rhodopsin exists in two forms in equilibrium resulting from the accessibility of two stable conformational states separated by a small difference in free energy.

We have studied the kinetics of rhodopsin decay reactions using flash photolysis of detergent-solubilised rhodopsin containing less than 0.2 mol phospholipid per mol rhodopsin. The kinetics of meta I → meta II were determined from absorbance measurements at 380 nm and of lumirhodopsin → meta I from absorbance measurements at about 415 nm, which is near the isosbestic point of meta I and meta II. In each case the absorbance against time function could be fitted by a sum of two exponential decay functions of the form $\Sigma A_i \exp(-t/\tau_i)$. We used a phospholid-free preparation so that we could exclude phospholipid heterogeneity of the digitonin micelle as a source of multiexponential kinetics.

We have assumed that these kinetics result from the independent decay of two forms of each of these species, and thus that the amplitudes of the two exponential terms are proportional to the initial concentrations of these two forms. Figure 1 shows that for meta I → meta II a change in temperature from 20 to 37 °C results in a change in the ratio of these two amplitudes, but not in their sum (Fig. 1*a* and *b*). Furthermore, this change is reversible (Fig. 1*c*). Such kinetics are caused by two components which are interconvertible, but only on a time scale which is long compared with the time constants for decay of meta I. Thus, these two components exist in equilibrium, but this equilibrium is established before the appearance of meta I.

We also found that the kinetics of decay of the earlier intermediate, lumirhodopsin, at 20 and 30 °C were consistent with double-exponential kinetics with approximately the same relative amplitudes as those determined from the meta I decay kinetics at these two temperatures. Presumably, the decay kinetics of bathorhodopsin, the earliest of the intermediates, would also be double exponential with the same ratio of amplitudes in these conditions, but we have as yet made no attempt to test this experimentally. Bathorhodopsin does, however, decay with double-exponential kinetics in rod outer segment (ROS) suspensions[6]. Thus, all of these intermediates display the same type of double-exponential decay kinetics. This suggests that rhodopsin itself exists in two forms before photolysis.

According to this interpretation, the ratio of the two amplitudes determined from the meta I → meta II kinetics is equal to the equilibrium constant for the transition between these two forms of rhodopsin. The values of the thermodynamic parameters for this hypothetical transition were calculated from the temperature dependence of the equilibrium constant in the range 20–37 °C (Fig. 2). For phospholipid-free rhodopsin solubilised in 2% (w/v) digitonin in distilled water, $pH \simeq 6$, $\Delta H^0 = 9.8$ kcalorie, $\Delta S^0 = 32$ e.u., and at 37 °C, $\Delta G^0 = -0.2$ kcalorie. These values are of approximately the same magnitude as those reported for transitions between some conformational substates of α-chymotrypsin[11,12]. Two components in these kinetics could also be due to the availability of two different environments in the digitonin micelle, but we have recently obtained similar results with ROS solubilised with cetyltrimethylammonium bromide, Emulphogene BC720, Amonyx-LO and with detergent-free suspensions of sonicated ROS. Thus, it seems likely that the heterogeneity inferred from these kinetics resides in the state of the protein itself.

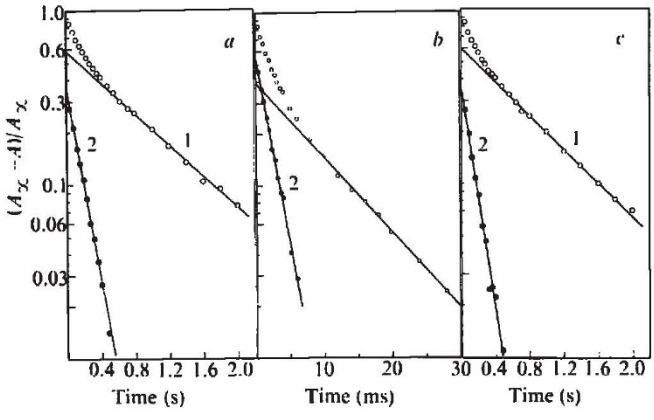

Fig. 1 Kinetics of change in A_{380} with phospholipid-free rhodopsin solubilised in 2% (w/v) digitonin in distilled water, $pH \simeq 6$. A_{380}, as a function of time, is relative to the preflash absorbance of the solution. Time constants and amplitudes were extracted from the data by linear least squares fitting and the 'peeling off' procedure. ○, $(A_\infty - A)/A_\infty$; ●, difference between these points and line 1. *a*, Sample photolysed at 20 °C; $A_1 + A_2 = 0.95$, $A_2/A_1 = 0.62$. *b*, Sample photolysed at 37 °C; $A_1 + A_2 = 0.96$, $A_2/A_1 = 1.4$. *c*, Sample held at 37 °C for 3 min, and photolysed at 20 °C; $A_1 + A_2 = 0.95$, $A_2/A_1 = 0.59$.

