justed to get an optical density around 1.5 at 355 nm and that of the olefinic acceptor was in the range 0.02–0.06 M in order to get a fast energy transfer.

The synthesis of several compounds has been described in previous papers: **1** and **12** in ref 2, **2** in ref 8, and **5**–**8** in ref 18. The others were prepared by reaction of 2-lithiobiphenyl (or 4-lithiofluorenyl) with the appropriate ketones and dehydration of the resulting alcohols with P_2O_5.

Registry No. 1, 57704-78-6; **2**, 83283-82-3; **3**, 98509-50-3; **4**, 75925-36-9; **5**, 98509-51-4; **6**, 10468-66-3; **7**, 98509-52-5; **8**, 98509-53-6; **9**, 98509-54-7; **10**, 98509-55-8; **11**, 98509-56-9; **12**, 57704-79-7; **13**, 34662-94-7.

Application of the H.T.-n Mechanism of Photoisomerization to the Photocycles of Bacteriorhodopsin. A Model Study†

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Abstract: The H.T.-n process, recently introduced to account for the primary process of vision, has now been incorporated into a scheme for the two photocycles of bacteriorhodopsin. The current analysis is based on molecular model construction as well as photochemical and bioorganic reasoning. Specific molecular structures are proposed for BR^t, K^t, L^t, M-I, M-II, Photo-M, L′, and M′ in the trans-cycle. The pathways connecting these species are H.T.-14 for photochemical processes and conformational relaxation and B.P.-14,16 for thermal reactions. The latter also leads to relocation of the iminium hydrogen, hence initiating the proton pumping activity. The proposed structures are consistent with the known spectroscopic properties of these species. Two possible schemes are considered for the minor BR^c photocycle.

In an earlier report we introduced a new mechanism for geometric isomerization of polyenes which involves translocation of a single H atom from one side of the polyene chain to another with concerted rotation of the two adjacent C,C bonds. This motion was dubbed the H.T.-n process (Figure 1).[1] Application of the mechanism to the primary process of vision led to the proposal of the 10-*s*-*cis*,*all*-*trans* structure for bathorhodopsin. This structure satisfactorily accounts for all known spectroscopic and chemical properties of the primary photoproduct. The H.T.-n process is not believed to be competitively favored relative to the conventional one-bond rotation process of isomerization in unconstrained molecules, e.g., those in solution. However, for chromophores in which translational and rotational motions are restricted (e.g., by specific protein–substrate interactions at the two termini and by the limited space available due to the surrounding protein), the H.T.-n process could become the dominant isomerization pathway because the alternative one-bond-rotation process is more volume demanding. Bacteriorhodopsin (BR), like rhodopsin, contains a retinyl chromophore in a membrane protein binding site.[2] A close examination of the possible application of the H.T.-n mechanism to the known chemistry of the isomeric pigments and their intermediates is, therefore, of obvious interest.

The presence of two independent photocycles and the large number of intermediates suggest that BR is mechanistically more complex than rhodopsin. However, on the basis of the same approach of model construction and photochemical and mechanistic considerations that was successfully applied to rhodopsin, we can now formulate a complete and logical scheme for early stages of the BR photocycles. This scheme is the subject of this report.

Background on the Photocycles of the BR System

The photobleaching and dark regeneration processes of BR have been studied in great detail by fast kinetics and low-temperature spectroscopy by many research groups. The topic has been reviewed in several excellent articles.[2] The key intermediates for the photocycles are indicated in Figure 2, and a brief review of the experimental results is presented below.

The configurational integrity of the 13,14 bond of the retinyl chromophore is not maintained at room temperature, giving approximately equal amounts of *all*-*trans*- (BR^t) and 13-*cis*-BR (BR^c).[2e] Light adaptation results in a slow 13-*cis* to *all*-*trans* conversion. At ≤0 °C where the dark adaptation process is suppressed, the light conversion to the BR^t becomes complete. Light excitation generates a series of intermediates. From BR^t the primary photoproduct is usually considered to be the red-shifted K^t intermediate, although more recently a shorter-lived precursor (J) of K^t has been detected in fast kinetic studies.[4] Sequentially, K^t is converted to the blue-shifted L^t and the much more blue-shifted M-I. It is during the L^t to M-I transition that the physiologically important proton-pumping activity is believed to originate, the product (M-I) being a deprotonated chromophore[5] containing the 13-*cis* geometry.[6] From kinetic arguments, M-I is believed to be in equilibrium with an isochromic M-II.[7] A slower, probably catalytic process follows, resulting in the formation of the red-shifted O intermediate, containing the all-trans geometry.[8] To complete the photocycle, O is converted to BR^t.

† Photochemistry of Polyenes 23. For part 22 in the series, see ref 1, to which readers should consult for a more general discussion of the H.T.-n process. Abbreviations used in this paper: H.T.-n, hula-twist at center n (also known as C.T.-n: concerted twist at center n);[1] B.P.-14,16, bicycle-pedalling at bonds 14 and 16; BR, bacteriorhodopsin; BR^t, *all*-*trans*-bacteriorhodopsin; BR^c, 13-*cis*-bacteriorhodopsin.

(1) Liu, R. S. H.; Asato, A. E. *Proc. Natl. Acad. Sci. U.S.A* **1985**, *82*, 259–263.

(2) For recent reviews see: (a) Hess, B.; Kuschmitz, D.; Engelhard, M. In "Membrane and Transport"; Martonosi, R. A., Ed.; Plenum: New York, 1982; Vol. 2, pp 309–318. (b) Ottolenghi, M. *Methods Enzymol.* **1982**, *88*, 470–491. (c) Lewis, A. *Methods Enzymol.* **1982**, *88*, 561–617. (d) Stoeckenius, W.; Bogomolni, R. A. *Annu. Rev. Biochem.* **1982**, *52*, 587–616. (e) Dencher, N. A. *Photochem. Photobiol.* **1983**, *38*, 753–768. (f) Honig, B.; Ebrey, T. G. *Methods Enzymol.* **1982**, *88*, 462–470.

