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Supporting Information

69451 Weinheim, Germany

Crystallographic Analyses of the Primary Visual Photochemistry

Hitoshi Nakamichi, and Tetsuji Okada

Supporting Text

Data collection

The tetragonal rhodopsin crystals are rod-shaped with the dimensions of 0.1 x 0.1 x 0.4 mm in average. Because of the anisotropic alignment of the retinal dipole moment which is nearly perpendicular to the longest dimension (c-axis) of the crystal, the degree of photoactivation significantly depends on the angle between the direction of activating light and the crystal axis. For both the microspectroscopy and x-ray diffraction, the measuring beam penetrates the smaller dimension (0.1 mm, optical density 0.5) whereas the activation laser light direction has to be as parallel as possible to the longest axis (0.4 mm). The minimal intensity of the laser light (2 mm diameter) required to produce the clear difference electron density map was 2 mW mm^{-2}. X-ray data sets were processed with HKL2000 (1). Of the two data sets presented in Supporting Table 1, set 2 contains only negligible twinning, which appears to provide the most accurate difference electron density map (Fig. 2). Although the amount of twinning in the set 1 is not trivial, the difference map calculated after detwinning resembled that of set 2. To avoid possible inaccuracies due to the detwinning procedure, structure refinement of bathorhodopsin with set 1 has been performed with CNS (2) taking the twinning ratio into account as described previously (3). Data set 1 and 2 are both illuminated from separate crystals. The data statistics of the corresponding dark data sets are similar or slightly better.

Model building and Refinement

The model of 9-cis-rhodopsin was constructed by energy minimization of the ground state rhodopsin structure with a weak dihedral restraint at C9=C10 of the retinal without x-ray terms. In this model, the

position of the β-ionone ring was almost the same as in rhodopsin, suggesting that the formation of 9-cis-rhodopsin does not give rise to the large positive/negative difference electron densities around the ring. We also performed energy minimization of the model of bathorhodopsin against the illuminated data set without any dihedral restraints around the C9=C10 bond to see if this angle has preference for the cis configuration. It was found that the structure of bathorhodopsin is not dependent on the restraints around this bond. These examinations, comparison of data sets taken with different light intensities and the experimental condition where excitation wavelength (488 nm) is very close to the absorption maximum of 9-cis-rhodopsin (485 nm) support the assumption that our F_{light} data can be approximately analyzed with only two components (rhodopsin and bathorhodopsin). These considerations are also supported by the previous study of the proportion of 9-cis-rhodopsin in the photostationary mixture (4), which suggests our illumination condition giving 35 % bathorhodopsin can yield no more than several % 9-cis form.

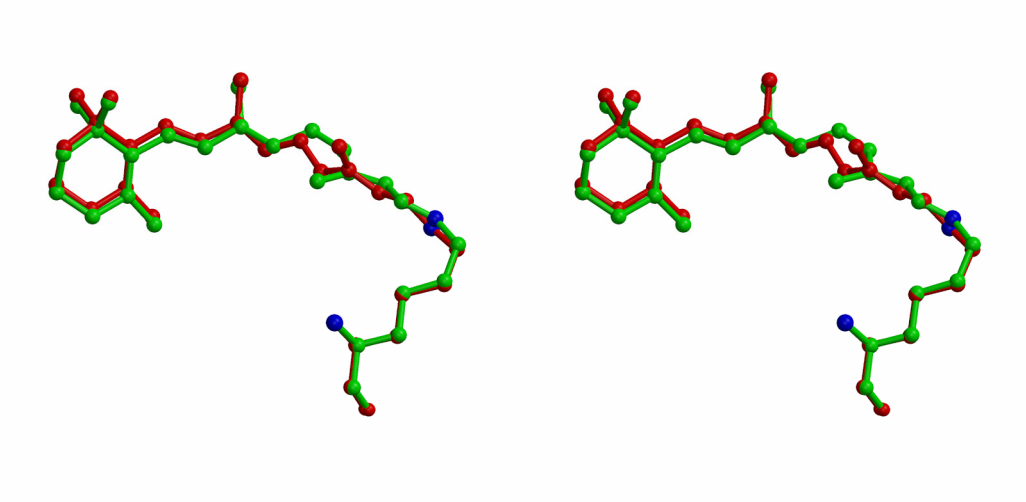
References

1. Otwinowski, Z., & Minor, W. (1997) *Methods Enzymol.* **276**, 307-326.
2. Brünger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, et al. (1998) *Acta. Crystallogr.* **D54**, 905-921.
3. Teller, D. C., Okada, T., Behnke, C. A., Palczewki, K., & Stenkamp, R. E. (2001) *Biochemistry* **40**, 7761-7777.
4. Oseroff, A. R., & Callender, R. H. (1974) Biochemistry 13, 4243-4248.

Supporting Table 1. X-ray diffraction data and refinement statistics.

	Data 1	Data 2
Data statistics		
Resolution, Å	50-2.6	50-2.7
Unit cell (a=b, c), Å*	96.49, 150.4	96.54, 150.4
Twin fraction	0.352	0.003
Mosaicity, °	0.45	0.54
Total observations	107422	96860
Unique observations	37672	33940
R_{merge}, % (outer shell)	11.5 (53.8)	12.2 (76.6)
Completeness, % (outer shell)	89.2 (52.7)	89.6 (43.5)
I / σ (I), (outer shell)	9.2 (1.1)	8.0 (1.0)
Wilson B factor, $Å^2$	59.7	55.4
Refinement statistics		
R_{cryst} , %	17.8	22.3
R_{free} , %	18.1	22.3
rmsd of bonds, Å	0.012	0.013
rmsd of angles, °	1.42	1.44

*The space group is $P4_1$. †The final refinements included dual conformations of the selected residues as described in the text. $R_{merge}= \Sigma_{hkl} \Sigma_i | I_i(hkl) - \langle I(hkl) \rangle | / \Sigma_{hkl} \Sigma_i I_i(hkl)$. $R_{cryst} =\Sigma_{hkl} | F_o-F_c | / \Sigma_{hkl} | F_o |$. R_{free} were calculated from a set of 5 % randomly selected reflections, which were omitted from refinement.



Supporting Figure 1. Stereo view of the retinal linked to Lys296 in rhodopsin (green) and bathorhodopsin (red). Nitrogen atoms are coloured in blue.