Wavelength regulation in iodopsin, a cone pigment

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ABSTRACT The opsin shift, the difference in wavenumber between the absorption peak of a visual pigment and the protonated Schiff base of the chromophore, represents the influence of the opsin binding site on the chromophore. The opsin shift for the chicken cone pigment iodopsin is much larger than that for rhodopsin. To understand the origin of this opsin shift and the mechanism of wavelength regulation in iodopsin, a series of synthetic 9-*cis* and 11-*cis* dehydro-and dihydro-retinals was used to regenerate iodopsin-based pigments. The opsin shifts of these pigments are quite similar to those found in bacteriorhodopsin-based artificial pigments. On the basis of these studies, a tentative model of wavelength regulation in iodopsin is proposed.

INTRODUCTION

Visual pigments consist of a chromophore, 11-*cis* retinal, covalently bound to different aproproteins (opsins) via a Schiff base to the ϵ-amino group of a lysine (Wald, 1968, Ovchinnikov, 1982; Hargrave et al., 1983). Resonance Raman spectra indicate that the Schiff base is protonated in rhodopsin (Oseroff and Callender, 1974; Mathies et al., 1977). The absorption maxima of the vertebrate pigments range from ~430 to 580 nm because the different opsins induce different shifts in the energy levels of the bound chromophore. However, because the protonated Schiff base of retinal in solution absorbs at ~440 nm, additional factors must be invoked to explain the longer absorption maximum of visual pigments. Several groups have proposed that charged amino acids positioned around the retinal chromophore might be responsible for the observed shifts (Kropf and Hubbard, 1958; Blatz and Mohler, 1975; Honig et al., 1979). In addition twisting about single bonds and variation in the counter ion-protonated Schiff base distance (Kakitani et al., 1985) can be important determinants of opsin shifts (Spudich et al., 1986; Lugtenburg et al., 1986). Spectroscopic measurements of 9-*cis* dihydro-retinals and the resulting opsin-based pigments led Honig et al. (1979) to propose a specific electrostatic two charge model of wavelength regulation. In this model, in addition to the negative counterion that was assumed to be in close association with the Schiff base, a second negative charge was placed near C-12 of the 11-*cis* chromophore of bovine rhodopsin. Dihydroretinals have also been used to study wavelength regulation in bacteriorhodopsin, halorhodopsin, and sensory rhodopsin, all from *Halobacterium halobium* (Spudich et al., 1986; Lanyi et al., 1988). Here we report our results using 9-*cis* and 11-*cis* dehydro and dihydro-retinals to study wavelength regulation of the red cone pigment, iodopsin (λ_{max} = 562 nm).

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MATERIALS AND METHODS

Chicken retina pigment extracts

Fresh chicken heads were obtained from a local poultry slaughter house and transported to the lab on ice in light-tight containers. The chicken eyes were removed in dim red light and kept at −80°C until needed. They were then thawed at room temperature for 3h and the eyes hemisected in dim red light. The rear half of the eye cup was cut into small fragments and put into ice cold buffer A (50 mM histidine pH 6.5; 50 mM NaCl). After shaking with white sand (100 retinas in 300 ml with 100 gms of sand) and filtering through cheese cloth, the solution was centrifuged at 20 K (J2-21 centrifuge, JA-20 rotor; Beckman Instruments, Inc., Palo Alto, CA) for 40 min and the supernatant discarded. The pellet was washed several times with buffer A until oil droplets from the retina no longer appeared in the supernatant. The pellet was then mixed with 45% sucrose in buffer A and centrifuged at 20 K for 40 min. The resulting supernatant was diluted three times with buffer A, then centrifuged at 20 K for 40 min. The pellet was washed several times until the supernatant became clear, then extracted with 5% digitonin overnight at 5°C. The extract was centrifuged at 20 K for 20 min. and the supernatant saved. 1 to 2 mg of iodopsin and similar amount of rhodopsin were extracted from 100 chicken retinas.

