Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7083–7087, November 1984
Biophysics

On the protein (tyrosine)-chromophore (protonated Schiff base) coupling in bacteriorhodopsin

(proton pump/pH dependence/temperature)

JANE H. HANAMOTO, PAUL DUPUIS, AND M. A. EL-SAYED

Department of Chemistry and Biochemistry, University of California, Los Angeles, 405 Hilgard Avenue, Los Angeles, CA 90024

Contributed by M. A. El-Sayed, July 16, 1984

ABSTRACT **The kinetics of formation of both the tyrosinate ion (from its absorption at 296 nm) and the deprotonated Schiff base (M_{412}) (from its absorption at 404 nm) are studied simultaneously at different pH values (7–11) and temperatures (5–25°C). Two formation rates are observed for M_{412} in agreement with previous observations. The slow one is dominant under physiological conditions and is found to be slightly faster than that for the tyrosinate formation. This is in disagreement with the proposal that the tyrosinate formation is a prerequisite to the deprotonation of the Schiff base (M_{412}). The ratio of the amplitudes of the fast and slow components is found to be sensitive to pH and, at any pH, it can be used to calculate an amino acid pK_a value of 9.6. This is explained by proposing the existence of two sites for the protonated Schiff base within the protein. In one site, the Schiff base is near the neutral form of an amino acid residue with a pK_a value of 9.6 (giving rise to the slow component), while in the other, it is near its conjugate base. The formation of the tyrosinate ion as well as the formation of the slow and fast components of M_{412} all have activation energies that are comparable to H-bond energies. A model is suggested to account for this and the comparable deprotonation rates of tyrosine and the slow component of the protonated Schiff base. It involves the reduction of their pK_a by their exposure to a positively charged species.**

Bacteriorhodopsin (bR) is a protein found in the cell membrane of the halophile *Halobacterium halobium* that transforms visible light into chemical energy (1–4). It is composed of one molecule of retinal covalently bound to the ε-amino group of a lysine residue in the surrounding protein via a protonated Schiff base linkage (5, 6). When bR absorbs light, it undergoes the following simplified photochemical cycle:

$$bR_{570} \rightarrow K_{610} \rightarrow L_{550} \rightarrow M_{412} \rightarrow O_{640} \rightarrow bR_{570}.$$

Following the $L_{550} \rightarrow M_{412}$ transformation, protons are ejected from the cell, thus generating an electrochemical gradient. This proton gradient directly drives some metabolic processes such as ATP synthesis and other endergonic processes (1–3, 7–10).

The exact mechanism of this proton pump still remains unclear. However, accumulated evidence indicates that the M_{412} intermediate plays a key role in the photochemical cycle of bR (1, 11). M_{412} is unique in the unprotonated nature of its retinal-lysine Schiff base bond, as compared with the protonated structure characterizing the parent pigment and all other intermediates (1, 12–16). Some studies have inferred that the Schiff base deprotonation is closely associated with the proton pump mechanism (17, 18). Studies of the effect of chemically modified tyrosines on the rate of decay of M_{412} suggest a coupling between some tyrosine residues and M_{412} (19–22).

Information relating to the molecular aspect of M_{412} formation has been obtained by Kalisky *et al.* (17). Their low temperature photolysis studies showed that at temperatures below −80°C, the yield of M_{412} was markedly increased by a change in the pH of the external medium. They proposed that the effect was due to the catalytic action of a protein group with a pK of ≈10 on the rate of the L_{550}-to-M_{412} process. These results, taken along with previous evidence for a deprotonation of a tyrosine residue during the L_{550}-to-M_{412} transition (23–25), suggested that the formation of a tyrosinate ion is a prerequisite for the deprotonation of the Schiff base.

The purpose of this work is to test this proposal by examining simultaneously the rise time of M_{412} and tyrosinate ion at physiological temperature and pH as well as under different perturbations of these.

