Salt effects on the conformational stability of the visual G-protein-coupled receptor rhodopsin

Arfaxad Reyes-Alcaraz, Marlet Martínez-Archundia, Eva Ramon and Pere Garriga

Supplementary Information

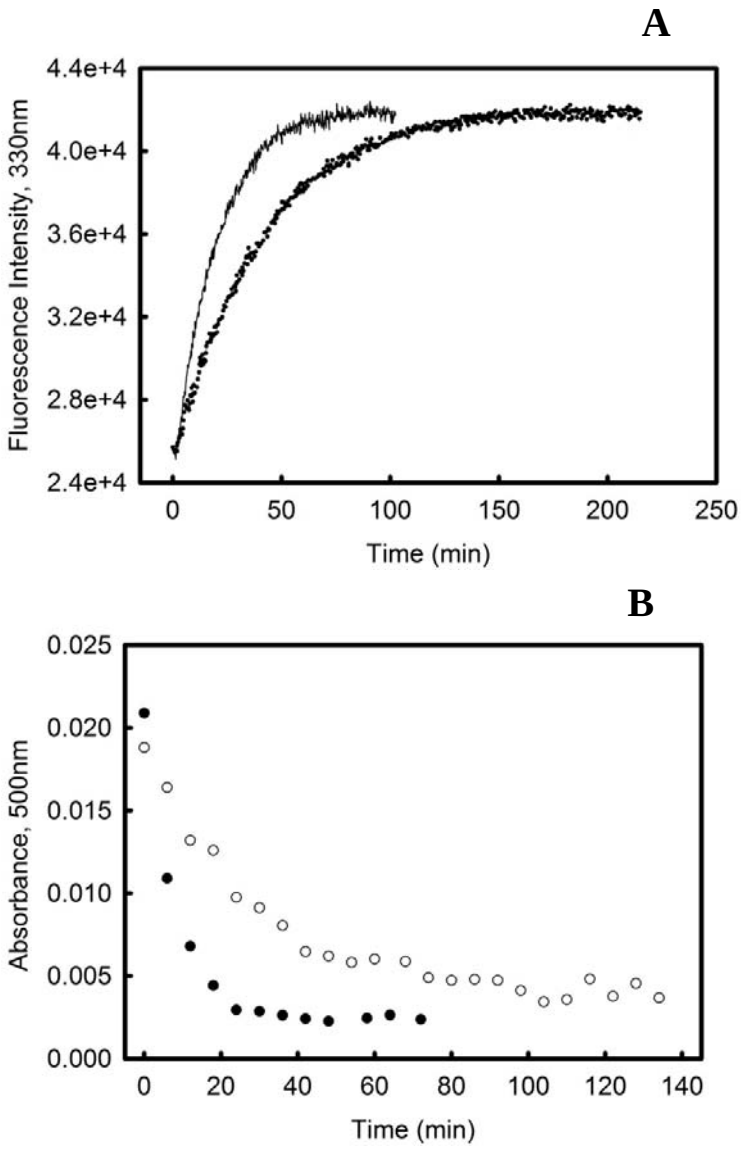

Figure S1. Rho Thermal decay studied by using fluorescence and UV-Visible spectroscopies. Rho in 100 mM HEPES pH 7.4 containing 0.05%DM was incubated at 55ºC and thermal stability was studied by using two spectroscopic techniques. A) Fluorescence spectroscopy was used to follow specific changes in Trp fluorescence from Rho due to retinal release, (retinal acts as a quencher of Trp fluorescence in Rho) (1), when treated at 55ºC in the absence (─) or in the presence of 3.5M NaCl (•••). B) UV/Visible spectroscopy was applied in order to monitor the effect of temperature to Rho chromophore. Here, the isomerization and Schiff base hydrolysis are followed over time at 55ºC in the absence (●) and in the presence of 3.5 M NaCl (○).

