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# Quantum Mechanical Studies on the Crystallographic Model of Bathorhodopsin**

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Rhodopsin is the pigment that mediates light/dark vision in the eyes of vertebrates. After photoexcitation, the chromophore of rhodopsin, 11-*cis*-retinal, isomerizes in an extremely fast reaction to form the all-*trans* isomer, thus starting a sequence of dark events that finally reaches the signaling state of the protein and activates the visual cascade. Formation of the first photoproduct, photorhodopsin, is complete within 200 fs and captures more than 50% of the photon energy, which is expended during the subsequent events. Within a few picoseconds photorhodopsin relaxes to bathorhodopsin, which is the first thermally equilibrated intermediate of the rhodopsin photocycle and can be trapped at cryogenic temperatures.[1,2] The preceding paper reports the first crystallographic analysis of this intermediate.[3] The main alteration is found to be the configurational change of the chromophore from the twisted 11-*cis* form in rhodopsin to the distorted all-*trans* form, with distinct but smaller changes in the chromophore environment.

Theoretical calculations can help to assess and improve protein X-ray data. Modeling the protein matrix is usually reliable owing to the use of standard computer software during the refinement process. The treatment of an active center, such as the chromophore embedded in the protein, may not reach the same level of accuracy as a result of its specific electronic structure, and the use of rigorous quantum mechanics may be required.[4] Herein, we describe the results of a theoretical study of the bathorhodopsin chromophore based on the X-ray crystal structure. The stability of the chromophore geometry inside the binding pocket was tested with DFTB,[5] a self-consistent charge density-functional tight-binding method. On the basis of the optimized geometry, DFT calculations were performed for the analysis of specific Raman bands and multiconfigurational CASPT2[6] calculations were performed to obtain UV/Vis and circular dichroism (CD) spectral data. Details of the DFTB and the excited-state calculations are provided in the Experimental Section. The results are discussed with reference to an analogous treatment of rhodopsin.[7]

A comparison of the X-ray crystal structure and the optimized chromophore structure is shown in Figure 1. The pattern of alternating bond lengths which is typical for the rhodopsin chromophore[7] is preserved in the batho intermediate. The calculated alternation is weaker than that determined experimentally, as is commonly observed for methods that include electron correlation.[8] Calculated bond angles agree well with experiment; a discrepancy is noted only for the bond angles at C7, C8, and C9. Previous crystal models of rhodopsin at different resolution[7] showed differences of