(3) Tokunaga, F.; Iwasa, T. *Methods Enzymol.* **1982**, *88*, 163–167.

(4) (a) Applebury, M. L.; Peters, K. S.; Rentzepis, P. M. *Biophys. J.* **1978**, *23*, 375–382. (b) Shichida, Y.; Matuoka, S.; Hidaka, Y.; Yoshizawa, T. *Biochim. Biophys. Acta* **1983**, *723*, 240–246.

(5) Braiman, M.; Mathies, R. *Biochemistry* **1980**, *19*, 5421–5428.

(6) Tsuda, M.; Glaccum, M.; Nelson, B.; Ebrey, T. G. *Nature (London)* **1980**, *287*, 351–353.

(7) (a) Eisenbach, M.; Bakker, E. P.; Korenstein, R.; Caplan, S. R. *FEBS Lett.* **1976**, *71*, 228–232. (b) Hess, B.; Kuschmitz, P. *FEBS Lett.* **1977**, *74*, 20–24. (c) Groma, G. I.; Helgerson, S. L.; Wolber, P. K.; Beece, D.; Dancshazy, Zs.; Keszthelyi, L.; Stoeckenius, W. *Biophys. J.* **1984**, *45*, 985–992.

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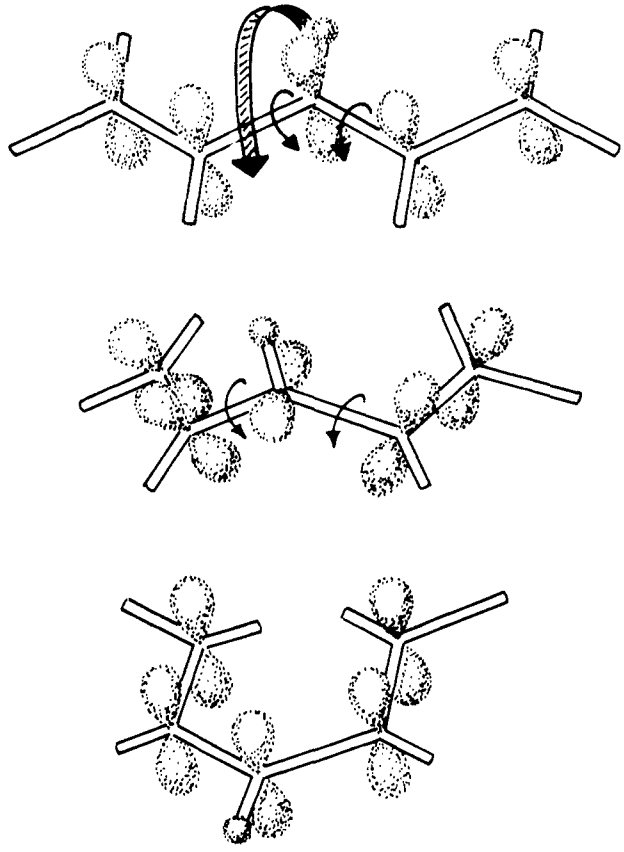


Figure 1. The H.T.-n process as demonstrated by a five-atom fragment. The two middle bonds of the W shaped (top) planar specie undergo a concerted rotation. The consequence is the flipping of the central C–H moiety from an inward orientation first to a vertical (middle) then to an outward orientation (bottom). The remaining atoms slide in a general direction of the plane of the fragment. The transition state (middle) contains an orthogonal, sp^2-hybridized methine group and the bottom lobes of the p-orbitals of atoms 2,4 point toward each other (homoconjugation). The net result of the H.T.-n motion is the conversion of the C_5 fragment from a "W" shape (top) to a "U" shape (bottom).

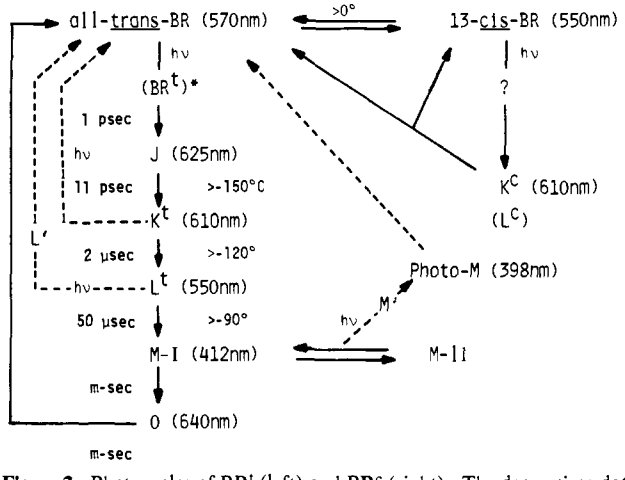


Figure 2. Photocycles of BR^t (left) and BR^c (right). The decay time data are those from Ottolenghi[2b] and the low-temperature data are from Tokunaga.[3] The early intermediate J was not detected in low-temperature experiments. The absorption maxima are listed in parentheses, and approximate lifetimes are given on the left. The dashed lines are those of secondary photochemical reactions of the K, L, and M intermediates. L' and M' are primary photoproducts from L^t and M detected in low-temperature experiments.[10]

Other important experimental data are the following. Low-temperature spectroscopic studies showed that K^t,[9] L^t,[10] and

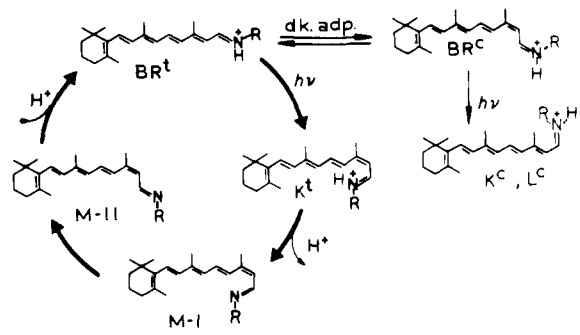


Figure 3. The Schulten model[17] for photocycles of BR. The proposed geometry for the intermediates are the following: K^t and M-I, 13-*cis*,14-*s*-*cis*; M-II, 13-*cis*,14-*s*-*trans*; BR^c, 13-*cis*,15-*syn*; K^c, 13-*trans*,14-*s*-*cis*,15-*syn*. Proton pumping occurs during the conversion of K^t to M-I.

