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X-ray Crystallographic Study of Archaerhodopsin

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Archaerhodopsin (aR), a light-driven proton pump found in *Halorubrum sp. aus*-1, was crystallized into an octahedral crystal belonging to the space group $P4_32_12$. It is shown that aR is composed of 7 helical segments and an anti-parallel β sheet. The main-chain structure of aR is nearly identical to that of bacteriorhodopsin, but a significant structural difference is observed in the protein surface, especially at lipid binding sites.

Key words : protein crystallization, retinal, proton pump, light reaction

INTRODUCTION

Archaerhodopsin (aR) is a light-driven proton pump found in an extremely halophilic archaebacterium (*Halorubrum sp. aus-1*), which was collected from a salt lake in western Australia [1]. The amino-acid sequence of aR exhibits 60% homology to the sequence of bacteriorhodopsin (bR), a proton pump found in the purple membrane of *Halobacterium salinarium* (Fig. 1) [2]. Like bR, aR molecules aggregate to form claret patches (claret membrane) in the cell membrane. At low ionic strength, this claret membrane is isolated as a sheet with a thickness of 5 nm and a diameter of 200 - 600 nm. It has been suggested from spectroscopic data of claret membrane that the second chromophore bacterioruberin interacts with aR in a specific manner [3]. But, little is known about the organization mechanism of this membrane. To determine the structure of aR, we have performed an X-ray crystallographic analysis using a three-dimensional crystal.

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                        1        10        20        30
aR-1 :  -MDPIALTAAVGA DLLGDGRPET LWLGIGTLLM LIGTFYFIVK
bR   :  MLELLPTAVEGVS QAQITGRPEW IWLALGTALM GLGTLYFLVK
       40        50        60        70        80
GWGVTDKEAR EYYSITILVP GIASAAYLSM FFGIGLTEVQ VGSEMLDIYY
GMGVSDPDAK KFYAITTLVP AIAFTMYLSM LLGYGLTMVP FGGEQNPIYW
       90       100       110       120       130
TGLVGALSHT ARYADWLFTT PLLLLDLALL AKVDRVSIGT LVGVDALMIV
ARYADWLFTT PLLLLDLALL VDADQGTILA LVGADGIMIG TGLVGALTKV
      140       150       160       170       180
PLARYTWWLF STCMIVVLY FLATSLRAAA KERGPEVAST FNTLTALVLV
YSYRFVWWAI STAAMLYILY VLFFGFTSKA ESMRPEVAST FKVLRNVTVV
      190       200       210       220       230
LWTAYPILWI IGTEGAGVVG LGIETLLFMV LDVTAKVGFG FILLRSRAIL
LWSAYPVVWL IGSEGAGIVP LNIETLLFMV LDVSAKVGFG LILLRSRAIF
      240
GDTEAPEPSA GAEASAAD-
GEAEAPEPSA GDGAAATSD
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Figure 1. Amino-acid sequences of aR and bR

MATERIALS AND METHODS

Claret membrane of *Halorubrum sp. aus-1* was purified according to [4]. Crystals of aR were obtained under a similar condition to that used for preparation of the *P622* crystal of bR [5]. Namely, a mixture of claret membrane (5 mg/ml), 5 mg/ml octylthioglucoside, 1.0 M ammonium sulfate, 0.3% heptane-triol, 0.04 % NaN_3 and 40 mM Na-Citrate (pH 5.2) was concentrated at 10 °C by the sitting-drop vapor diffusion method.

X-ray diffraction measurements were performed at the

beamline SPring8-BL41XU, where a frozen crystal at 100 K was exposed to a monochromatic X-ray beam at wavelength of 1.0 Å. Diffraction data were collected using a CCD camera (marccd 615). Indexing and integration of diffraction spots were carried out with Mosflm [5]. The scaling of data was done using SCALA in the CCP4 program suites [6]. For structural determination, the molecular replacement analysis was applied, using our structural model of bR (PDB code 1iw6) as a starting model. Structure refinement was performed using CNS [7] and XtalView [8]..

RESULTS AND DISCUSSION

When aR was crystallized in the presence of octylthioglucoside and ammonium sulfate, an octahedral crystal was obtained (Fig. 2). This crystal exhibits a strong linear dichroism; i.e., it becomes almost transparent when the polarization plane of the measuring light is parallel to the 4-fold axis of the crystal, suggesting that the absorption dipole moment of the retinal chromophore orients in perpendicular to the 4-fold axis.