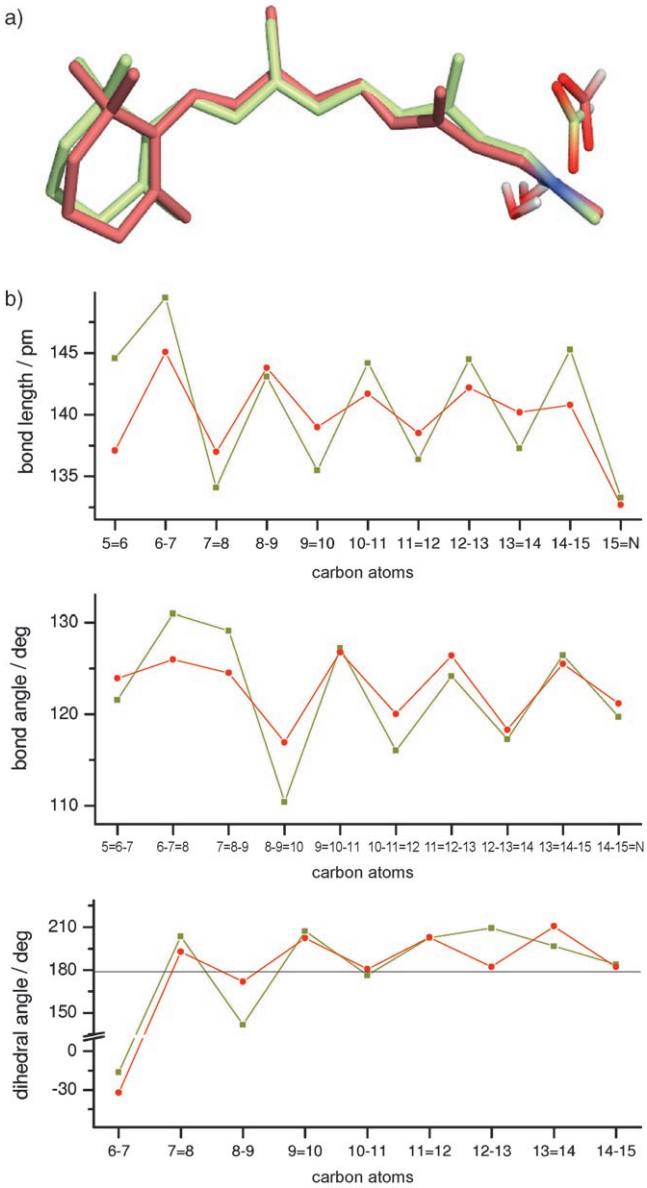


***Figure 1.*** The bathorhodopsin chromophore including Glu113 and wat2b before (green) and after quantum mechanical optimization (red) inside the binding pocket. a) Overlay of the chromophore structures. b) Comparison of the internal coordinates.

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more than five degrees in a few bond angles; further improvement of the X-ray crystal structure of bathorhodopsin may provide bond angles that are closer to the values obtained from the current quantum mechanics model.

The dihedral angles exhibit a pattern which is more obvious in the calculated than in the experimental structure: except for the sterically hindered C6–C7 bond, the conformation about all single bonds is almost planar-*trans* with deviations between 0 and 8°. In contrast, all double bonds are strongly twisted, from 19° (the C15–N16 bond) to 36° (the C13–C14 bond). This same alternation is also evident in the experimental structure except for the C8–C9 and the C12–C13 bonds. Substantial twist of the C12–C13 single bond obtained from the X-ray model is consistent with results from recent resonance Raman spectroscopy,[9] but a planar-*trans* conformation fits the vibrational data as well (see below). The twist at the C8–C9 bond remains to be confirmed. We conclude that the highly distorted X-ray chromophore model is reasonably stable and shows only small changes after DFTB optimization.

Preferential torsion of the double bonds in bathorhodopsin has been found in molecular dynamics simulations of the rhodopsin photoreaction[10,11] and in a minimum energy path calculation.[12] Out-of-plane deformation of double bonds is more costly compared to that of single bonds and may be relevant with respect to the mechanism of energy storage in the photoproduct. The resulting shape of the chromophore is remarkably simple. With all bonds twisted from the planar all-*trans* geometry in the same (positive) direction, the chromophore adopts an elongated corkscrew-like structure with right-hand screw sense (positive helicity).

Resonance Raman spectroscopy can be used to probe the local conformation of the chromophore. In particular, the hydrogen out-of-plane (HOOP) wagging modes at C11 and C12 have been taken as evidence for the distorted C11=C12 *trans* geometry of retinal in bathorhodopsin.[13] These modes are strongly coupled in rhodopsin at 969 $cm^{-1}$; they uncouple in bathorhodopsin and appear as a C11 HOOP mode at 920 $cm^{-1}$ and as a C12 HOOP mode at 858 $cm^{-1}$. We calculated the energies of these modes based on the model structures by using density functional theory (Figure 2). With the generally used scaling factor for the frequencies,[9] we found the C11-H/C12-H mode at 987 $cm^{-1}$ in rhodopsin which decouples to a mainly C11 HOOP mode at 956 $cm^{-1}$ and a nearly isolated C12 HOOP mode at 881 $cm^{-1}$ in bathorhodopsin, with absolute energies and the energy splitting in good agreement with experimental data.

Figure 3 shows the bathorhodopsin and rhodopsin chromophores after optimization within their respective binding pockets. There is a large clockwise rotation (as viewed from the Schiff base nitrogen) of the C13–C20 bond by 140°, from −17° in rhodopsin to −157° in bathorhodopsin, with resulting large displacements of the C12 and C14 hydrogen atoms. Rotation of the C11=C12 bond within the restricted binding pocket is possible because the other bonds, in particular the double bonds, rotate in the opposite direction (Figure 3b).

The mechanism of photoisomerization of rhodopsin has been discussed in the past. To account for the fast rate and the high efficiency of the reaction, Warshel proposed a volume-conserving process in which the *cis* link of the C11=C12 bond in rhodopsin shifts to the C15=N16 bond in bathorhodopsin in a so-called "bicycle-pedal motion".[14] According to molecular dynamics studies,[15] the isomerization starts locally at the C11=C12 bond and spreads rapidly to rotations of the other double bonds in a bicycle-pedal-like motion until it is aborted as the molecule returns to the ground state. From a comparison of the two conformations before and after isomerization, it appears that the batho chromophore structure is determined by the same requirements that govern the dark-state structure of the chromophore:[16] to provide for the least-strained conformation of the configurationally inverted unsaturated chain that connects the two ends fixed in the protein matrix.