M[7a,10,11] can all be photochemically converted back to BR^t. And more recently different rates of formation of M's have been observed, hence the appearance of the terms of fast M (M^f) and slow M (M^s).[12] The suggestion of the two forms of M originating from two different forms of L has also been made.[12b] The Resonance Raman[13] and FT-IR spectra[14] of K^t were shown to exhibit features different from those exhibited by normal 13-*cis* chromophore in spite of its known 13-*cis* configuration. And Keszthelyi and Ormos[15] were able to measure the extent of charge migration during the photocycle from a small change from BR^t to K^t (1.3 Å), to a negligible amount from K^t to L^t (0.2 Å), and to a large one from L^t to M (5 Å). All these facts must be incorporated into a complete model for the photocycle of BR^t.

The photochemistry of BR^c is less involved. Only one intermediate, K^c (610), has been unambiguously established although the possible existence of another transient has been suggested on kinetic grounds.[11b] Decay of K^c leads to regeneration of BR^c (major) and formation of BR^t (minor),[9] the latter being responsible for the slow light adaptation process eventually giving entirely BR^t. The BR^c cycle does not exhibit any proton-pumping activity.[2d]

There are many other models in the literature[2b,c,16] attempting to make assignments to the intermediates observed in the photocycles or emphasizing absorption properties and energy storage.[2f] Those of Schulten[17] and Birge and Pierce[18] discuss most specifically molecular structures and mechanistic details. Schulten's model, shown in Figure 3,[17] does not consider the role of the protein. The postulated *all-trans* to 13-*cis*,14-*s*-*cis* transformation for the

(8) Smith, S. O.; Pardoen, J. A.; Mulder, P. P. J.; Curry, B.; Lugtenburg, J.; Mathies, R. *Biochemistry* **1983**, *22*, 6141–6148.

(9) (a) Lozier, R. H.; Bogomolni, R. A.; Stoeckenius, W. *Biophys. J.* **1975**, *15*, 955–960. (b) Iwasa, T.; Tokunaga, F.; Yoshizawa, T. *Photochem. Photobiol.* **1981**, *33*, 539–545.

(10) Hurley, J. B.; Becher, B.; Ebrey, T. G. *Nature (London)* **1978**, *272*, 87–88.

(11) (a) Kalisky, O.; Goldschmidt, C. R.; Ottolenghi, M. *Biophys. J.* **1977**, *19*, 185–189. (b) Sperling, W.; Karl, P.; Rafferty, C. N.; Dencher, N. A. *Biophys. Struct. Mech.* **1977**, *3*, 79–88.

(12) (a) Li, Q.-Q.; Govindjee, R.; Ebrey, T. G. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 7079–7082. (b) Hamamoto, J. H.; Dupuis, P.; El-Sayed, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 7083–7087.

(13) (a) Pande, J.; Callender, R. H.; Ebrey, T. G. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7379–7382. (b) Braiman, M.; Mathies, R. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 403–407. (c) Hsieh, C.; Nagumo, M.; Nieol, M.; El-Sayed, M. A. *J. Phys. Chem.* **1981**, *85*, 2714–2717.

(14) (a) Rothschild, K. J.; Zagaski, M.; Cantore, W. A. *Biochem. Biophys. Res. Commun.* **1981**, *103*, 438–489. Rothschild, K. J.; Merrero, H. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 4045–4049. (b) Bagley, K.; Dollinger, G.; Eisenstein, L.; Singh, A. K.; Zimanyi, L. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 4972–4976. (c) Siebert, F.; Mantele, W. *Eur. J. Biochem.* **1983**, *130*, 565–573.

(15) Keszthelyi, L.; Ormos, P. *Biophys. Chem.* **1983**, *18*, 397–405.

(16) Smith, S. O.; Myers, A. B.; Pardoen, J. A.; Winkel, C.; Mulder, P. P. J.; Lugtenburg, J.; Mathies, R. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 2055–2059.

(17) (a) Schulten, K.; Tavan, P. *Nature (London)* **1978**, *272*, 85–86. (b) Orlandi, G.; Schulten, K. *Chem. Phys. Lett.* **1979**, *64*, 370–374. (c) Schulten, K. In "Energetic and Structure of Halophilic Microorganisms"; Caplan, S. R., Ginzburg, M., Eds.; Elsevier: Amsterdam, 1978; pp 331–334.

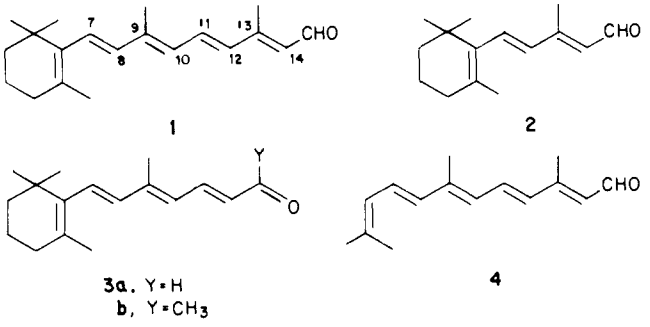
(18) Birge, R. R.; Pierce, B. M. In "Photochemistry and Photobiology"; Zewail, A. H., Ed.; Harwood Academic: New York, 1983; pp 841–855.

primary photochemical process was supported by results of CNDO calculations.[17b] Carbon rehybridization (sp^2 to sp^3) was alluded to in the formation of the transition-state structure.[17c] Proton loss was assumed to take place directly from K^t.[17a] An unusual structure containing the 15-*syn* geometry was postulated for BR^c, which after light excitation gives the 14-*s-cis*,15-*syn* structure for K^c.[17c] Birge and Pierce's model[18] assumed that the α-carbon and the cyclohexenyl ring are rigidly anchored to the protein. For the primary process, they proposed an extended bicycle-pedal model.[19] However, for it to be operative, the 15,16 geometry for BR^t was arbitrarily chosen to be *syn*, a feature not supported by experimental evidence. Below we offer an alternative model which emphasizes changes in the chromophore in the BR cycles.

