

# A Bioorganic View of the Chemistry of Vision: H.T.-n and B.P.-m,n Mechanisms for Reactions of Confined, Anchored Polyenes<sup>†</sup>

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Two decades ago, primarily through the efforts of George Wald and his associates R. Hubbard, A. Kropf, and P. K. Brown much of the foundation of the chemistry of vision was established.<sup>1</sup> Among the important contributions made by this remarkable group were development of the concept of geometric isomerization for the primary process of vision, identification of the specific geometric isomers of vitamin A in the visual cycles (e.g. see Figure 7), demonstration of the high stereospecificity of the binding site of opsin, the apoprotein of the visual pigment rhodopsin, and proof of the existence of photobleaching intermediates. In spite of recent feverish research activities on the chemistry of vision, many of the ideas developed by this group are still believed to be basically valid. Unfortunately this has also meant that many aspects of the visual process including the exact nature of the primary process of vision and the molecular structures of the bleaching intermediates<sup>2</sup> have remained rather vaguely defined.

On the other hand, considerable new information about the structure of visual protein has been obtained, including the amino acid sequence of rhodopsin<sup>3</sup> and of a partially resolved crystal structure of frog rhodopsin<sup>4</sup> and a postulated tertiary structure.<sup>3b,5</sup> Earlier studies on the more stable and readily available purple membrane, the vitamin A containing protein in halobacteria,<sup>6</sup> led to determination of its low-resolution crystal structure<sup>7</sup> and protein sequence,<sup>8</sup> the postulation of a tertiary structure,<sup>9</sup> and voluminous data from varied spectroscopic techniques.<sup>10</sup>

For some years, the research group at Hawaii has been studying photochemistry of polyenes in the vitamin A series.<sup>11</sup> The effort led to the completion of the preparation of all 16 possible geometric isomers of vitamin A,<sup>12</sup> more exactly defining their interaction with opsin,<sup>13</sup> and identifying factors controlling the regioselectivity of isomerization of polyenes.<sup>14</sup> The latter has led to the recent postulation of a new mechanism of photoisomerization for a polyene confined in a rigid medium: H.T.-n, Hula Twist at center n.<sup>15</sup> This mechanism has provided new insight into the chemistry of the two retinal-containing proteins. In this paper, we present a complete summary of our bioorganic,

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mechanistic approach to these and related topics.

## Primary Process of Vision (Background)

Bathorhodopsin is generally considered to be the primary photoproduct of vision.<sup>1,16</sup> Its exact structure and the mechanism of its formation are of great interest. Wald first postulated cis-trans isomerization as the primary process of vision.<sup>1</sup> However, the observation that the primary photoproduct was formed within a few picoseconds after excitation led to doubts that such a volume-demanding process can occur in such a short time.<sup>17a</sup> Hydrogen migration was later suggested instead.<sup>18</sup> However, recent mounting evidence does not

<sup>†</sup> Bioorganic Studies of Visual Pigments. 3. Parts 1 and 2 ref 13a and 13b. See text for complete names for the two mechanisms.

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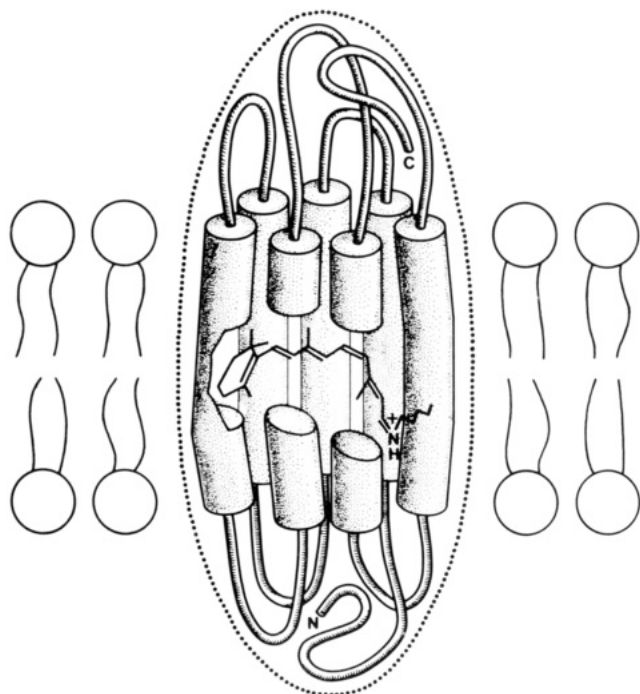
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**Figure 1.** Hargrave model for the tertiary structure of rhodopsin<sup>5</sup> with sections of those helices in the front cut out for better viewing of the retinal chromophore (provided by Prof. Hargrave). Note: Current evidence favors a 15-anti geometry instead of the 15-syn shown.

seem to support this suggestion.<sup>19</sup> Among other proposed models in the literature,<sup>20</sup> the bicycle pedal mechanism<sup>21</sup> is worthy of special mention because it suggested for the first time the possibility of a volume-conserving alternative mechanism for cis-trans isomerization.