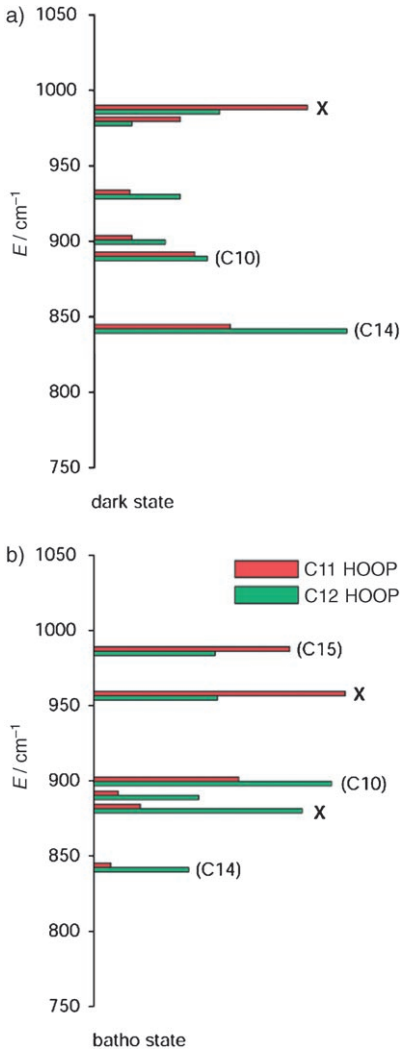


***Figure 2.*** Calculated energies and relative contributions of the C11 and C12 HOOP vibrations to the normal modes of the optimized rhodopsin (a) and bathorhodopsin (b) chromophores. Crosses refer to modes that are discussed in the text; in the other modes, the HOOP vibration indicated in parentheses is a major contributor.

Table 1 summarizes the calculated energies and spectral parameters. The ground-state energy of the chromophore–glutamate complex of bathorhodopsin is raised by 12.7 kcal $mol^{-1}$ relative to rhodopsin. The increase in the distance to

  

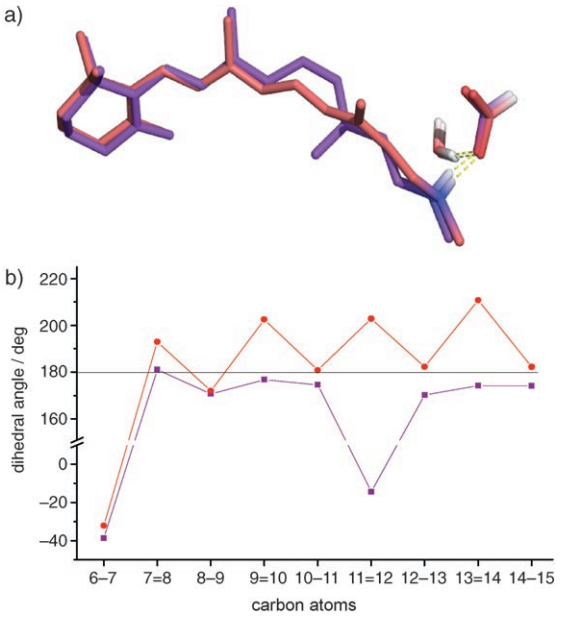


***Figure 3.*** Change in the structure of the chromophore including Glu113 and wat2b from rhodopsin (blue) to bathorhodopsin (red). a) Overlay of the two structures. b) Dihedral angles along the retinal chain (see also Supporting Information).

***Table 1:*** Calculated energies and spectral parameters of retinal chromophores.

| Structure | State | $E_{CASPT2}$[a] | $f$[b] | $R$[c] |
|---|---|---|---|---|
| 11-*cis*-retinal pSb[d] | $S_0$ | −870.7110 | | |
| | $S_1$ | 1.88 (659) | 0.93 | 0.16 |
| Rhodopsin[e] | $S_0$ | −1136.3419 | | |
| | $S_1$ | 2.52 (492) | 0.82 | 0.29 |
| Bathorhodopsin[e] | $S_0$ | −1136.3217 | | |
| | $S_1$ | 2.35 (528) | 0.81 | −0.39 |

[a] $S_0$ energies [au]; $S_1$ energies [eV] relative to $S_0$ (values in parentheses are given in nm). [b] Oscillator strength. [c] Rotatory strength [au]. [d] The chromophore without counterion; pSb = protonated Schiff base. See Ref. [18] for more details. [e] Chromophore including glutamate and wat2b according to Figure 3.