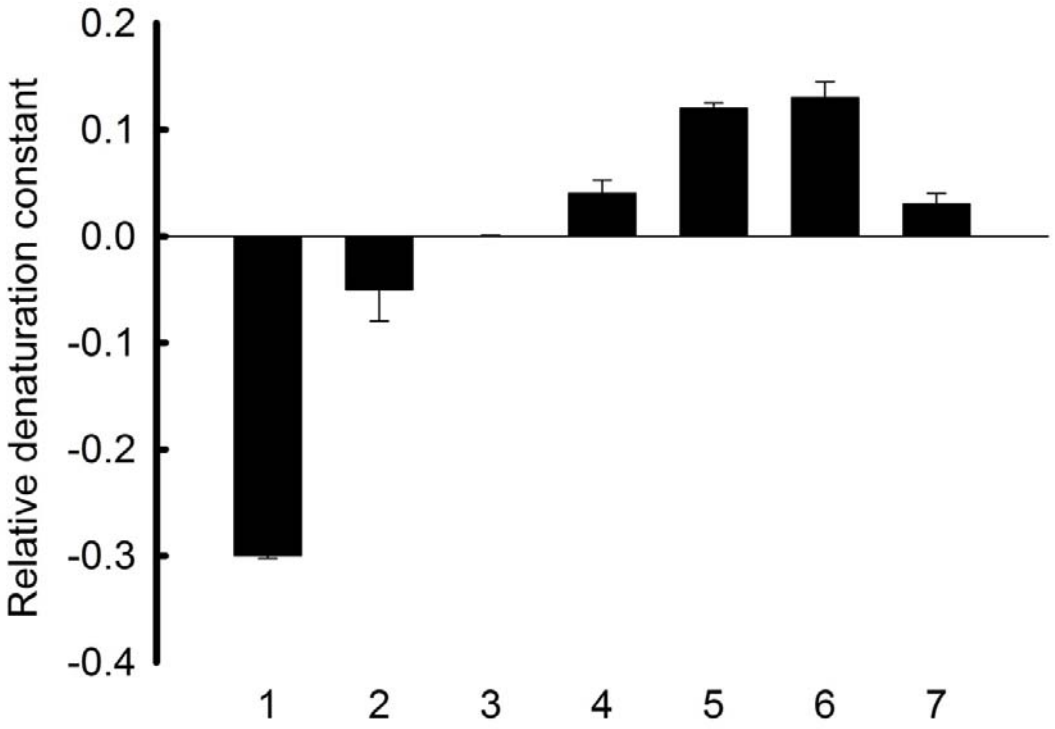

Figure S2. Effect of different salts on rhodopsin thermal stability. Rho thermal stability in 10mM MOPS pH7, 0.12% DM, and in the presence of 0.1M NaI (1), 0.5M LiCl (2), 3M NaBr (4), 3M KCl (5), 0.95M Na_2SO_4 (6) and 2M $MgCl_2$ (7) compared to the control sample (3), was followed by monitoring the loss of the absorbance at λ 500nm over time at 50ºC. Scans were collected every 5 min and data were normalized and fit to a single exponential function using Sigma Plot to derive the decay constant (k). Denaturation constants were calculated from (lnk(salt)-lnk(control))/lnk(control)). Data represent mean values from 3 independent experiments.

Different anions, such as bromide, chloride, iodide and sulfate were tested on Rho treated at 50ºC. The results suggest that the stability depends on the anion chemical nature in the aqueous environment as seen, for instance, by the strong effect of NaI as compared to other sodium salts at higher concentrations. On the other hand, lithium chloride instability is supposed to be explained by the specific effect of lithium binding to Rho (unpublished data).

References

1. Farrens, D. L., and H. G. Khorana. 1995. Structure and Function in Rhodopsin: Measurement of the Rate of the Metarhodopsin II Decay By Fluorescence Spectroscopy. J. Biol. Chem. 270: 5073-5076.