Purification of iodopsin

Purification of iodopsin and rhodopsin was based on the methods by Fager and Fager (1981) with some modifications. A column packed with 3 ml of Con-A sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) was washed with buffer B (50 mM histidine, pH 6.5, 50 mM NaCl, 3 mM $CaCl_2$, 3mM $MgCl_2$, 3 mM $MnCl_2$, and 0.1% digitonin). The chicken retina extract (containing ~0.5 mg of iodopsin) was loaded with a peristaltic pump and the column was subsequently washed with buffer B. The iodopsin was eluted with 20 ml of 1 mM methyl-D-mannoside in buffer B. The eluted fractions (~1 ml each) were

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evaluated by their absorption spectra. The fractions containing iodopsin (λ_{max} = 562 nm) and free of rhodopsin (λ_{max} = 500 nm) were collected and concentrated by ultrafiltration membrane cones (CF-25); Amicon Corp., Danvers, MA). For some pigment regeneration experiments a partially purified mixture of iodopsin and rhodopsin was used. The mixture was prepared by a procedure similar to that used for iodopsin purification but iodopsin and rhodopsin were eluted together with 50 mM of methyl-D-mannoside in buffer B, and then concentrated by ultrafiltration membrane cones.

Artificial chromophores

3,4-Dehydro, 5,6-dihydro, 7,8-dihydro, 9,10-dihydro, and 11,12-dihydro 11-*cis* retinals were synthesized as described elsewhere (Arnaboldi et al., 1979; Koutalos et al., 1989).

RESULTS

We determined the absorption maxima of the dehydro and dihydro pigments regenerated with the apoprotein of iodopsin by selectively bleaching the iodopsin and then recording the spectrum of the pigment regenerated after chromophore addition. To regenerate the 3,4-dehydro 11-*cis* retinal iodopsin-based pigment, a digitonin extract of chicken retina (containing both rhodopsin and iodopsin) was used for both sample and reference. The iodopsin in the sample cuvette was selectively bleached by a projector through a 2-64 cut off filter; Corning Glass

