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9-Demethylrhodopsin: Theoretical Evidence for a Relaxed Batho Intermediate[†]

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ABSTRACT: The 9-methyl group of retinal is crucial for the photoreaction of rhodopsin. On the basis of the results of QM/MM simulations, we propose that the primary function of the methyl group is not to properly align the chromophore in the ground state, but that it is a prerequisite for the peculiarly twisted and strained chromophore observed in the batho state. With the methyl group firmly anchored in the protein binding pocket the protein, at the cost of the incipient photon energy, manages to increase the strain energy stored in the chromophore by 25%, which may be crucial for driving the subsequent transformations.

The extraordinary ease and efficiency of the photoconversion of rhodopsin to bathorhodopsin, the primary step in the chemistry of vision, is the subject of ongoing debate (*1*). In a reaction which occurs in the femtosecond range with very high stereoselectivity and efficiency (the quantum yield of the photoreaction is higher than 70%), the chromophore of rhodopsin, which is 11-*cis*-retinal bound as a protonated Schiff base to the ε-amino group of K296 (Figure 1), is converted to distorted all-*trans*-retinal, which drives the protein toward the active state, effects binding of the G-protein transducin, and starts the visual cascade.

Central to the question of how the information of the light signal is passed to the different intermediates are energetic aspects. From a highly stabilized structure in the dark state (11-*cis*-retinal is an inverse agonist for receptor activation and effectively inhibits dark-state noise), the chromophore is converted into a full agonist upon light excitation retaining some 35 kcal/mol of the absorbed photon energy (*2*). How is this energy stored, and how is the protein prevented from diffusing immediately into the chromophore environment?

The study of 9-demethylrhodopsin (9-dm) has yielded valuable insights into this problem. The strongly reduced ability of this rhodopsin derivative to activate transducin (*3*) has been discussed in terms of a specific steric interaction of the C9 methyl group with the protein, the so-called steric trigger for rhodopsin activation (*4*).

More recent studies invoked a shift in the crucial MetaI−MetaII equilibrium preceding rhodopsin activation toward the inactive MetaI state caused by entropy changes of the species involved (*5*). A key observation appeared to be the strongly blue shifted UV−vis absorbance of the mutant (465 nm) relative to the wild type (498 nm) (*6*), from which it was concluded that the methyl group acts as a structural anchor for the proper docking of the chromophore in the dark state (*5*). Our results which are based on QM/MM simulations agree only partly with these conclusions. Our findings suggest that the 9-methyl group plays no significant role in the dark state; instead, it locks and supports the unusual and strained conformation of the first photointermediate, bathorhodopsin, raising through steric interactions with key amino acid side groups its internal energy by more than 30%.

Models of dark-state 9-dm and its photoproduct were built on the basis of wild-type models of rhodopsin (*7*) and bathorhodopsin (*8*) and subsequent molecular dynamics (MD) simulation of the complete protein using the SCC-DFTB/CHARMM platform (*9*) (see the Supporting Informa-

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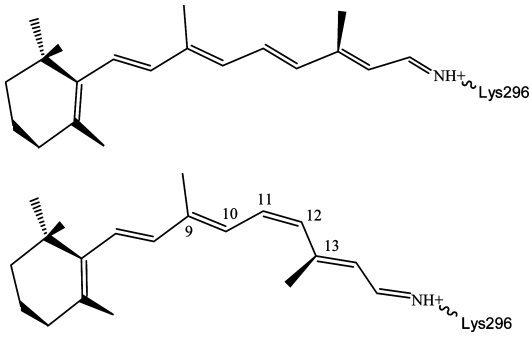

FIGURE 1: Conformation and atomic numbering of the 11-*cis*-retinal-protonated Schiff base chromophore of rhodopsin, in the dark or resting state (bottom) and in the first photointermediate state, bathorhodopsin (top).

