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Structural Role of Bacterioruberin in the Trimeric Structure of Archaerhodopsin-2

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Archaerhodopsin-2 (aR2), a retinal protein–carotenoid complex found in the claret membrane of *Halorubrum* sp. *aus-2*, functions as a light-driven proton pump. In this study, the membrane fusion method was utilized to prepare trigonal *P*321 crystals ($a=b=98.2$ Å, $c=56.2$ Å) and hexagonal $P6_3$ crystals ($a=b=108.8$ Å, $c=220.7$ Å). The trigonal crystal is made up of stacked membranes in which the aR2 trimers are arranged on a honeycomb lattice. Similar membranous structures are found in the hexagonal crystal, but four membrane layers with different orientations are contained in the unit cell. In these crystals, the carotenoid bacterioruberin [5,32-bis(2-hydroxypropan-2-yl)-2,8,12,16,21,25,29,35-octamethylhexatriaconta-6,8,10,12,14,16,18,20,22,24,26,28,30-tridecaene-2,35-diol] binds to crevices between the subunits of the trimer. Its polyene chain is inclined from the membrane normal by an angle of about 20° and, on the cytoplasmic side, it is surrounded by helices AB and DE of neighbouring subunits. This peculiar binding mode suggests that bacterioruberin plays a striking structural role for the trimerization of aR2. When compared with the aR2 structure in another crystal form containing no bacterioruberin, the proton release channel takes a more closed conformation in the *P*321 or $P6_3$ crystal; i.e., the native conformation of protein is stabilized in the trimeric protein–bacterioruberin complex. Interestingly, most residues participating in the trimerization are not conserved in bacteriorhodopsin, a homologous protein capable of forming a trimeric structure in the absence of bacterioruberin. Despite a large alteration in the amino acid sequence, the shape of the intratrimer hydrophobic space filled by lipids is highly conserved between aR2 and bacteriorhodopsin. Since a transmembrane helix facing this space undergoes a large conformational change during the proton pumping cycle, it is feasible that trimerization is an important strategy to capture special lipid components that are relevant to the protein activity.

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Introduction

Recent crystallographic studies have shown that a large number of membrane proteins are able to form oligomeric structures.[1] Advantages of protein oligomerization include (1) acquisition of a sophisticated transport mechanism such as a peristaltic transport mechanism proposed for the drug-efflux transporter ArcB[2] or a membrane rotary ion pump,[3] (2) formation of an ion-transporting pore[4] or ion channel,[5] (3) cooperative regulation of the transport activity,[6] (4) high-density protein arrangement in the cell membrane[7] and so on. Recent high-resolution structural analyses of oligomeric membrane proteins show that the subunit–subunit interactions are mediated by specific lipids.[8–15] Such lipids are believed to be physiologically important because they can fill a space between the hydrophobic protein surfaces in a complementary manner or, alternatively, they can provide a flexible environment around a functional protein that undergoes a series of conformational changes for ion transport or

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Abbreviations used: aR2, archaerhodopsin-2; aR1, archaerhodopsin-1; bR, bacteriorhodopsin.

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signal transduction.[16,17] For a membrane protein of medium size, oligomerization is an economic and effective strategy to recognize and capture special lipid components that are relevant to the protein activity. This advantage may have been utilized by microorganisms inhabiting such harsh environments that the lipid composition of the cell membrane is frequently adjusted for their survival.

Archaerhodopsin-2 (aR2) is a light-driven proton pump found in the claret membrane of *Halorubrum* sp. *aus-2*, which was collected from a salt lake in Western Australia.[18] This membrane protein shares 56% sequence identity with bacteriorhodopsin (bR), a homologous protein found in the purple membrane of *Halobacterium salinarum* (Fig. 1b). The retinal chromophore is contained commonly in these archaeal rhodopsins and its photoisomerization around the C13–C14 double bond is utilized to initiate the proton pumping cycle.[19,20] In the unphotolysed state, the retinal chromophore alone absorbs visible light maximally at 550–570 nm, exhibiting purple. The significant colour difference between purple membrane and claret membrane is due to the presence of the reddish pigment bacterioruberin [5,32-bis(2-hydroxypropan-2-yl)-2,8,12,16,21,25,29,35-octamethylhexatriaconta-6,8,10,12,14,16,18,20,22,24,26,28,30-tridecaene-2,35-diol] in the latter membrane (Fig. 1a). Most members of *Halobacteriaceae*, including *H. salinarum*, possess C_{50} carotenoids of bacterioruberin groups.[21,22] No trace of carotenoid is present, however, in the purple membrane of *H. salinarum*.[23] Because bacterioruberin is abundantly present in other cell membrane regions of *H. salinarum*, one needs to postulate a special lipid-recognition mechanism by which bacterioruberin is excluded from the crystalline lattice of purple membrane. Since previous spectroscopic data of claret membrane have shown that archaerhodopsin interacts with bacterioruberin in a specific manner,[19,24] it would be expected that elucidation of the binding manner of bacterioruberin to aR2 provides an insight into lipid-recognition mechanisms.