Other experimental facts related to the primary process that must be accounted for are the following. The formation of the bathorhodopsin is accompanied by an ~45-nm red-shift of the absorption maximum of the visual pigment<sup>22</sup> and unusual features in its vibrational spectra, obtained first by the resonance Raman technique<sup>23</sup> and subsequently by FT-IR.<sup>24</sup> They are different from those of *all-trans*-retinal and its derivatives in spite of the allegedly identical *all-trans* geometry in both cases. The Raman spectrum has an unusual shift of the peak corresponding to the hydrogen out-of-plane (HOOP) bending mode of H<sub>12</sub>.<sup>25</sup> When irradiated at low temperature bathorhodopsin reverts only to rhodopsin and 9-*cis*-rhodopsin.<sup>22</sup>

### Evolution of the H.T.-n Mechanism

Three factors led to the evolution of the H.T.-n model for photoisomerism of polyenes. First, a model for the chromophore region of rhodopsin has become available

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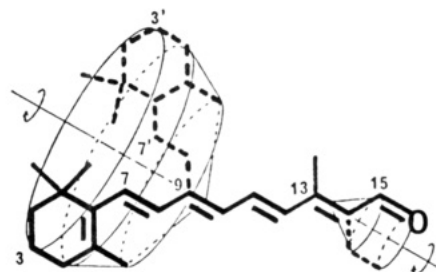
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**Figure 2.** Different spatial requirements for isomerization of *all-trans*-retinal to 13-*cis* (right) and to 9-*cis* (left). The alternative process of rotating C<sub>10</sub> to the 9-*cis* isomer is not shown.

through Hargrave's proposed tertiary structure of rhodopsin<sup>5</sup> (Figure 1). In this structure the chromophore is sandwiched between two layers of protein, each consisting of a set of three helices. The rapid primary photochemical process must therefore be of the form that can take place within the plane between the helices.

Another important structural feature of rhodopsin is the anchored nature of its chromophore. It is bonded to opsin at Lys-296 through a protonated Schiff base linkage.<sup>26</sup> The polyene chain is therefore linked to a rigid  $\alpha$ -helix by the butyl tether of the lysine (Figure 1). The presence of a hydrophobic pocket in opsin for the trimethylcyclohexenyl ring is suggested by studies of interaction of retinal analogues with opsin<sup>27</sup> and inhibition of rhodopsin regeneration by compounds such as  $\beta$ -ionone.<sup>28</sup>

Second, the extended effort of studying photochemistry of polyenes has led to a better understanding of factors controlling the photoisomerization process.<sup>11</sup> It is well-known that *all-trans*-retinal in hexane (or other nonpolar solvents) photoisomerizes regioselectively at the 13,14 and the 9,10 double bonds, giving the 13-*cis* (major) and 9-*cis* (minor) isomers.<sup>29,30</sup> In contrast, bathorhodopsin, which is also supposed to have an *all-trans* chromophore in a nonpolar pocket, isomerizes instead at the 11,12 (major) and the 9,10 (minor) bonds.<sup>22</sup> Two years ago, an explanation for these contrasting facts emerged through the search for a unified explanation for many examples of regioselective and regiospecific photoisomerization of polyenes in the vitamin A series.<sup>14a</sup>

Preferential isomerization of *all-trans*-retinal and its derivatives in a nonpolar solvent at the two trisubstituted double bonds is readily rationalized in terms of the larger steric repulsion at these centers in comparison to that around the disubstituted 7,8 or 11,12 bond. The dominance of the 13-*cis* isomer, however, needs additional explanation. Geometric isomerization at any double bond requires rotation of only one terminal carbon. For the 13,14 bond in the vitamin A series, it is obvious (Figure 2) that rotation for the C<sub>14</sub> portion should be preferred because a smaller volume of reaction, hence the displacement of fewer solvent molecules, is involved. On the other hand rotation of either end of the 9,10 double bond results in a much larger

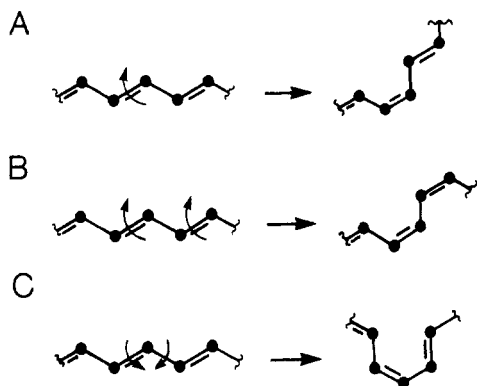
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(30) Product distribution in a polar solvent, hence incompatible to the nonpolar environment of the visual chromophore, is quite different.<sup>14b</sup>



**Figure 3.** Three possible ways of isomerization as demonstrated by a W array of five atoms: upper, one-bond rotation; middle, bicycle pedaling; lower, hula twist.

sweeping cone. Preferential formation of the 13-cis isomer is thus expected.

