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Do Cation-π Interactions Exist in Bacteriorhodopsin? *

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Metal ions are essential to the structure and physiological functions of bacteriorhodopsin. Experimental evidence suggests the existence of specific cation binding to the negatively charged groups of Asp85 and Asp212 via an electrostatic interaction. However, only using electrostatic force is not enough to explain the role of the metal cations because the carboxylate of Asp85 is well known to be protonated in the M intermediate. Considering the presence of some aromatic amino acid residues in the vicinity of the retinal pocket, the existence of cation-π interactions between the metal cation and aromatic amino acid residues is suggested. Obviously, introduction of this kind of interaction is conducive to understanding the effects of the metal cations and aromatic amino acid residues inside the protein on the structural stability and proton pumping of bacteriorhodopsin.

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Cations bind to the π face of an aromatic structure, such as benzene, phenylanine (Phe), tyrosine (Tyr) and tryptophan (Trp), through a noncovalent force termed the cation-π interaction. This interaction has been established on the basis of the studies of organic model systems and biological structure, and some representative examples that illustrate this phenomenon were recently reviewed by Dougherty.[1] Several features distinguish the cation-π interaction from other noncovalent binding forces (hydyogen bonding, hydrophobic interaction, van der Waals force and electrostatic interaction) and make it especially well suited to novel types of biological binding. A great deal of direct evidence indicates that cation-π interactions are important in a variety of proteins that bind cation ligands or substrates.[1] However, until now, there has been no direct evidence to show that cation-π interactions exist in bacteriorhodopsin (bR) in which those usual noncovalent forces are demonstrated to be of importance to maintaining its structural stability and fulfilling its proton pumping function.

Bacteriorhodopsin is a protein-chromophore complex that serves as a light-driven proton pump in the purple membrane of Halobacterium salinarium.[2] At a high salt concentration and low oxygen tension, the organism grows a purple membrane, which contains the protein named bacteriorhodopsin. After absorption of a photon, the protein undergoes a photocycle which pumps protons across the membrane from the inside of the cell to the outer medium. The resulting proton gradient generates a proton-motive force that is used by Salinarium to synthesize ATP from inorganic phosphate and ADP.[3]

The molecular mechanism of the proton pump of bR is an important subject for understanding the relationship between a biological structure and its functions. A great number of studies on bR show that its proton pumping is perfectly guaranteed due to various kinds of interactions, which include chemical bond (the Schiff base between retinal and Lys 216), ion pair interaction,[4] hydrophobic effect,[5] hydrogen bonding[6] and van der Waals forces.[7] Although the above covalent and noncovalent intermolecular forces in bR have been extensively studied and discussed, but the functioning of bR as a proton pump requires the presence of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions which bind at specific locations inside the protein and on the surface of the protein. On the one hand, the removal of these cations produces the blue membrane ($\lambda_{max} \approx 603$ nm), bacteriorhodopsin which lacks a photocycle and does not pump protons. On the other hand, the spectroscopic and photochemical properties of the protein can be restored by adding either Ca^{2+} or Mg^{2+} to an aqueous solution of the blue membrane.[8] Thus it can be seen that the metal cations in bR are essential to ensure the proton pumping function of the protein.

In an effort to understand the role of metal cations in the bR function, several research groups determined the binding characteristics of these metal cations and the location of their sites. However, different binding sites and binding numbers of these cations have been reported by different research groups up to now.[9] Jonas and Ebrey observed the correlation between colour changes and one bound Ca^{2+}, which suggested that this Ca^{2+} might be located in the retinal pocket with a specific binding involving Asp85, Asp212, Tyr57, Tyr185, Arg82 and protonated Schiff base.[10] Titration studies with a Ca^{2+}-specific electrode led to a proposal that the high-affinity binding sites are stabilized by the negative charges of Asp85 and Asp212.[8,11] Furthermore, the studies of Birge *et*

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al.[12] indicated that the hydrated metal cation occupies a dynamic site involving two aspartic residues (Asp85 and Asp212) and two tyrosine residues (Tyr57 and Tyr185). Therefore, based on the above experimental results, the location of the metal cation binding sites in bR is gradually revealed, e.g. the high affinity binding site of metal cation is located in the vicinity of the retinal pocket. Consequentially, what does a specific binding involve between Asp85, Asp212, Tyr57, Tyr185, Arg82, protonated Schiff base and the metal cations mentioned by Jonas and Ebrey?[10]

This question could first be answered through the work by Tan *et al.*[13] They added a series of organic cations replacement of the metal cations to the cation-depleted blue membrane and observed that these cations regenerate the purple colour and restore the photocycle and proton pumping ability of bR. Their MNDO/PM3 molecular orbital theory and semiclassical MM2 force field calculations suggested that those large organic cations can replace the metal cations to stabilize Asp212 and Asp85 anion by electrostatic interactions during the O-to-bR reaction.

Recent extended x-ray absorption fine structure (EXAFS) studies provided evidence for an octahedral coordination for the high-affinity cation-binding site with six oxygen atoms.[14,15] More recently, the magnetic susceptibility technique provided an independent means of corroborated EXAFS results. A possible structure of the strong binding site was proposed and its feasibility was tested by quantum mechanical calculations. In this model, an Mg^{2+} cation interacts with one oxygen atom of the side chain of Asp85, with both oxygen atoms of Asp212 and with three water molecules. One of these water molecules is hydrogen bonded to both the nitrogen of the protonated Schiff base and the Asp85 oxygen. It could serve as a shuttle for the Schiff base proton to move to Asp85 in the L–M transition.[16] The partial structure of the model was corroborated by recent high-resolution diffraction analysis although the location of the cation-binding site was not yet observed directly.[17]

Indeed, the most possible cation-binding structures in bR are the side chains of aspartic acids (Asp212 and Asp85) which contain carboxylic acids that are documented to ionize to anionic carboxylates during the O-to-bR reaction. However, it is obvious that the carboxylic acid of Asp-85 will not be ionized during the formation of the M intermediate.[18] Here, bacteriorhodopsin might need a different kind of cation-binding site, a "hydrophobic anion" for which the obvious candidates are the side chains of Tyr57, Trp86 and Tyr185, to stabilize the structure of bR. In other words, cation-π interactions exist between Ca^{2+} or Mg^{2+} and aromatic amino acid residues in the retinal pocket of bR besides the electrostatic interactions between the metal cation and anionic carboxylates of aspartic acids.