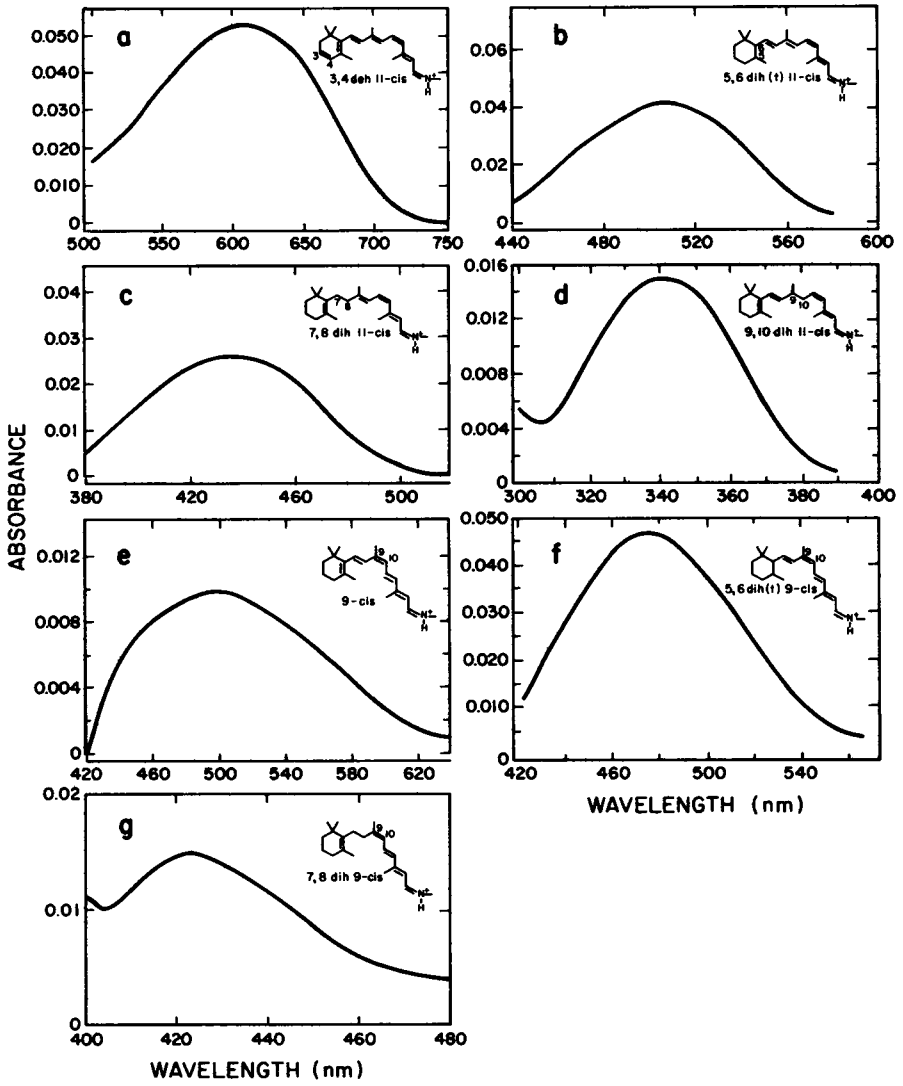

FIGURE 1 Absorption spectrum of *a* 3,4-dehydro iodopsin, λ_{max} = 613 nm; *b* 5,6-dihydro (*trans*) iodopsin, λ_{max} = 505 nm; *c* 7,8-dihydro iodopsin, λ_{max} = 440 nm; *d* 9,10-dihydro iodopsin, λ_{max} = 341 nm; *e* isoiodopsin, λ_{max} = 500 nm; *f* 5,6-dihydro (*trans*) isoiodopsin, λ_{max} = 475 nm; *g* 7,8-dihydro isoiodopsin, λ_{max} = 423 nm.

Works, Corning, NY ($\lambda > 640$ mm) and the baseline spectrum was taken. Then an amount of the chromophore (measured by optical density) equal to the iodopsin before bleaching was added to both the sample and reference cuvettes and absorption spectra taken at 3 min intervals until regeneration was complete. The absorption spectrum of the regenerated pigment was obtained by subtracting the base line from the spectrum after 9 min regeneration. The spectrum of the 3,4-dehydro retinal analogue of iodopsin absorbs maximally at 613 nm. (Fig. 1 *a*). A similar method was also used to determine that the absorption maxima of 9-*cis* isoiodopsin (Fig. 1 *e*), 5,6-dihydro (Fig. 1 *f*), and 7,8-dihydro-isoiodopsin (Fig. 1 *g*), are at 500, 475, and 423 nms, respectively.

Purified iodopsin was used to regenerate the 5,6-dihydro and 7,8-dihydro iodopsin analogues. Iodopsin was bleached and the retinal analogues added as before. Regeneration was fast and complete after 20 min; the sample was then bleached sequentially with a series of cutoff filters starting from long wavelengths and going to shorter ones until the regenerated pigment was bleached. The absorption spectra of the regenerated pigments were obtained by subtracting the spectrum after bleaching from that before bleaching. The 5,6-dihydro (*trans*) iodopsin absorbs maximally at 505 nm. (Fig. 1*b*) while the 7,8-dihydro iodopsin's maximum is at 440 nm.

The Con-A purified iodopsin/rhodopsin mixture was used for 9,10-dihydro iodopsin regeneration for both sample and reference because digitonin extracts of chicken retina absorb strongly where the 9,10 dihydro pigment absorbs. The iodopsin in the sample cuvette was selectively bleached with light of wavelengths >640 nm, and a baseline spectrum was taken. A threefold excess of 9,10-dihydro retinal was added to both sample and reference, and the spectrum taken at hour intervals until the regeneration was completed. The absorption spectrum of regenerated pigment was obtained by subtracting the base line from the spectrum after 12 h regeneration. The pigment absorbs maximally at 343 nm. (Fig. 1 *d*).

