Proc. Natl. Acad. Sci. USA
Vol. 75, No. 2, pp. 549–553, February 1978
Chemistry

The molecular mechanism of excitation in visual transduction and bacteriorhodopsin

(rhodopsin photochemistry/protein dynamics/dipolar states and allosteric mechanisms in biology/proton translocation/ion gate molecular regulation)

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Communicated by Harry B. Gray, November 7, 1977

ABSTRACT **An electronic theory of excitation is proposed and described in terms of a three-dimensional excited/ground-state energy surface which elucidates the photochemical and excited-state dynamics of rhodopsins. In this theory the primary action of light is to produce significant electron redistribution in the retinal, thereby generating new interactions that vibrationally excite and perturb the ground-state protein conformation. Thus, light energy causes charge redistribution in the retinal and induces transient charge-density assisted bond rearrangements (such as proton translocation) in the protein structure which is stabilized by subsequent retinal structural alteration. In this theory the isoprenoid chain of the retinal is considered a structurally pliable molecular entity that can generate charge redistributions and can subsequently achieve intermediate conformations or various isomeric states to minimize the energy of the new protein structure generated by light. Thus, the 11-*cis* to all *trans* isomerization of the retinylidene chromophore is not considered a primary mechanism of excitation. An alternate biological role for this molecular process (which is eventually completed in all photoreceptors but not in bacterial rhodopsins) is to provide the irreversibility needed for effective quantum detection on the time scale of a neural response. Finally, it will be demonstrated that this mechanism, which readily accounts for the photophysical and photochemical data, can also be restated in terms of the Monod, Wyman, and Changeux terminology suggesting that aggregates of these pigments may function allosterically.**

This paper proposes a generalized mechanism of excitation in visual transduction and bacteriorhodopsin that accounts for the spectral similarities observed in all rhodopsin-like systems while accounting for their functional diversity. Unlike previous descriptions of the excitation mechanism (1), this theory is based on the effect the excited state of retinal has on the conformational state of the protein. The result of this approach, which views a protein's conformation as a dynamically fluctuating and responding entity, elucidates the photochemical and excited-state dynamics of rhodopsins in terms of a unique excited-state/ground-state energy surface. In essence, the theory not only explains a large fraction of the recent data on rhodopsin* and bacteriorhodopsin,† but also demonstrates how the energy used in the subsequent steps of transduction is stored in the photochemical event.

Experimental observations

There are several experimental results that must be accounted for in any mechanism of excitation. In both rhodopsin and bacteriorhodopsin, absorption of a photon produces a high energy species (19, 20) that has a red-shifted absorption maximum relative to the parent pigment (21–23) (Fig. 1). This red-shifted species is produced in <6 psec (24, 25) in both these systems. A kinetic argument of Rosenfeld *et al.* (26), based on the data of Kropf *et al.*(27), has shown that all batho intermediates lie at least 13 kcal higher in energy than their respective rhodopsins.

In addition to the above constraints, the discovery of light emission from bacteriorhodopsin (28) has shown (28, 29) that the observed emission emanates from a minimum in the excited-state surface that can be populated from bacteriorhodopsin. However, the above experiments have not been able to demonstrate that this minimum can be populated from the batho intermediate. Furthermore, room temperature (30) and low temperature (77 K) (29) picosecond emission spectroscopy and picosecond absorption spectroscopy (25, 31, 32) have shown that the photochemistry occurs directly from the state produced by vertical excitation, whereas emission occurs by a parallel competing pathway from a state or states that are entered by molecules that do not proceed to the batho intermediate.

The final constraint governing rhodopsin's photochemistry is the important observation that in both rhodopsin and bacteriorhodopsin the sum of the forward (ϕ_{PF}) and reverse (ϕ_{PR}) photochemical quantum yields is one, even though the individual yields differ considerably in these pigments (26, 33, 34). This is a characteristic of many photochemical reactions, including proton transfer, isomerization, etc., and *does not* point to a specific mechanism. What it does indicate, however, is that all other parallel competing pathways of decay are small in comparison to photochemistry and that the two species rhodopsin/bacteriorhodopsin and batho have a common excited state. At first sight this latter constraint seems to contradict the observation that the chromophore in the batho intermediate exhibits different emission behavior. However, our model readily accounts for all these observations.

