

RHODOPSIN - STRUCTURE, SPECTROSCOPY AND DYNAMICS OF A CHROMOPHORE IN A TRULY HETEROGENEOUS ENVIRONMENT

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We report *ab initio* calculations of the ground and several excited states of protonated 11-*cis*- and all-*trans*-retinal Schiff base, the chromophores of the visual pigment rhodopsin and its photoproduct, bathorhodopsin, respectively. A supramolecule in which a formate anion and water have been attached to the Schiff base is described as a first attempt to model part of the complex environment of the chromophore. For highly coiled geometries of the retinals CD spectral data have been calculated. The comparison with experimental data suggests that a revision of the presently accepted structures may be necessary.

1 Introduction

Protonated 11-*cis*-retinal Schiff base is the chromophore of rhodopsin, the visual pigment which is responsible for light/dark vision in the retina of the vertebrate eye (Fig. 1, left). As the first step in a series of events that finally leads to the perception of light in the brain, the molecule is isomerized by photons of suitable wave length to the all-*trans*-isomer (Fig. 1, right), a reaction which occurs with a very high quantum yield of 65%¹ and is complete within 200 femtoseconds.²

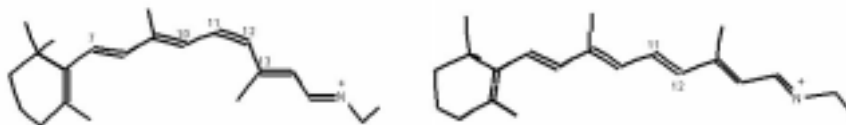


Figure 1. Configuration of protonated retinal Schiff base before (left) and after photochemical isomerization (right) of the C11-C12 double bond.

The stereospecificity of this photoreaction is remarkable: Of all double bonds in the molecule which can theoretically undergo *cis/trans* isomerization only the 11-12-bond is affected by the action of light. This, and the high efficiency of the isomerization reaction, can probably only be understood if proper account is taken of the effects of the environment on the structure and dynamics of the chromophore.

In rhodopsin the retinal chromophore is embedded in a pocket formed by seven so-called transmembrane helices which form the main part of the visual pigment. (Fig. 2). Chemically, rhodopsin is a protein, a macromolecule consisting of about 350 amino acids linearly connected into a molecular chain. While the primary structure of rhodopsin, i.e. the sequence of the constituent amino acids, is known for various animal species, knowledge about the three-dimensional structure of the protein, i.e. the atomic arrangement in space, is limited. Models of rhodopsin have been developed based on chemical experience and with reference to the structure of closely related proteins such as bacteriorhodopsin, a retinal-based proton pump of certain bacteria. In addition, experimental evidence, in particular spectroscopic studies and studies of mutant species, has increased our understanding how the interplay between the retinal molecule and its protein surrounding brings about the unique properties of the pigment.

Since the photoisomerization of the chromophore involves electronically excited states, electronic structure theory, that means quantum theory, has to be employed rather than force-field theory which describes the molecule as an ensemble of atoms interacting through mechanical forces. However, subjecting the whole molecule with thousands of heavy atoms to an *ab initio* treatment is out of the question for many years to come.

We have shown that the isolated chromophore can be calculated to any degree of accuracy using either Hartree-Fock or Density Functional Theory.³ By adding what will hopefully turn out to be functionally important pieces of the environment to the chromophore at a level that is computationally feasible now, and proceeding from there with increasing complexity we hope to arrive eventually at a sufficiently sophisticated model for the protein binding pocket of rhodopsin. In the following we present, as a first step in this direction, a minimal model of rhodopsin consisting of the chromophore, water and the carboxylate group of one amino acid. We will also discuss evidence for two highly folded chromophore models of rhodopsin and bathorhodopsin based on Circular Dichroism (CD) spectroscopy. The calculated CD spectra for these systems may require the revision of the presently accepted structures.