Chemical Properties Related to the Binding Site Structures of BR

The photoisomerization of retinal (**1**) and its derivatives in nonpolar solvents is highly selective at the more crowded trisubstituted double bonds. Furthermore, it shows regioselective preference for the 13,14 bond.[20] The BR system, on the other hand, isomerizes **specifically** at the 13,14 bond.[2] This medium directed regiospecificity[21] implies that the protein and substrate interact rather loosely around the 13,14 bond but more tightly around all other double bonds. The failure to form other isomeric BR's[2d] is in agreement with this conclusion. Previously, the same line of reasoning, when applied to rhodopsin, led to the conclusion that relatively loose protein–chromophore interactions occur near the C-10 and C-11 of the chromophore in the visual pigment.[1]

The primary amino acid sequence of BR is known.[22] The binding interaction between the retinyl chromophore and the protein has been established to be the protonated Schiff base linkage at Lys-216.[23,24] Evidence for the existence of a secondary interaction between the trimethylcyclohexenyl ring and a possible hydrophobic pocket in the binding site is less direct but abundant. From analogue studies it has been shown that the two chain shortened homologue of retinals (the C_{15} aldehyde, **2**, the C_{17} aldehyde, **3a**, and the C_{18} ketone, **3b**)[25] inhibit pigment formation between *all-trans*-retinal and the *apo*-purple membrane. On the other hand, acyclic retinal analogues such as the linear pentaenal **4**, which retains part of the nonpolar ring, are known to form pigments of low stability.[26] That other analogues such as phenyl



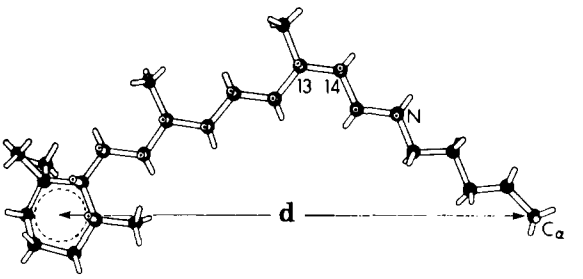


Figure 4. A molecular model of the protonated Schiff base of the 13-*cis*-retinyl chromophore bonded to the butyl side chain of a lysine unit (Lys-216). The sp^2 atoms are marked by vertical rods terminating with concentric circles showing orientation of the p orbitals. The chromophore assumes the most relaxed *all-s-trans* conformation (except for 6-*s-cis*) and the butyl group the relaxed staggered conformation. The α-carbon of the lysine unit is affixed to the surface with a nail, and the cyclohexenyl ring is then loosely fixed by passing it over a concentric cylinder, which is in turn taped to the surface.[32] The size of the cylinder (dotted circle) was chosen to be slightly smaller than the area within the hexagon. Therefore, the distance between the center of the ring and the C_α (d) is fixed in a very loose sense. The model is then converted to the *all-trans* chromophore (Figure 5A) for subsequent manipulations.

and ring-substituted derivatives form stable BR analogues[27] also agrees with the presence of a hydrophobic pocket, albeit relatively nonspecific, in the *apo*-purple membrane.[28] This notion is also supported by fluorescence studies.[29] Therefore, as with rhodopsin,[30] it appears reasonable to assume that the two ends of the chromophore are restricted within the binding site of BR, i.e., anchored at both ends. It is worth noting that the same conclusion was earlier independently reached by Birge and Pierce[18] and Towner et al.[25c] in their studies. This structural restriction implies that **the rapid early processes of BR can take place only in those forms not requiring substantial changes of the longitudinal distance between the anchors.**

For consideration of the longitudinal requirement of the chromophore in relation to the binding site,[30b,31] we must emphasize once again the need to include the tether-like butylamino group of Lys-216.[1,32] Examination of models of the **anchored**, tethered chromophore of BR after photoexcitation immediately leads to the conclusion that **BR^t cannot have the fully extended *all-s-trans*-polyenyl and the *all-staggered*-butyl conformation.** This is because such a conformation when constrained at both ends cannot undergo any form of isomerization to an, inevitably, shortened chromophore without significant and unlikely protein reorganization. Consideration of the absorption properties of BR^t and the relative ease in twisting a polyene vs. an alkane suggests that the polyene portion is likely to be relatively planar, leaving the butyl group to assume a gauche-like twisted conformation. This "curled" butyl group provides the necessary tethering flex-

