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Review Article

Mechanism of the light-driven proton pump of bacteriorhodopsin based on the consistency principle

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According to the consistency principle, a design principle for protein tertiary structures, all interactions that maintain a protein's structure are consistent with each other. We assume that proteins satisfy the consistency principle. The specific local structures that form are consequences of the consistency principle. The specific local structures and the global conformation become interdependent. We assume that protein function is a consequence of the interdependency and the breaking of consistency. We applied this idea to the light-driven proton-pump mechanism of bacteriorhodopsin. Bacteriorhodopsin has two distinct conformers: one in which the proton channel opens toward the extracellular side, and another in which the channel opens toward the cytoplasmic side. Important reactions involved in proton pumping are protonation of D85 from the retinal Schiff base and reprotonation of the Schiff base from D96. To recruit a key water molecule, a characteristic pentameric hydrogen bond network is formed around the D85 and Schiff base, but is lost during proton pumping. These reaction components can be explained by active consistency-breaking and processes that either establish new consistency or restore the original consistency. Thus, the consistency principle can be expanded from structure to guide our understanding of protein function. This hypothesis is applicable to other functional proteins with two distinct conformers.

Key words: two distinct conformations, conformation change, pentameric hydrogen bond network, consistency-breaking

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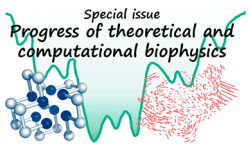
The consistency principle states that all of the interactions required for the maintenance of a protein's native conformation are consistent with each other [1]. According to this principle, only native interactions are required for protein folding and stability. Perfect consistency means that all interactions are independent. One example of consistency can be found between short-range and long-range interactions [1]. Interactions between atoms just a few residues apart along the peptide chain are defined as short-range, whereas those between atoms that are far apart in the primary sequence but near one another in space are defined as long-range [1]. Short-range interactions mainly contribute to secondary structure, whereas long-range interactions are important for global conformation.

The consistency principle effectively describes an important property of proteins: the unique stable tertiary structure of each protein is defined by its amino acid sequence. It has

◀ ***Significance*** ▶

In this paper, we expand on the consistency principle, originally proposed to explain protein structure formation, to explain the light-driven proton pumping mechanism of bacteriorhodopsin. We propose that consistency-breaking is a trigger for protein function, and that the rearrangement of intramolecular interactions to establish new consistency or restore the original consistency provides the driving force for function. This hypothesis is applicable to other functional proteins that have two distinct conformers. Thus, the consistency principle provides general principles for understanding complex systems.



become clear, however, that many proteins do not assume unique tertiary conformations, but instead have fluctuating conformations; such proteins are called intrinsically disordered protein (IDP) [2,3]. Many IDPs assume defined structures when they form complexes with their partner proteins; this is referred to as coupled folding and binding [2,3]. The resultant structures may satisfy the consistency principle. We assume that IDPs lack some of the native interactions that are required for folding [4]; hence, we will not discuss this class of proteins further in this manuscript.

Another important property of proteins is that each protein has a specific function. Go [5] stressed that spatiotemporally ultrahigh-resolution structures are required in order to describe the molecular mechanisms underlying a protein's specific functions, and argued that computer simulations are the best way to obtain such structures. Because protein function can be described as a sequence of chemical reactions, ultrahigh-resolution structures of all functional steps will lead to a detailed understanding of the overall mechanism [6–8]. Using this approach, however, we can only say that one protein works one way, and that another protein works another way. Is it impossible to derive general design principles of protein function? Go raised an important point: When consistency is partially unsatisfied, it may be constructive to ask why it is imperfect [9]. We propose that the answer to this question has to do with the fact that each protein has a specific function. The purpose of this article is to interpret the mechanism of light-driven proton pump of bacteriorhodopsin (bR) with the consistency principle.

Incomplete consistency and protein function

In real proteins, consistency often seems imperfect. We often observe that some intramolecular interactions in proteins are cooperative or coupled. The consistency principle dictates that all types of intramolecular interactions are independent of each other. Therefore, the concepts of cooperative or coupled are never the consequences of the consistency principle. The fact that alanine scanning is generally successfully in studies of folding mechanisms and stability suggests that the consistency principle seems to be satisfied to some extent in real proteins. However, a few residues are sensitive to alanine substitution, and their replacement causes the protein to lose foldability. These key residues must be responsible for the cooperative or coupled interactions involved in folding. In fact, Dobson stated that folding is determined by such key residues [10]. The residues sensitive to alanine substitution are likely to be responsible for many different types of intramolecular interactions simultaneously, including both short-range and long-range interactions. Thus, interdependency is established in the final structure, which satisfies the consistency principle. We assume that interdependency can be understood as a consequence of the consistency principle.