The excitation mechanism

The energy surface that explains the above experimental facts and defines our mechanism is seen in Fig. 2. The molecule R in its ground state (left of the diagram) absorbs a photon. As has

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Abbreviation: PDC, protein deformation coordinate.

* *Rhodopsin is a quantum detector* and is the protein in photoreceptor cells that absorbs a single photon, initiating the processes in these cells that generate a neural response. It is composed of 11-*cis*-retinal complexed to a membrane glycopolypeptide aqueous matrix called opsin (Fig. 1A) by a protonated Schiff base linkage (2–8) to a lysine residue (9, 10). All photoreceptor rhodopsins produce an all *trans* chromophore *at the end* of the sequence of events depicted in Fig. 1A. Thus, on the basis of this observation, it was suggested that the 11-*cis* to all *trans* isomerization is the molecular mechanism of excitation in rhodopsin and is the only action of light (1).

† *Bacteriorhodopsin is an energy converter* (11–13) and was discovered in *Halobacterium halobium* (14). It acts as a light-driven proton pump. The molecule in the proton pumping cycle is initially composed of all *trans*-retinal (15) complexed to a membrane polypeptide aqueous matrix called bacterio-opsin (Fig. 1*B*) by a protonated Schiff base linkage (16) to a lysine residue (14). There is no evidence for an 11-*cis* to *trans* or a *trans* to 11-*cis* isomerization (17, 18).

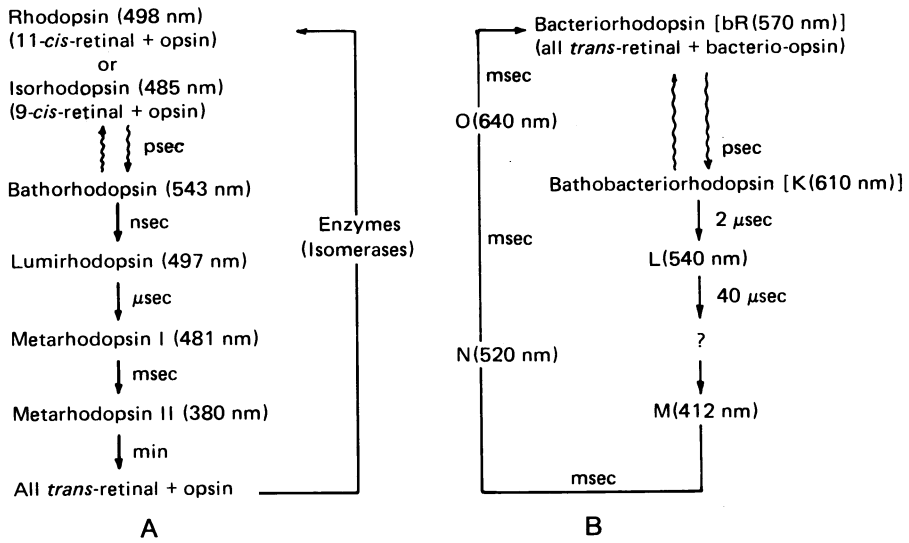


FIG. 1. The photochemically induced (wavy arrows) thermal sequence of events in (*A*) bovine rhodopsin and (*B*) bacteriorhodopsin. After absorbing a single photon, visual pigments either in the 11-*cis* (rhodopsin) or 9-*cis* (isorhodopsin) form all follow the sequence shown in *A* to metarhodopsin I. At that point, invertebrate rhodopsins have a slightly altered thermal sequence. Photochemical back reactions to rhodopsin, isorhodopsin, or bacteriorhodopsin from any of the thermal intermediates occur in all pigments, although for invertebrates relative percentages of isorhodopsin and rhodopsin produced by such a process vary depending on the intermediate in question. Furthermore, bacteriorhodopsin exhibits an additional feature not found in visual pigments. Bacteriorhodopsin displays thermal reversibility whereas visual pigments display irreversibility on the time scale of a neural response, which is msec.

