The unusual pK_a of the rhodopsin chromophore

Is this how nature minimizes photoreceptor noise?

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The active site of the visual pigment, rhodopsin, contains a retinyl polyene chromophore that is bound to the protein backbone via a protonated Schiff base linkage to lysine 296 (Fig. 1). When this chromophore absorbs light, it undergoes photochemistry (from an 11-*cis* to an 11-*trans* conformation) and initiates a complex series of dark reactions which ultimately generate a nerve impulse (for a recent review see reference 1). The efficiency of this process is quite high (the quantum yield for converting a photon of light into a nerve impulse is about 67%). This high efficiency contrasts with the extremely low rate of thermal activation of the protein, which in the human visual system, generates a false (dark) signal every ~0.01 s for each photoreceptor cell. Because each photoreceptor cell contains $\sim 10^9$ rhodopsin molecules, the dark noise rate is impressively low, $\sim 10^{-11}$ events rhodopsin^{-1} s^{-1}. This characteristic is in part responsible for the ten log units of operating range that is achieved by the eye. The question of how nature controls dark noise is a subject of active debate (1, 8–14).

An article by Steinberg et al. (15) in this issue presents a detailed study of the pK_a of the Schiff base proton on the chromophore within the binding site of the visual pigment, rhodopsin. By using a series of model retinal chromophores with electron-withdrawing substituents, these authors concluded that the apparent pK_a of the protonated Schiff base is 16 or greater. This pK_a value is significantly larger than corresponding values in model compounds or related proteins (see below). Although it was not possible to definitively rule out the possibility that the Schiff base linkage is not accessible for titration from the aqueous bulk medium, more recent studies of proton exchange rates in rhodopsin suggest that this linkage is accessible (R. Callender et al., manuscript in preparation). In this article we explore the potential relevance of a high pK_a value on photoreceptor noise.

There is growing evidence that the mechanism for thermal activation of rhodopsin is a two step process (1, 14). The first step is deprotonation of the 11-*cis* protonated Schiff base chromophore. The second step is thermal 11-*cis* to 11-*trans* isomerization of the chromophore.

$$\mathbf{R}(\mathrm{RNH}_{11\text{-}cis}) \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathbf{R_d}(\mathrm{RN}_{11\text{-}cis}) \overset{k_2}{\rightarrow} \mathbf{B_d}(\mathrm{RN}_{\text{all-}trans})$$

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We note that $\mathbf{B_d}$ (deprotonated bathorhodopsin) generated via the above mechanism would be quite similar to Meta II which is generated via the light induced photobleaching sequence, and would be expected to behave in a similar fashion with respect to activation of transducin. Activation of transducin initiates hyperpolarization of the plasma membrane and the generation of a nerve impulse. Simple reaction rate theory suggests that the total rate of this process can be described (very approximately) by the following equation (16):

$$k_{\mathrm{tot}} = \frac{k_1 k_2}{k_{-1}} \cong \frac{kT}{h}\left(\frac{10^{(\mathrm{pK_a^A} - \mathrm{pK_a^{PSB}})}}{1 + 10^{(\mathrm{pK_a^A} - \mathrm{pK_a^{PSB}})}}\right) \times \left(\frac{1 - \exp\left(\frac{-h\nu_{\mathrm{isom}}}{kT}\right)}{\exp\left(\frac{E_2}{kT}\right)}\right), \quad (1)$$