One example of interdependency is the case of β-lactoglobulin (βLG). Although βLG is composed of β-strands, secondary structure prediction of this protein also reveals some helical regions, and indeed, non-native α-helices form during folding of βLG [11]. On this issue, Go commented, "the degree of imperfection is small, because such segments assume either structure with very similar propensity" [9]. Because Go [1] used a simple but unique lattice model for proteins, these interactions can be reduced to inter-residual interactions. The contents of long-range interactions are mainly atomic packing, as well as hydrophobic interactions. By contrast, the contents of short-range interactions influence the secondary structure of each residue. On the other hand, the secondary structure propensity is an empirical parameter derived from the solved structures that is affected by the microenvironment of the position. In such a case, global conformation and the formation of secondary structure elements are rather interdependent. In this context, we consider that secondary structure propensity is not an ideal parameter. Another important determinant of secondary structure is hydrogen-bonding between main chain CO and NH. According to Go's definition, hydrogen bonds in an α-helix are short-range and periodic, whereas those in a β-sheet encompass both short-range and long-range interactions. Incorporation of these different properties into one term, the propensity, may be responsible for the apparent discrepancy with the consistency principle. Even with this discrepancy, however, we assume that the principle holds for the native conformation of βLG.

Many functional proteins have two distinct conformations. Typical examples are allosteric transition of allosteric proteins such as the T and R states of hemoglobin, and the open and closed forms of various receptor proteins. The existence of such distinct conformers is closely related to the specific function of a protein, e.g., the deoxidized and oxidized forms of hemoglobin and the unliganded and liganded states of receptors. We consider both states to satisfy the consistency principle. Ligand binding affects the interactions around the active site, which leads to consistency-breaking. Rearrangements of interactions should satisfy the consistency principle, resulting in a conformational change to the other stable conformer. The conformational transition may be accompanied by further changes in interactions that are aspects of the chemical reactions involved in specific functions. Therefore, the consistency principle, along with consistency-breaking upon ligand binding, provides the driving force for conformation change and function. We will apply this idea to the proton-pump mechanism of bR.

Bacteriorhodopsin

BR is a light-driven proton pump found in *Halobacterium salinarum* [12] that functions as a single molecule. bR assembles in a two-dimensional hexagonal lattice to form purple membrane regions in the cell membrane of the bacterium [12]. Figure 1 shows the structure of bR and the proton pump

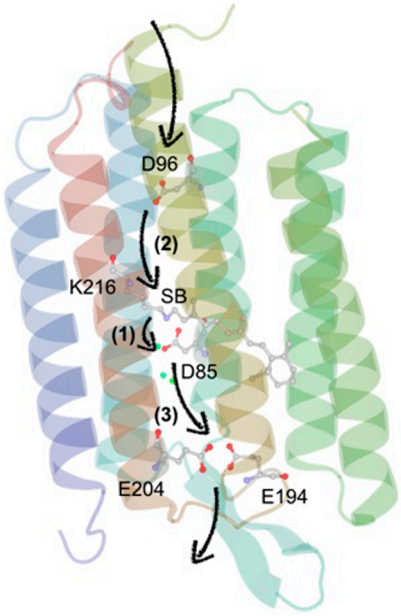


Figure 1 Molecular structure of bacteriorhodopsin and proton-pump pathway.

pathway. bR is composed of seven α-helical segments and a chromophore, all-*trans* retinal, which is covalently bound to K216 via a protonated Schiff base (SB). Upon light absorption, bR undergoes a photocycle with several distinct photointermediates (J, K, L, M, N, and O) that is initiated by isomerization of retinal all-*trans* to 13-*cis* retinal, and this cycle is associated with proton pumping. The intermediates can be characterized spectroscopically [13,14]. The proton pathway was identified by the sophisticated Fourier Transform Infrared spectroscopy (FTIR) and X-ray crystallographic studies (Fig. 1) [7,8,13–18]. The essential components are proton transfers 1) from SB to D85 (L-to-M transition), 2) from D96 to SB (M-to-N transition), and 3) from D85 to E204 and/or E194 (N-to-O transition). During the final stage of the photocycle, D96 is protonated from the aqueous cytoplasmic milieu. These local proton transfers are accompanied by a global conformational change that moves the site of channel opening from the extracellular to the cytoplasmic side [15]. Hereafter, we refer to these conformations as the E-conformation and C-conformation, respectively. Although a recent femtosecond crystallography study by X-ray free electron laser (XFEL) revealed no substantial global conformational changes [7], a large change can be visualized by high-speed AFM [19].