Glu113 by 0.3 Å which is found experimentally (but not theoretically) in the conversion of rhodopsin to bathorhodopsin would raise the energy by another 7.3 kcal mol$^{-1}$. Compared to the increase in energy of bathorhodopsin by 36 kcal mol$^{-1}$ relative to rhodopsin,[17] this result implies that between 23.3 and 16 kcal mol$^{-1}$ is stored as strain between the chromophore and the binding pocket as both adjust to the new configuration of the chromophore.

The remaining data in Table 1 refer to the properties of the excited state of the chromophore in different configurations and surroundings. For the bare 11-*cis*-retinal pSb without counterion, the calculated $S_1$ energy corresponds to a $\lambda_{max}$ value of 659 nm.[18] Recently, the absorbance of all-*trans*-retinal pSb in the gas phase was determined and found to peak at 610 nm.[19] This result is in very good agreement with the value of 600 nm which we calculate for the totally relaxed all-*trans* chromophore.[20] Additionally, the red shift to be expected from the configurational change and bond torsion[19] renders the calculated value for the *cis* chromophore very reasonable.

The presence of the charged Glu113 residues shifts the absorbance close to the experimental value of rhodopsin (498 nm) despite the fact that no charges and polarization of the protein are considered. In calculations reported elsewhere,[11] the glutamate-induced blue shift of the chromophore agrees with that observed herein. However, the bare chromophore is blue-shifted strongly relative to that observed by us, possibly a consequence of the different basis set and the chromophore geometry employed.

The position of the Glu113 residue affects not only the ground state of the chromophore but also the $S_0 \rightarrow S_1$ gap. We calculated the excitation energy as a function of the glutamate distance for a model chromophore (see Supporting Information) and extrapolated a red shift of 20 nm for the rhodopsin chromophore when the counterion is moved from 2.7 to 3.4 Å, the experimentally observed distance. The calculated shift for rhodopsin to bathorhodopsin (36 nm) is in very good agreement with that determined experimentally (44 nm). Note that the position of Glu113 does not change in the two quantum mechanical structures. If one allows for the experimentally determined increase in the counterion distance by 0.3 Å, the absorbance of bathorhodopsin is shifted by only 7 nm to 43 nm relative to the dark state. We conclude that movement of the counterion is not a major contributor to the red shift of the intermediate.

Circular dichroism spectroscopy can be used to monitor the overall shape of an inherently chiral chromophore; specifically, the absolute conformation of enantiomeric or pseudoenantiomeric geometries may be distinguished. For the α band in rhodopsin which peaks at 495 nm, the rotatory strength is approximately +0.1 au; in bathorhodopsin, the band inverts and is red-shifted by 45 nm, with a rotatory strength of −0.4 au.[21] The α bands of rhodopsin and bathorhodopsin calculated on the basis of the quantum mechanical geometries agree in sign and magnitude with those determined experimentally. Both the sign inversion of the rotatory strength and its marked increase in bathorhodopsin, a consequence of configurational change and the prominent helical geometry of the chromophore, are correctly reproduced by the calculations.

In summary, quantum mechanical calculations on the first crystallographic model of bathorhodopsin have provided additional structural details of the chromophore. On the basis of these structures reasonable agreement was found between calculated and experimental resonance Raman, UV/Vis, and CD spectral properties. These studies are a step towards a comprehensive picture of the molecular mechanism for the highly efficient photodetection in rhodopsin.

## Experimental Section

Computational model: Of the two molecules in the asymmetric unit of the crystal, chain B of bathorhodopsin[3] was chosen as the basis for the model. The chain consists of the chromophore, 28 selected amino acid residues, and two water molecules close to the counterion. Twenty-seven of the amino acids were employed in our optimization of the rhodopsin binding pocket,[8] and we introduced Tyr192, which

  

forms a hydrogen bond to Glu181. Except for Glu113, which is negatively charged, all amino acids were considered neutral; the chromophore carries a positive charge. The complete amino acid residues were considered. Where the backbone chain was interrupted, hydrogen atoms were filled in to saturate the free valences. During the computations, the backbone atoms were kept fixed in space.