Displacement of solvent molecules is normally a negligibly small factor in controlling the course of a chemical reaction. However, for very fast processes associated with short-lived intermediates this factor becomes significant. In fact by increasing the viscosity of the medium, the role of the solvent can be magnified as revealed by the excited-state properties of retinal<sup>31</sup> and other organic molecules.<sup>32</sup>

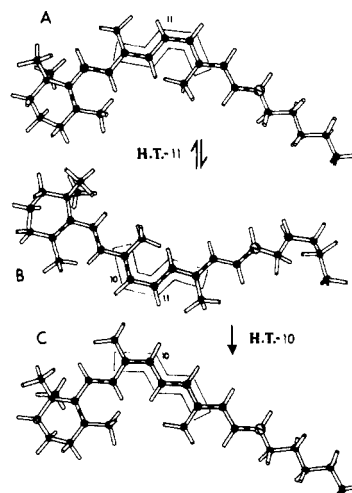
For the visual polyene chromophore embedded in the more rigid protein matrix, one may logically anticipate a greater medium effect. Hence, the different regioselective photoisomerization of bathorhodopsin must be a reflection of the specific protein structure around the polyene. Again, recalling that isomerization about any double bond requires rotation of only one end of each double bond, the combined regioselectivity (i.e., exclusive formation of the 11-cis and 9-cis isomers) suggests that the protein loosely surrounds the polyene near carbons 10 and 11, and more tightly restricts the remaining portion of the polyene.

Third, the use of molecular models greatly assisted conceptualization of the H.T.-n model. Figure 3 shows three possible isomerization processes for five trigonal planar atoms representing a section of a polyene chain. The one-bond rotational mechanism for cis-trans isomerization (A) involves a large reaction volume and significant changes of the orientation of the termini of the polyene chain that seem incompatible with a fast reaction of an anchored, confined polyene. The bicycle-pedaling process (B) with two C-H bonds rotating out of the plane requires a much smaller reaction volume and virtually no reorientation of the termini of the polyene chain. The stereochemical consequence, however, is in disagreement with the experimental results of one-photon-one-bond isomerization for the visual process. A cis,cis geometry is formed from the trans,trans structure, implying one-photon-two-bond isomerization.

Instead of rotating two alternate bonds simultaneously, the H.T.-n process (C) involves concerted rotation of two adjacent bonds. With only one C-H bond projecting out of the plane, an even smaller reaction volume is required. The termini of the polyene remain in the original plane and are only slightly reoriented. Addi-

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**Figure 4.** Molecular models showing H.T.-n transformations of the chromophore of rhodopsin. A: The 11-cis chromophore bonded to the butylamino group of Lys-296. The  $\alpha$ -carbon and the cyclohexenyl ring were partially immobilized in a manner described in the text. After H.T.-11, structure A was transformed to B with the boxed C<sub>9</sub>-C<sub>13</sub> carbons being converted from an inverted sickle to an upright sickle. B: The all-trans,10-s-cis structure in bathorhodopsin. C: 9-cis-Rhodopsin after application of H.T.-10 to B.

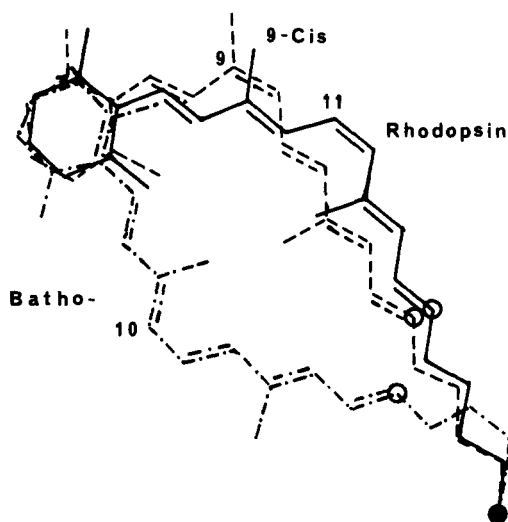
tionally, the trans,trans geometry is converted to a cis,trans geometry, in agreement with the observed one-photon-one-bond isomerization. The newly formed s-cis conformation is compatible with the red-shifted primary product. For example, the Woodward rules for UV absorption require that 39 nm be added to a homoannular (hence, s-cis) diene in calculating its absorption maximum,<sup>33</sup> agreeing with the bathochromic shift of the primary photoproduct. Also, the inherent instability of the s-cis conformation could easily account for the ensuing facile conversion to an intermediate with the more stable s-trans conformation that is consistent with the blue-shift in intermediate subsequent to bathorhodopsin. On theoretical ground there are also reasons to believe that the two-bond concerted motion of the H.T.-n process is a low-energy process.<sup>15</sup>