EXPERIMENTAL

Master slants of the ET1-001 strain of *H. halobium* were generously provided by R. Bogomolni and W. Stoeckenius. The procedure for the growth and purification of the purple membrane was a combination of those outlined by Oesterhelt and Stoeckenius (26) and Becher and Cassim (27).

The experimental arrangement used to monitor the UV and visible absorption changes in bR under the same conditions is basically the same as the one described in ref. 28 without the use of an electromechanical shutter. The only other modification consisted in adding a 0.3 optical neutral density filter acting as a beam splitter and allowing 30% of the probe light to enter a reference RCA 1P28A photomultiplier tube, while the remaining light was focused onto the sample cell. The dynode chain of the photomultiplier was that described by Fenster *et al.* (29). Photolysis of the sample was accomplished with a focused 6-nsec, 532-nm beam obtained from the frequency-doubled output of a neodymium:yttrium/aluminum garnet laser (Quanta Ray, Mountain View, CA). Peak photolysis powers were kept at 50 mW by attenuating the laser beam with a 041 Corning filter.

In the early experiments, we took single-shot photographs of an oscilloscope displaying the transient absorbance. In a second procedure, the output from the oscilloscope was connected to a Biomation 8100 Waveform recorder that was interfaced to a Tracor Northern Digital Signal Analyzer. The output was then recorded on an XY recorder. Our latest experiments involve the output of the scope being connected to a Biomation 805 Waveform recorder that was interfaced to an Apple II computer. This computer was used to signal-average the data and store it on magnetic floppy discs. For the tyrosinate signal, 512 shots were averaged, while the M_{412} signal required only 128 shots. These data, after correction

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: bR, bacteriorhodopsin.

for the baseline, were then sent to a VAX computer on which it was analyzed and plotted via a special computer program. Most of the data were analyzed by this latter method.

For both the temperature and pH studies, the concentration of bR was ≈22 μM in a 1.0-cm pathlength cuvette, assuming an extinction coefficient of 63,000 $M^{-1}\cdot cm^{-1}$ at 570 nm. The temperature was kept constant by using a temperature-controlled cell connected to a water-bath circulator. The temperature was monitored by using a Markson pH/TEMP meter. For the pH studies, the bR was suspended in 0.1 M KH_2PO_4 buffers, which were adjusted to the desired pH by addition of 0.2 M NaOH. The pH values of all of the buffers were checked prior to the experiment. The temperature was maintained at 22.0°C. For the temperature studies, bR was suspended in water without any buffer. In all cases identical conditions were maintained with respect to the bR concentration and laser pulse intensity.

RESULTS AND DISCUSSION

Deprotonation Kinetics of the Schiff Base and Tyrosine. Both the M_{412} and tyrosinate absorption at time t were normalized to the maximum absorbance change observed at 404 and 296 nm, respectively. If the normalized absorption is proportional to the mole fraction of the transient, (X_t), then $1 - X_t$ is the mole fraction of the precursor (L_{550} for M_{412} and tyrosine for tyrosinate), for which:

$$\ln(1 - X_t) = -kt. \quad [1]$$

The constant k is the decay constant of the precursor to the daughter whose absorption is being monitored or, conversely, the rise rate constant for M_{412} or tyrosinate.

Figs. 1 and 2 show these plots for the tyrosinate and M_{412} formations, respectively, at different pH values. Both figures show the presence of more than one component. In the case of the tyrosinate ion, the very fast component in the figure corresponds to changes in the retinal absorption, which are also detectable at 296 nm (30). The positive spike is due to scattering from the incident laser beam which was not totally filtered. The slower component corresponds to the tyrosinate signal and is fitted to Eq. 1. The results are summarized in Table 1.

An attempt was made to fit the results of Fig. 2 to an equation of the form:

$$\ln(1 - X_M) = \ln[C_1\exp(-k_{M\ fast}t) + C_2\exp(-k_{M\ slow}t)], \quad [2]$$

which involves two independent components. The results are also summarized in Table 1. They show that the amplitudes rather than the rate constants of each component seem to change with pH.