(19) Warshel, A. *Nature (London)* **1976**, *260*, 679–683.
(20) (a) Liu, R. S. H.; Asato, A. E. *Tetrahedron* **1984**, *40*, 1931–1969. (b) Rao, V. J.; Fenstemacher, R. J.; Liu, R. S. H. *Tetrahedron Lett.* **1984**, *25*, 1115–1118.
(21) Liu, R. S. H.; Asato, A. E.; Denny, M. *J. Am. Chem. Soc.* **1983**, *105*, 4829–4830.
(22) (a) Ovchinnikov, Yu. A.; Abdulaev, N. G.; Feigina, M. Yu.; Kiselev, A. V.; Lobanov, N. A. *FEBS Lett.* **1979**, *100*, 219–224. (b) Khorana, H. G.; Gerber, G. E.; Herlihy, W. C.; Gray, C. P.; Anderegg, R. J.; Nihei, K.; Biemann, K. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5046–5050.
(23) (a) Bayley, H.; Huang, K. S.; Radhakrishan, R.; Ross, A. H.; Takagaki, Y.; Khorana, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2225–2229. (b) Lemke, H. D.; Oesterhelt, D. *FEBS Lett.* **1981**, *128*, 255–260. (c) Mullen, E.; Johnson, A. H.; Akhtar, M. *FEBS Lett.* **1981**, *130*, 187–193.
(24) The slight disagreement in the BR sequencing results led to different numbering of this key lysine residue as 215[22a] or 216.[22b] In this paper, we follow Khorana's designation of Lys-216.
(25) (a) Tokunaga, F.; Govindjee, R.; Ebrey, T. G.; Crouch, R. *Biophys. J.* **1977**, *19*, 191–198. (b) Ovchinnikov, Yu. A.; Shkrob, A. M.; Rodionov, A. V.; Mitzner, B. I. *FEBS Lett.* **1979**, *97*, 15–19. (c) Towner, P.; Gaertner, W.; Walckhoff, B.; Oesterhelt, D.; Hopf, H. *Eur. J. Biochem.* **1981**, *117*, 353–359.
(26) (a) Crouch, R. K.; Or, Y. S.; Ghent, S.; Chang, C.-H.; Govindjee, R.; Ebrey, T. G.; Callender, R. H.; Pande, A. *J. Am. Chem. Soc.* **1984**, *106*, 8325–8327. (b) Rao, V. J.; Zingoni, J.; Crouch, R.; Denny, M.; Liu, R. S. H. *Photochem. Photobiol.* **1985**, *41*, 171–174.
(27) (a) Balogh-Nair, V.; Nakanishi, K. *Methods Enzymol.* **1982**, *88*, 496–506. (b) Sen, R.; Singh, A. K.; Balogh-Nair, V.; Nakanishi, K. *Tetrahedron* **1984**, *40*, 493–500. (c) Sheves, M.; Baasov, T.; Friedman, N.; Ottolenghi, M.; Feinmann-Weinberg, R.; Rosenbach, V.; Ehrenberg, B. *J. Am. Chem. Soc.* **1984**, *106*, 2435–2437. (d) Maeda, A.; Asato, A. E.; Liu, R. S. H.; Yoshizawa, T. *Biochemistry* **1984**, *23*, 2507–2513.
(28) The formation of a stable analogue with the lengthened C_{22}-hexaenal[25a] is not inconsistent with the concept of a fixed distance between the anchors. Model shows that such a chromophore can be accommodated after conformational changes of the butyl tether. Also, it should be noted that the argument used for the BR-cycles here can equally be applied to that of an analogue even if there should be a slight change of the distance between the anchors in an analogue.
(29) Kouyama, T.; Kimura, Y.; Kinoshita, K.; Ikegami, A. *J. Mol. Biol.* **1981**, *153*, 337–359.
(30) (a) Matsumoto, H.; Yoshizawa, T. *Nature (London)* **1975**, *258*, 523–526. (b) Matsumoto, H.; Yoshizawa, T. *Vision Res.* **1978**, *18*, 607–609.
(31) Matsumoto, H.; Liu, R. S. H.; Simmons, C.; Seff, K. *J. Am. Chem. Soc.* **1980**, *102*, 4259–4262.
(32) Liu, R. S. H.; Asato, A. E.; Denny, M.; Mead, D. *J. Am. Chem. Soc.* **1984**, *106*, 8298–8300.

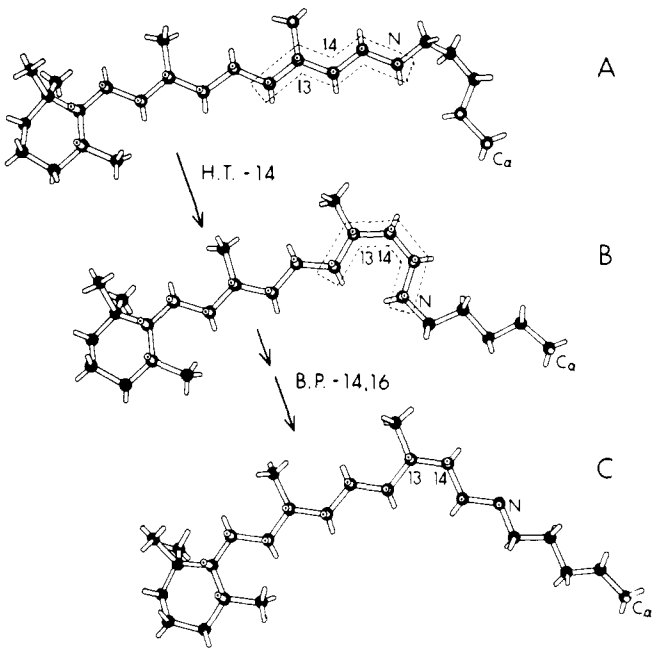


Figure 5. (A) A molecular model showing the anchored chromophore of BR^t, as constructed in a manner described in Figure 4. The polyene chromophore was adjusted to the relaxed *all-s-trans*,6-*s-cis* conformation. Restricted by the fixed anchors, the butyl side chain now exists in a twisted conformation. The area enclosed assumes an inverted "W" shape. (B) Application of the H.T.-14 motion to the model in part A yielded the structure shown: 13-*cis*,14-*s-cis*. This is believed to be K^t. The area enclosed assumes an inverted "U" shape. L^t is a relaxed K^t (see text) retaining the same gross polyene structure. (C) Application of the B.P.-14,16 motion to the structure in part B yielded the structure shown: 13-*cis*-14-*s-trans*. After the loss of the imino proton, the structure is believed to be that of M-I.

ibility for the photochemical and subsequent transformations. By the same token, one reaches the conclusion that BR^c also has a "curled" butyl group (see below).