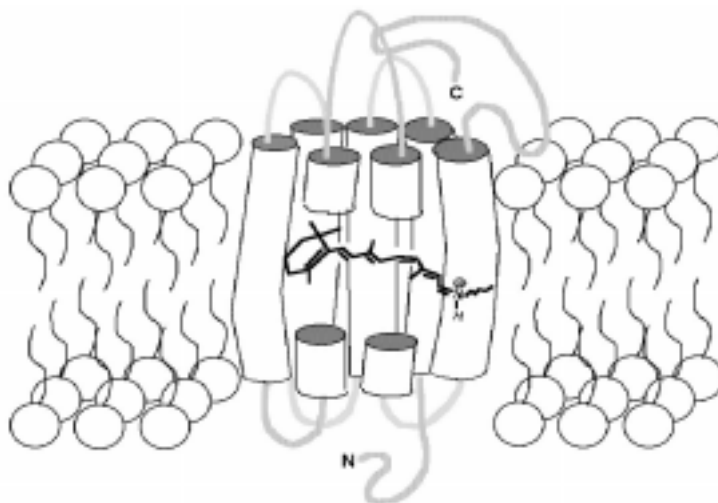


Figure 2. Cartoon of rhodopsin, with the retinal chromophore inside, embedded in a lipid bilayer membrane. The grey thread is the aminoacid chain, with the so-called N- (for nitrogen) and C- (for carbon) terminus. The chain runs through the whole protein molecule (in effect it *is* the molecule) and is coiled in seven regions into helical structures, the so-called transmembrane helices.

2 A minimal rhodopsin complex

According to density functional calculations employing a high quality atom centered basis set auf Gaussian functions (B3LYP/6-31G**) the protonated 11-*cis* retinal Schiff base chromophore is planar from C6 to N⁺³ (see Fig. 1 for the numbering of the chromophore). There is a sterically congested area, from C10 to C13, which is the most likely place for a non-planar deformation; however, the gain in steric strain when the molecule is twisted about the right bonds is obviously not enough to offset the loss in conjugation energy as a consequence of decreased π -overlap.

It is generally assumed that the retinal chromophore becomes twisted when bound to the protein and buried inside the binding pocket - a result of electronic and/or steric interactions. Apart from the most conspicuous observation which is the optical activity of rhodopsin and which will be discussed in section 3, evidence for distortion of the chromophore comes among others from solid state magic angle spinning NMR spectroscopy. By determining with this technique the distances between the methyl group at C13 and C10

and C11, respectively, it has been possible to arrive at a quantitative estimate, 44° , of the over-all twist between the two halves of the molecule.⁴ There is evidence that in rhodopsin one amino acid, Glu113, is the primary counter-ion of the Schiff base (Glu stands for glutamic acid; the index indicates that it is the 113th amino acid counting from the N-terminus). In addition ^{13}C chemical shift differences between the free and the protein bound chromophore suggest that one of the oxygens of the glutamate ion is close to C12. We have geometry-optimized a molecular complex consisting of protonated 11-*cis* retinal Schiff base, a water molecule and a negatively charged formate ion, HCOO^- , with density functional theory (B3LYP/6-311G**). The resulting geometry is shown in Fig. 3.

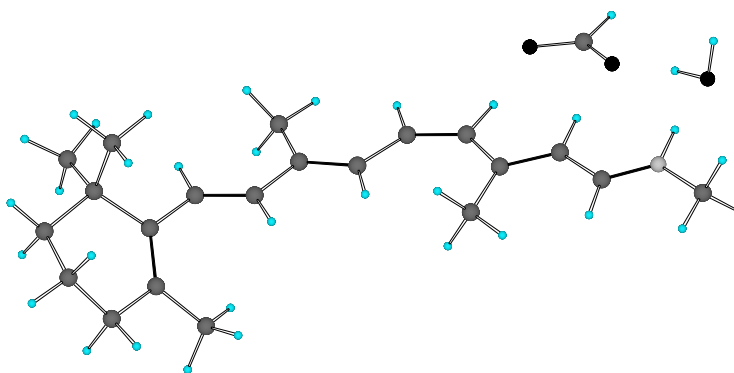


Figure 3. DFT-optimized geometry of the complex between protonated 11-*cis*-retinal Schiff base, formate ion and a water molecule. Oxygen atoms are in black.

We note close non-bonded interactions (distances 215.5 and 194.3 pm) between the formate oxygens and the hydrogens at C12 and C14, respectively. The position of the water is such that it can act as a relay for proton transport: Upon deprotonation of the Schiff base it accepts the hydrogen from N^+ and donates another one to the formate ion. To study the electronic effects of complexation, we take a closer look at the bond distances along the conjugated carbon chain of the chromophore as it changes from the free to the complexed state (Table 1). For comparison, the bond lengths of the unprotonated Schiff base are included also. Perusal of Table 1 reveals the following: Protonation of the Schiff base (last row to first row) decreases bond length alternation: All double bonds become larger, all single bonds shorter, a consequence of the

Table 1. Bond lengths along the conjugated carbon chains of 11-*cis*-retinal protonated Schiff base, its complex with water and formate ion and its deprotonated form (bond lengths in pm; first and last row entries calculated with B3LYP/6-31G**, the second row with B3LYP/6-311G**). (a) 11-*cis*-retinal, protonated SB, (b) 11-*cis*-retinal, protonated SB, complexed with water and formate, (c) unprotonated 11-*cis*-retinal SB

