# Does the Chromophore's Ring Move after Photoexcitation of Rhodopsin?

Thomas G. Ebrey and Masato Kumauchi
Department of Biology, University of Washington, Seattle, Washington 98195

ABSTRACT By comparing the shift of the absorption maxima when a visual pigment is converted to its lumirhodopsin photointermediate for two classes of pigments, we can infer whether or not the pigment's $\beta$-ionone ring has left its binding site. We compare this shift for the long-wavelength sensitive visual pigment of chicken iodopsin ($\lambda$max = 571 nm), which has polar residues in the ring binding site that interact with the ring, with that for three pigments, which do not. We conclude that by the time the Lumi product of the pigment is formed, the ring has moved away from the ring binding site.

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Address reprint requests and inquiries to Thomas G. Ebrey, Tel: 206-685-3550; E-mail: tebrey@u.washington.edu.

What is the action of light on the chromophore of rhodopsin? There is agreement that light causes a rapid *cis-trans* isomerization (reviewed in (1)), but does this lead to large movements of the chromophore itself with respect to its binding site? Recently Borhan et al. (2) has presented evidence from photoaffinity labeling experiments that the $\beta$-ionone ring of the retinylidene chromophore of rhodopsin (see Fig. 1) moves substantially in going from the initial, unphotolyzed state of the pigment to an early photointermediate, lumirhodopsin (Lumi).

We believe there is another set of experiments that also suggests that the $\beta$-ionone ring undergoes significant movement during this change. These experiments are based on the observation that the absorption spectrum of a visual pigment can be greatly red shifted by alteration of three amino acid residues (at positions 164, 261, and 269, using the residue numbers of bovine rhodopsin) (3,4). These changes are primarily responsible for the spectral shift from a ''green cone'' pigment (for humans 531 nm) to that of a ''red cone'' (561 nm) pigment. When these residues are changed from an apolar residue to a polar one, Ala-164-Ser, Phe-261-Tyr, and Ala-269-Thr, the spectrum is red shifted by $\sim$1070 $cm^{-1}$ (average of seven pairs of pigments). Changes at two of the three residues, 261 and 269, account for most of the change (5,6).

The x-ray structure of rhodopsin (7,8) showed that these two residues were part of the retinal binding site and close to the $\beta$-ionone ring (see Fig. 1). The shift to longer wavelengths depends on the strength of interaction and so the distance between the ring and the two polar residues. If the ring moves away from its usual binding site in forming its Lumi intermediate, there should be an anomalous blue spectral shift for this intermediate compared to a visual pigment that does not rely on the two residues to shift its absorption spectrum. Bovine rhodopsin ($\lambda_{max}$ = 498 nm) is an example of the type where both residues are in their nonpolar form and so their interaction with the ring is less important. Another example is a chicken pigment, P508, in the RH2 family (for visual pigment families see (9,10)). The chicken long-wavelength sensitive cone pigment iodopsin is an example of the type where both residues are in their polar form, and that, along with the effect of chloride binding, shifts the spectrum out to 571 nm. A third example with nonpolar residues is a mid-wavelength pigment from gecko, P521, which is in the same visual pigment family as iodopsin (9,11). The gecko pigment, like iodopsin, binds a chloride ion to help shift its absorption maxima to longer wavelengths (reviewed in (10,11)).

We first calculated the wavelength shift upon the formation of the first photointermediate, bathorhodopsin, for these four pigments using the data presented in references (12,13). In all four pigments the bathoproduct is shifted $\sim$1400 $cm^{-1}$ to the red of the unphotolyzed pigment and so there is no anomalous difference in the spectral shifts for the two types of pigments, suggesting that the ring had not moved away from the two residues at this stage. In forming the next photointermediate, Lumi, bovine rhodopsin is shifted back to a value near its initial absorption maximum (497 nm, so almost no net shift) and the Lumi's of the chicken P508 and the gecko P521 pigments had very similar small shifts when they were formed. However, the Lumi product of iodopsin (535 nm) is shifted by a much larger amount, 1510 $cm^{-1}$, and to the blue of its initial absorption maximum. This is the result predicted if the ring moved away from the two hydroxyl-containing residues in forming Lumi. Such a large shift might also occur if the chloride were to be released when the Lumi intermediate of iodopsin is formed, but this cause is excluded because the gecko pigment has the chloride, but not the binding site shifting residues, and its Lumi shifts just like the nonchloride binding pigments.

These results imply a striking difference between the effects of light on visual pigments compared to bacteriorhodopsin. There are several similarities in the photochemistry


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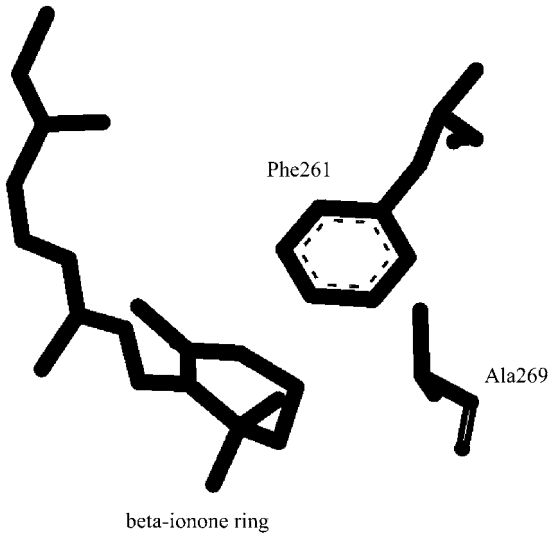


**FIGURE 1 The special relationship of the two wavelength-shifting residues at positions 261 and 269 of visual pigments to the $\beta$-ionone ring of the retinylidene chromophore. Based on the data of reference (7) (Protein Data Bank code 1F88).**

of these two classes of retinal pigments such as photoisomerization, in a temperature-independent process, leading to a high free-energy, red-shifted primary photoproduct. However, for bacteriorhodopsin x-ray structures of its primary bathoproduct, K, and the product formed by warming K, L, and L's thermal decay product M, all have the $\beta$-ionone ring unmoved from its initial site in the pigment (14). Thus the movement of the $\beta$-ionone ring of a visual pigment as lumirhodopsin is formed represents a fundamental difference between these two types of retinal-based pigments in the steps that occur after photoisomerization.

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