The H.T.-n Model for the Main Trans Cycle

To aid the current analysis, a molecular model is used which is similar to the one[1,32] used for the rhodopsin system.[33] In it the α-carbon of the bonded lysine group was rigidly fixed while the ring was loosely anchored in a manner described in Figure 4. The rotational freedom of the trimethylcyclohexenyl ring allows the model to mimic the nonspecific, nonbonded hydrophobic interaction at this end of the chromophore. For facile interconversion of geometric as well as conformational isomers (and an averaged representation of both the excited and the ground states of the polyene, which have different π-bond orders), the sp^2 atoms of the chromophore are connected by single bonds only. The distance between the α-carbon and the center of the trimethylcyclohexenyl ring (i.e., the distance between the two anchors) is important even though it does not have to be rigidly fixed.[1] From the above discussion, it must be shorter than those of the fully extended BR^t and BR^c. We have chosen this distance to be that of the relaxed conformer of the butyl Schiff base of 13-*cis*,14-*s-trans*,15-*anti* (i.e., one with the staggered conformation of the butyl group and the *s-trans* conformation in the polyene chain except for the 6,7 single bond, Figure 4). After fixing the distance in a manner as described in Figure 4, the model was then converted to that of the *all-trans* isomer containing *all-s-trans* conformation throughout the polyene chain. By necessity, the butyl group is no longer in the stretched out (staggered) conformation. It is adjusted to an *all-gauche*-like conformation in order to minimize eclipsing interactions. This structure is illustrated in Figure 5A.

(33) A minor modification, suggested to us by Professor R. Birge, is having the α-carbon more rigidly fixed in order to minimize the pivotal motion of the β-carbon about the α-carbon.[32]

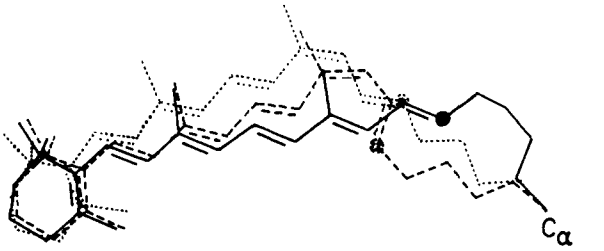
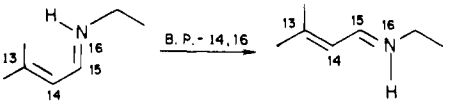


Figure 6. The superimposed bond-line structures of BR^t (—), K^t (---), and M-I (···) for highlighting the relative positions of the atoms of the chromophore during the course of chemical changes taken place between the anchors. The positions of the imino nitrogen are marked by circles.

To account for the dynamic processes of BR and its intermediates, we further introduce the following assumptions.

1. All photochemical processes involving the polyene chromophore encapsulated within the binding site are H.T.-14. These include the secondary photochemical processes of the intermediates. The small volume requirement for H.T.-n makes the process especially suitable for the rapid photochemical reactions. Its end result is the same as that described by Schulten. During the H.T.-n process, the carbons are not expected to deviate significantly from the sp^2 (Figure 1). The advantages of the H.T.-n process in situations where the motion of atoms is restricted have been discussed above and elaborated on earlier.[1]

2. The principal driving force for subsequent dark processes is relief of steric crowding derived from light-induced changes in the chromophore. This can be either in the form of protein relaxation (a faster process) or the loss of the *s-cis* conformation to the corresponding *s-trans* form (a slower process). For the latter we propose specifically the less volume demanding bicycle-pedal process.[19] For BR^t, the principal process is B.P.-14,16, i.e.,



Consideration of the relative π-bond orders at these centers suggests that the bicycle-pedalling process is only possible as a ground-state process.

Structure of K^t. Application of the H.T.-14 motion to the anchored structure of BR^t leads to the new structure shown in Figure 5B. The W to U conversion associated with such a process (Figure 1) is clearly shown in the enclosed C_{12}–N_{16} fragments in parts A and B of Figure 5. The chromophore contains the 13-*cis* geometry and also the 14-*s-cis* conformation, which, in accordance with the Woodward Rule for UV absorption of conjugated systems,[34] should contribute to the red shift in the absorption spectrum of K^t.[35] This structure is the same as that proposed by Schulten. The Resonance Raman[13] and FT-IR[14] spectra of K^t should, therefore, exhibit features distinctively different from those of simple 13-*cis* model compounds in the retinyl system. Furthermore, that K^t is photochemically reversible to BR^t[9] is readily accounted for by the reverse H.T.-14 process.

The dark reactions subsequent to the formation of the primary photoproduct K^t must be, by necessity, exothermic, a condition readily satisfied by the known high-energy content of K^t (15.8 ± 2.5 kcal/mol above BR^t).[37] The structures of L^t and M are deduced from their photochemical property of being able to revert to BR^t. This is because when again assuming that H.T.-14 is responsible for the protein-bound polyenes in L^t and M, one

(34) (a) Fieser, L. F.; Campbell, W. P. *J. Am. Chem. Soc.* **1938**, *60*, 139–170. (b) Woodward, R. B. *J. Am. Chem. Soc.* **1942**, *64*, 72–75.

(35) The discussion on the effect of *s-cis* linkage on absorption characteristics is only intended to point out one structural feature not previously considered for the intermediates. It does not exclude other protein-directed effects[2c] such as the double point charge model.[36]

(36) Nakanishi, K.; Balogh-Nair, V.; Arnaboldi, M.; Tsujimoto, K.; Honig, B. *J. Am. Chem. Soc.* **1980**, *102*, 7945–7947.

(37) Birge, R. R.; Cooper, T. M. *Biophys. J.* **1983**, *42*, 61–69.

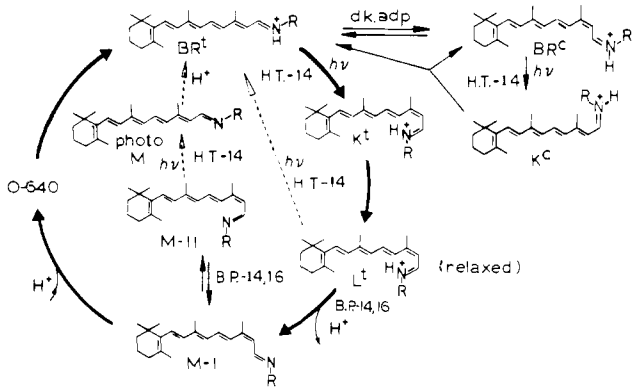


Figure 7. The H.T.-n model for the photocycles of BR in bond-line structures. See text for a discussion of how such structures may be misleading.

reaches the conclusion that both species must have the common 13-*cis*,14-*s*-*cis* geometry, i.e., **identical with that of K^t.**

Structures of L^l and M. The answer to the question of the nature of structural differences between the two 13-*cis*,14-*s*-*cis* structures for K^t and L^t has been provided by Hurley et al., in their work of low-temperature photochemical behavior of L^t and M.[10] The photochemical processes were found to be possible even at −196 °C, while subsequent conversion of the photoproducts (L′ and M′) to BR^t took place only after warming the samples to −90 °C. For these observations, they suggested that the slower processes correspond to protein conformational changes made necessary by the geometrical changes of the chromophore in the light step.