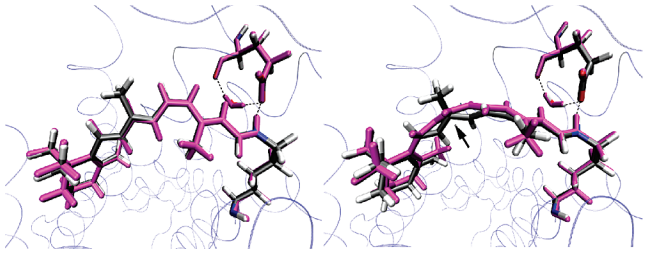

FIGURE 2: Overlay of the wild type (gray) and the 9-dm chromophores (magenta) in rhodopsin (left) and bathorhodopsin (right). Part of the counterion and water 2b are also shown. Note the large forward rotation of the C9−H fragment in the photoproduct of 9-dm (arrow) compared to the almost stationary C9-CH_3 group of the wild type.

tion for more details). Overlays of chromophores of the wild type and the 9-dm analogue within their respective binding sites are shown in Figure 2. In the dark or inactive state (left), the two chromophore structures are virtually identical, the root-mean-square distance between all pairs of atoms being 0.17 Å. We note, in particular, the close agreement between the two calculated C11=C12 twist angles (−18° in the wild type vs −14° in 9-dm). This twist which is essential for the unusual photochemistry of rhodopsin (*9*) is presumably caused by opposite torques acting on the β-ionone and the Schiff base terminus of the chromophore in the binding pocket (*10*), and the 9-methyl group is not a necessary prerequisite for the creation of this twist.

Contrast this with the corresponding batho state (Figure 2, right), in particular the geometry of the unsaturated bridge from C8 to C12 which differs remarkably in the wild type and the 9-dm chromophores. Note especially C9, which with its attached methyl group does barely move in the wild type but rotates strongly clockwise (viewed from the β-ionone ring) in the mutant where the methyl group is missing. This suggests that it is not the dark state of the mutant which is disturbed but rather the batho state, which as a consequence of the missing methyl group is not as firmly anchored in the binding group as the wild type. This confirms what has been concluded from Fourier transform (*5*) and resonance Raman IR spectroscopy (*12*), viz. the lack of chromophore distortion in the batho state of 9-dm.

To quantify the extra strain energy stored in bathorhodopsin as a consequence of the locked methyl group, we have calculated the energies of the wild type and the 9-dm chromophore using second-order perturbation theory (CASPT2) with an atomic natural orbital (ANO) basis set (*13*). In the

Table 1: CASPT2 Calculated Ground- and Excited-State Energies S_0 and S_1 of the Wild Type and of the 9-dm Chromophores[a] in the Dark and Batho States

		wild type	9-dm
dark state	S_0[b]	−1136.4586	−1097.2753
	S_1[c]	57.2 (500)	60.3 (474)
batho state	S_0[d]	−1136.4342 (15.3)	−1097.2565 (11.7)
	S_1[c]	51.3 (557)	54.4 (526)

[a] The "chromophores" consist of the properly twisted retinal-protonated Schiff bases, the counterion, and two water molecules, water 2a and water 2b. [b] Energy in arbitrary units. [c] Energy relative to S_0, in kilocalories per mole (absorbance, in nanometers, in parentheses). [d] Energy in arbitrary units (energy relative to the corresponding ground state, in kilocalories per mole, in parentheses).

wild type, the energy increase of the chromophore from the dark to the batho state is 15.3 kcal/mol compared to only 11.7 kcal/mol in the 9-dm analogue (Table 1). Thus, the first photointermediate of the rhodopsin wild type stores some 3.6 kcal/mol more photonic energy than the 9-dm mutant, an amount that may be critical for the subsequent interconversion to the MetaI state and the shift of the equilibrium toward the active MetaII state.

If the wild type and the 9-dm chromophores fit the rhodopsin binding pocket equally well, resulting in almost identical geometries, what, then, is the reason for the strongly blue shifted absorbance of the latter species? Table 1 includes the results of our excited-state calculations on the retinal chromophore in the dark and batho state, both with and without the 9-methyl group. Turning to the dark states first, we find absorbances which are in very good agreement with the experiment. The blue shift arises since in the excited state negative charge is moved from the β-ionone terminus of the chromophore toward the imine nitrogen. This leaves the odd-numbered carbon centers from C7 to C11 with additional positive charges which are stabilized by electron-donating methyl groups. Calculations employing model compounds show that this effect is sufficient to cause blue shifts of the observed order when a methyl group is removed from a retinal-protonated Schiff base (see Table S3 of the Supporting Information for numerical details).