been shown experimentally (35) and theoretically (36, 37), absorption of a photon causes significant electron redistribution in the retinal, increasing the positive charge density near the ring of the retinylidene chromophore—the absorbing species of the protein. Our mechanism suggests that the protein's active site becomes vibrationally excited as a result of the electronic excitation of the retinal and begins to deform in response to the new interactions generated by the significant electron redistribution in the chromophore. In effect, the transient charge-density in the retinal chromophore induces bond rearrangement in the protein structure. Such a protein deformation (e.g., proton translocation from one group in opsin to another, bond rotation, etc.) lowers the energy of the excited retinal-protein complex along a third dimension in Fig. 2, the protein deformation coordinate (PDC). Simultaneously, as this protein deformation lowers the energy of the excited retinal along PDC it also raises the energy of the ground state along PDC. This occurs because the original protein conformation is the lowest energy structure for the ground state of the system before electronic excitation. The result of lowering the excited-state energy and raising the ground-state energy creates at some protein deformation coordinate, P_x, a crossing between the ground- and excited-state surfaces. Up to this point, all the molecular rearrangement has occurred in the protein along PDC as a result of the light-induced electronic rearrangement in the retinal. However, once the retinal crosses at P_x back onto the ground-state surface, it has two choices: it can relax into either the minimum labeled R or batho. If the retinal chooses to return to its original electron distribution in R, then the protein relaxes along PDC to its original structure. On the other hand, if the retinal chooses to relax into the batho form, then the retinal has to adjust its conformation along the Retinal reaction coordinate to minimize the new protein structure (P′). Such an alteration in the retinal structure would act to relax the vibrationally excited protein into the batho minimum at P′, R_3. Similarly, if a molecule is excited from the batho form, it reaches the same crossing at P_x very effectively. Therefore, ϕ_{PF} and ϕ_{PR} are defined solely by the interaction of the excited- and ground-state surfaces at P_x and not by whether the molecule is excited from R or the batho form.

Now let us consider the fate of those molecules that finally emit light and do not proceed by the wavelength-independent surface along PDC to photochemistry. These molecules (1 in 10,000 at room temperature, significantly higher as the temperature is lowered) are deflected by any "roughness" in the PDC surface and enter states near zero PDC that are not accessible to molecules in which protein deformation to P_x has taken place. Thus, the emitting state (or possibly even states), with a lifetime of 15 psec at physiological temperatures (30, 31) or 40 psec at 77 K (29), is drawn to indicate zero PDC and, as is observed (31), has dynamic properties that are not correlated to the rate of production of the photochemical product at physiological temperatures or at 77 K. This is clearly understood in terms of our three-dimensional illustration in Fig. 2. The excited state (or possibly even states) at zero PDC can be populated from R and can emit light, while the excited chromophore in a protein conformation corresponding to batho cannot effectively enter these emitting states even though R and the batho intermediate have a common excited state.

Insights and predictions

The above mechanism can be viewed as a simple extension of the ideas that have very successfully explained the color of visual pigments (5, 35, 38–41). These ideas are based on the suggestion that the protein stabilizes the vertically excited state of the chromophore in which light induces significant charge redistribution. Our mechanism extends this idea and suggests that the excited-state charge redistribution in the retinal induces a protein conformational change which eventually produces the batho intermediate. Thus, the altered protein environment of the chromophore could readily explain at least part of the "red shift" observed in going from rhodopsin to bathorhodopsin. Furthermore, our model also allows the retinal to assume intermediate conformations to minimize the energy of the new protein structure in bathorhodopsin. Therefore, this ground-state destabilization of the retinal and the protein could account for the 13 kcal of energy stored in the batho intermediate. It is significant that part of the energy absorbed by the retinal is stored in the protein in which the subsequent steps in the transduction process occur.

