

Photoisomerization by Hula-twist. Photoactive biopigments^{*,†}

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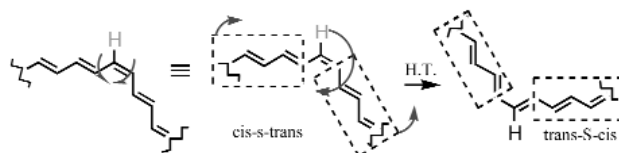
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Abstract: A review of literature on photoisomerization of bilirubin and photoactive yellow protein suggests possible involvement of the volume-conserving Hula-twist (HT) reaction mechanism in their primary photochemical processes. Additional definitive experiments to verify their involvement are proposed. Information related to photoproducts of bacteriorhodopsin, rhodopsin, and phytochrome are reviewed. For carotenoids, because of participation of the longer lived triplet state in, for example, the photosynthetic apparatus, the involvement of HT is probably less likely.

INTRODUCTION

Geometric isomerization is a common reaction for carotenoids [1]. In this paper, we wish to consider a new reaction mechanism for photoisomerization, its scope in reactions of photoactive biopigments and its possible involvement in photochemistry of carotenoids. It is the Hula-twist process.

Hula-twist (HT) was introduced in 1985 as a volume-conserving reaction mechanism to rationalize the rapid isomerization of the 11-*cis*-retinyl chromophore in rhodopsin following light excitation [2]. It involves sweeping translocation of a single C–H unit with the net result of simultaneous conformational and configurational changes of two adjacent bonds.

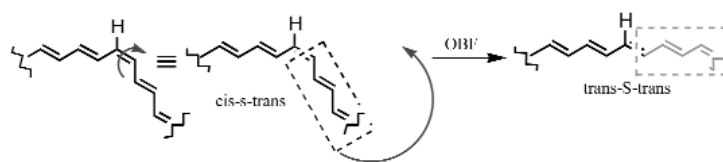


It was 13 years later that the first examples of such a two-bond photoisomerization were reported in an investigation of irradiation of isomers of pre-vitamin D in a frozen medium [3].

In late 2000, Liu and Hammond proposed a general mechanistic scheme for all photoisomerization reactions brought by direct irradiation [4]. In it, the conventional one-bond-flip (OBF) process is believed to be involved under unconstrained conditions (commonly in fluid solutions or in the vapor phase), and the volume-conserving HT process in solid solutions or under other constrained conditions. A subsequent review of the literature revealed many previously reported cases of photoisomerization that are now more consistently explained by the HT process [5,6]. New examples of HT are also becoming known. In this paper, the cases of photoactive biopigments that involve photoisomerization in their primary processes are summarized. In a separate paper, examples of simple organic systems were discussed [7].

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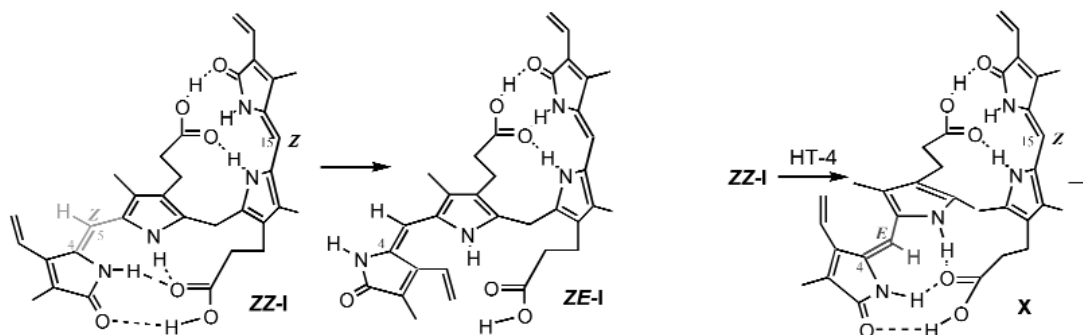
[†]Dedicated to Prof. Silvia Braslavsky on the occasion of her 60th birthday.



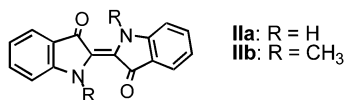
EXAMPLES OF PHOTOISOMERIZATION BIOPIGMENTS

Bilirubin or phototherapy of jaundice

The photochemistry of bilirubin in phototherapy of jaundice was resolved in an elegant study by Lightner and McDonagh [8]. The *Z,Z* isomer (*Z,Z*-I) exists in approximately two symmetrical halves, each containing a network of three internal H-bonds [9]. In this way, all polar functional groups are masked, making the isomer lipophilic. Upon light absorption, photoisomerization gives first the *E,Z* isomer and a simultaneous disruption of part of the H-bonds and then the *E,E* isomer with breakage of more H-bonds. The exposed polar functionalities make the photoproducts water-soluble, thus allowing them to be excreted from the jaundiced infant [8].