Table 1 summarizes the absorption maxima of the artificial iodopsins and their opsin shifts. The absorption maxima of the artifical iodopsins are all red-shifted relative to their protonated Schiff base chromophores. The opsin shift is the difference in wavenumber between a pigment and its protonated Schiff base chromophore (Nakanishi et al., 1980). The opsin shift of 3,4-dehydro iodopsin is 4,950 cm^{-1}, similar to that of iodopsin. As the double bonds at C-5, C-7, and C-9 of 11-*cis* retinal are sequentially hydrogenerated the opsin shifts of the regenerated pigments are gradually reduced from 3,250 cm^{-1} to 1,900 cm^{-1}. The opsin shifts of isoiodopsin, 5,6-dihydro (*trans*), and 7,8-dihydro isoiodopsin are also reduced sequentially from 2,750 cm^{-1}, 2,500 cm^{-1}, to 1,850 cm^{-1}; these shifts are smaller than for the corresponding 11-*cis* analogues.

TABLE 1 Chicken cone (iodopsin) based pigments

Chromophore		Protonated Schiff base	Pigment	Opsin shift
		nm	*nm*	*cm^{-1}*
3,4 deh	11-*cis*	471	613	4,900
	11-*cis*	440	562	4,950
5,6 dih(t)	11-*cis*	433	505	3,300
7,8 dih	11-*cis*	392	440	2,800
9,10 dih	11-*cis*	320	341	1,900
3,4 deh	9-*cis*	471	575*	3,750
	9-*cis*	440	500	2,750
5,6 dih(t)	9-*cis*	425	475	2,500
7,8 dih	9-*cis*	392	423	1,850

*Data from Wald et al. (1953).

DISCUSSION

Fig. 2 compares the opsin shifts of iodopsin analogs with those of the bovine rhodopsin (Koutalos et al., 1989) and bacteriorhodopsin (Spudich et. al., 1986) analogues. The opsin shifts of 9,10-dihydro and 7,8-dihydro analogues of iodopsin are similar to those of the bovine rhodopsin, but the opsin shifts of 5,6-dihydro, natural retinal, and 3,4-dehydro are much larger. Thus, the binding sites of the rod and cone pigments interact very differently with the retinal analogues.

In contrast, the opsin shifts of the iodopsin-based pigments are very similar to those seen for the comparable analogues of bacteriorhodopsin (Fig. 2). The exception is 5,6-dihydro (*trans*) iodopsin which has a larger opsin

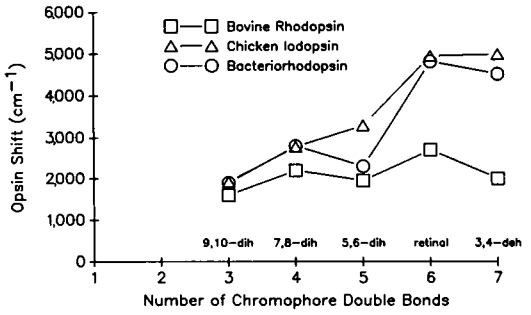

FIGURE 2 Comparison of opsin shifts of dehydro and dihydro pigments among iodopsin, bovine rhodopsin, and bacteriorhodopsin.

shift in iodopsin than in bacteriorhodopsin. These data suggest that the mechanism of wavelength regulation in iodopsin and in the bacterial pigments is similar. The factors that have been implicated for the bacterial pigments and thus, by assumption, for iodopsin are: (*a*) a reduced negative potential in the vicinity of the Schiff base, (*b*) a planarized ring-chain conformation, and (*c*) an ion pair near the β-ionone ring with the negative charge near C-6 and the positive charge near C-7 (Spudich et al., 1986; Lugtenburg et al., 1986; Lanyi et al., 1988).