Conversion to the batho state is associated with a calculated red shift of 52 nm for the 9-dm species, a value similar to the one calculated for the wild type (57 nm). The main contribution to the bathochromic shift observed in bathorhodopsin is steric, especially dihedral distortion (*14*). These distortions are differently distributed along the carbon chain in the wild type and in the mutant, but their sums are surprisingly similar: 60° single-bond distortion from C6 to N16 for the wild type versus 71° for the 9-dm chromophore. The corresponding values for the sum of double-bond distortions are 111° and 105°, so the agreement between the two spectral shifts is not surprising. Since the calculated bathorhodopsin absorbance lies somewhat to the red of the experiment (542 nm), one might assume a similar error for 9-dm bathorhodopsin and arrives at a predicted absorption of ∼515 nm.

The experimental evidence of the 9-demethylbathorhodopsin UV−vis absorption is rather thin. There are several reports of a slightly red shifted photoproduct of 9-dm (*3, 4*). The next intermediate which absorbs at 470 nm is assigned already the MetaI state which is in equilibrium with the strongly red shifted MetaII state (*5a*). So where does this

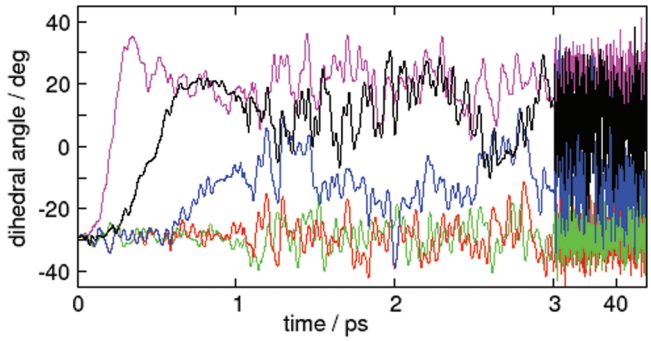

FIGURE 3: Time evolution of the C6−C7 dihedral angle of the retinal chromophore in the batho state as a response to different mutations: magenta for C9-CH3 to 9-dm, black for Y191A/Y268A, blue for Y268A, red for Y191A, and green for T118G. All mutations are switched on at time zero.

leave the lumirhodopsin state? Note that the FTIR spectrum of the "slightly red shifted species" is almost identical to that of the lumi intermediate (*3*), in contrast to the corresponding wild-type spectra, where the transition from the batho to the lumi state involves distinct spectral changes.

Following this line of reasoning, another view of the succession of intermediates following the photoconversion of 9-dm would be transient formation of a strongly blue shifted batho intermediate, formation of the more stable slightly blue shifted lumi intermediate, and then formation of MetaI. With 9-demethylbathorhodopsin being, as our calculations show, sterically less fixed in the binding pocket, one could think of a number of reasons why it does not exhibit the stability of wild-type bathorhodopsin.

What are the constraints that prevent the 9-methyl group of wild-type rhodopsin from rotating upon conversion to the batho state? We have performed MD simulations to see how the chromophore reacts to the mutation of some key amino acids which are known from IR spectroscopy (*15*) to interact closely with this group.

One consequence of the rotation of C9 is the sign change of the C6−C7 dihedral angle. This angle which measures the orientation of the β-ionone ring with respect to the carbon chain is negative in rhodopsin and bathorhodopsin (−42° and −29°, respectively) but becomes positive when 9-dm (−44°) is converted into the batho state (+20°). We have used this angle to monitor a possible conformational switch of the wild-type batho chromophore as a consequence of single-site and multiple-site mutations. The results are shown in Figure 3.

Removal of the 9-CH_3 group causes the immediate conversion of the wild-type chromophore to the geometry observed in 9-dm. Of the three single-site mutations that were studied, T118G, Y191A, and Y268A, the last one has the largest effect on the amplitude of this angle, but neither of them brings about the sign change observed for the 9-dm geometry. Contrast this with the double mutation Y268A/Y1991A, the angle of which rapidly switches and fluctuates around +10°, definitely in a 9-dm-type geometry.

