Proton-pump mechanism in retinal Schiff base: On the molecular structure of the M-state

Ayan Datta, Swapan K. Pati *

Theoretical Sciences Unit and Chemistry and Physics of Materials Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur PO, Bangalore 560064, India

Abstract

Theoretical characterizations of the various intermediates in the proton-pump cycle of the retinal Schiff base in the *Halobacterium salinarium* have been performed. Contrary to the general belief over the years that the most stable intermediate, the M-state, is a non-protonated *cis*-isomer, we find that the M-state is a polarized *cis*-isomer stabilized due to interactions of the dissociating proton with the π-electrons. The role of proton in the pump cycle is found to be profound leading to the stabilization or in certain cases destabilization of the intermediates. We propose the chemical structure of the M-state for the first time.
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Bacteriorhodopsin (bR), a transmembrane photosynthetic protein, is found in the purple membrane of *Halobacterium salinarium* [1,2]. On being excited by visible light, a proton-pump is triggered and the protein undergoes a cycle of events in which a proton is transferred from the cytoplasmic side to the extracellular side of the membrane, thereby creating a proton gradient across the membrane. Crystal structure reveals that this protein contains seven transmembrane helices embedding the light absorbing chromophore, an all-*trans* retinal, covalently attached to Lys-216 by a protonated Schiff base linkage [3]. The important steps in the proton-pump in bR involve an all-*trans* to 13-*cis* isomerization in the chromophore upon the excitation by light. In the next few steps, the proton is dissociated from this *cis*-isomer producing a stable intermediate, called M-state. After the release of the proton from the M-state, there is a conformational change in the chromophore involving a rotation along a single bond to produce the late M-state. This late M-state then accepts a proton from extracellular side and transforms to the ground state or the initial all-*trans* configuration, thereby completing the cycle.

However, a proper understanding of the processes in the membrane proteins remains elusive because of their difficult crystallization conditions [4]. Even the recent structural resolution of 1.5 Å is not sufficient to clearly show the complete atomistic picture of the various intermediates and particularly the position of the light atoms like hydrogen [5,6]. Most of the theoretical efforts till now have taken as input the structures at various stages of the pump from these poor resolution X-ray patterns for further quantum mechanical or molecular dynamics calculations [7–9]. A full ab-initio calculation for the whole protein is not quite practical. As a result, there is no clear understanding of the essential factors that govern the proton-pump cycle.

In this paper, we are able to show that a clear under-standing of the whole pump cycle can be derived through a single universal parameter: the N· · ·H distance. We find that the electrostatic interaction of the dissociating proton from this bond has a very important role in the generation of various intermediates in the proton-pump cycle and can cover the essence of the otherwise very complicated phenomenon in this biological membrane system. In particular, we explain the reason for the initial isomerization process of the ground state conformation in bR since generally it is known that the light induces a photoexcitation. The reason for the surprising fact that the *cis*-isomer is stable

* Corresponding author. Tel.: +91 80 22082829; fax: +91 80 22082767.
  E-mail address: pati@jncasr.ac.in (S.K. Pati).
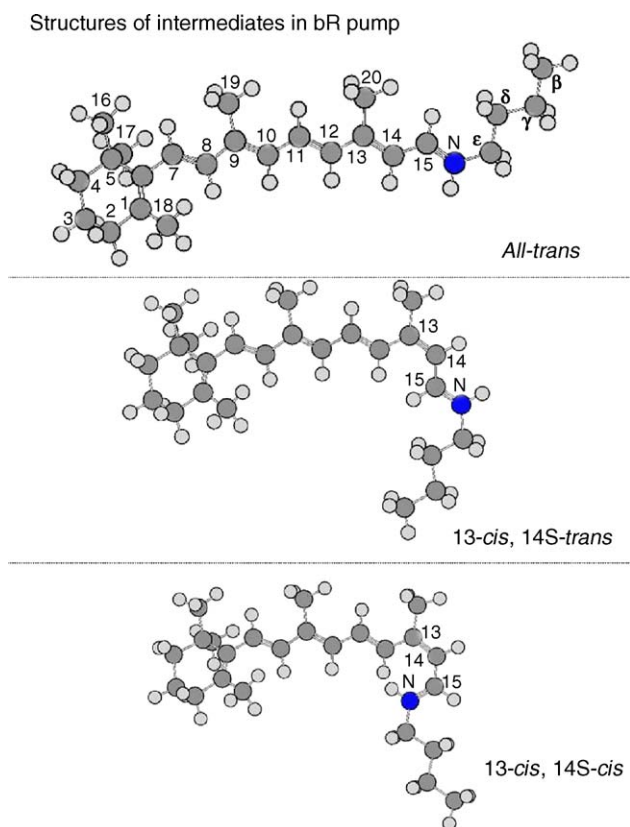
Structures of intermediates in bR pump