Comparison of the rate constants of M_{412} formation with the tyrosinate formation shows that the tyrosinate signal is always slightly slower than the M_{412} formation. This is in disagreement with previous results (28), which were not as precise as the measurements done in this work, and with the results at −1.5°C (24) before correction for changes in retinal absorption at 296 nm (30).

The M_{412} signal was found to decay faster than the tyrosinate signal. Since our semilogarithmic plots are normalized to the absorbance maximum, this could make the M_{412} formation appear to be faster than it actually is. To correct for this decay at long time, we computer-fitted the M_{412} original curves to a biphasic formation and a simple decay through the method of nonlinear least squares (the assumed scheme is $A,B \rightarrow C \rightarrow D$). Although there is evidence that the decay of M_{412} is also biphasic (31–34), the slow decay rate is about 1 order of magnitude smaller than the faster one. This is the reason for taking only one decay rate into consideration. This correction resulted in the same value for the fast rate constant but in a smaller value for the slow rate constant (e.g., $1.16 \times 10^4\ sec^{-1}$ at $7.0 \leq pH \leq 9.6$). This is still slightly larger than the rise constant for the tyrosinate formation. The rest of the discussion in this paper will then be based on the results on Table 1.

The Two Components of M_{412}. As shown in the previous section, the deprotonation of the Schiff base has two components with rate constants independent of pH. Only their relative amplitudes change significantly with pH. Ort and Parson (33) also have observed that the ratio of the fast component to the slow component increased with pH.

Rosenbach *et al.* (18) have proposed a mechanism in

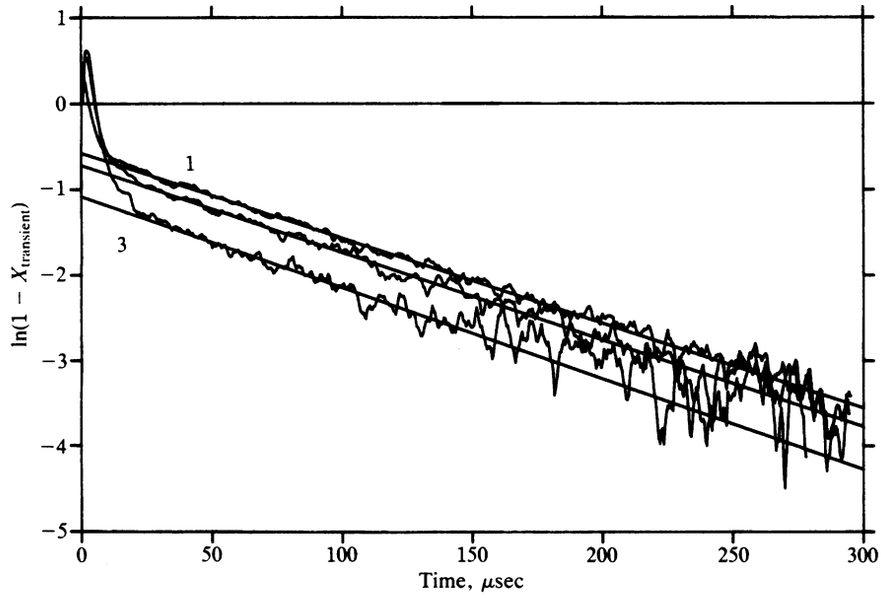

FIG. 1. A plot of $\ln(1 - X_t)$ versus time for the absorbance change at 296 nm (22°C) at pH 9.4 (trace 1), 9.8 (trace 2), and 10.2 (trace 3). The fast component corresponds to changes in the retinal absorption (30). The positive spike is caused by the scattering of the incident laser.