The high-resolution crystal structure of bR reveals the characteristic pentameric hydrogen bond network around SB (Fig. 2) [20,21]. In the pentameric structure, three water molecules (Wat401, Wat402, and Wat406) are recruited by hydrogen bonds. Wat402, which is crucial for the proton-pump mechanism, is hydrogen-bonded to SB, D85, and D212 [20,21]. Wat401 is hydrogen-bonded to D85 and

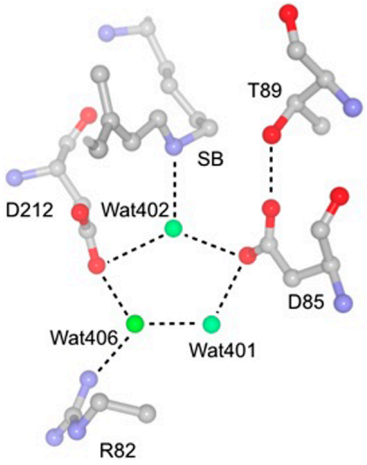


Figure 2 Pentameric hydrogen-bond network at the active site [19].

Wat406, and Wat406 is hydrogen-bonded to D212 and R82. The pentameric structure is thought to determine the properties of microbial rhodopsins [13,14]. During the photocycle, Wat402 is displaced and ultimately disappears, eliminating the pentameric structure.

Femtosecond time-resolved X-ray crystallography by XFEL beautifully revealed the reaction process of retinal isomerization and proton transfer from SB to D85 [7,8]. The protonated SB is directed toward D85, which is directed to the opposite side by isomerization. Hydrogen bonding to D85 via Wat402 is broken by isomerization, which causes the displacement of Wat402 [7]. The new water molecule (Wat452) is recruited to form a hydrogen bond with SB [7]. Wat452 is then hydrogen-bonded to D85 via T89 [7]. During the rearrangement of this hydrogen bond network, D85 is protonated from SB. Unfortunately, current XFEL technology cannot observe hydrogen atoms directly, and arguments persist regarding the protonation mechanism of D85 [7,13,14].

Recent advances in sophisticated experimental techniques have enabled spatio-temporally ultrahigh-resolution structural analyses of bR, which in turn have revealed each step of the proton pump mechanism. However, the previous model of early 2000s based on low resolution structure is not far from today's understanding. We proposed a conformation-controlled conformation change model for the proton pumping mechanism of bR (Fig. 3) [22]. Our model is based on the experimental observations that protonation of D85 and deprotonation of SB brings about the E-to-C conformation change [23], and that the C-conformation affects the pK_a of D96, thereby promoting proton transfer to SB [24]. We also revealed that the local electrostatic interactions determine the global conformation [25]. Therefore, reprotonation of SB causes the C-to-E conformation change [22]. Finally, the E-conformation affects the environment of D85 to be deprotonated. We realized that our model can be reasonably interpreted as a rational extension of the consistency principle.

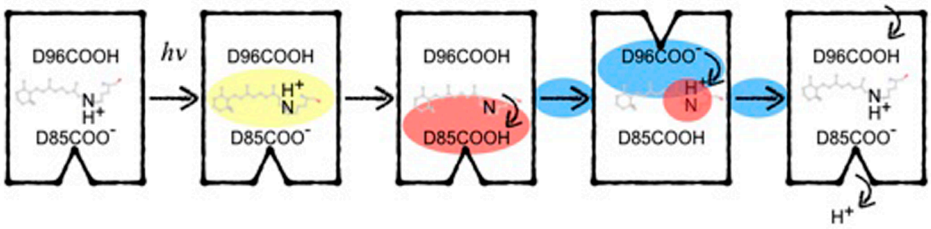


Figure 3 Light-driven proton-pump mechanism based on the consistency principle. Characteristic structures without pentameric hydrogen bond network are depicted schematically. Channel opening direction is depicted by a cleft. Yellow represents the reaction triggered by light. Red indicates the active consistency-breaking site. Changes that satisfy the consistency principle are indicated in blue.