Indeed, the conversion of BR^t to K^t involves considerable displacement of several atoms of the chromophore (Figure 6). Therefore, we also believe that L^t is a relaxed form of K^t, with the relaxation process involving primarily protein reorganization and secondarily minor chromophore conformational changes.[38] Hence, charge migration is expected during the BR to K^t step but not for the K^t to L^t transition.[15] The blue shift of the absorption spectrum of L^t must then be due to a combined effect of nonplanarity in the chromophore and reoriented protein perturbation.[2c,36]

The reversible photochemical reaction of M is more complicated because of the presence of an equilibrium of two forms of M (M-I, M-II).[7] Considering the combined results of the proton-pumping activity during the L^t to M-I transition, the associated charge migration, and the photochemical behavior, we propose the following mechanistic pathways. The conversion of L^t to M-I involves a volume-conserving B.P.-14,16 process for the tethered chromophore. Associated with the process is the more extensive molecular reorganization; hence, it is slower than that from K^t to L^t. The driving force is relief of repulsive interaction in the *s*-*cis* conformation of the chromophore rather than the protein–substrate interactions in K^t.

The B.P.-14,16 process converts L^t to a new 13-*cis*,14-*s*-*trans* structure for M-I. The drastic change of the direction of the NH group is consistent with the large charge migration (5 Å) as determined by Keszthelyi and Ormos.[15] In fact, since M-I is already deprotonated,[5] the fast proton migration process must have taken place during the sweeping (180°) motion of the B.P.-14,16 process that brought the imino proton close to the opening of a proton channel,[39] thus initiating the proton pumping activity.[40]

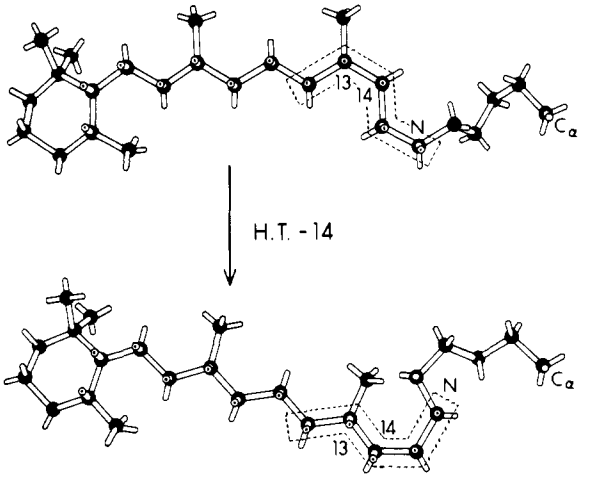


Figure 8. (A, top) A molecular model showing an anchored chromophore of BR^c. The polyene geometry is 13-*cis*,15-*syn*. The same distance (*d*) defined in Figure 4 has been retained. When the chromophore assumes the relatively planar, relaxed conformation, the butyl group is again twisted. The enclosed area resembles an inverted sickle. (B, bottom) Application of H.T.-14 to the structure in part A yielded the new structure shown: 13-*trans*,14-*s*-*cis*,15-*syn*. This is believed to be K^c. The enclosed area now resembles an upright sickle.

As emphasized in the discussion of the primary process of vision,[1] an important feature of the H.T.-n process is the small reaction volume required for flipping of the CH group. The in-plane motion of the remaining conjugated system (see Figure 1) is not expected to involve any substantial concurrent changes of protein structures because the chromophore is known to occupy a relatively free space between the helices of the protein.[41] In Figure 6, where the bond-line structures of BR^t and K^t are superimposed, this in-plane displacement is clearly shown. Also shown is the structure of M-I, making evident the extent of reorientation of the iminium nitrogen.[42]

The equilibrium between two forms of M[7] can be most economically explained by interconversion of the 13-*cis*,14-*s*-*cis* (M-I) and the 13-*cis*,14-*s*-*trans* (M-II) structure via the B.P.-14,16 process. Reversible photoreaction of M, therefore, originates from M-II. After a H.T.-14 process, an intermediate with the 13-*trans*,14-*s*-*trans* structure (M′)[10] is produced which upon protein relaxation gives first photo-M[11a] and then upon protonation regenerating BR[1 10,11a] (see Figure 7).

Formation of intermediates later than M probably involves substantial reorganization of the protein.[43] The basic assumptions invoked in our model for the anchored chromophore are probably no longer valid. Furthermore, the M to O conversion might involve catalytic processes between the protein and the substrate. This subject should be treated separately.

The proposed BR^t photocycle is summarized in Figure 7 with conventional bond-line structures. This figure is presented mainly for the purpose of comparison with that of Schulten's model. It should be emphasized that such structures do not reflect protein-imposed structural details; thus they misleadingly suggest free motion of the α-carbon to which the butylamino group is attached. It is interesting to note that in spite of starting from a different viewpoint (e.g., inclusion of the role of the protein here but not in Schulten's), our scheme (Figure 7) shows considerable similarity

(38) The same line of reasoning has led to the assignments of structures for the later intermediates in the visual cycle, including the reassignment of lumirhodopsin to that of a relaxed *all-trans*-10-*s*-*cis* chromophore: Liu, R. S. H.; Matsumoto, H.; Asato, A. E.; Mead, D. *J. Am. Chem. Soc.*, submitted.

(39) Hess, B.; Kuschmitz, D. *Front. Biol. Energ.* [*Pap. Int. Symp.*] **1978**, *1* 257–264.