The cation-π interaction can be considered not only as an electrostatic effect that involves the quadrupole moment of the aromatic residues, but also as a hydrophobic effect because the aromatic acids are composed of hydrocarbon units.[1] This combination of properties is especially well suited to the interior of bR and the purple membrane, in which cation binding by conventional ion pairing may not be feasible. In addition, Tan's calculations indicated clearly the existence of Van del Waals contacts between the cation and the aromatic side chains of Tyr and Trp. Therefore, the cation-π interactions between the metal cations (Ca^{2+} or Mg^{2+}) and Trp86, Tyr57, and Tyr185 should exist at least in the retinal pocket according to their calculations.

Subsequently, what effects will be brought about if the cation-π interaction in bR is introduced? Firstly, the role of metal cations in bR can be easily understood. The electrostatic forces between the metal cations and anionic carboxylates of aspartic acids, as well as the cation-π interactions between the metal cations and aromatic amino acid residues, are crucial to maintain the structure stability and the proton pumping function of bR. Obviously, these interactions will disappear in the blue membrane in which Ca^{2+} and Mg^{2+} ions are removed. Secondly, the cation-π interactions will be considered when one carries out molecular dynamics calculations of bR. Thirdly, the roles of aromatic amino acid residues in bR, especially Trp86, Tyr57 and Tyr185, will be reconsidered. Previous studies indicated that single substitutions of Tyr by Phe did not affect folding, retinal binding, and proton pumping of bR.[19] Because Phe, like Tyr, also interacts with the metal cation by the cation-π interaction, it could not completely exclude the effect of Tyr on the proton translocation process of bR. However, Y57D, unlike the substitutions of Tyr by Phe, lacks the cation-π interaction and thus blocks the M intermediate and Schiff base deprotonation, although an altered photocycle and a proton transport do occur.[20] An analogous phenomenon was observed in the case of the Y185F. The Y185F produces an O-like species. In contrast to the purple species, the red-shifted species has a photocycle involving a red-shifted K intermediate and a second long-lived intermediate possibly similar to N.[21] However, neither its protonated Schiff base undergoes deprotonation nor its tryptophan fluorescence undergoes quenching while Asp-85 is protonated.[22] On the one hand, this means that previous measurements[19] of Y185F did not differ the O-like species from the purple species. On the other hand, the mutation of Y185F may weaken the cation-π interaction with the metal cation. This is in agreement with the order of this kind of interaction, Trp>Tyr>Phe.[1] The third case is the Eu^{3+}-regenerated bR. An emission decay measurement of bound Eu^{3+} indicated that one Eu^{3+} binding site is

near the retinal and its luminescence is completely quenched.[23] It is interesting to note that the difference spectrum of bR fluorescence upon Eu^{3+} binding exhibited a minimum at 304 nm in addition to an expected maximum at 330 nm.[24] Both experiments suggest that Eu^{+} and one aromatic amino acid, e.g. Trp86, in the vicinity of the Schiff base would be close enough so that there is an appreciable overlap between their orbits. In other words, Eu^{3+} could bind to the π face of the aromatic amino acid.

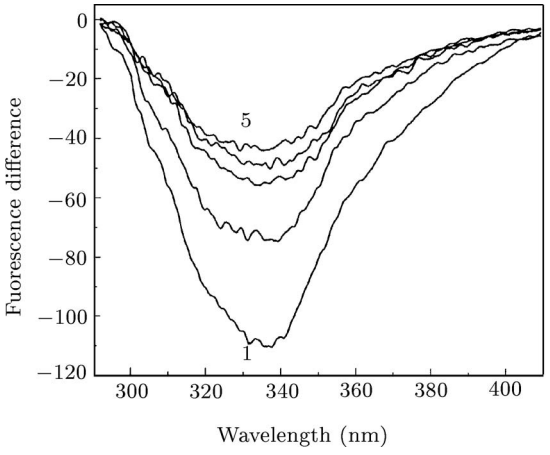

Fig. 1. Fluorescence difference spectra of the deionized blue membrane upon addition of alkaline earth metal cations Mg^{2+} (2), Ca^{2+} (3), Sr^{2+} (4), Ba^{2+} (5). The first difference spectrum (1) is between the native purple membrane and the deionized membrane.

Although the refined x-ray diffraction structure of bR has failed to determine the location of the metal cation-binding sites until now, a great deal of evidence shows that the metal cation exists in the vicinity of the retinal pocket of bR. Therefore, it is reasonable to suggest that the cation-π interactions between aromatic amino acid residues and Ca^{2+} or Mg^{2+} are among many noncovalent forces that contribute to the structure and function of bR. Of course, it must be determined by the experimental test whether the cation-π interactions exist in bR. When alkaline earth metal cations are added to the deionized blue membrane suspension, the quenched tryptophan emission intensity exhibits a 1/R dependence, where R is the ionic radius of the corresponding metal ion (Fig. 1). This feature very closely resembles a cation-π interaction. Therefore, the cation-π interaction may exist in bR.

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