#### H.T.-11 and H.T.-10

For application of the H.T.-n mechanism to the primary process of vision, a molecular model of the pigment was constructed (Figure 4). The 11-cis-retinyl chromophore bonded to a butylamino group was adjusted to a relaxed conformation: all-s-trans conformation for the polyene chain and staggered conformation for the butyl group. To mimic the rigid environment of a helix the C $_{\alpha}$  carbon, to which the butyl group is bonded, was held immobile. The cyclohexenyl ring was partially immobilized by placing it around a cylinder, which was in turn fixed in position.<sup>13b,15</sup> Thus, the ring was allowed to pivot around the cylinder, an approximation of the nonbonded interaction in the hydrophobic pocket. The five key carbon atoms involved in the H.T.-11 motion (C<sub>9</sub> to C<sub>13</sub>) are boxed in Figure 4.

When the anchored chromophore of rhodopsin (Figure 4A) is subjected to the H.T.-11 process, a structure (4B) containing the all-trans configuration and the 10-s-cis conformation is generated. The latter should

(33) Woodward, R. B. *J. Am. Chem. Soc.* **1942**, *64*, 72.



**Figure 5.** Superimposed structures of the anchored rhodopsin (—), bathorhodopsin (---), and 9-*cis*-rhodopsin (· · ·) during photoisomerization.

produce a red-shift in the UV-vis absorption of this intermediate and makes it structurally different from *all-trans*-retinal and its derivatives, which may account for the unique vibrational spectrum of bathorhodopsin. Moreover, the structure provides a ready explanation for the unusual shift of the  $H_{12}$  HOOP band, i.e. internal steric crowding between the 9-methyl hydrogens and  $H_{12}$ . A particularly relevant observation is the resonance Raman data for the bathorhodopsins of two rhodopsin analogues. For 5-demethylrhodopsin, a similar shift of the  $H_{12}$  HOOP band was observed while for 9-demethylrhodopsin such a shift was absent.<sup>34</sup> The occurrence of 10-*s-cis* structures readily accounts for these puzzling observations.

The current model also readily accounts for the secondary photochemical equilibrium between bathorhodopsin, rhodopsin, and 9-*cis*-rhodopsin. The return to rhodopsin simply follows the identical but reverse H.T.-11 process. However, application of H.T.-10 converts bathorhodopsin to 9-*cis*-rhodopsin in the conformationally stable form (Figure 4C). This form should, as observed,<sup>22</sup> exhibit spectral characteristics identical with that formed from direct combination of 9-*cis*-retinal and opsin. On the other hand, the same H.T.-10 process is not available to rhodopsin because it would lead to a much shortened and severely crowded 9-*cis*,10-*s-cis*,11-*cis* structure.

When structures 4A-C are superimposed at the anchor points (Figure 5), it becomes clear that substantial motion of the carbons in the polyene chain would take place during the rhodopsin to bathorhodopsin to 9-*cis*-rhodopsin conversions. All atoms, however, move generally within the plane of the chromophore, which is allowed in the Hargrave model of rhodopsin. The only exceptions to this generalization are the neighboring  $C_{11}$  and  $C_{10}$  hydrogens. The displacement of the polyene chain (Figure 5) also suggests possible relocation of the counterion, the "second point charge",<sup>35</sup> and other perturbations<sup>36</sup> during the photochemical trans-

formations. Therefore, contribution to the red-shifted absorption could be due to changed protein perturbations of the  $\pi$  system as well as the presence of the *s-cis* conformation.

#### Dark Intermediates: B.P.-m,n

Bathorhodopsin is highly unstable. In vertebrates, it successively cascades to *all-trans*-retinal and opsin by way of a series of intermediates (lumirhodopsin, metarhodopsin I, metarhodopsin II, pararhodopsin),<sup>1,22</sup> for many years, characterized only by their UV-vis spectra. But in fact, the chromophoric structures of these intermediates are suggested by their secondary photochemistry. For example, lumirhodopsin is known to revert to rhodopsin and 9-*cis*-rhodopsin.<sup>37</sup> Invoking the same mechanistic considerations applied to bathorhodopsin, one concludes that the chromophore of lumirhodopsin is likely to be identical both configurationally and conformationally with that of bathorhodopsin.<sup>38</sup>

The substantial lateral displacement of the polyene chain during the earlier transition from rhodopsin to bathorhodopsin should induce compensatory motion of those protein side chains around the polyene chromophore. However, since such conformational changes may require more than the  $\sim 20$ -ps duration of the photochemical transformation,<sup>17b,c</sup> the primary photoproduct must be one with a nonrelaxed protein environment. Hence, lumirhodopsin is probably a protein-relaxed form of bathorhodopsin in which the polyene chain may also have undergone minor conformational readjustment.<sup>38</sup> The blue-shift of the absorption spectrum of lumirhodopsin is presumably due to the above-mentioned modified protein perturbation of the chromophore.