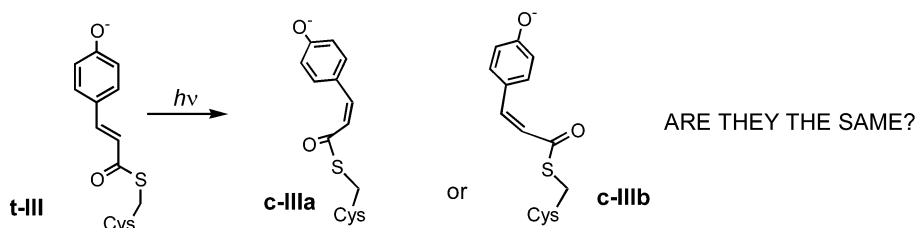
The primary photochemical process of bilirubin, however, remains unclear. In fact, the success of the phototherapy becomes somewhat puzzling when one considers the lack of photoreactivity of the indigo dye **IIa** [10]. In the latter case, the double internal H-bonds are sufficient to obviate the torsional relaxation process of the central double bond. In agreement, the corresponding *N*-methylated pigment **IIb** photoisomerizes readily [11]. How then can the triply H-bonded bilirubin photoisomerize so readily? A closer examination of the two pigments revealed an obvious difference. The presence of a vinyl hydrogen in bilirubin, but not in the indigo dye, is likely to be the key feature. Such a partial structure is necessary for HT [6,12]. Hence, it has been proposed that the primary process of excited bilirubin involves *E,Z* isomerization via HT but not OBF, yielding initially a primary product (**X**) in which the H-bonds remain intact. Since HT is believed to be a diabatic process, the strained product should be in the ground state. Thus, disruption of the H-bonds is likely to be a sequential ground-state process leading eventually to the final stable *E,Z* isomer. A second photon that converts the *E,Z* isomer to the *E,E* isomer is likely to initiate the same sequence of events. However, pico-sec time-resolved absorption studies at room temperature failed to detect any transients other than that attributable to excited bilirubin [13].



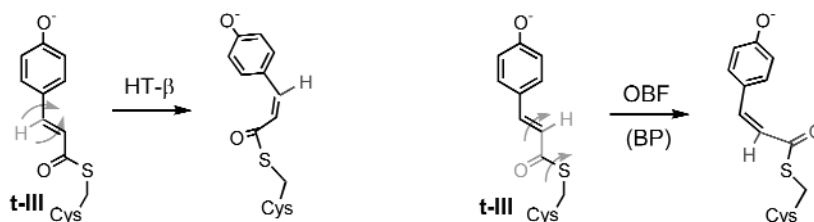
Photoactive yellow protein (PYP)

For the photophobic PYP (photoactive yellow protein) pigment, its photochemistry and structure have been examined in detail [14]. The chromophore is the *trans*-*p*-thiocinnamate, *t*-**III**, shown below. The photoproducts including the dark intermediates have been examined by time-resolved UV-vis and FT-IR spectroscopies and low-temperature spectroscopic techniques.

By nano-second time-resolved X-ray crystallography at room temperature, the first detectable photoproduct of PYP (MW^a 14 000) was determined [15], and the *cis*-chromophore is shown (structure *c*-**IIIa**) below. The conversion from the pigment to the photoproduct was described as a “crank-shaft” motion. By steady state irradiation at 100 K, the crystal structure of the PYP photoproduct (*c*-**IIIb**) was also determined [16], apparently different from *c*-**IIIa** (relative to the virtually stationary protein residues). The *cis* chromophore in *c*-**IIIb** obviously was forced fit into the reaction space available within the frozen host cavity with the *cis* double bond twisted at an amazing 80°. These two products were likely products along two different pathways (photocycles) detected first in low-temperature spectroscopy, then by time-resolved fast kinetics at room temperature [17].



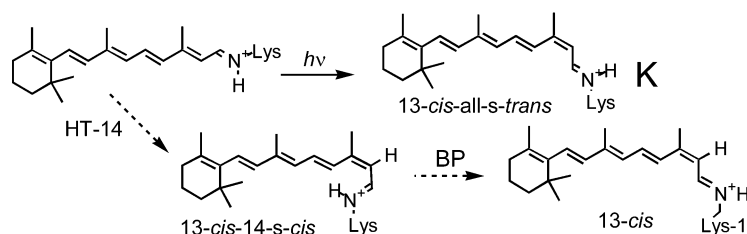
It occurred to us that there are two separate volume-conserving pathways of isomerization that can lead to different products if conducted under different conditions. Structure *c*-**IIIa** is the one expected from the HT process around the β -CH unit (with respect to the thioester). This implies no flipping of the phenoxide ring (not detectable) nor the thioester unit [15]. Structure *c*-**IIIb**, on the other hand, is one expected from an OBF process (the preference at the smaller thioester end was emphasized [16]). The presence of the carbonyl group, or more importantly the nearby single bond, now allows OBF via a unique volume-conserving bicycle-pedal (BP) process [18] (above, right). In this case, the BP process involves a double and a single bond, thus unlike two double bonds in a formal BP process [18], that makes it energetically possible as a photochemical process. Therefore, PYP is unique in the sense that the free volume available for isomerization is different for solubilized protein versus that of frozen protein. This difference apparently led to the different regiospecific volume-conserving processes [15a]. The use of an analog involving an unsymmetrically labeled phenoxide ring could verify the absence of ring flip during either photoisomerization pathway.



