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Molecular modelling study of the biological role of the retinal Schiff base in bacteriorhodopsin

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Different structural and conformational aspects of the retinal Schiff base and the effect of the protein environment on these properties have been investigated. During these studies, we have gained a vast experience about the chemistry and physics of the retinal analogues, about the inclusion of the effects of the protein environment into the calculations, and about the appropriate methodologies for treatment of such systems. On the basis of the results obtained from *ab initio* calculations and MD simulations, structural aspects of retinal Schiff base which influence the pK_a of the system are revealed and a new mechanisms by which the protein environment influences the structure and pK_a of the chromophore are proposed.

Ab initio methods have been used for the calculation of proton affinity of several Schiff base models with different lengths of the polyene backbone, and at different *cis*/*trans* isomerization states. The results confirm that the π-electrons are the most important part of the chromophore, and that the polyene is necessary for maintaining the pK_a of the Schiff base group in retinal at functional levels. Different isomerization states, however, did not demonstrate any significant effect on proton affinity. We have also studied the effect of methyl substitutions at different positions of the polyene chain on the structure, charge distribution and proton affinity of retinal Schiff base.

After examination of different computational methodologies, the isomerization barriers to the rotation around all conventional single and double bonds in the retinal Schiff base were calculated in a model including the same number of conjugated double bonds as retinal. This part provided the first isomerization barriers for all of the single and double bonds calculated in a realistic model of retinal Schiff base, and at a high level of theory. In complete agreement with the experimental findings, the C=N and the C_{13}=C_{14} bonds of the protonated species were found to have the lowest isomerization barriers among the double bonds. In the neutral species, however, high barriers to these rotations were predicted. Therefore, protonation is essential for any ground state isomerization of the double bonds in the chromophore. The opposite effects of the isomerization around single bonds or double bonds on the pK_a of retinal Schiff base was also shown. On the basis of the obtained results, we proposed, for the first time, that the protein environment may take advantage of these opposite effects to, at least partly, adjust the pK_a of the chromophore.

The difficulties involved in the extrapolation of the results obtained from gas phase calculations motivated us to set up a series of calculations including the environment as dielectric medium and to see how this might influence the shape of the potential energy surface and proton affinity of the studied molecules. The results showed that even slightly

modifying the dielectric response of the micro-environment can significantly influence the protonation state of the Schiff base group. Therefore, the protein environment can very efficiently adjust the pK_a of the chromophore by modifying local screening effects in the vicinity of the retinal Schiff base, and control the process of the proton transfer. Extension of these calculations to the complete set of models showed that representing the protein/solution environment by a continuum model, although improving the results, can only partly simulate the effects of the protein on the molecule, and that explicit consideration of the environment is necessary for a complete description of the system.

Then, we set up a series of computations, explicitly including the binding pocket of the retinal into the calculations. This was the first reported study, where some parts of the protein environment have been explicitly included in the *ab initio* calculations of retinal Schiff base. In agreement with proposed counter-ion(s) of the protonated retinal Schiff base in bacteriorhodopsin the results showed that only Asp_{85} and Asp_{212}, which are present in the form of negatively charged groups, have significant effects on the structure and electronic configuration of both unprotonated and protonated model Schiff bases.

After extensive exploration of the structure and electronic properties of retinal Schiff base, we examined our new proposed mechanism for the pK_a adjustment of the chromophore by protein, with studying the *in situ* structure of the chromophore. By setting up a series of molecular dynamics simulations with different sets of parameters and starting structures, the probability and the relevance of the existence of a twisted chromophore in bacteriorhodopsin were examined. Very interestingly, and in complete relevance to our proposed mechanisms, the largest deviations from a planar structure were observed only for the C_{13}=C_{14} and C_{15}=N_{16} double bonds in retinal Schiff base structure. The results of the simulations of different mutants showed that, the side chain of Trp_{86} has the largest impact on the planarity of retinal. The results suggested the importance of the bulky residue of Trp_{86} in the isomerization process, in both ground and excited states of the chromophore, and in fine-tuning of the pK_a of the retinal protonated Schiff base in bacteriorhodopsin. The obtained results confirmed our previous conclusions about the likelihood of a twisted chromophore inside the protein environment, and large coupling of steric and electrostatic interactions of the chromophore and the protein environment.