	C9=C10	C10-C11	C11=C12	C12-C13	C13=C14	C14-C15	C15-N
(a)	139.7	140.6	139.6	141.3	140.9	138.7	133.3
(b)	137.2	142.9	137.2	143.8	138.5	140.5	131.3
(c)	136.9	143.9	136.6	145.6	136.6	145.0	128.3

chromophore changing from a polyene-type systems, with strongly alternating double bonds, to a cyanine type system, with more equalized bond lengths. This is a well-documented fact: Protonation causes, among others, the large shift of the retinal Schiff base absorption maximum to longer wave lengths. Complexation of the protonated form with water and the formate gegenion (first row to second row) reverses this effect, though not complete. Throughout the carbon chain, bond lengths are between those of the protonated and the unprotonated Schiff base. Obviously the charge on the chromophore becomes localized due to the interaction with the negative anion and subsequent loss of conjugation.

Complexation at this level does not induce non-planarity into the chromophore, which is not surprising considering that both the formate ion and the water molecule position themselves in the plane of the chromophore.

The corresponding complex with the chromophore in the all-*trans* configuration is shown in Fig. 4. Similar to the 11-*cis* case just discussed, conjugation is decreased relative to the free chromophore, a fact which should be of significant consequences for the energies of the excited state. The stabilization of the all-*trans* isomer relative to 11-*cis* in the ground state increases upon complexation from 5.25 to 6.68 kcal mol⁻¹.

3 Structure and CD-spectroscopy

One of the most direct manifestations of the non-planar geometry of the protein-bound chromophore is the Circular Dichroism, or CD, observed in the absorption spectrum of rhodopsin: In a CD-spectrum the difference in absorptivity of an optically active medium for left and right circular polarized light, expressed as the so-called ellipticity θ , is plotted against the wave length. In the spectrum of rhodopsin, there is a positive CD-band with maximum at 500 nm and another stronger one at 320nm (Fig. 5). After irradiating

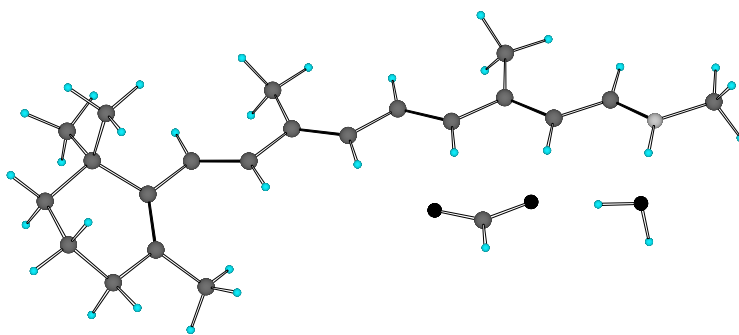


Figure 4. DFT- optimized geometry of the complex of protonated all-*trans* retinal Schiff base, formate ion and a water molecule.

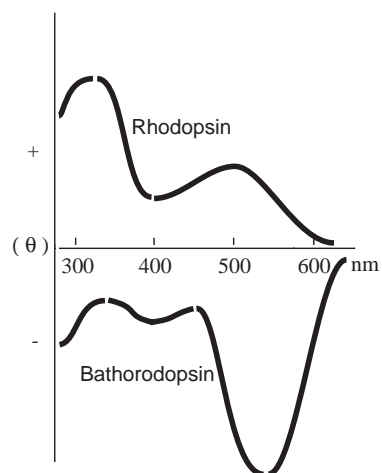


Figure 5. CD-spectra of rhodopsin and of first photoproduct (after Ref. ⁵).

with blue light at 437 nm at 77 K (to slow down consecutive reactions and stabilize the intermediate) the resulting photosteady state mixture has been analyzed to give the CD spectrum of bathorhodopsin, which is rhodopsin with the retinal chromophor in the all-*trans*-geometry. This spectrum, also shown

in Fig. 5, displays a prominent negative band at 520 nm followed by smaller bands towards shorter wave lengths.

The sign and the magnitude of the different CD bands allow conclusions to be drawn pertaining to the three-dimensional geometry of the chromophore on the condition that the excited states causing the bands can be identified. For the retinal protonated Schiff base in rhodopsin which represents a perturbed cyanine type chromophore this condition is easily met: the intense long wave length absorption at 500 nm which is responsible for the color of the protein corresponds to the strongly allowed $\pi\pi^*$ -absorption of the conjugated double bond system.

