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Sensitizing Pigment in the Fly*

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Abstract. The sensitizing pigment hypothesis for the high UV sensitivity in fly photoreceptors (R1–6) is further substantiated by measurements of the polarisation sensitivity in the UV. The quantum yield of the energy transfer from sensitizing pigment to rhodopsin was estimated by electrophysiological measurements of the UV sensitivity and the rhabdomeric absorptance (at 490 nm) in individual receptor cells. The transfer efficiency is ≥ 0.75 in receptors with an absorptance in the rhabdomeres of 0.55–0.95. This result suggests that the sensitizing pigment is bound in some way to the rhodopsin. A ratio of two molecules of sensitizing pigment per one rhodopsin is proposed.

Key words: Sensitizing pigment – Fly photoreceptor – UV sensitivity – Polarization sensitivity – Energy transfer

A. Evidence for the Sensitizing Pigment Hypothesis

The spectral sensitivity of fly photoreceptors R1–6 is dual-peaked with one maximum in the near UV at about 350 nm and the other in the visible near 500 nm as first measured by Burkhardt (1962). Based on the finding that the extinction of the rhabdomeres is due to a single visual pigment and a photostable extinction in the UV, Kirschfeld et al. (1977) explained the high UV sensitivity by the hypothesis of a sensitizing pigment, which absorbs UV quanta and transfers excitation energy to the rhodopsin. As the mechanism for the sensitization process the dipole-dipole energy transfer according to Förster (1951) was proposed.

In principle two alternative explanations for the high UV sensitivity are still possible: *1.* it may be due to relative enhancement of short wavelength extinction

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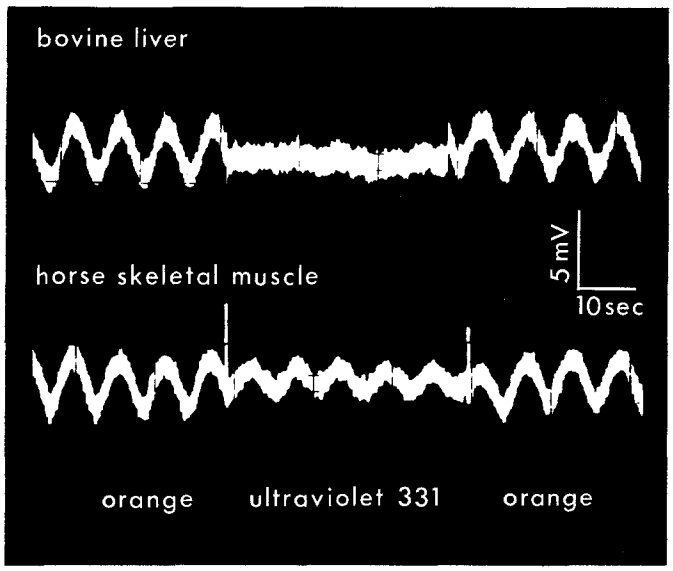

Fig. 1. Response of receptor cells (1–6) to light delivered through a continously rotating polarisation filter. First orange light was applied and then the colour was switched to UV light (331 nm), adjusted in intensity to evoke approximately the same mean response. While there is no modulation during the UV in the fly raised on bovine liver (upper trace), a response to the angle of the e-vector of the light is obvious in the carotenoid deprived fly, raised on horse skeletal muscle. Note the absence of a phase change in the lower trace

by waveguide effects (Snyder and Pask 1973) and *2.* it may be due to an unusually enhanced β-peak of the rhodopsin (Paulsen and Schwemer 1979). A waveguide effect as the underlying basis for the high UV sensitivity is in fact already eliminated by older experiments (e.g., Goldsmith and Fernandez 1968) in which the spectral sensitivity of white eye mutants has been measured by using the ERG. Since in these mutants and with the kind of illumination chosen, rhabdomeres are stimulated primarily with light crossing the rhabdomeres obliquely, waveguide effects cannot explain the measured high UV sensitivity.

The following observations give evidence for the sensitizing pigment hypothesis and are not in accordance with the assumption of an enhanced β-peak of the visual pigment.

1. Carotenoid deprivation in flies leads to a higher reduction of UV sensitivity in comparison to the visible, as first measured by Goldsmith et al. (1964). This is not expected if the sensitivity in the UV and visible is due to a single pigment. The sensitizing pigment concept, however, allows an easy explanation of this finding.