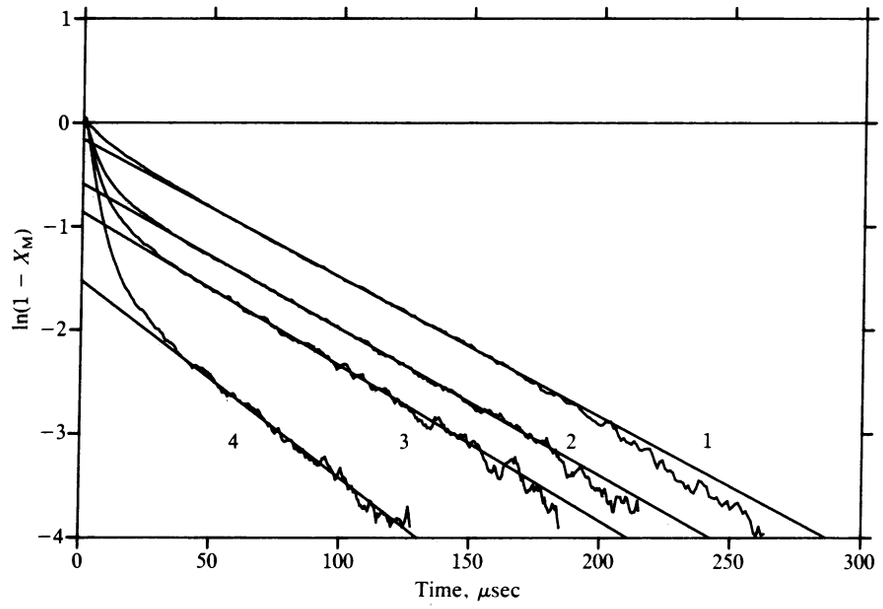

FIG. 2. A plot of ln(1 − X_M) versus time for the absorbance change at 404 nm (22°C) of M_{412} at pH 7.0 (trace 1), 9.4 (trace 2), 9.8 (trace 3), and 10.2 (trace 4). Note that the amplitude of the fast component becomes greater as the pH increases, but the slopes (rate constants) are rather insensitive to pH.

which a tyrosine and a tyrosinate ion are involved in the M_{412} formation as follows:

$$L_{550}[=N\overset{+}{H}—, AH] \rightarrow L'_{550}[=N\overset{+}{H}—, A^-] \rightarrow M_{412}[=N—, A^-],$$

where $=N\overset{+}{H}—$ and AH denote the protonated Schiff base of retinal and the side chain of a tyrosine residue, respectively. According to this model, the L_{550}-to-M_{412} transformation takes place in two sequential steps. In the first, the tyrosine residue deprotonates to form the L'_{550}. This is followed by the deprotonation of the Schiff base (M_{412}). Thus, the deprotonation of a tyrosine residue is a prerequisite to the deprotonation of the Schiff base. The fact that, at physiological temperature and pH, the deprotonation of the Schiff base is found in our work to be faster than the tyrosinate formation sheds doubt on this mechanism.

The presence of two components for the deprotonation of the Schiff base whose rate constants are independent of pH might best be described in terms of two parallel paths. Since the presence of a tyrosinate ion could explain that the M_{412} formation rate at low temperature increases with pH (17), this suggests to us that the two paths might still involve the tyrosine–tyrosinate system but in another manner. We propose that the protonated Schiff base exists at two different sites within the protein. In one site, it is located near a tyrosine (AH), and it slowly deprotonates during the photocycle. In the other site, the protonated Schiff base is near a tyrosinate ion (A^-) and deprotonates rapidly. The rate of the AH $\rightleftharpoons$ A^- + H^+ equilibration is assumed to be slower than the rate of both the L_{550} and M_{412} formations.

In our mechanism, the observed increase in the amplitude of the fast M_{412} component, as the pH increases, is a result of the larger concentration of sites for the fast-deprotonating Schiff base due to an increase in the concentration of the tyrosinate ion. This is different from the model of Rosenbach *et al.* (18) in which the rate of deprotonation is proposed to increase as the pH increases.

