structures of bacterial cytochromes $c_2$ from *Rhodospirillum rubrum* and from *Rhodobacter capsulatus* have already been determined [7-9]. While the bacterial cytochromes $c_2$ are structurally similar to the mitochondrial cytochromes $c$, they are functionally distinct in their dual roles as electron carriers: the former transfers electrons to the reaction center in photosynthesis, while the latter transfers them to the cytochrome oxidase in respiration. We have solved and refined the crystal structure of cytochromes $c_2$ from *Rps. viridis* at 2.7 Å resolution. This paper deals with the comparison of this structure with those of the other cytochromes $c$, especially in regard to the environment around the heme binding pockets.

2. Materials and methods

2.1. Crystallization and data collection

The crystals of cytochrome $c_2$ from *Rps. viridis* belong to the trigonal space group P321 with unit cell dimensions of $a = b = 76.13 \text{Å}$ and $c = 40.40 \text{Å}$ [10]. X-ray diffraction data were collected at room temperature by an automated oscillation camera system equipped with an imaging plate (DIP100; MAC Science Co., Japan) [11,12] on a rotating anode generator operated at 50 kV, 250 mA with CuKα radiation. The crystal was oscillated by 1.5° or 2.0° for each frame around the $a^* + b^*$ axis. A complete intensity data set at 2.7 Å resolution was collected using one crystal before it was damaged by X-ray irradiation. The total exposure time was 3.5 h with a rotation range of 90° covered by 60 frames of the imaging plates.

Intensity data were evaluated by the program system ELMS [11] and processed by the program package PROTEIN [13]. A total of 3,130 independent reflections were obtained after scaling and merging of
13,116 measurements with the merging R-value of 0.082 at 2.7 Å resolution, which corresponds to 76% of the total theoretical number of observations.

2.2. Structure refinement

The crystal structure was solved by the molecular replacement procedure on the basis of the structure of tuna cytochrome c as reported previously [14]. Refinement using the present native data set at 2.7 Å resolution was performed by the simulated annealing procedure with the program X-PLOR [15] and by the restrained least-squares method with the program PROLSQ [16]. The molecular dynamics simulations where the temperature was raised to 3,000 K and then slowly cooled to 300 K in steps of 25 K with subsequent energy minimization were performed with gradual extension of resolution to 2.7 Å. Successively, refinements consisting of several cycles of PROLSQ and manual refitting of the model to a difference Fourier map were carried out. The 2Fo-Fc, Fo-Fc, and fragment-deleted ('omit') difference Fourier electron density maps were calculated using PROTEIN [13] and inspected on an Evans and Sutherland PS390 graphics system using the program FRODO [17]. The heme group was restrained to an ideal planar geometry with an Fe-N(pyrrrole) distance of 1.96 Å. The heme-to-ligated histidine and methionine distances were restrained with Fe-N = 2.00 Å and Fe-S = 2.30 Å, respectively. The vinyl groups of heme-to-cysteine distances were restrained at 1.81 Å. Water molecules were assigned if peaks in the Fo-Fc maps were situated at positions to give at least one hydrogen bonding to protein atoms. They were refined as neutral oxygen atoms with individual temperature factors in the same manner as protein atoms.

The current model comprises all 107 residues, one heme and 52 water molecules with an overall R-factor of 0.216 for 2,852 observed reflections in the resolution range from 10 to 2.7 Å. The model has good stereochemistry with rms deviations from an ideal geometry of 0.011 Å for the covalent bond distances, 0.037 Å for the interbond angle distances, 0.036 Å from the planar groups and 0.020 Å for the peptide bond torsional angles. Individual temperature factors were assigned for all non-hydrogen atoms with the mean value of 16.5 Å². The computations were performed on a VAXstation 4000 (model 90).