The reduced negative potential postulated for iodopsin probably is due to a weakened protonated Schiff base nitrogen-counter ion interaction as in bacteriorhodopsin. This would cause more delocalization of the positive charge and an increased red shift in iodopsin and the dihydro analogues. This weaker nitrogen/counter-ion interaction could result from a greater nitrogen/counter-ion distance (Blatz and Mohler, 1975), or from a relatively high dielectric constant near the Schiff base region (Sheves et al., 1985), or from the positioning of the counter-ion lying beneath the plane of the chromophore (Birge et al., 1988).

A second factor that could contribute to the opsin shift is twisting about single bonds, specifically the 6,7 bond. The large opsin shift of 3,4-dehydro retinal and the natural chromophore could be rationalized if the conformation of β-ionone ring of the retinal in iodopsin was 6-s-*trans*. The absorption maximum for the s-*trans* conformer is calculated to be red-shifted by 16–32 nm from that of the s-*cis* one (Honig et al., 1976), and a locked 6-s-*trans* protonated Schiff base analogue of retinal is found to absorb at 465 nm, red-shifted by 25 nm compared with the 6-s-*cis* analogue (Lugtenburg et al., 1986). Unlike the other wavelength control mechanisms, there is no evidence for this in iodopsin. If present, it could make a contribution of up to 1,200 cm^{-1} to the opsin shift.

A third factor which can induce large opsin shifts for the 3,4-dehydro, native, and 5,6-dihydro pigments is the presence of a negative charge (accompanied by a more distant positive counterion) located near the ring. As the saturated bond is moved toward the terminus of the side chain, i.e., from retinal to 5,6-dihydro to 7,8-dihydro to 9,10-dihydro iodopsin, the opsin shift decreases. These results lead to a simple interpretation since the magnitude of the shift induced by external charges would depend on their proximity to the conjugated π-electron system. If a negative charge were present in the vicinity of the β-ionone ring as in bacteriorhodopsin, this would have a maximal effect on the natural chromophore with six double bonds and the effect would successively decrease as the location of the double bond which was saturated approaches the side-chain terminus. While it is possible to obtain a large opsin shift for iodopsin without postulating an electrostatic interaction near the ring, the monotonic increase of the opsin shift in the dihydro retinal series strongly suggests that such an interaction is present. The difference in the opsin shift between bacteriorhodopsin and iodopsin pigments for the 5,6-dihydro chromophore could result from a different orientation of the positive charged counterion which has been placed near C-7 in the bacterial pigment.

Additional evidence for a second negative charge along the polyene chain comes from the smaller opsin shifts for the 9-*cis* pigments compared with the 11-*cis* ones (Table 1). A simple explanation for this difference is that with a second negative charge near the ring, the 11-*cis* chromophores are in an optimal conformation to shift the absorption maximum to longer wavelengths while with the nonphysiological 9-*cis* chromophores, the polyene chain is not as close to the second negative charge, leading to blue shifted isorhodopsin pigments.

A model of color regulation in iodopsin is shown in Fig. 3. A weakened nitrogen-counter ion interaction is assumed. The second negative charge near the ring is postulated to account for the gradually increasing opsin shift for the dehydro and dihydro chromophores while, in contrast to bacterioprhodopsin, the accompanying posi-

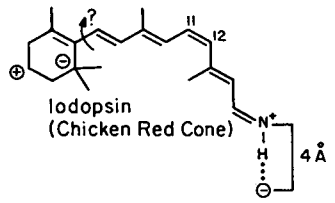

FIGURE 3 A model of color regulation in iodopsin. The negative counterion is placed slightly further away from the protonated nitrogen (4 Å) than is found in solution. A second negative charge is placed near C-7; its positive counterion is placed distant from the conjugated chain. The 6-7 bond is tentatively taken as s-*trans*.

tive charge is placed so as to minimize its interaction with the chain. The ring is tentatively taken to be the planar s-*trans* which is the most likely conformation in bacteriorhodopsin (Harbison et al., 1985; Lugtenburg et al., 1986). However, it should be emphasized that at present there is no evidence for an altered ring conformation in iodopsin.

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