2. Gemperlein et al. (1980) recently measured the spectral sensitivity from the ERG with refined resolution using the technique of Fourier interferometry. They found a fine structure in the UV with three distinct peaks (332, 350, and 369 nm). We confirmed this spectrum by microspectrophotometry of single R1–6 cells and Hardie measured this fine structure by intracellular recordings

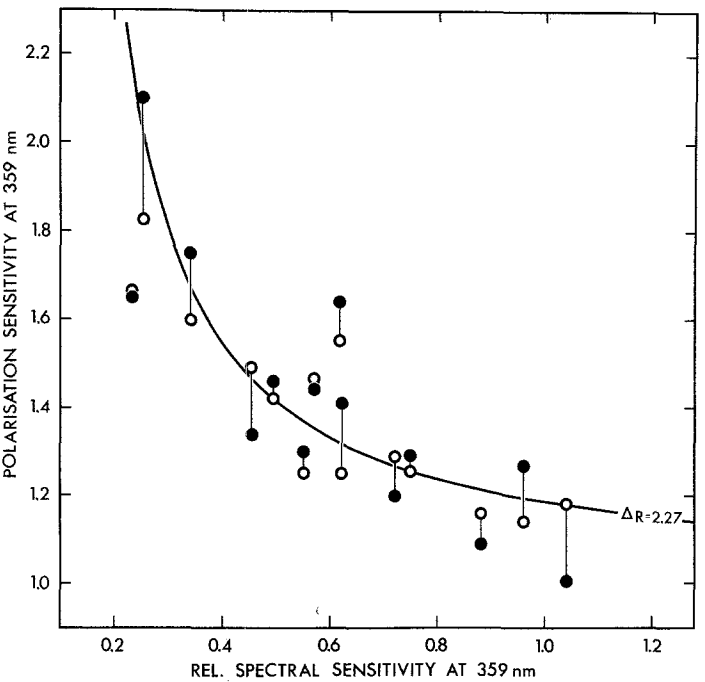

Fig. 2. Polarisation sensitivity in the UV versus relative UV spectral sensitivity of receptors type R1–6 for unpolarised light in carotenoid deprived flies. Dots represent measured values from different receptors of different flies, the corresponding circles denote theoretical values of the UV polarisation sensitivity predicted from the respective PS in the visible range. The assumptions for the calculation have been: a constant dichroism due to the rhodopsin over the whole spectral range and no dichroism due to the sensitising pigment. The formula (valid for small pigment concentrations, actually present in the measured receptors) used was: $PS = (S + D)/(S - D)$; where $S = S(359)$ is the relative UV spectral sensitivity for unpolarised light and $D = a(\Delta_R - 1)/(\Delta_R + 1)$; with Δ_R the dichroism due to the rhodopsin, and a is the relative extinction coefficient of rhodopsin, a (359) = 0.22 (see appendix). The relatively small deviation of the predicted values show that the assumptions are reasonable. The curve predicts the polarisation sensitivity in the UV if the mean PS in the visible $\Delta_R = 2.27$ is introduced into the calculation

with a more conventional illumination (Kirschfeld et al. 1983). A fine structure like this is not known in any rhodopsin.

3. The photoreceptors exhibit polarisation sensitivity (PS) in the visible but not in the UV (Fig. 1; Hardie 1979; Guo 1981). This finding may be interpreted as due to a sensitizing pigment whose orientation in the microvillus membrane results in no rhabdomeric dichroism. However, as it stands this argument is not conclusive. The absence of PS in the UV could also be due to scattering or a different orientation of the transition dipoles of the rhodoposin's β- and α-peak with respect to the membrane. Thus for example 15-15′-cis-β-carotene incorporated in a stretched polyethylen film exhibits different signs of dichroism if measured in the visible and UV respectively (Eckert and Kuhn 1960).

To get more detailed information we therefore measured the PS together with the spectral sensitivity for unpolarised light in receptors of carotenoid

deprived flies by intracellular recording. As shown in Fig. 1 (lower trace) in the UV a PS is obvious in carotenoid deprived flies and the modulation of the receptor response exhibits no phase change with respect to the visible. Furthermore, it can be shown that the measured PS in the UV is larger the smaller the UV sensitivity of the photoreceptor (Fig. 2). Thus the measured PS in the UV is to a good approximation predictable from the relative UV sensitivity assuming that the rhabdomeric dichroism due to rhodopsin is the same in the visible and in the UV and that the sensitizing pigment introduces no dichroism.