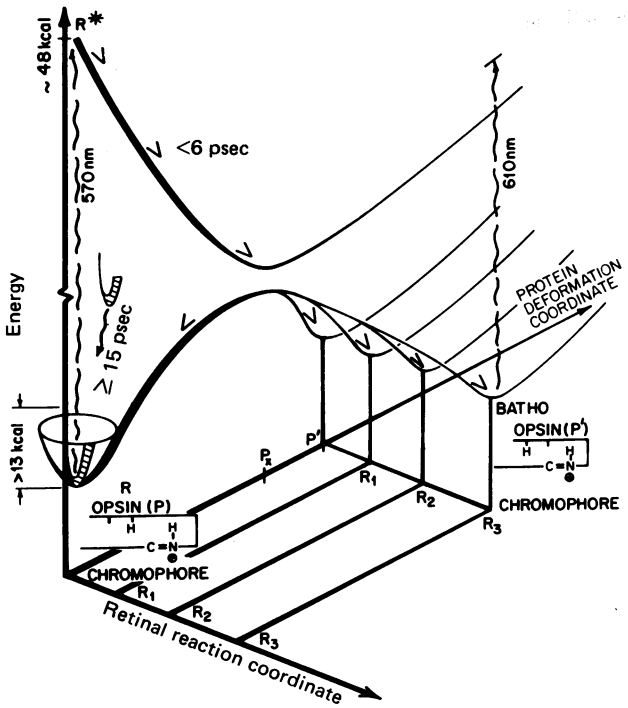


FIG. 2. The three-dimensional ground-state and excited-state energy surface that defines the molecular mechanism of excitation in rhodopsin and bacteriorhodopsin. The surface shown is described in *The excitation mechanism* and is drawn with time scales and absorption maxima observed in bacteriorhodopsin. The wavy arrows indicate photon absorption or emission; V indicates the path of photochemistry. Emission occurs from a state near zero Protein deformation coordinate defined only by the axes Energy and Retinal reaction coordinate. Photochemistry to the batho intermediate from R* occurs first along the Protein deformation coordinate and then, after the minimum at P′ is reached, along the Retinal reaction coordinate to R_3. Diagrams depicting *one* possible protein deformation are drawn alongside R and Batho. The nature of the protein deformation will be determined by the protein structure and may vary in rhodopsin and bacteriorhodopsin. The surfaces along the Protein deformation coordinate are not necessarily smooth. In bacteriorhodopsin, at room temperature, the roughness of this surface (probably caused by the surrounding protein structure) deflects 1 in 10,000 molecules to the emitting state near zero PDC with essentially no effect on the photochemistry. However, at temperatures approaching 4 K this roughness may affect the evolution of the batho intermediate.

It is also encouraging that this mechanism of excitation can be directly related to the transduction process. The primary protein conformational change to P′ could initiate in bacteriorhodopsin a series of proton translocations along a stepladder of proton-binding groups with different pKs *eventually* deprotonating the Schiff base proton. In fact, using kinetic resonance Raman spectroscopy, we were able to demonstrate (42) that the state of protonation of the protonated Schiff base is altered with a time constant of ~20 μsec at physiological temperatures and pH, whereas the rise time of the batho intermediate occurs in a picosecond (25, 31).

In photoreceptor pigments, however, the primary excitation mechanism depicted in Fig. 2 does not imply that rhodopsin, like bacteriorhodopsin, is a light-driven proton pump. This is suggested by the data of Hagins and Yoshikami (43), who failed to detect light-induced pH changes in rod outer segments but did observe changes in the intracellular Ca^{2+} ion concentration. These observations may suggest that the new protein structure results in a release of certain transmitter substances such as Ca^{2+}. In addition, resonance Raman results have shown that in both squid (4, 41) and bovine (6) rhodopsin the vertically excited state charge redistribution in the chromophore is maintained to a degree in the batho intermediate. This result can be explained by our protein deformation model since the protein deforms as a result of the excited state electron distribution in the chromophore and can partially stabilize this redistribution. Thus, such a protein deformation, which can be viewed as either stabilizing the excited state polarity of the chromophore or inducing polarity in the chromophore's ground state charge distribution, may also control the ion movements in the vicinity of the disc membrane initiated by the new protein conformation, P′.

In addition to the above insights, our model also provides understandable answers to other puzzling experimental data. For example, it explains why various rhodopsins and bacteriorhodopsin analogs, which do not seriously alter the ability of retinal to undergo excited-state electron redistribution and do not directly modify opsin, do form functioning pigments (44) and can form batho intermediates (45). It also predicts that the timescale for transforming rhodopsin to bathorhodopsin may not be <6 psec in all pigments. This timescale will be defined by the nature of the opsin conformation surrounding the chromophore. It may therefore vary in different species and will be unrelated to such processes as isomerization of the retinal. Furthemore, the protein conformation will define not only the kinetic transformation from rhodopsin to bathorhodopsin, but will also affect the timescales of all subsequent transformations. Therefore, by genetically altering opsin, a species could easily regulate not only the absorption maximum of the pigment, but also the rate of appearance of the various intermediates, thereby controlling the time for relaxing the charge redistribution in the chromophore and affecting the times for transduction and generation of a neural response.