Determination of the pK_a of Tyrosine. If the idea of two independent chromophore populations, one near a tyrosine and the other near a tyrosinate, is valid, then the dissociation constant of the tyrosine residue could be determined from the rise curve of M_{412} as follows.

The dissociation constant of the amino acid residue, K_a, is given by

$$K_a = [H^+][A^-]/[HA].$$

This would result in the following equations:

Table 1. The pH dependence of the rate constants* for the M_{412} and tyrosinate formations at 22°C

	$k \times 10^4$ sec^{-1}			
pH	M fast	M slow	Tyr	pK_a†
7.0	15 ± 3 (0.12)	1.3 ± 0.1 (0.88)	0.92 ± 0.04 (0.65)	—
9.0	16 ± 4 (0.40)	1.4 ± 0.1 (0.60)	0.97 ± 0.04 (0.58)	9.2
9.2	15 ± 3 (0.44)	1.4 ± 0.1 (0.56)	0.98 ± 0.04 (0.58)	9.3
9.4	18 ± 3 (0.45)	1.5 ± 0.1 (0.55)	1.0 ± 0.1 (0.55)	9.5
9.6	18 ± 2 (0.55)	1.6 ± 0.1 (0.45)	0.99 ± 0.04 (0.50)	9.5
9.8	17 ± 3 (0.58)	1.7 ± 0.1 (0.42)	1.1 ± 0.1 (0.48)	9.7
10.0	18 ± 2 (0.70)	1.8 ± 0.2 (0.30)	1.1 ± 0.1 (0.39)	9.6
10.2	17 ± 3 (0.77)	2.1 ± 0.2 (0.23)	1.2 ± 0.2 (0.31)	9.7
10.4	22 ± 3 (0.83)	3.4 ± 0.4 (0.17)	1.2 ± 0.1 (0.22)	9.7

*In parentheses are the relative amplitudes for the two M_{412} components and the fraction of the total absorbance change at 296 nm corresponding to the tyrosinate formation.
†pK_a is calculated from Eq. 3 in the text.

$$pK_a = pH - \log[A^-]/[HA]$$
$$= pH - \log C_1/C_2, \quad [3]$$

where C_1 and C_2 are the constants given in Eq. 2 corresponding to the mole fractions of the chromophore near tyrosinate and tyrosine, respectively. C_2 can be determined by extrapolating the long-time-linear least-mean-squares line in Fig. 2 to time $t = 0$. Subtraction of this value from unity gives C_1. The results of using Eq. 3 are shown in Table 1. The pK_a value obtained in this manner is found to be ≈9.6, which is smaller than the value of ≈10 obtained by Fukumoto *et al.* (28) and by us from a plot of the fraction of tyrosinate formed (Table 1) versus pH.

A number of reasons might account for the observed difference in the pK_a values obtained from the two methods. The use of mole fractions instead of activities and the assumption that the absorption spectra of M_{412} in the two sites are identical could introduce errors in the method that monitors the relative amplitudes of the slow and fast components. However, if the difference between the two pK_a values is significant, it might suggest that they correspond to two tyrosines in two slightly different protein environments (with different pK_a values). It is reasonable to assume that the tyrosine(s) that ionize(s) during the cycle is (are) located near the chromophore. It is also reasonable to assume that the tyrosine–tyrosinate couple whose equilibrium is sensed by the protonated Schiff base is near that group too. However, it is possible that a slight difference could be present, sufficiently to produce a deviation of about 0.4 pK_a unit in the two values determined by the two methods.