3. Results and discussion

The three-dimensional folding of the cytochrome c2 polypeptide chain is shown in Fig. 1. The present structure was compared with those of two bacterial cytochromes c2 (from Rb. capsulatus [7] and Rs. rubrum [8,9]) and a mitochondrial cytochrome c (from tuna in the oxidized and reduced forms [18,19]). Atomic coordinates were obtained from the Brookhaven Protein Data Bank [20]. The overall molecular foldings of these cytochromes c are very similar to each other except for amino acid insertions on the molecular surface in Rb. capsulatus and Rs. rubrum. The amino acid sequence of Rsps viridis cytochrome c2 is more similar to that of tuna cytochrome c than those of two other bacterial cytochromes c2 in terms of the length of polypeptide chain and the sequence homology.

It is notable that the similarity between the cytochromes from Rsps viridis and tuna is more remarkable also in their three-dimensional structures, especially those around the heme binding pocket. In mitochondrial cytochromes c, it is known that a buried water molecule located within the heme binding pocket is evolutionarily conserved and seems to play a role in the mechanism of electron transfer reactions [18,19]. In Rsps viridis cytochrome c2, a water molecule is also found which is positioned within hydrogen bond distances of the hydroxyl groups of Tyr-66 and Thr-77 and the carboxyl oxygen of Asn-51 (Fig. 2A). This water molecule occupies the same position as those found in the mitochondrial cytochromes c, as shown in Fig. 2B, where hydrogen bonds with three amino acid residues (Tyr-67, Thr-78 and Asn-52) exist in the heme binding pocket in the same manner as in Rsps viridis. It should be mentioned that these water molecules in cytochromes from Rsps viridis and tuna have no hydrogen bondings with the carboxylate oxygen of the heme propionate group.

On the other hand, as shown in Fig. 2C and D, the environment around this conserved water molecule in the heme pocket in cytochromes c2 from Rb. capsulatus and Rs. rubrum is somewhat different from those in the above mentioned cytochromes c. Asn-51 in cytochrome c2 from Rsps viridis is replaced by Ile-57 in cytochrome c2 from Rb. capsulatus, in which the water molecule located at the slightly different position is hydrogen bonded to the heme propionate group (Fig. 2C). In cytochrome c2 from Rs. rubrum, this water molecule does not exist and its position is occupied by the hydroxyl group of Tyr-52 that is also hydrogen bonded to the heme propionate group as shown in Fig. 2D. Considering the atomic position and the hydrogen bond geometry around these water molecules in the heme pocket, cytochrome c2 from Rsps viridis is more similar to tuna cytochrome c than to cytochromes c2 from Rb. capsulatus and Rs. rubrum. With respect to not only the homology in the amino acid sequences but the structural similarity of the vicinity of the heme group, Rsps viridis cytochrome c2 is closer to the mitochondrial cytochromes c than the other two bacterial cytochromes c2.

![Fig. 1. A ribbon diagram of the three-dimensional folding of cytochrome c2 from Rsps viridis generated by the program MOLSCRIPT [24]. The heme group is represented by a ball-and-stick model.](image-url)
Fig. 2. Stereoviews of the hydrogen bonding pattern around the buried water molecule in the heme binding pockets. The water molecule is represented by a filled circle with hydrogen bondings indicated by dashed lines. The entry names in the Protein Data Bank are shown in square-brackets for B–D. (A) Rps. viridis cytochrome c₁ (this work); (B) tuna cytochrome c in the reduced form [SCYT]; (C) Rb. capsulatus cytochrome c₁ [IC2R]; (D) Rs. rubrum cytochrome c₁ in the reduced form [3C2C].

It is also known that this water molecule takes on a significant positional shift upon a change in the oxidation states in mitochondrial cytochromes [18,19]. Interestingly, the cytochromes from Rps. viridis and tuna have similar oxidation-reduction potentials of +285 and +260 mV, respectively [21], although two bacterial cytochromes from Rb. capsulatus and Rs. rubrum have higher oxidation-reduction potentials of +340 and +350 mV, respectively [22]. The structural similarity in the heme pocket environment around this conserved water might correspond to the values of the oxidation-reduction potentials in these cytochromes. However, the precise role of this water molecule in the mechanism of electron transfer remains uncertain.

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