Fig. 1. Structures of the intermediates in bR. Note that the proton release from the N-atom in the 13-*cis*, 14S-*cis* occurs in the M-state.
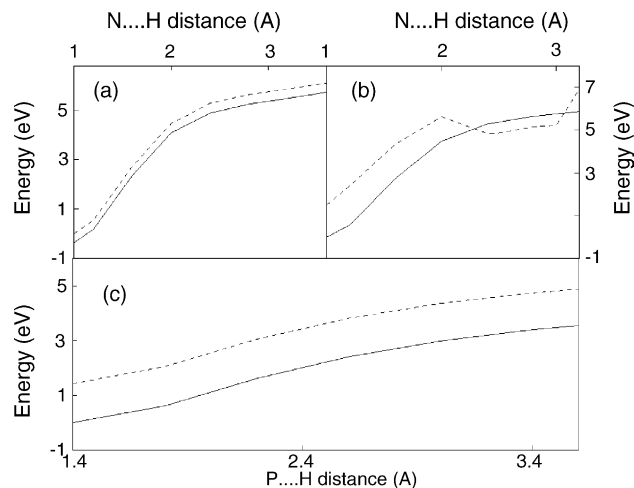

Fig. 2. Energy profiles as a function of proton distance for all-*trans* (solid line) and (a) 13-*cis*, 14S-*trans* (dashed line), (b) 13-*cis*, 14S-*cis* (dashed line), (c) all-*trans* (solid line) and 13-*cis*, 14S-*trans* (dashed line) isomers of P-ylide. Energies are scaled as $E = E + 23784.5\,eV$ for (a) and (b) and as $E = E + 31601.6\,eV$ for (c).

over the *trans* for the M-state, while in most cases a *trans*-isomer is generally found to be energetically stable, is explained. We sequentially explain the reasons behind each event as the Schiff bases pass through the pump cycle.

We optimize the structures for the chromophores by substituting the Lys-216 residue by a butyl group. We have used the 6-31G(d, p) basis set and electron correlation was accounted by the Becke's three parameter hybrid method and the Lee–Yang–Parr correlation functional (B3LYP) [10]. Although our results are with butyl, we have verified that substituting butyl by lysine would give the same results with a very little quantitative difference. These geometries were used to compute the energies at various N···H distances.

In Fig. 1, the structures of various intermediates in bR cycle are shown. The first step in the process involves all-*trans* to 13-*cis*, 14S-*trans* isomerization with a π-twist along the C(13)−C(14) bond which is carried out by the absorption of photon energy. We find that the lowest optically allowed state is almost 2.3 eV above the all-*trans* ground state. This is calculated as the vertical absorption gap from the optimized ground state geometry of the all-*trans* isomer. A conformational isomerization (from optimized all-*trans* to π-twisted 13-*cis*, 14S-*trans* isomer); however, requires only 0.17 eV. In room temperature, although there will be a Boltzmann distribution of both the isomerized and photoexcited states, the number of bR molecules undergoing isomerization is quite large compared to the photoexcited state of the all-*trans* isomer. It is interesting to note

that the energy required for this isomerization process in the protonated case is 0.17 eV while it is 0.25 eV for the deprotonated case. This is because to twist the double bond for the protonated case requires less energy as the formal +ve charge on the N drags the electrons and in the process the double bond attains a resonating single bond character. Starting from the 13-*cis*, 14S-*trans* isomer, we mimic the proton release process by elongating the N−H bond until the proton can be assumed to be free. At every N···H distance, we examine the change in energy due to the 'pulling' of proton from the N. The same profile is also examined for the all-*trans* isomer. We compare these two profiles in Fig. 2(a). The all-*trans* isomer is always stable over the 13-*cis*, 14S-*trans* form, although the energy difference is quite small at all distances. Thus, the dissociating proton does not stabilize the 13-*cis*, 14S-*trans* state.

It has been suggested by Schulten et al. that after the twist along the C(13)−C(14) double bond, the bR under goes a 14S-*trans* to 14S-*cis* twist along a single bond to reach the 13-*cis*, 14S-*cis* (M-state) [11]. However, we find that the energy difference between these two structures is 1.3 eV. Therefore, it appears surprising that such a step would occur, since it amounts to introducing steric repulsion in the system. To understand the reason behind the stability of the M-state and the actual conformation of the M-state, we compare the stability of this 13-*cis*, 14S-*cis* isomer with respect to the all-*trans* isomer for every N···H distance. The 'pulling' of proton for the 13-*cis*, 14S-*cis* case has been carried out by computing the lowest energy conformation at every N···H distance. We find that upto the N···H distance of 2 Å and after 3 Å, the most stable conformation is when the proton is out-of-plane of the molecule. In the intermediate distances, the stable conformation leads to proton being in the molecular plane [12]. Fig. 2(b) describes the profiles for the all-*trans* and the minimum energy 13-*cis*, 14S-*cis* form at every N···H distance. It is quite clear from the figure that the all-*trans* isomer is lower in energy than the 13-*cis*, 14S-*cis* isomer for the associated as well for the dissociated N···H distances. But,