(40) The alternative suggestion of L^l being the protonated form of M-I suffers from the difficulty of finding a direct photochemical route for L^l to return to BR^l. However, we cannot rule out the possibility of the existence of an equilibrium between the two protonated forms (14-*s*-*cis* and 14-*s*-*trans*) of L, a possible explanation for the presence of two forms of L.[12b]

(41) (a) Engelman, D. M.; Henderson, R.; McLachlan, A. D.; Wallace, B. A. Proc. *Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2023–2027. (b) Wallace, B. A. *Methods Enzymol.* **1982**, *88*, 447–462.

(42) From the model, a structure containing identical geometry of the chromophore but a different conformation of the lysine side chain (hence a different orientation of the polyene chain) can also be constructed for BR^l. We arbitrarily chose the one with the least displacement of the polyene chain for the subsequent photochemical process.

(43) Hoffmann, W.; Graca-Miguel, M.; Barnard, P.; Chapman, D. *FEBS Lett.* **1978**, *95*, 31–34.

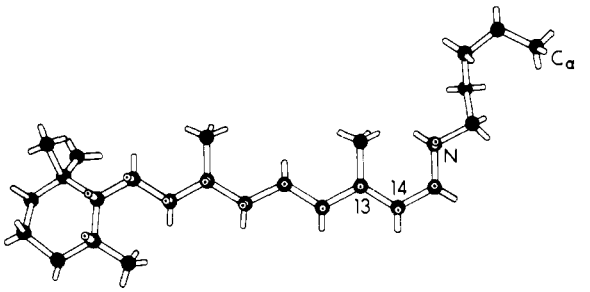


Figure 9. An alternative structure for K^c: 13-*trans*,14-*s*-*cis*,15-*anti*, based on a 13-*cis*,15-*anti* structure for BR^c (see text for details).

with that of Schulten's (Figure 3).

The BR^c Photocycle

Using the same distance between the cyclohexenyl ring and the α-carbon as defined in Figure 4, we constructed the 13-*cis*,15-*syn* structure proposed by Schulten[17] and embraced by Harbison et al.[44] and Smith et al.[16] (Figure 8A). As with BR^t, the butyl group of BR^c is twisted, thus serving dual functions in permitting the planar chromophore to fit within the confined cavity and subsequently accommodating a shorter chromophore in the photoproduct. Application of the H.T.-14 motion to BR^c yielded the structure in Figure 8B for K^c. It contains the 13-*trans*,14-*s*-*cis*,15-*syn* geometry, which is identical with that proposed by Schulten.[17c]

The 15-*syn* geometry makes K^c chemically different from K^t with potentially a different rate of relaxation. The 15-*syn* geometry no longer allows the chromophore to undergo the B.P.-14,16 process, thus ruling out the proton-pumping activity.

Molecular model constructions also suggest another possible interpretation for the *cis* photocycle. If one starts out with the 13-*cis*,14-*s*-*trans*,15-*anti* structure for the chromophore (i.e., similar to that in Figure 4), H.T.-14 leads to the 13-*trans*,14-*s*-*cis*,15-*anti* structures for K^c and L^c (Figure 9). A subsequent B.P.-14,16 process leads to the 13-*trans*,14-*s*-*trans*,15-*anti* geometry which is identical with BR^t, thus accounting for the reversal to BR^t.[45] However, the combined ^{13}C NMR[44] and Raman[16] data on BR^c do not seem to support this structure.[45]

(44) Harbison, G. S.; Smith, S. O.; Pardoen, J. A.; Winkel, C.; Lugtenburg, J.; Herzfeld, J.; Mathies, R.; Griffin, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1706–1709.

(45) It is perhaps worth noting that the NMR evidence alone does not unambiguously argue for the 15-*syn* geometry. The upfield shift of carbon-14 in BR^c in the ^{13}C NMR experiments[44] is also consistent with a 14-*twisted*,15-*anti* geometry. Such shifts are well documented in the literature, e.g., in several nonplanar vitamin A isomers.[46]

(46) Englert, G. *Helv. Chim. Acta* **1975**, *58*, 2367–2390.

Conclusions

The H.T.-n process, introduced earlier to account for all seemingly unrelated and unexplained experimental facts related to the early stages of the photobleaching process of the visual pigment, has now been applied to the bacteriorhodopsin system. The process has been assigned to the primary processes of BR's and secondary photochemical reactions of the intermediates. With the aid of a molecular model, we have constructed a complete and consistent picture concerning the photocycles of BR, which successfully accounts for the following experimental facts: (1) the rapid ease of the primary and secondary photochemical processes of the encapsulated polyene chromophore; (2) unusual spectroscopic data of K^l (Raman, IR, and UV); (3) charge migration during transitions between intermediates; and (4) proton relocation (hence proton-pumping activity) between L^t and M. Additionally specific molecular structures have been proposed for BR^t, BR^c, K^t, L^t, M-I, M-II, L′, M′, and photo-M, and mechanistic pathways connecting these intermediates have also been outlined.

It is interesting to note that the current model was constructed in ways totally different from earlier work. Nevertheless, it retains some of the key features in the models proposed by Schulten,[17] and concepts presented by Hurley et al.[10] The strength of the approach lies in its simplicity in stressing the use of molecular models in testing validity of the concepts. It provides specific molecular details not available from other published models. Such information, though partly speculative, can be subjected to future experimental and theoretical verification. We hope that this sequence of essays[1,32] on the chemistry of confined and anchored chromophores will stimulate new investigations into properties of the visual pigment as well as BR. At the same time, we wish to caution that the simplified approach is likely to lead to the sacrifice of meaningful detailed information. In particular, the reduction of the binding site to a two-dimensional projection is clearly a gross simplification. Therefore, the current results should in no way discourage other more exact and realistic approaches such as binding site mapping, e.g., through refined molecular mechanics calculations coupled with real time computer simulation.[47]

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(47) Beddell, C. R. *Chem. Soc. Rev.* **1984**, *13*, 279–319.