Another reason that might account for the difference between the two pK_a values is the possibility that they correspond to two different amino acids. The one determined from the absorption at 296 nm can only be assigned to a tyrosine (with aromatic absorption in this region), whereas the one determined from the rise times of the two M_{412} components could be for a lysine. Both of these amino acids have pK_a values of >10 in aqueous solution that could be reduced inside the protein. Although this is indeed a possibility that cannot be discounted from our data, the experiments showing the definite effects of chemical modifications of tyrosine on the proton pump cycle (19–22) favor the tyrosine system. In the discussion below, we shall attribute the presence of two types of Schiff bases to a tyrosine, but we must keep in mind that a lysine is also a possible candidate.

Temperature Studies. Table 2 gives the activation energies and the rate constants as a function of temperature for the formation of the tyrosinate ion and the two components of M_{412}. The formation of the latter seems faster than the tyrosinate formation at all temperatures studied. However, the difference becomes smaller as the temperature decreases. It is interesting that the observed values for the activation energy of each transient are similar to hydrogen bond energies. This suggests the involvement of hydrogen bonds, and it might or might not support theories involving hydrogen-bonded chain structures to transport protons across the membrane during the photocycle (35).

Table 2. The activation energies and the rate constants for the M_{412} and tyrosinate formations in water (no buffer used)

T, °C	M fast	M slow	Tyr
	$k \times 10^4$ sec^{-1}		
25	13 ± 2	1.8 ± 0.1	1.4 ± 0.1
20	8.1 ± 0.4	1.3 ± 0.1	0.99 ± 0.04
15	6.3 ± 0.4	0.93 ± 0.05	0.76 ± 0.04
10	4.5 ± 0.2	0.66 ± 0.04	0.56 ± 0.04
5	—	0.48 ± 0.04	0.42 ± 0.04
	E_a, kJ/mol		
	42 ± 3	47 ± 3	39 ± 3

The fact that the rate constant of the fast component of M_{412} is an order of magnitude larger than that for the slow component must be attributed to a difference in entropy rather than energy of activation.

SUMMARY OF IMPORTANT RESULTS AND POSSIBLE CONCLUSIONS

The important results and possible conclusions of this research can be summarized as follows.

(*i*) The kinetics of M_{412} formation show two components with a 1-order-of-magnitude difference in their rise times.

(*ii*) The slow component of M_{412} constitutes the largest fraction of M_{412} formed at physiological temperature and pH.

(*iii*) The formation rate of the slow component of M_{412} is slightly faster than that for the tyrosinate ion at all of the pH and temperature ranges studied. This eliminates the hypothesis that the formation of the tyrosinate ion is a prerequisite for the deprotonation of the Schiff base.

(*iv*) The rate constants of the fast component of M_{412} formation is independent of pH. The "apparent" rate constant of the slow component seems to increase slightly with the increasing pH. This is explained by our inability to resolve the two components completely as the amplitude of the fast component increases with increasing pH.

(*v*) The ratio of the amplitudes of the fast and slow components of M_{412} is found to be sensitive to pH and can be used to calculate a pK_a value of about 9.6 at each pH studied. This suggests that the two rise times for M_{412} are for protonated Schiff bases in two different environments (sites), one located near an amino acid with a pK_a of about 9.6 and the other near its conjugate base. In bacteriorhodopsin, the only amino acids with a pK_a value near 10 are tyrosine and lysine.

(*vi*) The activation energy for the formation of the tyrosinate ion or any of the two components of M_{412} is about 40 kJ/mol, which is comparable to hydrogen bond energies. This might suggest the involvement of hydrogen bonds [in chains (35, 36) or otherwise] in the deprotonation process of the protonated Schiff base and tyrosine.

(*vii*) The large difference between the rates of formation of the slow and fast components of M_{412} is attributed to a large difference in their entropy of activation, which could result from the structural difference between the environment around the tyrosine and that around the tyrosinate ion near the chromophore.