The loss of the crowded 10-*s-cis* conformation must then occur after the lumi stage. Metarhodopsin was reported to be photostable at  $-65$  °C,<sup>37</sup> a result consistent with the presence of an altered polyene structure in its chromophore. A delayed *s-cis* to *s-trans* conformational change is consistent with an activation energy associated with rotation of formal single bonds in a conjugated polyene. The added blue-shift is consistent with the presence of the 10-*s-trans* conformation. At room temperature, metarhodopsin I was reported to revert photochemically back to rhodopsin.<sup>39</sup>

Metarhodopsin can exist in two forms: the protonated meta I and the deprotonated meta II. Formation of meta II involves loss of the iminium proton and extensive opening of the protein pocket.<sup>40</sup> Most interestingly, irradiation of meta II was found to produce a transient with an absorption maximum at 467 nm that is believed to be 13-*cis*-rhodopsin.<sup>41</sup> Based on the above discussion of the photochemical properties of the unconfined *all-trans* chromophore, we now indeed expect the major photoproduct to be the 13-*cis* isomer!

The loss of the crowded 10-*s-cis* geometry is proposed to occur at the lumirhodopsin to metarhodopsin stage.

(37) Hubbard, R.; Brown, P. K.; Kropf, A. *Nature (London)* 1959, 183, 442.

(38) Earlier lumi was incorrectly assigned with the 10-*s-trans* conformation.<sup>15</sup> The current conclusion parallels that of Hubbard et al.<sup>37</sup> except that their argument, presented before the establishment of bathorhodopsin, was directed toward lumirhodopsin and metarhodopsin.

(39) Baker, B. N.; Williams, T. P. *Vision Res.* 1971, 11, 449.

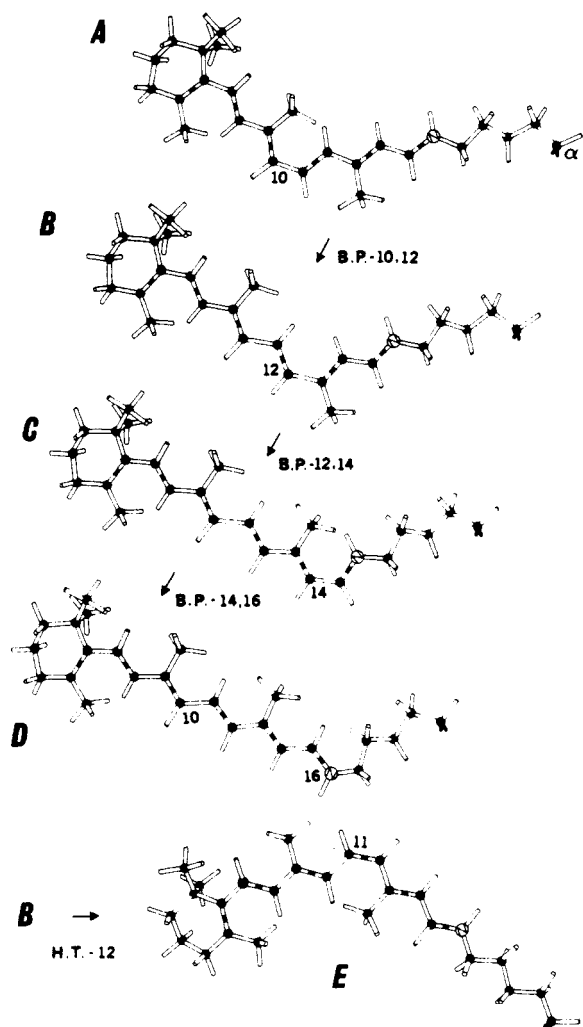
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(36) Kakitani, M.; Kakitani, T.; Rodman, H.; Honig, B. *Photochem. Photobiol.* 1985, 41, 471.