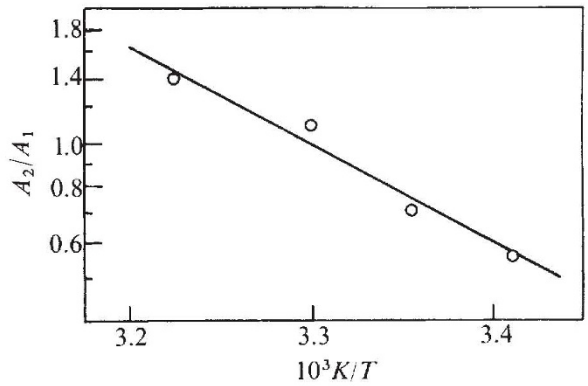

Fig. 2 Van't Hoff plot for the proposed reaction $R_1 = R_2$ over the range 20–37 °C. At each temperature, A_1 and A_2 were determined as described in Fig. 1. A straight line was fitted to the data points by linear least squares, giving $\Delta H^0 = 9.8$ kcalorie and $\Delta S^0 = 32$ e.u.

This work was supported by grants from the NIH, NSF and ERDA.

JAMES G. STEWART
BARBARA N. BAKER
THEODORE P. WILLIAMS

Institute of Molecular Biophysics, The Florida State University, Tallahassee, Florida 32306

Received June 4; accepted August 21, 1975.

1 Matthews, R. G., Hubbard, R., Brown, P. K., and Wald, G., *J. gen. Physiol.*, **47**, 215–240 (1963).
2 Linschitz, H., Wulff, V. J., Adams, R. G., and Abrahamson, E. W., *Archs biochem. Biophys.*, **68**, 233–236 (1957).
3 Wulff, V. J., Adams, R. G., Linschitz, H., and Abrahamson, E. W., *Ann. N.Y. Acad. Sci.*, **74**, 281–290 (1958).
4 Abrahamson, E. W., Marquisee, J., Gavuzzi, P., and Roubie, J., *Z. Elektrochem.*, **64**, 177–180 (1960).
5 Grellmann, K-H., Livingston, R., and Pratt, D., *Nature*, **193**, 1258–1260 (1962).
6 Pratt, D. C., Livingston, R., and Grellmann, K-H., *Photochem. Photobiol.*, **3**, 121–127 (1964).
7 Marquisee, J., thesis, Case Western Reserve Univ., Cleveland, Ohio (1966).
8 Rapp, J., Wiesenfeld, J. R., and Abrahamson, E. W., *Biochim. biophys. Acta*, **201**, 119–130 (1970).
9 Williams, T. P., Baker, B. N., and McDowell, J. H., *Expl Eye Res.*, **18**, 69–75 (1974).
10 Applebury, M. L., Zuckerman, D. M., Lamola, A. A., and Jovin, T. M., *Biochemistry*, **13**, 3448–3458 (1974).
11 Lumry, R., and Biltonen, R., in *Structure and Stability of Biological Macromolecules* (edit. by Timasheff, S., and Fasman F.), (Dekker, New York, 1969).
12 Kim, Y. D., and Lumry, R., *J. Am. chem. Soc.*, **93**, 1003–1013 (1971).

Erratum

In the article "Seismic evidence for local undulation of olivine–spinel phase boundary", by S. K. Dey-Sarkar and R. A. Wiggins (*Nature*, **257**, 572; 1975) the last two sentences of the first paragraph should read: While analysing seismograms between 14 and 18°, a group of anomalous data was observed localised in a specific region which could be explained only by a local undulation of the 410-km discontinuity. Here we present these observations and give a speculative explanation for the undulation.