POSSIBLE MODEL

In the model proposed by Rosenbach *et al.* (18), the deprotonation of the Schiff base takes place as a result of a reduction of its pK_a. If one assumes this to be true, the fact that the deprotonation of the Schiff base and that of the tyrosine occur on a similar time scale might suggest the involvement of a third species that reduces their pK_a to the value of the physiological pH (around 7). During the cycle, the relative distance between a positively charged species and the protonated Schiff base, as well as its nearby tyrosine, could be reduced. This would result in a decrease in the pK_a value of the Schiff base because of the repulsion with its positive charge* and a decrease in the pK_a of tyrosine as a result of the attraction to the negatively charged tyrosinate ion that is produced.*

The rate constants of the deprotonation of the Schiff base and tyrosine are either determined by the rate of the relative

*The change in the pK_a is assumed to result from a change in the ΔH of the ionization reactions; the change in ΔS is not presently known.

motion of the positive charge toward the Schiff base and tyrosine or the rate by which proton acceptors (in a chain or free) position themselves appropriately with respect to the proton donors. Both mechanisms undoubtedly would involve the protein motion, which implies the breaking and making of H bonds, accounting for the observed activation energies.

The larger entropy of activation for forming the fast M_{412} component could result from the difference in the organizational entropy, when the positive charge approaches an environment having a tyrosinate ion or one having a neutral tyrosine. A tyrosinate ion would tend to have a stronger interaction with the surrounding medium than would the neutral amino acid residue. Thus, the change in entropy produced by the incoming positive charge could be rather different in both cases.

As to the identity of the positively charged species, it can be an amino acid like arginine or a bound inorganic cation—e.g., Ca^{2+}, Mg^{2+}, ... In support of the bound inorganic cation is the work of Chang *et al.* (37), which shows that the removal of these cations eliminates the formation of M_{412}. Their work also suggests that the form of bR present without these cations is similar to the blue membrane present at very low pH (38–42). The batho form of the latter has recently been found to form in 30 psec and to decay within the same time scale as that for normal bR (43). If so, then the L_{550}-to-M_{412} part of the cycle might not take place in the absence of metal cations.

We thank Drs. B. Honig, T. Ebrey, S. Seltzer, and R. Mathies for stimulating discussions. This work is supported by the Department of Energy, Office of Basic Sciences. P.D. thanks the Natural Sciences and Engineering Research Council of Canada for a postdoctoral fellowship.