Bacteriorhodopsin

The X-ray crystal structure of the 2.8 KD bacteriorhodopsin (bR) is known [19]. The structure of the primary photoproduct K has also been elucidated recently [20]. The results are similar to that of PYP in the sense that there is no significant movement of the protein residues during isomerization, i.e., photochemistry again took place within the reaction volume available to the chromophore. It clearly shows

that the predicted 14-*s-cis*-13-*cis* structure [20,21] that requires a significant two-dimensional change in shape, could not possibly take place. In fact, the crystal structure of K shows that the chromophore contains the 14-*s-trans*-13-*cis* structure [20].



However, the stable K intermediate does not rule out the possibility that the primary photochemical process started with HT-14. The rigid protein structure would not allow the process to proceed to completion. The motion could lead to a highly strained 13-*cis*-geometry (e.g., equivalent to the 80° twisted photoproduct of PYP) in the ground state. The butyl tether of the lysine anchor should allow an immediate bicycle pedal motion (BP-14,16), thereby transferring the *s-cis* linkage to the alkyl side chain and in the process relieving much of the strain. The possible sequence of events is depicted by the dotted arrows. Whether J, the precursor of K, plays a role in the conformational relaxation process is a point of interest.

Other photoactive biopigments

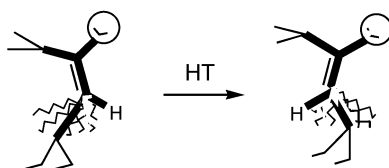
The X-ray crystal structure of *rhodopsin* is now known [22]; however, that of bathorhodopsin, the first stable photoproduct, is still not available. Hence, the exact nature of its photochemistry remains speculative. If one assumes a similar absence of protein movement and limited reaction volume available for the primary process as in PYP and bR, then the proposed structure of 10-*s-cis*-all-*trans* [1] or 12-*s-cis*-all-*trans* [23,24] for bathorhodopsin is not likely to be correct. A more likely one that involves a minimum volume change from rhodopsin is that of 10-*s-trans*-all-*trans*. To reach that structure, we would like to suggest an initial motion of HT-12 [23] (observed in a time-resolved RR experiment) [25] followed by sequential BP motions (BP-10,12, BP-12,14, and BP-14,16) to transfer the 12-*s-cis* linkage to the butyl tether. The net consequence is the same as a twist and shear mechanism suggested by Kakitani [26]. It will be of interest to determine the role of photorhodopsin, the unstable precursor of batho, in this proposed mechanistic scheme.

The structures of *phytochrome* containing a tetrapyrrole near-infrared absorbing pigment and its primary photoproduct are less well characterized [27]. However, it has been pointed out that a BP motion cannot be involved in its photochemical process. Instead, HT was suggested.

Lastly, a few words on photochemistry of carotenoids appear appropriate. For bound carotenoids, photochemistry has been studied in most detail in the photosynthetic apparatus. Its role is believed to be central in protecting the system from any destructive reactions of singlet oxygen via triplet-triplet energy transfer [28]. Hence, the 15-*cis*/15-*trans* isomerization is likely a triplet state reaction. The longer triplet lifetime [29] would mean that the rigidity of the protein matrix plays a less important role, minimizing possible involvement of HT. For photochemistry of other bound carotenoids [30], their transformations are less well understood but certainly should be examined in light of the new photochemical concepts that are now available.

APPENDIX

The volume-conserving nature of the Hula-twist motion and the absence of changing sidedness of bulk of the molecule can be described, perhaps more clearly, by the following cartoon figures.



ACKNOWLEDGMENT

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Note added in proof. Because of the revised time-resolved X-ray crystal structure of PYP photoproduct [Z. Ren, B. Perman, V. Srajer, T.-Y. Teng, C. Pradervand, D. Bourgeois, F. Schotte, T. Ursby, R. Kort, M. Wolff, K. Moffat. *Biochemistry* **40**, 13788–13801 (2001)], the mechanistic process described above for the primary photochemical process of PYP will have to be modified. An expanded and modified view is available elsewhere: Y. Imamoto, M. Kataoka, R. S. H. Liu, submitted to *Photochem. Photobiol.*