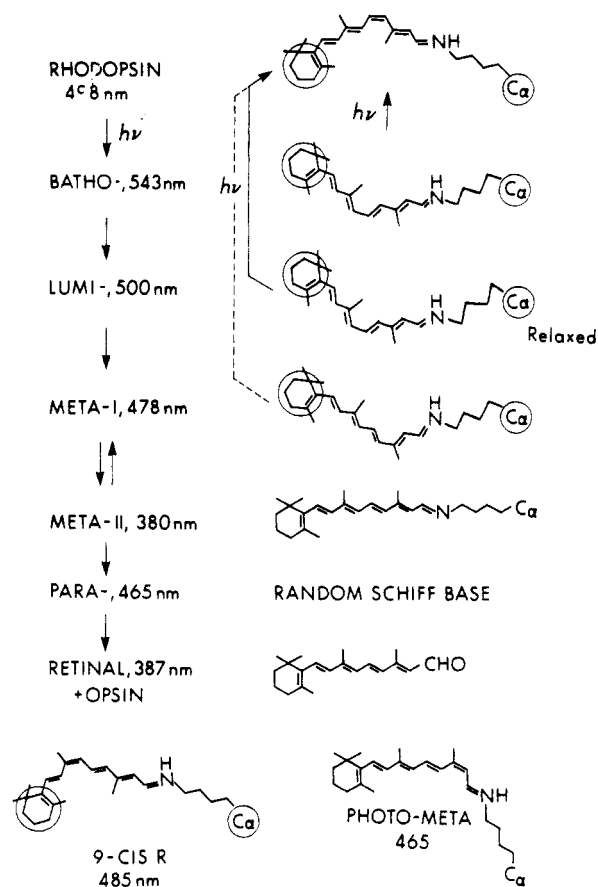
**Figure 6.** Molecular structures showing transitions between lumi (A), meta I (B), and meta II via B.P.-m,n processes. The 14-s-cis structure (C) should have only a transient existence (see text). Structure D after the loss of the iminium proton and the tertiary protein structure is meta II. Meta I upon irradiation gives rhodopsin (E).

Since both the lumi and meta I are still enclosed by the protein, the reaction path should continue to be dictated by the shape of the cavity.<sup>42</sup> Following the same arguments presented for H.T.-n, it is clear that the normal one-bond rotational process for the s-cis to s-trans conformational change is not likely to be in operation.<sup>43</sup> Of the two volume-conserving processes, H.T.-n is for excited polyenes, while B.P.-m,n (bicycle pedaling at bonds m,n) when used for twisting single bonds can be a ground-state process. However, the first B.P.-10,12 process (Figure 6) only results in the transfer of the cis linkage from the 10,11 position to the 12,13. Two subsequent, successive B.P.-12,14 and B.P.-14,16 processes result in the eventual transfer of the cis linkage to the butyl tether (Figure 6).

A close examination of the model reveals that the first B.P.-10,12 process should be relatively easy because it involves rotation of two C-H bonds out of the plane of the molecule. But, the second B.P.-12,14 process should be more sterically inhibited because of the 13-methyl

(42) Any minor protein changes including slight altering of the distance between the anchors should have little effect on the course of reactions of the confined polyene.

(43) Liu, R. S. H.; Matsumoto, H.; Asato, A. E.; Mead, D. J. *Am. Chem. Soc.*, in press.



**Figure 7.** Structures of the intermediates in the visual cycle that involves additional steps of dark isomerization of all-trans-retinal to 11-cis followed by pigment regeneration. The circles emphasize the anchored nature of the chromophore. Lower: Secondary photochemical products.

group. This slow step is followed by a less inhibited B.P.-14,16 process. Therefore, the observation of two forms of metarhodopsins could be explained on these kinetic grounds. Furthermore, since the last step involves relocation of the charged iminium group, significant disruption of the protein structure is expected to follow. Therefore, it is not surprising that at this stage the hydrophobic pocket is opened.<sup>40</sup> Also this charge relocation is probably related to the early receptor potential (ERP),<sup>44</sup> known to occur at the meta II stage.

The above mechanistic considerations allow one to propose specific molecular structures for all key intermediates in the visual cycle (Figure 8).

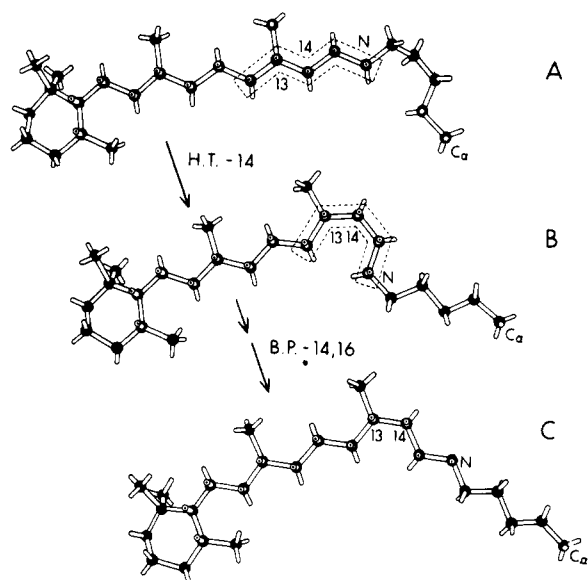
#### Vertebrates and Invertebrates: H.T.-12<sup>43</sup>

The invertebrate visual pigments<sup>45</sup> are different from those of vertebrates in several ways. Chemically most striking is the stability of the metarhodopsins in invertebrates. Instead of spontaneously dissociating to retinal and opsin, they regenerate upon standing or more efficiently by irradiation the corresponding rhodopsins.<sup>45</sup>