1. Stoeckenius, W., Lozier, R. & Bogomolni, R. A. (1979) *Biochim. Biophys. Acta* **505,** 215–278.
2. Henderson, R. (1977) *Annu. Rev. Biophys. Bioeng.* **6,** 87–109.
3. Honig, B. (1978) *Annu. Rev. Phys. Chem.* **29,** 31–57.
4. Caplan, S. R. & Ginzburg, M., eds. (1978) *Energetics and Structure of Halophilic Microorganisms* (Elsevier/North-Holland Biomedical, New York).
5. Oesterhelt, D. & Stoeckenius, W. (1971) *Nature (London) New Biol.* **223,** 149–152.
6. Bridgen, J. & Walter, I. D. (1976) *Biochemistry* **15,** 792–798.
7. Dencher, N. & Wilms, M. (1975) *Biophys. Struct. Mech.* **1,** 259–271.
8. Lozier, R., Bogomolni, R. A. & Stoeckenius, W. (1975) *Biophys. J.* **15,** 955–962.
9. Belliveau, J. W. & Lanyi, J. K. (1977) *Arch. Biochem. Biophys.* **178,** 308–314.
10. Stoeckenius, W. & Bogomolni, R. A. (1982) *Annu. Rev. Biochem.* **51,** 587–616.
11. Ottolenghi, M. (1980) *Adv. Photochem.* **12,** 97–200.
12. Lewis, A., Spoonhower, J., Bogomolni, R. A., Lozier, R. H. & Stoeckenius, W. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4462–4466.
13. Marcus, M. & Lewis, A. (1977) *Science* **195,** 1328–1330.
14. Aton, B., Doukas, A. G., Callender, R. H., Becher, B. & Ebrey, T. G. (1977) *Biochemistry* **16,** 2995–2999.
15. Braiman, M. & Mathies, R. (1980) *Biochemistry* **19,** 5421–5428.
16. Bagley, K., Dollinger, G., Eisenstein, L., Singh, K. & Zimanyl, L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4972–4976.
17. Kalisky, O., Ottolenghi, M., Honig, B. & Korenstein, R. (1981) *Biochemistry* **20,** 649–655.
18. Rosenbach, V., Goldberg, R., Gilon, C. & Ottolenghi, M. (1982) *Photochem. Photobiol.* **36,** 197–201.
19. Konishi, T. & Packer, L. (1978) *FEBS Lett.* **92,** 1–4.
20. Scherrer, P., Packer, L. & Seltzer, S. (1981) *Arch. Biochem. Biophys.* **212,** 589–601.
21. Lemke, H.-D. & Oesterhelt, D. (1981) *Eur. J. Biochem.* **115,** 595–604.
22. Lam, E., Seltzer, S., Katsura, T. & Packer, L. (1983) *Arch. Biochem. Biophys.* **227,** 321–328.
23. Bogomolni, R. A., Stubbs, L. & Lanyi, J. (1978) *Biochemistry* **17,** 1037–1041.
24. Hess, B. & Kuschmitz, D. (1979) *FEBS Lett.* **100,** 334–340.
25. Bogomolni, R. A. (1980) in *Bioelectrochemistry*, ed. Keyzev, H. (Plenum, New York), pp. 83–95.
26. Oesterhelt, D. & Stoeckenius, W. (1974) *Methods Enzymol.* **31,** 667–678.
27. Becher, B. & Cassim, J. Y. (1975) *Prep. Biochem.* **5,** 161–178.
28. Fukumoto, J. M., Hanamoto, J. H. & El-Sayed, M. A. (1983) *Photochem. Photobiol.* **39,** 75–79.
29. Fenster, A., LeBlanc, J., Taylor, W. & Johns, H. (1973) *Rev. Sci. Instrum.* **44,** 689–690.
30. Kuschmitz, D. & Hess, B. (1982) *FEBS Lett.* **138,** 137–140.
31. Korenstein, R. & Hess, B. (1977) *Nature (London)* **270,** 184–186.
32. Korenstein, R., Hess, B. & Kuschmitz, D. (1978) *FEBS Lett.* **93,** 266–270.
33. Ort, D. R. & Parson, W. W. (1978) *J. Biol. Chem.* **253,** 6158–6164.
34. Ohno, K., Takeuchi, Y. & Yoshida, M. (1981) *Photochem. Photobiol.* **33,** 573–578.
35. Nagle, J. F. & Tristram-Nagle, S. (1983) *J. Membr. Biol.* **74,** 1–14.
36. Nagle, J. F. & Morowitz, H. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 298–302.
37. Chang, C.-H., Suh, C.-K., Govindjee, R. & Ebrey, T. (1984) *Biophys. J.* **45,** 210 (abstr.).
38. Edgerton, M. E., Moore, T. A. & Greenwood, C. (1978) *FEBS Lett.* **95,** 35–39.
39. Tsuji, K. & Rosenheck, K. (1978) *FEBS Lett.* **98,** 368–372.
40. Mowery, P. C., Lozier, R. H., Chae, Q., Tseng, T. W., Taylor, M. & Stoeckenius, W. (1979) *Biochemistry* **18,** 4100–4107.
41. Muccio, D. D. & Cassim, J. Y. (1979) *J. Mol. Biol.* **135,** 595–609.
42. Maeda, A., Iwasa, T. & Yoshizawa, T. (1980) *Biochemistry* **19,** 3825–3831.
43. Kobayashi, T., Ohtani, H., Iwai, J.-I., Ikegami, A. & Uchiki, H. (1983) *FEBS Lett.* **162,** 197–200.