THE PRIMARY EVENT IN VISION INVESTIGATED BY TIME-RESOLVED FLUORESCENCE SPECTROSCOPY

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ABSTRACT The picosecond fluorescence kinetics and quantum yield from bovine rhodopsin were measured in the 5–40°K range. The fluorescence rise and decay times are faster than our resolution of 15 ps (full width at half maximum) over this entire temperature range. The size of the observed emission was also temperature independent, and we find that the upper limit of rhodopsin's fluorescence quantum yield to be $\phi_f \approx 10^{-5}$. Replacing all of rhodopsin's exchangeable protons with deuterons by suspending rhodopsin in D_2O had no effect on either the kinetics of the emission or the value of the quantum yield. Our data provide strong confirmation of the idea that the first step in the visual process is an excited-state *cis*-to-*trans* isomerization about the C11–C12 double bond of retinal.

INTRODUCTION

The visual pigment, rhodopsin, consists of a single chromophore, 11-*cis* retinal, covalently bound in the form of a protonated Schiff base to the ε-amino group of lysine-321 in the apoprotein opsin (1). Vision is initiated when the chromophore absorbs a photon, and rhodopsin is transformed to its primary photoproduct, bathorhodopsin. There are a number of remarkable properties in the primary photophysics of visual proteins. The formation quantum yield of bathorhodopsin from rhodopsin (0.67) is very high, indicative of an extremely efficient process. Also, some two-thirds of the incident photon's energy is converted to chemical energy in the rhodopsin-to-bathorhodopsin phototransition, since the enthalpy of bathorhodopsin is some 35 kcal/mol higher than that of rhodopsin's (2,3). This energy is used to drive subsequent thermal reactions, ultimately resulting in visual perception.

The rhodopsin-bathorhodopsin transformation has been extensively studied. The in situ retinal chromophore isomerizes, being converted from an 11-*cis* isomer to a *trans*-like species (1). The bathorhodopsin formation time is very fast, <6 ps, at room temperature, being as yet unresolved in picosecond absorption spectroscopic measurements. At much lower temperatures near liquid helium, the formation time has been measured. Peters et al. (4) found that the rise time of bathorhodopsin followed a non-Arrhenius temperature dependence and increased markedly when measured for samples suspended in D_2O. These results prompted their suggestion that a proton(s) translocation process is the rate limiting step in bathorhodopsin formation. Thus, there are at least two steps in the rhodopsin-bathorhodopsin transformation, isomerization of the retinal chromophore and proton motion of an unspecified, but exchangeable opsin proton. The ordering of these steps is unclear.

Using picosecond fluorescence techniques, we recently determined the fluorescence quantum yield, ϕ_f, of bovine and squid rhodopsins at room temperature (5). Both pigments yielded the same results within our signal-to-noise ratio, where we found $\phi_f = 1.2\ (\pm 0.5) \times 10^{-5}$. From $\phi_f = \tau_n/\tau_r$, we estimated that the nonradiative lifetime of the process that competes with fluorescence to be on the order of 0.1 ps. The rise and decay times of the emission signal were faster than our resolution of 15 ps. In this paper, we extend these measurements by studying the fluorescence properties of bovine rhodopsin at very low temperatures, in the 5–40°K range, and for samples suspended in D_2O as well as H_2O. We find the measured fluorescence quantum yield and kinetics to be unaffected by either changes in temperature or sample deuteration. We conclude that the proton-translocation rate limiting step in bathorhodopsin formation is too slow to be the process involved in that governing rhodopsin's fluorescence properties. Since previous results (5, 6) from our laboratory strongly suggest that the nonradiative decay process competing with emission in rhodopsin does involve rotational motion about retinal's 11C-12C bond, we conclude that the first step in forming bathorhodopsin is a fast subpicosecond 11-*cis*-to-*trans* photoisomerization. We described below constraints that our data places on the relevant potential surfaces describing such rapid rotational motion.