The unique chemistry of the invertebrate visual process is understandable on the grounds of a more restrictive pigment binding site. The recently deter-

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**Figure 8.** Molecular structures showing conversion of BR<sup>t</sup> (A) to K or L (B, see text) and M-I via (C) the H.T.-14 and B.P.-14,16 processes.

mined protein sequence for flies<sup>46</sup> reveals a substantial difference of its structure in comparison to those of the vertebrates,<sup>3,47</sup> suggesting a possible genetic origin for a tighter protein binding site. The stable metarhodopsins in invertebrates probably retain the all-trans,12-s-cis structure produced by the B.P.-10,12 process, with the subsequent volume-demanding B.P.-12,14 process obviated by protein-induced steric inhibition. Therefore, no meta II and protein disruption are detected in the invertebrates. Irradiation of the encapsulated metarhodopsin should result in hula twist again. For the 12-s-cis structure, the only available pathway is H.T.-12, giving the 11-cis,12-s-trans structure, i.e. regenerating the corresponding rhodopsin, in agreement with the known property of invertebrate metarhodopsin. One may further speculate that the same H.T.-12 process is involved in the photochemical regeneration of the 11-cis geometry in the unique cephalopod pigment retinochrome.<sup>48</sup> An obvious implication of this conjecture is that retinochrome selectively interacts with the 12-s-cis conformer of *all-trans*-retinal that, incidentally, is the reactive form in the Diels-Alder reaction.<sup>49</sup>

#### Bacteriorhodopsin: H.T.-14 and B.P.-14,16<sup>50</sup>

Bacteriorhodopsin, BR<sup>t</sup>, exhibits some chemical properties similar to those of rhodopsin.<sup>20</sup> For example, geometric isomerization is also involved in the photo-process, converting an all-trans chromophore to the 13-cis. A short-lived, red-shifted primary photoproduct (K) is also followed by decay to a series of intermediates (L, M-I, M-II, and O). Bacteriorhodopsin also exhibits features different from those of rhodopsin in that the dark intermediates spontaneously return to BR<sup>t</sup> and

that the L to M step is accompanied by proton pumping with the net result of converting solar energy to an electrical gradient across the membrane.

The regiospecific (13-trans to 13-cis) photochemical property suggests that the BR protein with the exception of those groups around C<sub>14</sub> surrounds the polyene chromophore rather closely. However, since the all-trans chromophore is the longest among all the retinal isomers, it is obvious that for an anchored chromophore the butyl group must exist in a twisted (coiled)<sup>50</sup> conformation so that its subsequent extension during the photochemical process can permit formation of a shortened chromophore. Arbitrarily, the distance between the two anchors, the  $\alpha$ -carbon of Lys-216<sup>51</sup> and the hydrophobic pocket,<sup>52</sup> was chosen to be that of the 13-cis isomer.<sup>50</sup> With the distance fixed, the structure was converted to the all-trans geometry (Figure 8A).

Following the same rationale outlined for the visual pigment, the primary process of BR<sup>t</sup> was proposed to be H.T.-14,<sup>50</sup> which converts BR<sup>t</sup> to the structure shown (Figure 8B), corresponding to K<sup>t</sup>. It contains the 13-cis configuration and the 14-s-cis conformation. Therefore, its vibrational spectra would be expected to exhibit features different from that of 13-cis-retinal and its derivatives.<sup>53</sup> The s-cis conformation would also contribute to the observed bathochromic shift.

Both K and L upon irradiation at low temperature revert back to BR<sup>t</sup>.<sup>54</sup> Following the same argument presented for lumirhodopsin, one would conclude that L must also have the 13-cis,14-s-cis geometry. Therefore, as has been suggested<sup>54</sup> the difference between K and L must be protein based: K being the protein unequilibrated primary photoproduct, while in L the protein side chains near the chromophore are equilibrated. The accompanying change in absorption maxima from K to L would then be primarily due to modified protein perturbation of the polyene chromophore.<sup>55</sup>

The transition from L to M leads to proton release presumably to the outside of the bacteria membrane. At the molecular level the chromophore is known to convert from the protonated to the unprotonated Schiff base form.<sup>56</sup> Therefore, it is logical to associate the proton loss at the Schiff base site with the proton-pumping phenomenon. A complicating factor is the possible existence of two forms of M, first designated as M-I and M-II<sup>57</sup> and more recently as M<sup>f</sup> (fast) or M<sup>s</sup> (slow)<sup>58</sup> present in equilibrium or formed subsequently. On the basis of the anchored, tethered nature of the chromophore a bicycle-pedaling process at bonds