These results exclude a depolarisation by scattering consistent with MSP-data (Kirschfeld and Vogt, in preparation) and indicate that the rhabdomeric dichroism due to rhodopsin is about the same in the visible and UV. This, again provides strong evidence for a sensitizing pigment.

Although the sensitizing pigment has not yet been extracted from the eyes, in view of the cumulative evidence we regard the sensitizing pigment hypothesis as confirmed in the following.

B. Determination of the Quantum Efficiency of Energy Transfer

In the foregoing we considered only the spectral sensitivity which is the product of the absorption and the quantum efficiency. Thus a high UV sensitivity does not necessarily indicate a high quantum efficiency since a lower quantum efficiency may be canceled by a higher absorption. In the case considered quantum efficiency means the transfer efficiency (γ_S) from sensitizing pigment to rhodopsin and can thus depend on both the concentration of sensitizing and visual pigment. γ_S therefore cannot be regarded a priori as a constant and its definition makes sense only for a given concentration of both pigments, i.e., for an individual photoreceptor.

Usually transfer efficiencies are determined by measuring the fluorescence quantum yields (or life times) in the presence and absence of the acceptor, respectively. This was done for example in cattle rhodopsin labeled with fluorescent probes in the bleached and unbleached form, respectively (Wu and Stryer 1972). Unfortunately, this approach is not possible in fly photoreceptors since rhodopsin illumination does not result in bleaching but in thermostable metarhodopsin (570) which is as good an acceptor as rhodopsin (indicated by its high UV photosensitivity, Minke and Kirschfeld 1979). However, we can define γ_S as the reciprocal of the number of quanta which in the mean have to be absorbed by a sensitizing pigment molecule to evoke the same response of the receptor cell as one quantum absorbed by the rhodopsin itself (see Fig. 3). Formally the relative [S (490) $\equiv$ 1] spectral sensitivity of a red adapted receptor with sensitizing pigment is given by

$$S(\lambda) = \frac{\gamma_R E_R(\lambda) + \gamma_R \gamma_S E_S(\lambda)}{E_R(\lambda) + E_S(\lambda)} \cdot \frac{1 - 10^{-[E_R(\lambda) + E_S(\lambda)]}}{\gamma_R [1 - 10^{-E_R(490)}]} \tag{1}$$

with E_R, extinction by rhodopsin; E_S, extinction by sensitizing pigment [E_S (490)

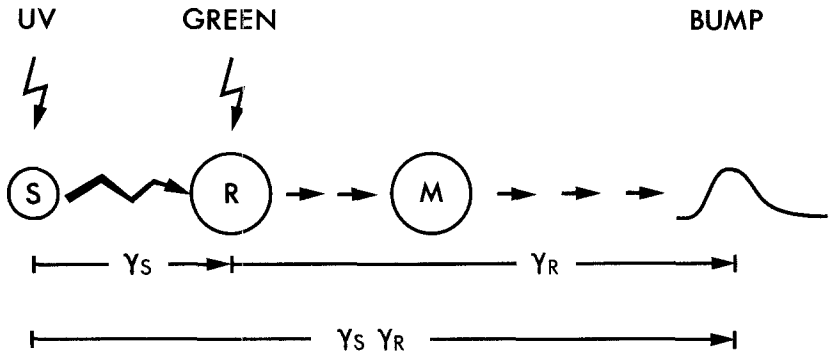

Fig. 3. Schematic drawing of the presumed transduction process in fly photoreceptors (R1–6). Direct absorption of a light quantum by rhodoposin (R) leads via several steps with a probability γ_R to an electrical response of the receptor cell. Absorption of a UV quantum by the sensitising pigment (S) leads (via an energy transfer mechanism) with a probability γ_S to the same excited state of rhodopsin as that caused by direct absorption. The probability that a quantum absorption by the sensitising pigment is followed by a response of the receptor is $\gamma_S \gamma_R$. In the text we use for γ_S the term transfer efficiency or quantum efficiency of energy transfer

$= 0]$ and γ_R, the quantum efficiency of production of an electrical response by rhodopsin; γ_R cancels (Vogt and Kirschfeld 1982).