where $\mathrm{pK_a^A}$ represents the pK_a of the principal proton acceptor group within the protein binding site, $\mathrm{pK_a^{PSB}}$ represents the pK_a of the protonated Schiff base chromophore, ν_{isom} is the frequency of the $C_{11} = C_{12}$ ground state torsional mode, E_2 is the activation energy of the isomerization step, h is Planck's constant, k is Boltzmann's constant, and T is the temperature. If we assume $\mathrm{pK_a^{PSB}} = 16$, $\mathrm{pK_a^A} = 7$, $\nu_{\mathrm{isom}} = 300\ \mathrm{cm}^{-1}$, $E_2 = 22$ kcal mol^{-1} (see reference 17) and $T = 310$ K (body temperature) we calculate $k_{\mathrm{tot}} = 1.5 \times 10^{-12}\ \mathrm{s}^{-1}$. Given the level of approximation inherent in Eq. 1 and the above assignments, the agreement with the observed dark noise rate of $\sim 10^{-11}$ events rhodopsin^{-1} s^{-1} is encouraging. For purposes of discussion, we can write the total rate as proportional to two factors:

$$k_{\mathrm{tot}} = \frac{k_1 k_2}{k_{-1}} \propto k_2 10^{-\mathrm{pK_a^{PSB}}}. \quad (2)$$

If the above model of photoreceptor noise is correct, it follows from Eqs. 1 and 2 that a high pK_a of the protonated Schiff base chromophore is important to the biological control of photoreceptor noise. Model protonated retinyl Schiff base chromophores in solution exhibit pK_a values ~7 (15, 18–20). The retinyl chromophore in bacteriorhodopsin, the light transducing protein in the purple membrane of *Halobacterium halobium*, exhibits a pK_a of ~13 (18). An increase in pK_a of six units upon incorporation of the retinyl chromophore in bacteriorhodopsin is impressive. The corresponding increase of

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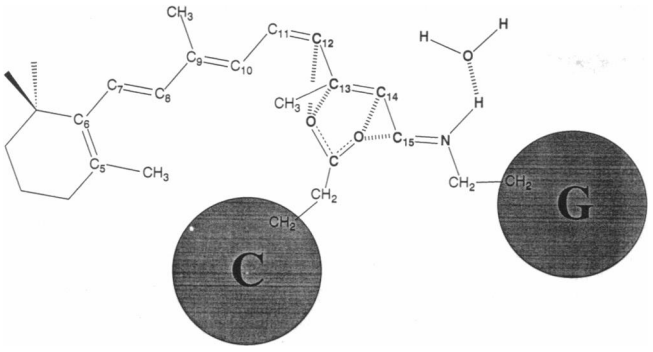

FIGURE 1 A model of the binding site of rhodopsin based on the available experimental data (adapted from reference 1). Two-photon spectroscopy demonstrates that the binding site is neutral (2), and site directed mutagenesis studies have identified the single counterion to be glutamic acid residue 113 on helix C (3–5). Spectroscopic and theoretical studies indicate that the glutamic acid counterion interacts primarily with the chromophore in the region C_{12}–C_{13}=C_{14}–C_{15} region of the chromophore and that at least one water molecule is hydrogen bonded to the imine proton (1, 2, 6, 7).

more than nine units in rhodopsin is extraordinary. Nature rarely explores the limits of physical phenomena without simultaneously improving the comparative advantage of the system. In this case, we hypothesize that the adjustment of the pK_a in rhodopsin is intimately related to the natural selection of a photoreceptor protein exhibiting minimal dark noise. For comparison, if the chromophore of rhodopsin had the same pK_a as that observed in bacteriorhodopsin, the visual pigment would have a dark noise rate three to four orders of magnitude larger.

Further experimental and theoretical work will be required before the mechanistic origins of the high pK_a of the rhodopsin chromophore can be established. We know from site directed mutagenesis studies that the replacement of the primary counterion, Glu_{113}, by glutamine modifies the apparent pK_a to a value close to those observed for model compounds in solution (3–5). Molecular orbital calculations (21) indicate that the strength of the Schiff base N—H bond increases as the counterion is moved down the chain towards atom C_{12} (see Fig. 1). Thus, the shift in pK_a appears to be associated at least in part with the nature of the chromophore-counterion interactions. What remains to be explained is how these specific interactions can generate such an unusual pK_a shift.

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