Mechanism of light-driven proton pumping based on the consistency principle

According to the consistency principle, all interactions involved in maintaining stable protein tertiary structure are consistent each other. We believe that the dark-state bR structure, the E-conformation, satisfies the consistency principle. The two-stage folding model has been proposed for the folding of bR: a first stage in which the seven transmembrane α-helices form, and a second stage involving the assembly and packing of these helices [26]. This model is an expression of the consistency principle for membrane protein folding. If the consistency were perfect, D96 would be deprotonated because of the intrinsic pK_a of glutamic acid. From this standpoint, it seems that the consistency principle is broken in bR. However, let us assume that the protonation of D96 is a consequence of the consistency principle. In the E-conformation, all of the consistent interactions determine the local environment of D96, which is highly hydrophobic; consequently, D96 is protonated in order to occupy the local energy minimum. We also consider that the pentameric structure around the active site including three water molecules (Fig. 2) [20,21] is the consequence of the consistency principle. During folding, all of the interactions may work independently. However, once the final stable conformation is established, the global conformation and local structure must be quite interdependent, because it is impossible to change short-range interactions without simultaneously altering long-range interactions. Therefore, it is rational to consider that the local structures of bR, such as the isomerization state of retinal, the protonation states of D85 and D96, and the pentameric structure around the active site, are strongly coupled with global conformation. Local structures unique to the E-conformation are all-*trans* retinal, protonated SB, deprotonated D85, protonated D96, and the stable pentameric hydrogen-bond network. Upon light absorption, bR can assume another metastable conformation, the C-conformation. The local structures coupled to the C-conformation are 13-*cis* retinal, deprotonated SB, protonated D85, and deprotonated D96, which are consequences of the consistency principle; the pentameric hydrogen-bond network around the active site is lost [7]. The local structures coupled to the global conformation are summarized in Table 1. Due to the consistency principle, we assume that the change in either one of the local structures or the global conformation into another structure induces changes in other local structures, as well as the global conformation. In the real photocycle of bR, photoisomerization of retinal and proton transfer from SB to D85 involve a series of reactions, as demonstrated by XFEL studies [7]. In order to maintain the consistency principle, this process would be a relaxation of local consistency breaking and it appears quasistatic. Ultimately, however, local consistency is broken to transfer a proton from SB to D85. This change induces the E-to-C conformation change in order to satisfy the consistency principle. The deprotonation of D96 is coupled with the conformation change. If the consistency is ideal, the C-conformation should be stable.

However, the proton released from D96 is transferred to SB, which is consistency-breaking for the C-conformation and preferable for the E-conformation. Consequently, the C-to-E change occurs in order to satisfy the consistency principle, which is coupled with isomerization of retinal, protonation of D96 from the cytoplasmic side (i.e., proton

Table 1 Features of the global conformation of bacteriorhodopsin in each of two postulated stable local structures

Global Conformation	E-Conformation	C-Conformation
Retinal	all-*trans*	13-*cis*
Schiff base	CNH^+ (protonated)	CN (deprotonated)
D85	COO^- (deprotonated)	COOH (protonated)
D96	COOH (protonated)	COO^- (deprotonated)
Pentameric structure around the active site	present	absent

uptake), and deprotonation of D85. The proton dissociated from D85 is released to the extracellular side through proton-releasing groups (i.e., proton release). In summary, light activation brings about consistency-breaking, i.e., the deprotonation of SB, which triggers the photocycle; it is the process of the photocycle itself that satisfies the consistency principle. At the N intermediate, another consistency-breaking, reprotonation of SB, occurs to restore the ground state, again due to the consistency principle.

Perspective

We tried to describe the molecular mechanism of light-driven proton pumping by bR, based on the consistency principle. The essential feature of this description is that both the consistency principle and active consistency-breaking provide the driving force for protein function. In order to predict the reaction pathway, we need to have detailed structural information about the two conformers. The consistency principle provides useful guidelines for the computer simulations of the proton-pump mechanism. Protein function should be a consequence of rearrangement of intramolecular interactions and the formation of intermolecular interactions, and these rearrangements should satisfy the consistency principle. This hypothesis is applicable to other functional proteins that have two distinct conformers.

We believe that the consistency principle sheds light to the hierarchical nature of protein structure. The consistency principle is equivalent to the claim that the different types of interactions are confined within individual levels of the hierarchy, respectively. However, in real proteins, there must be interactions across hierarchies. Such cross-hierarchical interactions may affect the interactions confined within each level of the hierarchy. When cross-interactions become predominant over confined interactions, the connected hierarchies become interdependent. Protein function is thus the consequence of rearrangements of intramolecular interactions and formation of intermolecular interactions, and the formation of interactions that connect different hierarchies is required for the function. In this sense, consistency-breaking is mainly brought about by interactions between different levels of the hierarchy. Protein function is triggered by an active breaking of consistency, which creates new interactions across levels. The consistency principle predicts that the rearrangement of interactions within each level, as well as across levels, occurs either to establish new consistency or to restore the original consistency.

Hierarchical nature is a characteristic property of not only protein structures, but also complex systems. An understanding of interactions across levels of hierarchy is a key to understanding complex systems. Thus, the consistency principle represents an important guideline for the understanding of complex systems.

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Conflict of interest

Both M. K. and H. K. declare that they have no conflict of interest.

Author contributions

The basic idea for this paper came from daily discussion between M. K. and H. K. M. K. and H. K. cowrote the manuscript.

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