Retinal's structure in bathorhodopsin

The lack of an 11-*cis* to all-*trans* isomerization in bacteriorhodopsin and the similarity of the spectral transformations observed in this pigment and all rhodopsins could be interpreted as strong evidence against the hypothesis that such an isomerization is responsible for the primary red shift in the spectrum when the batho intermediate is produced. This is supported by resonance Raman experiments on photoreceptor bathorhodopsins (5, 6, 41). These experiments show that the spectra of the batho species differs markedly from the spectrum of a model all-*trans* protonated Schiff base of retinal (5) even though the spectra of the later intermediates in photoreceptor rhodopsins are reproduced accurately by this model compound (5). However, in spite of the above evidence, there has been a reluctance to reevaluate the fundamental nature of the 11-*cis* to all-*trans* hypothesis. A primary reason was the observation that rhodopsin (containing the 11-*cis* isomer) and isorhodopsin (containing the 9-*cis* isomer) both reach the same batho intermediate (Fig. 1*A*). It was felt that this could only mean that the retinal in the batho form is *trans* since this was apparently the only common configuration to both the 9-*cis* and 11-*cis* isomer. We have reconsidered this last point in terms of our theory and the observed reduction of the C=C stretching frequency in the resonance Raman spectrum of the batho intermediate. In view of the above observation, that the C=C stretch is reduced in frequency, we suggest that only in photoreceptor rhodopsins [which combine with 11-*cis*- and 9-*cis*-retinal (46, 47) and, therefore, unlike bacteriorhodopsin (48), exhibit conformational freedom between C_9–C_{12}] do torsional motions occur around the weakened C_9–C_{10} and C_{11}–C_{12} retinal double bonds (Fig. 3). This torsional motion can occur subsequent to the light-induced protein structural change to minimize the conforma-

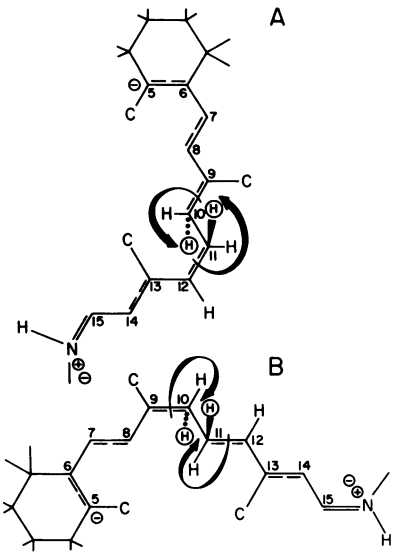


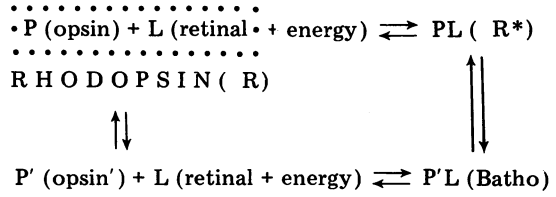
FIG. 3. A possible out-of-plane motion around the perturbed retinal double bonds at C_9-C_{10} and C_{11}-C_{12} in batho (photoreceptor) rhodopsins. This motion can eventually generate a common intermediate chromophore structure from retinal in either the (*A*) 11-*cis* or (*B*) 9-*cis* isomeric form. The motion can be simulated by rotating *as a unit* C_{10} and C_{11} out of the plane of the paper as depicted by the arrows through the C_9–C_{10} and C_{11}–C_{12} bonds. The arrows naturally also represent simultaneous rotation out of the plane of the paper of the Hs attached to C_{10} and C_{11}, which are depicted out-of-plane at possible new positions with circles around the Hs. A common position for C_{13} has to be chosen in *A* and *B* in order to get the same intermediate structure from both isomers. Double bonds are represented by either = or — to indicate variability in the strength of the double bonds in the batho form, as indicated by the C=C and C=N stretching frequencies in the resonance Raman spectra of this species. In addition, the above torsional motion may participate in generating the intense torsional modes observed between 800 and 900 cm^{-1} in the resonance Raman spectra of photoreceptor bathorhodopsins. Furthermore, subsequent rotations around C_{11}-C_{12} and then C_9-C_{10} can easily achieve an all-*trans* conformation by metarhodopsin I. The driving force for such rotations would be both the conformational energy of the protein and the desire of the chromophore to achieve planarity. Finally, the structure of the retinal in bathobacteriorhodopsin cannot be determined thus far from the resonance Raman spectra. According to our theory, however, it will be solely determined by the chromophore conformation that can minimize the energy of the new protein structure achieved as a result of electron redistribution in the excited state of the chromophore.