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We thus conclude that, of the two known processes leading to the photochemical formation of bathorhodopsin, photoisomerization about retinal's C11–C12 double bond proceeds proton translocation. The isomerization process is very fast and very efficient. Moreover, our results are quite consistent with previous work suggesting that the excited-state potential surface along the torsional motion is barrierless and fairly steep (11–13). This follows first from rhodopsin's extremely low emission quantum yield, which requires a fairly steep surface (5). Moreover, should there be any significant barrier to rotation in the first 45° of rotation, we would generally expect a strong temperature dependence to the emission, with a marked increase in fluorescence quantum yield at liquid helium temperature compared to room temperature. Such a conclusion is not, however, unambiguous. The retinal chromophore must be coupled strongly enough to its thermal bath environment so that significant thermal equilibration occurs within ~0.1 ps after excitation for there to be any temperature dependence of the emission properties measured here. This implies a rather strong coupling that may or may not exist. Finally, we point out that the excited-state potential energy surface slope, along the 11–12 torsional angle, is probably similar in steepness for angles close to 0° or *trans* (the bathorhodopsin side of the surface) as it is for 180° or 11-*cis* (the rhodopsin side). This follows from the observation above that bathorhodopsin's fluorescence quantum yield is as small or smaller than rhodopsin's, being $\leq 10^{-5}$, at least for temperatures $\geq$20°K.

We gratefully thank Prof. K. Nakanishi and Dr. J. Carriker for the use of facilities and assistance in preparing and characterizing the samples.

This work was supported by National Institutes of Health (NIH) grants EY02515 and EY03142 to City College and NIH grant EY01253 to Columbia University and by a City University Faculty Research Award Program (BPSC-BHE) grant.

Received for publication 25 September 1984 and in final form 15 January 1985.

REFERENCES

1. Ottolenghi, M. 1980. The photochemistry of rhodopsin. *In* Advances in Photochemistry. J. N. Pitts, Jr., G. S. Hammond, and D. Grosjean, editors. John Wiley and Sons, Inc., New York. 12:97–200.
2. Honig, B., T. G. Ebrey, R. H. Callender, U. Dinur and M. Ottolenghi. 1979. Photoisomerization, energy storage and charge separation: a model for light energy transduction in visual pigments and bacteriorhodopsin. *Proc. Natl. Acad. Sci. USA.* 76:2503–2507.
3. Cooper, A. 1979. Energy uptake in the first step of visual excitation. *Nature (Lond.).* 282:531–533.
4. Peters, K., M. L. Applebury, and P. M. Rentzepis. 1977. Primary photochemical event in vision: proton translocation. *Proc. Natl. Acad. Sci. USA.* 74:3119–3123.
5. Doukas, A. G., M. R. Junnakar, R. R. Alfano, R. H. Callender, T. Kakitani, and B. Honig. 1984. Subpicosecond excited state processes in visual pigments. Evidence for subpicosecond isomerization rates *Proc. Natl. Acad. Sci. USA.* 81:4790–4794.
6. Buchert, J., V. Stefancic, A. G. Doukas, R. R. Alfano, R. H. Callender, J. Pande, H. Akita, V. Balogh-Nair, and K. Nakanishi. 1983. Picosecond kinetic absorption and fluorescence studies of bovine rhodopsin with a fixed 11-ene. *Biophys. J.* 43:279–283.
7. Doukas, A. G., J. Buchert, and R. R. Alfano. 1982. Picosecond laser techniques and design. *In* Primary Events in Biology Probed by Ultrafast Laser Spectroscopy. R. R. Alfano, editor. Academic Press, Inc., New York. 347–418.
8. Papermaster, D. S., and W. J. Dreyer. 1974. Rhodopsin content in the outer segment membranes of bovine and frog retinal rods. *Biochemistry.* 13:2438–2444.
9. Suzuki, T., and R. H. Callender. 1981. Primary photochemistry and photoisomerization of retinal at 77°K in cattle and squid rhodopsins. *Biophys. J.* 34:261–265.
10. Stubbs, G. W., J. C. Saari, and S. Futterman. 1974. 11-*cis* retinal-binding protein from bovine retina. *J. Biol. Chem.* 254:8529–8533.
11. Rosenfeld, T., B. Honig, M. Ottolenghi, J. Hurley, and T. G. Ebrey. 1977. *Cis-trans* isomerization in the photochemistry of vision. *Pure Appl. Chem.* 49:341–351.
12. Birge, R. R., and L. M. Hubbard. 1980. Molecular dynamics of *cis-trans* isomerization in rhodopsin. *J. Am. Chem. Soc.* 102:2195–2205.
13. Birge, R. R., and L. M. Hubbard. 1981. Molecular dynamics of *trans-cis* isomerization in bathorhodopsin. *Biophys. J.* 34:517–534.