Crystal structure of the bromide-bound D85S mutant of bacteriorhodopsin: principles of ion pumping

M.T. Facciotti[1], V.S. Cheung[2], D. Nguyen[2], S. Rouhani[3], and R.M. Glaeser[1,2,3]
[1]Biophysics Graduate Group, University of California, Berkeley, California 94720, USA
[2]Department of Molecular and Cell Biology, University of California, Berkeley, California 94720, USA
[2]Life Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory,
University of California, Berkeley, California 94720, USA

INTRODUCTION

Active transport of ions across the cell membrane is a fundamental energy-driven process that establishes electrochemical gradients for driving a variety of processes, including ATP synthesis and metabolite uptake. Attempts to understand the mechanistic basis of ion pumping from a structural perspective have been hampered by the difficulty of crystallizing integral membrane proteins. As a means to gain more insight about the ion pumping process, we previously crystallized a halide-pumping mutant of bacteriorhodopsin, bR(D85S), in its halide-free state [1]. Here, we describe the crystal structure of bR(D85S) with a bromide ion bound in its ground-state binding site. In conjunction with the previously determined structure of the halide-free state [1], this study reveals structural changes that occur during the initial substrate-binding step of halide-ion transport, and suggests some design principles that may apply more generally to other ion pumps.

MATERIALS AND METHODS

Crystals of bR(D85S) were grown in a 1-monooleoyl-*rac*-glycerol (Sigma, St. Louis) gel. Mother liquor consisting of 100mM Sodium Acetate pH 4.6, 200mM KCl, and 10% PEG 4000 was layered on top of the hydrated gel. Crystals grew to a final size of 5□m by 30□m by 120□m in the period of 3 weeks. Individual crystals were then transferred to a solution containing no halide, 100mM Sodium Acetate, pH 4.6, 10% PEG 4000 to remove the bound chloride, after which they were transferred sequentially to cryo-protectant solutions containing 100mM Sodium Acetate, pH 4.6, 1.25M KBr, and 12%, 16% 20%, and 25% PEG 4000, respectively.

Crystals were flash frozen in liquid nitrogen and irradiated on beamline 8.3.1 of the ALS with a 30□m collimated x-ray beam. Five crystals were used to collect the halide data-set. Data reduction was performed by the Elves scripts [2]. Diffraction data were integrated with MOSFLM [3] and subsequently scaled with SCALA [4]. Molecular replacement using 1KGB [5] as the starting search model, without the retinal, water, and lipid molecules, was performed by the program CNS Version 1.1 [6]. Refinement with CNS and model building using the program O [7] together with annealed simulated omit, |Fo| - |Fc|, and 2|Fo| - |Fc| maps reduced the values of R and R_{free} to 21.5 and 23.5, respectively.

DISCUSSION

From our observations on bR(D85S), together with information from previously determined structures of pumps and channels, we propose that the active transport of an ion across a cell membrane consists of at least the following four functional transitions, summarized in figure 1. (i)

Three important events occur in the first transition that is shown in figure 1. In the first event, substrate entry into the binding site of an ion pump requires the transient conformational opening of a gate that provides access to an internal binding site. We believe that such a gate is formed by R82 in bR(D85S) and its homologue R108 in halorhodopsin. An intriguing possibility is that the guanidinium group of the arginine residue may also act as a Born-energy chaperone for the substrate anion. The internalized substrate ion must next form an ionic interaction with a charged residue in the binding site, a role that is played by the protonated Schiff-base in hR and bR(D85S). Finally, substrate binding must induce a conformational change that closes the entrance side of the pump, thereby making it more difficult for transient conformational openings to occur, which would allow backflow of the substrate ion. In bR(D85S), bromide binding induces significant repacking of helices on the extracellular side of the pump which could lock the side chain of R82 in its upward-facing (closed) conformation. (ii) Energy consumption must then be coupled to what needs to be no more than a small directional displacement of the charged group with which the substrate ion interacts as a binding partner. In both hR and bR(D85S), this step consists of photon-induced retinal isomerization, which is in effect another instance of rotamer switching. This structural change, although small, can nevertheless have a large effect on the spatial energy landscape for the transported ion, moving it vectorially within the membrane and raising it to a potential energy that is higher than what it will have when it is released on the opposite (in this case cytosolic) side of the cell membrane. The idea that small-scale conformational changes can have quite large energetic consequences with significant functional relevance has previously been suggested as a mechanism for signal transduction across the membrane by the aspartate chemotaxis receptor [8]. (iii) The substrate must then traverse the remaining portion of the protein. In the case of bR(D85S), it is reasonable to suppose that a repacking of helix F and helix G occurs on the cytoplasmic side of the protein, producing an N-like intermediate similar to that observed with the F219L mutant [9] or the constituitively N-like structure of the D96G/F171C/F219L triple mutant [10]. The helix packing observed in the N-like structures is believed to increase the access of water molecules to the cytoplasmic side of the Schiff base, and thus may open up a path for the diffusion of the halide ion out to the cytoplasmic side of the membrane. The suggestion that the extracellular side must open in order to create an exit for the halide-ion has also been proposed as a requirement for halorhodopsin's pumping cycle [11]. (iv) After the substrate has left, the protein will be free to return to its ground state conformation.

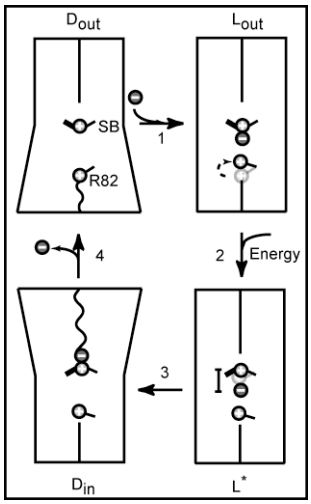

Figure 1 Functional steps in the ion-transport cycle of bR(D85S). The cartoon schematically shows, via sloping lines, an outward tilt for helices on either the extracellular side or the cytoplasmic side of the protein, in the dynamic (D_{out} and D_{in}) states respectively, corresponding to known structures of bacteriorhodopsin in which one or more helices tilt outward. The presumed increase in the permeability of the corresponding half of the protein is indicated by the transition from a straight to wiggly line crossing that half of the protein. In the first step, the extracellular half of the transport channel starts in a "dynamic" conformation, D_{out}. The side chain of R82 serves as a dynamic gate which, by fluctuating between inward facing and outward facing conformations, allows entry of a halide ion to the binding site. Substrate binding then induces a conformational transition to the "latched" state, L_{out}, in which R82 is held tightly in the upward facing position. The movement of R82 is indicated by a change in position of the schematic side chain and a curved dashed arrow. The initial position of the side chain is shown in faint blue. In the second step, photon energy is absorbed by the retinal chromophore and results in a transition to a high-energy state, L^*, in which a small displacement of the protonated Schiff base relative to the bound halide raises the potential energy of the halide ion by a large amount. The displacement of the Schiff base is represented in a similar fashion to that of R82, while a bar indicates that this displacement increases the distance between the halide ion and the Schiff base. The third step envisions a repacking of helices on the cytoplasmic side of the protein that yields a dynamic state, D_{in}, which allows the anion to exit the protein. Finally, in the fourth step, the protein relaxes back to its ground state, D_{out}.

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Principal investigator: Robert Glaeser, Life Sciences Division, Ernest Orlando Lawrence Berkeley National Laboratory. Email: rmglaeser@lbl.gov. Telephone: 510-642-2905.