tional energy of the protein. Thus, this could be the first step in a unique opsin-assisted retinal reaction surface in photoreceptor rhodopsins and may participate in generating at least one, if not both, of the intense 800 cm^{-1} torsional modes observed in the batho intermediate of photoreceptor rhodopsins (5, 6, 41). In fact, as is described in Fig. 3, it can easily be shown with space-filling molecular models that a retinal perturbed in such a fashion can initially be in either the 9-*cis* or 11-*cis* form and still reach a common batho conformation that is not all *trans*. Thus, these arguments demonstrate that even though the fundamental mechanism of excitation can be the same in both photoreceptor and bacterial rhodopsins, the different opsins in these two pigments can shape the reaction surface of the retinal in a way that either permits or denies an eventual 11-*cis* to all-*trans* isomerization.

The 11-*cis* to *trans* isomerization

What then is the biological role of the 11-*cis* to *trans* isomerization that is eventually observed in *all* photoreceptor pigments? To answer this question and to understand why such an isomerization does not occur in bacteriorhodopsin, we have to reconsider the widely differing biological roles of photoreceptor and bacterial rhodopsins. *Photoreceptor rhodopsins are quantum detectors, while bacteriorhodopsin is an energy converter.* An essential element of a quantum detector is irreversibility. The 11-*cis* to *trans* isomerization clearly provides the system with this molecular irreversibility on the timescale of visual transduction and explains the primacy of the 11-*cis* to *trans* isomerization in vision. On the other hand, an energy converter must use a molecular mechanism that minimizes such conformational changes and maximizes reversibility. Thus, bacteriorhodopsin has evolved a system to accomplish these fundamental molecular objectives of this unique biological solar energy converter with reversibility that finally results in reformation of bacteriorhodopsin in milliseconds and without unnecessary endoenergetic conformational changes. What is sacrificed in the process, by over a factor of two (33, 34), is the high quantum efficiency of photoreceptor pigments, which is also an essential aspect of a quantum detector.

In conclusion, the objective of this paper was to present a mechanism and an energy surface that could account for all the photophysical and photochemical data on rhodopsin and bacteriorhodopsin. In view of the above objective, it is interesting that the resulting molecular mechanism of excitation can be restated in the terminology of Monod, Wyman, and Changeux (49) that has effectively described many protein-mediated cellular processes. This relationship between our model for the excitation process and the Monod–Wyman–Changeux terminology can be seen in the illustration below:



where P is the R state and P′ is the T state in the Monod–Wyman–Changeux description (49). In essence, this states that the addition of light to R produces an active complex R* which induces a protein conformational change represented as P′L or batho. The subsequent intermediates produce P′ + L; then enzymes in photoreceptor rhodopsins or thermal energy in bacteriorhodopsin complete the cycle on different timescales. If, indeed, this (49) and other similar mechanisms (e.g., the Koshland–Nemethy–Filmer model (50) apply to rhodopsin, then our detailed energy surface also can be used to probe theoretically and experimentally these mechanisms in greater detail. Furthermore, what is particularly significant about this representation of our mechanism of excitation is the fact that it readily accounts for the rate of certain forms of dark adaptation (formation of P + L), which have been shown (51) to be proportional to the concentration of bleached rhodopsin (P′). This is exactly what the Monod–Wyman–Changeux representation would predict for a reaction in which the rate constant for formation of P + L is much larger than the rate constant for dissociation of P + L into P′ + L, as is the case in rhodopsin.

In these terms not only can we now understand such phenomena as the prolonged depolarizing after potential in invertebrates (52) (which is proportional to the concentration of acid metarhodopsin in the cell), but also, since the Monod–Wyman–Changeux mechanism was originally formulated to describe allosteric behavior in proteins, it is quite possible that a single photon inducing a P′L state in one rhodopsin could also induce a new structural state in many neighboring rhodopsins in the disc membrane. This new structural state may release

certain transmitters (e.g., Ca^{2+}), and it is experimentally known that light absorption causes detectable increases in the Ca^{2+} concentration in rod outer segments (43). It is also conceivable that this increase in Ca^{2+} concentration in the cell and other changes in cytochemistry may alter the conformation of the remaining rhodopsins in the cell, making subsequent photon absorption less effective in establishing these new transmitter-releasing structural states. Such an effect could readily explain observations on the loss of visual sensitivity (53). Finally, our molecular mechanism of charge redistribution followed by a protein conformational transition may also apply to other biological energy transduction processes, such as photosynthesis (reaction centers) and electron transport.