Once the electronic states involved in the CD have been identified it remains to choose a geometry on the Born-Oppenheimer potential energy surface from which the Franck-Condon- or vertical excitation takes place. Based on this geometry electronic wave functions and transition moments are calculated from which theoretical expressions for the rotatory strengths, which is the area enclosed by a CD band, can be obtained.

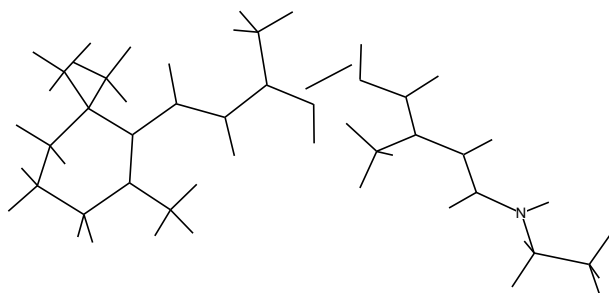


Figure 6. 11-*cis*-Retinal protonated Schiff base according to de Groot *et al.*⁶

For the geometries, we used the results of H. de Groot and co-workers who performed Car-Parrinello type molecular-dynamics calculations on the rhodopsin chromophore⁶ and have obtained, as a consequence of boundary conditions imposed on the chromophores, highly coiled structures (Figs. 6 and 7). Based on these geometries we have performed excited state calculations employing the MOLCAS-4⁷ and GAUSSIAN 94 program packages.⁸ A 3-21G basis set was employed for the wave functions. The (restricted) active space for the CI part (RAS2) consisted of 10 electrons in 10 orbitals.

The calculated energies and oscillator strengths indicate that the excited states we have chosen correspond to the bands observed in the spectrum.

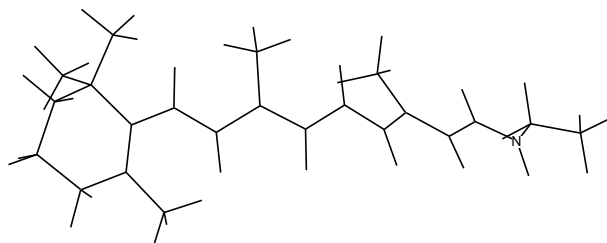


Figure 7. all-*trans*-Retinal protonated Schiff base according to de Groot *et al.*⁶

Table 2. Calculated relative energies, wave lengths λ , oscillator strengths f and rotary strengths R (in Debye-Bohr magneton) of the two lowest excited states of 11-*cis*- and all-*trans*-retinal protonated Schiff base.

	States	E (a.u.)	l (nm)	f	R (D μ_B)
11- <i>cis</i>	2A-1A	0.0841	543	3.1	-0.52
	3A-1A	0.1204	379	0.4	-0.46
all- <i>trans</i>	2A-1A	0.0875	522	3.1	2.43
	3A-1A	0.1.71	426	0.1	0.43

In particular, the high oscillator strengths of the 2A and the low oscillator strengths of the 3A states which moreover decreases when the chromophore configuration changes from 11-*cis* to all-*trans* attest to this. The calculated rotary strengths, -0.52 and +2.4 DmB, for the 2A-states of 11-*cis*- and all-*trans*-retinal protonated Schiff base, are to be compared with the experimental CD-spectra (Fig. 5), in which a positive and a negative CD band are observed for the long wave length absorptions of the corresponding species, rhodopsin and bathorhodopsin, respectively. The exact value of R depends somewhat on the method of preparation⁹ but is in the range of 0.5 DmB, in good agreement with the results of our computation, except for the sign.

We are not ready at this time to include the calculated results for the next higher 3A states. The theoretical description of this state which is composed of at least two different configurations is more difficult than the lowest excited state which involves mainly the excitation from the highest occupied to the lowest empty molecular orbital. We note, however, that all calculated rotary strengths have opposite signs compared to the experimental ones. These data suggest that the absolute configurations of the models on which the calculations of de Groot *et al.* are based (and which are used throughout

the relevant literature) may have to be revised.

4 Conclusion

The theoretical description of the interaction between the retinal chromophore and its environment inside the rhodopsin protein binding pocket has been termed a *grand challenge* for quantum chemistry.¹⁰ We have presented two aspects of our approach: modelling of a minimal complex consisting of the protonated chromophore, a water molecule at the (formally) positive nitrogen and Glu113 and calculation of the rotatory strengths expected for different geometries of the chromophore. The complex is planar, both in the 11-*cis* and in the all-*trans* configuration, and the delocalization of the chromophore π -system is intermediate between the protonated and the deprotonated form. The calculated CD data imply that the absolute configuration of the twisted chromophore inside the protein pocket may be opposite to the one generally assumed. This may be important for attempts to chemically model the binding site.

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