Supporting evidence for the borderline behavior of single-point mutations close to the 9-methyl group may be gained from the work of Janz and Farrens (*16*), who found that the dark-state absorbance of Y268F is insignificantly shifted, to 495 nm, compared to that of the wild type, while the G-protein activation rate is reduced 5-fold. In line with the arguments presented above, we think that the mutation does not prevent the proper alignment of the wild-type chromophore in the dark state but severely impairs formation of the strained batho intermediate.

In conclusion, we have demonstrated the role of the C9 methyl group in stabilizing the highly strained chromophore geometry in bathorhodopsin. When this pivotal group is missing, the photointermediate is free to assume a more relaxed structure compared to the wild type, with its internal energy reduced by 3.6 kcal/mol. Whether the almost quantitative agreement of this calculated energy loss with the free energy change calculated from the shift of the MetaI−MetaII equilibrium in 9-dm (*5b*) (4.3 kcal/mol) is more than accidental has to await the development of more realistic structural models.

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SUPPORTING INFORMATION AVAILABLE

Model building and computational methods, Tables S1−S4 with Cartesian and internal coordinates, and CASPT2 energies (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. (a) Mathies, R. A., and Lugtenburg, J. (2000) in *Handbook of Biological Physics* (Stavenga, D. G., DeGrip, W. J., and Pugh, E. N., Jr., Eds.) p 55, Elsevier, Amsterdam. (b) Okada, T., Ernst, O. P., Palczewski, K., and Hofmann, K. P. (2001) *Trends Biochem. Sci. 26*, 2759–2767. (c) Sakmar, T., Menon, S. T., Martin, E. P., and Awad, E. S. (2002) *Annu. Rev. Biophys. Biomol. Struct. 31*, 443–484. (d) Palczewski, K. (2006) *Annu. Rev. Biochem. 75*, 743–767.
2. (a) Cooper, A. (1979) *Nature 282*, 531–533. (b) Schick, G. A., Cooper, T. M., Holloway, R. A., Murray, L. P., and Birge, R. R. (1987) *Biochemistry 26*, 2556–2562.
3. Ganter, U. M., Schmid, E. D., Perez-Sala, D., Rando, R. R., and Siebert, F. (1989) *Biochemistry 28*, 5954–5962.
4. Han, M., Groesbeck, M., Smith, S. O., and Sakmar, T. P. (1998) *Biochemistry 37*, 538–545.
5. (a) Vogel, R., Fan, F. B., Sheves, M., and Siebert, F. (2000) *Biochemistry 39*, 8895–8908. (b) Vogel, R., Lüdeke, S., Siebert, F., Sakmar, T. P., Hirshfeld, A., and Sheves, M. (2006) *Biochemistry 45*, 1640–1652.
6. Blatz, P. E., Lin, M., Balasubramaniyan, P., Balasubramaniyan, V., and Dewhurst, P. B. (1969) *J. Am. Chem. Soc. 91*, 5930–5936.
7. Okada, T., Sugihara, M., Bondar, A. N., Elstner, M., Entel, P., and Buss, V. (2004) *J. Mol. Biol. 342*, 571–583.
8. Nakamichi, H., and Okada, T. (2006) *Angew. Chem., Int. Ed. 45*, 4270–4273.
9. Cui, Q., Elstner, M., Kaxiras, E., Frauenheim, T., and Karplus, M. (2001) *J. Phys. Chem. B 105*, 569–585.
10. Weingart, O. (2007) *J. Am. Chem. Soc. 129*, 10618–10619.
11. Sugihara, M., Hufen, J., and Buss, V. (2006) *Biochemistry 45*, 801–810.
12. Eyring, G., Curry, B., Mathies, R. A., Fransen, R., Palings, I., and Lugtenburg, J. (1980) *Biochemistry 19*, 2410–2418.
13. Pierloot, K., Dumez, R., Widmark, P. O., and Roos, B. O. (1995) *Theor. Chim. Acta 90*, 87–114.
14. Schreiber, M., and Buss, V. (2003) *Int. J. Quantum Chem. 95*, 882–889.
15. Nagata, T., Oura, T., Terakita, A., Kandori, H., and Shichida, Y. (2002) *J. Phys. Chem. A 106*, 1969–1975.
16. Janz, J. M., and Farrens, D. L. (2004) *J. Biol. Chem. 279*, 55886–55894.

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