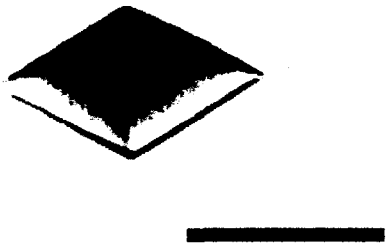

Figure 2. Octahedral crystal of aR

Diffraction data (Table 1) suggested that this crystal belongs to the space group $P4_12_12$ or $P4_32_12$. The latter space group provided a correct structural model of aR (Fig. 3). It is found that the asymmetric unit contains two aR molecules, which are related by a local two-fold symmetry. The dimeric structure of aR is stabilized by hydrogen-bonding

Table 1. Statistics of data collection and refinement

Data collection	
Space group	$P4_32_12$
Unit cell dimensions	a = b = 127.9 Å, c = 109.9 Å,
	$\alpha = \beta = \gamma = 90°$
Resolution	28.5 - 3.5 Å
Unique reflection	11838
Completeness	98.6 %
R_{sym}	9.0%
Refinement	
R-value	29.5%
R_{free}	34.4%

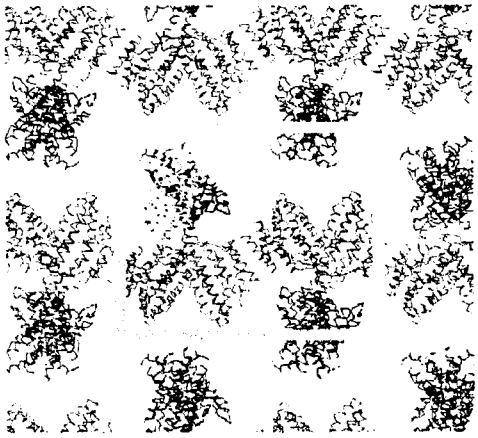

Figure 3. View of the crystal structure along the a-axis.

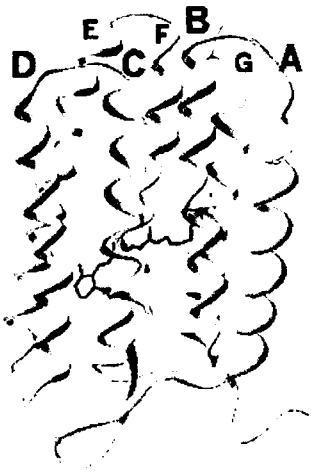

Figure 4. Ribon model of aR with retinal in a wire model.

interactions between Tyr135 OH of one monomer and the carbonyl oxygen of Val189 of the other monomer.

The polypeptide chain of aR is folded into 7 α-helices (A through G) and the loop between helices B and C forms an anti-parallel β-sheet (Fig. 4). All-*trans* retinal is bound to Lys216 located in the middle of helix G. In the crystal, the proteins are arranged in such a manner that the retinal polyene chain directs in perpendicular to the c-axis. This result is consistent with the linear dichroism of the crystal.

Structural comparison with bR shows that, except for the N-terminal region, the main-chain structure of aR is nearly identical to that of bR (Fig. 5). The functionally important amino-acid residues located inside the protein are conserved and they have the same conformations as seen in bR. Remarkable structural differences between aR and bR are observed in the protein surface, especially in the vicinity of the lipid binding site located at the crevice between two monomers within the trimeric structure of bR.

The present study shows that the aR molecules form the dimeric structure in the octahedral crystal. On the other hand, the X-ray diffraction pattern from a dried film of claret membrane shows that aR molecules are arranged in a hexagonal lattice, whose lattice constant is only slightly larger than that observed in purple membrane (our unpublished data). The diffraction pattern observed is best explained when aR trimers are arranged in the hexagonal lattice in a similar fashion as seen in purple membrane. Thus, claret membrane is structurally indistinguishable from purple membrane. But, the formation mechanism of the trimeric structure may be different for these two proteins, as there is a significant structural difference in the lipid binding site. It is possible that bacterioruberin has an important structural role in the organization of the trimeric structure of aR.

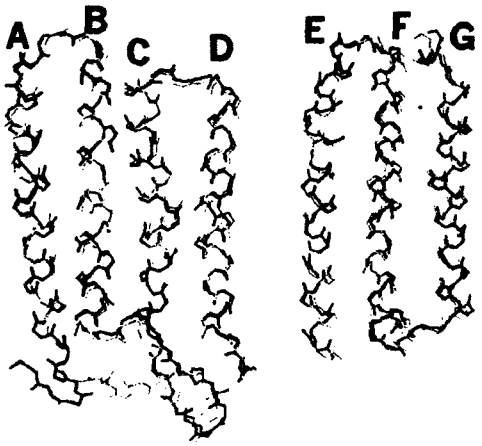

Figure 5. Structural comparison of aR (black) and bR (gray).

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