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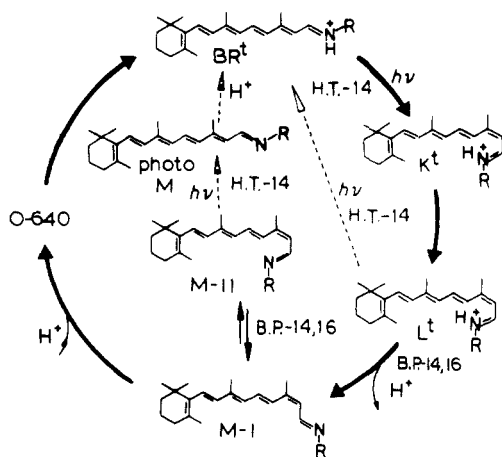
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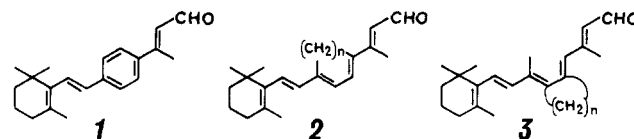
**Figure 9.** Proposed structures of the intermediates in the photocycle of bacteriorhodopsin. K and L differ in their protein structures (see text).

14,16 (B.P.-14,16) for the L to M conversion was proposed,<sup>50</sup> giving a 13-cis,14-s-trans structure (Figure 8C). The process involves semicircular motions of the C<sub>15</sub>-H and the N-H bonds. During this sweeping motion, the acidic iminium hydrogen is proposed to become proximal to the opening of a proton channel,<sup>59</sup> and deprotonation of the iminium group results. That M can photochemically revert back to BR<sup>t</sup>,<sup>54</sup> suggested the 13-cis,14-s-trans structure in equilibrium with the 13-cis,14-s-cis form, the requisite geometry for returning to BR<sup>t</sup> via the H.T.-14 process. These two conformers for M could be those detected in experiments.<sup>57,58</sup> On the other hand, the Raman spectra of the two forms of M were recently reported to be quite similar.<sup>60</sup>

These mechanistic considerations lead us to propose specific molecular structures for all intermediates in the photocycle of BR<sup>t</sup>. They are shown in Figure 9. However, when described in this form they do not show the anchored nature of the chromophore. Several years ago, Schulten, on the basis of calculations on a simplified chromophore, proposed a scheme<sup>61</sup> similar to that in Figure 9. His K is identical. No L was specified, and the two M's, based entirely on bond order arguments, were the reverse of ours. So even though based on an entirely different premise (i.e., not considering the effect of the protein), Schulten reached conclusions remarkably similar to those derived from the H.T.-n model.

### Model Studies for the Proposed Primary Products

While the proposals of the all-trans,10-s-cis and 13-cis,14-s-cis structures for the primary photoproducts of rhodopsin and bacteriorhodopsin would seem to lend themselves to ready experimental and theoretical verifications, we must caution possible difficulties. Considerable amounts of strain energy are present in bathorhodopsin (~35 kcal/mol)<sup>62</sup> and K<sup>t</sup> of BR (15.8 ± 2.5 kcal/mol).<sup>63</sup> They far exceed the 3–6 kcal/mol



**Figure 10.** Model compounds for proposed bathorhodopsin.

normally attributed to that of an s-cis conformation in a conjugated system.<sup>64</sup> The excess strain is likely protein induced (for example, when filling a compressed chromophore lengthened during the photochemical transformation<sup>15</sup> or due to the polyene chain pressing against the protein). Such additional strains are difficult to incorporate into model systems (Figure 10). Thus, the failure of analogue 1 (with the all-trans,10-s-cis bathorlike structure) to bind with opsin,<sup>65</sup> while consistent with the proposed structure for the bathorhodopsin, cannot safely be construed as a proof for the structure. Similarly, the spectral data of the analogues 2 and 3 and their protonated Schiff bases might not provide useful information on bathorhodopsin. Only after proper evaluation of all-protein-induced strains might one be able to interpret reliably experimental data based on model systems.

Additional evidence for or against the mechanistic processes proposed herein are potentially obtainable from calculations and experiments. In that regard it should be noted that the H.T.-14 process has already been shown by calculations to be a low-energy pathway in a model BR system (although not including any protein perturbation).<sup>61b</sup> And, steric inhibition of the hula twist process by structural modification at the reaction site has been demonstrated in several analogue studies.<sup>65</sup>

### Epilogue

This account shows that a relatively simple approach of model construction and mechanistic reasoning can provide a consistent picture explaining, without exceptions, numerous facts related to the chemistry of the visual chromophore that has not previously been provided from other more elaborate studies. Many of the specific molecular structures that emerged from the current deductive reasoning are potentially verifiable by experiments and/or calculations. Therefore, it is our hope that this work will stimulate efforts to test the specifics proposed herein and the implications derived from them, and that these efforts will in turn eventually lead to a complete molecular description of the visual process.

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