In a previous study, aR2 was crystallized into a 3-D crystal belonging to space group $C222_1$ and its structure was determined at 2.5 Å resolution.[25] One

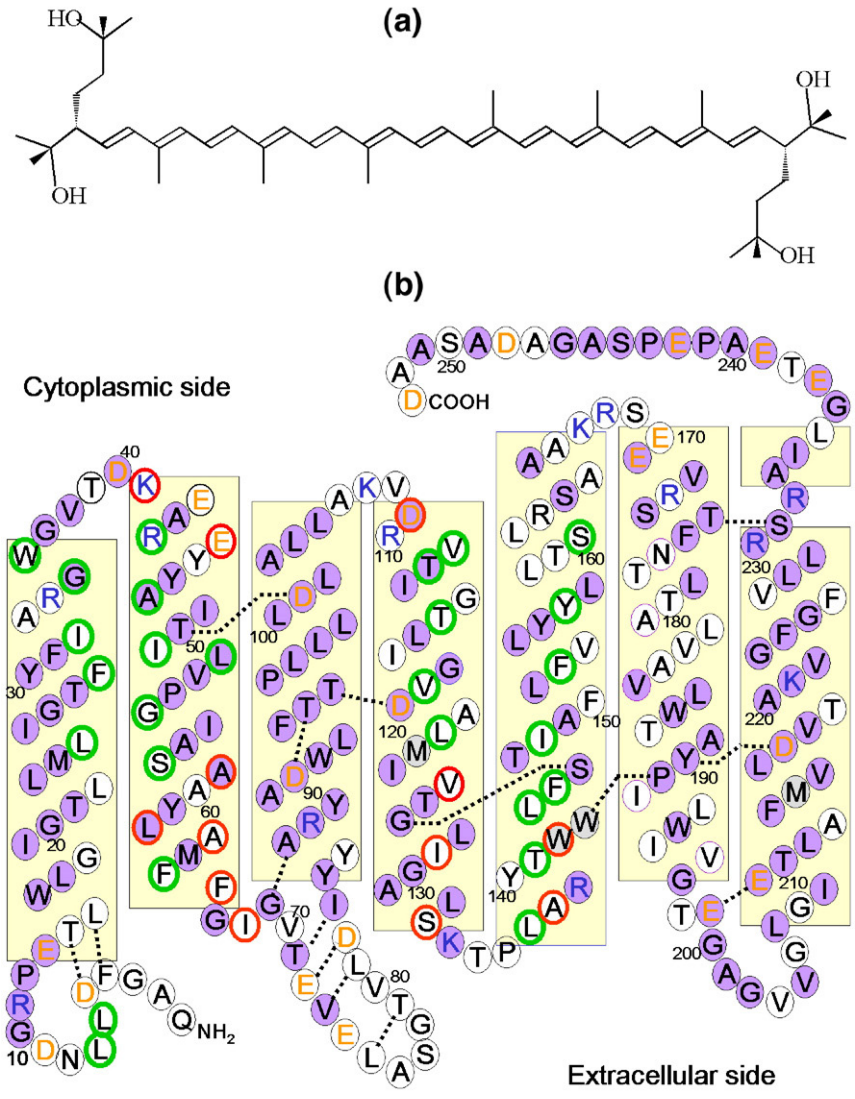

Fig. 1. (a) Chemical structure of bacterioruberin. (b) Schematic diagram of the topology of aR2 showing helices as rectangles. Residues contacting bacterioruberin and residues participating in intratrimer protein–protein interactions are marked with green and red circles, respectively. Violet circles represent residues conserved between aR2 and bR.

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