Supporting Information

Thermal Decay of Rhodopsin: Role of Hydrogen Bonds in Thermal Isomerization of 11-*cis* Retinal in the Binding Site and Hydrolysis of Protonated Schiff Base

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Experimental Section

Materials

All chemicals were purchased from Sigma-Aldrich, Inc., unless otherwise stated. The surfactant, *n*-dodecyl-*β*--D-maltoside (DM), was purchased from Anatrace, Inc.

Sample Preparation

Rhodopsin was expressed and purified as described previously.[1] After purification, the concentration of the rhodopsin sample was ~0.3 mM in buffer A (50 mM sodium phosphate, 0.1% DM, pH 6.5). The rhodopsin sample was further concentrated to ~2.5 mM by Amicon Ultra centrifugal filter device (30k MWCO, Millipore) for the kinetic experiments. The concentrated samples were added to a large volume of buffer A to give a final dilution factor of 10 prior to initiating the kinetic experiments. Assuming that DM in the form of micelles would be unable to pass through the filter during the concentration procedure, the concentration of DM in the final samples for kinetic measurements was calculated to be < 0.2%.

Sample Preparation for D_2O experiments

Two 500-μl samples of rhodopsin at ~0.3 mM in buffer A were concentrated to 20 μl individually in two Biomax-30K NMWL centrifugal tubes (0.5 ml, Millipore). After concentration, one sample was diluted with buffer B (50 mM sodium phosphate in H_2O, 0.02% DM, pH 6.5) back to ~0.3 mM and stored in buffer B overnight at 4^{o}C. Another sample was diluted with buffer C (50 mM sodium phosphate in D_2O, 0.02% DM, pH 6.5) back to ~0.3 mM and stored in buffer C. The following day, both samples were concentrated to ~2.5 mM for the kinetic experiments.

Thermal Decay Measurement

Thermal decay experiments were performed with a UV-vis spectrometer (Shimadzu UV-2450). Buffer A at a volume of 2.7 ml was equilibrated in a water-jacketed cuvette at 59°C, with temperature monitored by a thermal couple. At t = 0, 0.3 ml of concentrated rhodopsin at room temperature was added and the UV-Vis spectra were

taken at appropriate time points. After each measurement, 200 µl of sample was removed from the cuvette and immediately put in ice-cold glass vials to quench any thermal process and kept on ice. Samples were divided into two aliquots for the following kinetic measurements: (1) thermal isomerization and (2) hydrolysis of the protonated Schiff base.

Thermal Isomerization Measurement

Retinal was extracted from the aliquots removed during the thermal decay experiment. The procedure for retinal extraction and HPLC analysis was adapted from a previous protocol with minor modifications.[2] The extracted retinal was dried under argon and stored as thin films of retinaloximes at -80ºC until analyzed by HPLC. To perform the HPLC analysis, the dried samples were dissolved in hexane (25 µl) and subsequently injected into an HPLC (Beckman Coulter SYSTEM GOLD® 125 Solvent Module) with a silica column (Beckman Coulter Ultrasphere 4.6 × 250 mm). Absorbance at 360 nm was used for detection (Beckman Coulter SYSTEM GOLD® 168 Detector). The mobile phase was hexane supplemented with 8% diethyl ether and 0.33% ethanol.

Analysis and Fitting for the Thermal Isomerization Measurement

The peaks in each chromatograph were fitted to Gaussian functions to obtain the peak areas, which were calibrated to the corresponding extinction coefficients at 360 nm. The fraction of 11-*cis* retinal was obtained from the calibrated peaks of 11-*cis* retinaloxime divided by the sum of calibrated areas of all peaks in each chromatograph.

Hydrolysis of Protonated Schiff Base

To each 100-µl aliquot from the thermal decay experiment, 1 M HCl (4 µl) was added to decrease the pH to 1-2 to denature the opsin protein. The UV-Vis spectra were taken as in the thermal decay experiment.

References

(1) Yan, E. C. Y.; Epps, J.; Lewis, J. W.; Szundi, I.; Bhagat, A.; Sakmar, T. P.; Kliger, D. S. *J. Phys. Chem. C* **2007**, *111*, 8843-8848.

(2) Tsutsui, K.; Imai, H.; Shichida, Y. *Biochemistry* **2007**, *46*, 6437-6445.