Quantum mechanical platforms: The chromophore with the binding pocket around was optimized using the DFTB code.[5] For the calculation of UV/Vis and CD spectral data, CASPT2 calculations were performed on the chromophore including Glu113 and wat2b. The (12/12) active space included all π-type electrons and orbitals. For the basis, an atomic natural orbital (ANO)[22] set was employed contracted to 4s3p1d for the heavy atoms and 2s for hydrogen; the level shift was 0.3 au. Transition dipole moments were calculated in the velocity formalism and combined with CASPT2 energies. For the vibrational spectra, we used DFT (B3LYP//6-31G**).

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[1] "Structure and Mechanism of Vertebrate Visual Pigments": W. J. DeGrip, K. J. Rothschild in *Handbook of Biological Physics*, *Vol. 3* (Eds.: D. G. Stavenga, W. J. DeGrip, E. N. Pugh, Jr.), Elsevier Science Press, Amsterdam, **2000**, pp 1 – 54.
[2] T. Okada, O. P. Ernst, K. Palczewski, K. P. Hofmann, *Trends Biochem. Sci.* **2001**, *26*, 2759 – 2767.
[3] H. Nakamichi, T. Okada, *Angew. Chem.*, DOI: 10.1002/ange.200600595/*Angew. Chem. Int. Ed.*, DOI: 10.1002/anie.200600595.
[4] U. Ryde, K. Nilsson, *J. Am. Chem. Soc.* **2003**, *125*, 14232 – 14233.
[5] M. Elstner, D. Porezag, G. Jungnickel, J. Elsner, M. Haugk, T. Frauenheim, S. Suhai, G. Seifert, *Phys. Rev. B* **1998**, 58, 7260 – 7268.
[6] K. Andersson, P.-A. Malmqvist, B. O. Roos, A. J. Sadley, K. Wolinski, *J. Phys. Chem.* **1990**, *94*, 5483 – 5488.
[7] T. Okada, M. Sugihara, A. N. Bondar, M. Elstner, P. Entel, V. Buss, *J. Mol. Biol.* **2004**, *342*, 571 – 581.
[8] M. Sugihara, V. Buss, P. Entel, M. Elstner, T. Frauenheim, *Biochemistry* **2002**, *41*, 15259 – 15266.
[9] E. C. Y. Yan, Z. Ganim, M. A. Kazmi, B. S. W. Chang, T. P. Sakmar, R. A. Mathies, *Biochemistry* **2004**, *43*, 10867 – 10876.
[10] J. A. Gascon, V. S. Batista, *Biophys. J.* **2004**, 87, 2931 – 2941.
[11] U. F. Röhrig, L. Guidoni, A. Laio, I. Frank, U. Rothlisberger, *J. Am. Chem. Soc.* **2004**, *126*, 15328 – 15329.
[12] T. Andruniow, N. Ferre, M. Olivucci, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 17908 – 17913.
[13] I. Palings, E. M. M. van den Berg, J. Lugtenburg, R. A. Mathies, *Biochemistry* **1989**, *28*, 1498 – 1507.
[14] A. Warshel, *Nature* **1976**, *260*, 679 – 683.
[15] O. Weingart, unpublished results.
[16] M. Sugihara, J. Hufen, V. Buss, *Biochemistry* **2006**, *45*, 801 – 810.
[17] A. Cooper, *Nature* **1979**, *282*, 531 – 533.
[18] M. Schreiber, V. Buss, *Int. J. Quantum Chem.* **2003**, 95, 882 – 889.
[19] L. H. Andersen, I. B. Nielsen, M. B. Kristensen, M. O. A. El Ghazaly, S. Haake, M. Brondsted Nielsen, M. A. Petersen, *J. Am. Chem. Soc.* **2005**, *127*, 12347 – 12350.
[20] S. Sekharan, O. Weingart, V. Buss, *Biophys. J.*, DOI: 10.1529/biophysj.106.087122.
[21] S. Horiuchi, F. Tokunaga, T. Yoshizawa, *Biochim. Biophys. Acta* **1980**, *591*, 445 – 457.
[22] K. Pierloot, B. Dumez, P. O. Widmark, B. O. Roos, *Theor. Chim. Acta* **1995**, 90, 87 – 114.

  