Note Added in Proof. After this paper was submitted, a paper appeared that supports our suggestion of a protein deformation coordinate. Peters *et al.* (54) have experimentally observed a 7-fold decrease in the rate of appearance of the bathorhodopsin absorption in D_2O. They interpreted these data as evidence that the Schiff base proton, the only exchangeable proton on the chromophore, changes its position when bathorhodopsin is formed. As they noted, this would alter the C=N bond, making it more of a single bond. However, resonance Raman results, which show no change in the C=N frequency in going from rhodopsin to bathorhodopsin (19), argue strongly against the explanation of Peters *et al.* Thus, the critical exchangeable proton changing its position must be in the opsin matrix and must control the onset of bathorhodopsin's absorption.

I acknowledge the hospitality of the Division of Chemistry and Chemical Engineering at the California Institute of Technology. This work was completed while I was a visiting professor at that institution from January to June 1977. I also acknowledge stimulating discussions with Profs. A. Szabo, W. Goddard, M. Wolbarsht, S. Lewis and R. Hoffman. I also thank D. Collins, R. Cookingham, M. Marcus, G. Perreault, J. Spoonhower, M. Sulkes, A. Lemley, and B. Ehrenberg for experimental contributions during the past 6 years. This work was supported by Grant EY 01377 from the National Institutes of Health, the Alfred P. Sloan Foundation, Research Corporation, a Type G American Chemical Society Petroleum Research Fund grant, and a contract from the Naval Air Systems Command.

1. Wald, G. (1968) *Science* **162,** 230–239.
2. Lewis, A., Fager, R. S. & Abrahamson, E. W. (1973) *J. Raman Spectrosc.* **1,** 465–470.
3. Lewis, A. & Spoonhower, J. (1974) in *Neutron, X-ray and Laser Spectroscopy in Biophysics and Chemistry,* eds. Yip, S. & Chen, S. (Academic Press, New York), pp. 347–376.
4. Lewis, A. (1976) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35,** 51–53.
5. Sulkes, M., Lewis, A., Lemley, A. & Cookingham, R. E. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4266–4270.
6. Oseroff, A. R. & Callender, R. H. (1974) *Biochemistry* **13,** 4243–4248.
7. Callender, R. H., Doukas, A., Crouch, R. & Nakanishi, K. (1976) *Biochemistry* **15,** 1621–1629.
8. Mathies, R., Oseroff, A. R. & Stryer, L. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1–5.
9. Bownds, D. (1967) *Nature* **216,** 1178–1181.
10. Akhtar, M., Blosse, P. T. & Dewhurst, P. B. (1967) *Chem. Commun.* **13,** 631–632.
11. Oesterhelt, D. & Stoeckenius, W. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2853–2857.
12. Danon, A. & Stoeckenius, W. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1234–1238.
13. Racker, E. & Stoeckenius, W. (1974) *J. Biol. Chem.* **249,** 662–663.
14. Oesterhelt, D. & Stoeckenius, W. (1971) *Nature New Biol.* **233,** 149–152.
15. Oesterhelt, D., Meentzen, M. & Schumann, L. (1973) *Eur. J. Biochem.* **40,** 453–463.
16. Lewis, A., Spoonhower, J., Bogomolni, R. A., Lozier, R. H. & Stoeckenius, W. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4462–4466.
17. Jan, L. Y. (1975) *Vision Res.* **15,** 1081–1086.
18. Pettei, M. J., Yudd, A. P., Nakanischi, K., Henselman, R. & Stoeckenius, W. (1977) *Biochemistry* **16,** 1955–1958.
19. Lewis, A. (1976) *Biophys. J.* **16,** 204a.
20. Lewis, A. (1977) *in Electrical Phenomena at the Biological Membrane Level,* ed. Roux, E. (Elsevier, Amsterdam), pp. 543–563.
21. Yoshizawa, T. & Horiuchi, S. (1973) in *Biochemistry and Physiology of Visual Pigments,* ed. Langer, H. (Springer-Verlag, New York), pp. 69–81.
22. Lozier, R. H., Bogomolni, R. A. & Stoeckenius, W. (1975) *Biophys. J.* **15,** 955–962.
23. Kung, M. C., Devault, D., Hess, B. & Oesterhelt, D. (1975) *Biophys. J.* **15,** 907–911.
24. Busch, G., Applebury, M., Lamola, A. & Rentzepis, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 2802–2806.
25. Kaufmann, K. J., Rentzepis, P. M., Stoeckenius, W. & Lewis, A. (1976) *Biochem. Biophys. Res. Commun.* **68,** 1109–1115.
26. Rosenfeld, T., Honig, B., Ottolenghi, M., Hurley, J. & Ebrey, T. G. (1977) *Pure and Applied Chemistry* **49,** 341–351.
27. Kropf, A., Whittenberger, B., Goff, S. & Waggoner, A. (1973) *Exp. Eye Res.* **17,** 591–606.
28. Lewis, A., Spoonhower, J. P. & Perreault, G. J. (1976) *Nature* **260,** 675–678.
29. Alfano, R. R., Yu. W., Govindjee, R., Becher, B. & Ebrey, T. G. (1976) *Biophys. J.* **16,** 541–545.
30. Hirsch, M. D., Marcus, M. A., Lewis, A., Mahr, H. & Frigo, N. (1976) *Biophys. J.* **16,** 1399–1409.
31. Lewis, A., Marcus, M., Ippen, E. P. Shank, C. V., Hirsch, M. D. & Mahr, H. (1977) *Biophys. J.* **17,** 75a.
32. Lewis, A. (1977) *Biophys. Struct. Mech.* **3,** 97–100.
33. Becher, B. & Ebrey, T. G. (1977) *Biophys. J.* **17,** 185–191.
34. Goldschmidt, C. R., Kalisky, O., Rosenfeld, T. & Ottolenghi, M. (1977) *Biophys. J.* **17,** 179–183.
35. Mathies, R. & Stryer, L. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2169–2173.
36. Salem, L. & Bruckmann, P. (1975) *Nature* **258,** 526–528.
37. Salem, L. (1976) *Science* **191,** 822–830.
38. Kropf, A. & Hubbard, R. (1958) *Ann. N.Y. Acad. Sci.* **74,** 266–280.
39. Honig, B., Greenberg, A. D., Dinur, D. & Ebrey, T. G. (1976) *Biochemistry* **15,** 4593–4599.
40. Wolbarsht, M. L. & Yamanashi, B. S. (1977) *Biophys. J.* **17,** 17a.
41. Sulkes, M., Lewis, A. & Lemley, A. T. (1977) *Biophys. J.* **17,** 78a.
42. Marcus, M. & Lewis, A. (1977) *Science* **195,** 1328–1330.
43. Hagins, W. A. & Yoshikami, S. (1977) *Biophys. J.* **17,** 196a.
44. Marcus, M. A., Lewis, A., Racker, E. & Crespi, H. (1977) *Biochem. Biophys. Res. Commun.* **78,** 669–675.
45. Kropf, A. (1976) *Nature* **264,** 92–94.
46. Hubbard, R. & Wald, G. (1952–53) *J. Gen. Physiol.* **36,** 269–315.
47. Hubbard, R., Gregerman, R. I. & Wald, G. (1952–53) *J. Gen. Physiol.* **36,** 415–429.
48. Oesterhelt, D. & Schumann, L. (1974) *FEBS Lett.* **44,** 262–265.
49. Monod, J., Wyman, J. & Changeux, J. P. (1965) *J. Mol. Biol.* **12,** 88–118.
50. Koshland, D. E., Jr., Nemethy, G. & Filmer, D. (1966) *Biochemistry* **5,** 365–385.
51. Donner, K. O. (1973) in *Biochemistry and Physiology of Visual Pigments,* ed. Langer, H. (Springer-Verlag, New York), pp. 205–209.
52. Hochstein, S., Minke, B. & Hillman, P. (1973) *J. Gen. Physiol.* **62,** 105–128.
53. Rushton, W. A. H. (1961) in *Light and Life,* eds. McElroy, W. D. & Glass, B. (John Hopkins Press, Baltimore, MD), pp. 706–723.
54. Peters, K., Applebury, M. L. & Rentzepis, P. M. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3119–3123.