If we neglect spectral overlap in the UV for the present $[E_R(359) = 0]$ we can write for the relative spectral sensitivity in the UV

$$S(359) = \gamma_S \frac{A_S(359)}{A_R(490)};$$

with $A_S(359)$ and $A_R(490)$ as the absorptances ($A = 1 - 10^{-E}$, i.e., the fraction of light absorbed) due to sensitizing pigment and visual pigment, respectively. Since $A_S(359) \leq 1$, we arrive at

$$\gamma_S \geq S(359) A_R(490) . \tag{2}$$

Thus essentially we have to determine only the relative spectral sensitivity in the UV and the absorptance of the rhabdomere at 490 nm to get a minimal estimate of γ_S, even if we do not know the UV absorptance. One consequence of (2) is that at very high absorptance in the visible the relative spectral sensitivity in the UV can be equal to the transfer efficiency at most.

Both, A (490) and S (359) vary from fly to fly even if raised on the same diet, and we therefore measured the two factors in one and the same receptor by intracellular recording. The absorptance at 490 nm was determined using two different methods (details in Vogt and Kirschfeld, in preparation).

1. Rhodopsin Selfscreening

In white eyed *Calliphora* (chalky) after preceding red adaptation, the spectral sensitivity (unpolarised light) was measured using point source illumination whereby the light source was either aligned on the optical axis of the receptor or 30° off-axis. From the difference of the shape of the spectral sensitivity on the red flank the absorptance A_R (490) was calculated.

2. Metarhodopsin Screening

In wild type *Calliphora* the spectral sensitivity (unpolarised light) was measured on-axis after preadaptation with different colours. The absorptance A_R (490) can be calculated from the change in spectral sensitivity. (This method was previously used by Tsukahara and Horridge 1977; Stark et al. 1977; formalisms are given by Hamdorf and Schwemer 1975.)

For the calculations of the transfer efficiency γ_S we used Eq. (1) with E_S (359) $\leq$ 2 E_R (490) since microspectrophotometrically, we never measured an extinction in the UV larger than twice that in the visible. For E_R (359) we used 0.22 E_R (490) the value we found for the relative UV sensitivity in extremely carotenoid deprived flies. [Assuming E_S (359) = ∞ leads to nearly the same values for γ_S if A_R (490) > 0.7.]

In the 22 cells (22 flies) measured the absorption at 490 nm and the relative spectral sensitivity in the UV are in the range from A (490) = 0.54 with S (359) = 1.20 to A (490) = 0.96 with S (359) = 0.78. By using (1) the minimal transfer efficiency for 14 receptors is in the range γ_S = 0.75 to γ_S = 0.85 with only small deviations for the other cells (4 cells with $\gamma_S \geq 0.7$ and 4 cells with $\gamma_S \geq 0.85$). Although the mean of the minimal transfer efficiency is 0.82, in the following we use the very conservative estimate, $\gamma_S \geq 0.75$.

C. Conclusions

The high transfer efficiency of the sensitizing pigment requires an explanation within the scope of the dipole-dipole energy transfer mechanism. The most attractive explanation would be that the sensitizing pigment is not randomly distributed in the membrane but bound in some way to the opsin, thus guaranteeing a close proximity to the retinal chromophore.
This hypothesis is supported by the following arguments:
1. If we calculate the mean distance between the sensitizing pigment and the retinal chromophore required for the measured transfer efficiency using the β-lactoglobulin-retinol-complex (Fugate and Song 1980) as a model for the sensitizing pigment we arrive at a critical distance (where the transfer efficiency is 0.5) of

$$R_0 = 30 \text{ Å}.$$

For the experimentally obtained transfer efficiency of $\gamma_S \geq 0.75$ this leads to

$$R \leq 25\,\text{Å}\,.$$

This distance is smaller than the ca. 40 Å diameter of rhodopsin [assuming a globular protein with a molecular weight as measured by Ostroy (1978) for *Drosophila* rhodopsin] or the 80 Å diameter of the particles in the microvillar membrane as measured by Boschek and Hamdorf (1976), Harris et al. (1977) and Schwemer (1979).
According to Förster (1951) the critical distance in nm is given by

$$R_0 = 310(Ik^2\gamma_F n^{-4})^{1/6}$$

and

$$R = R_0(\gamma_S^{-1} - 1)^{1/6}\,;$$

I, the spectral overlap integral (in $\text{cm}^6 \cdot \text{mol}^{-1}$): I is $1.39 \cdot 10^{-10}$ using the emission spectrum of the β-lactoglobulin-retinol-complex (Fugate and Song 1980, Fig. 4A) and a rhodopsin spectrum (Schwemer 1979) with ε_{max} = 4,000 $\text{m}^2 \cdot \text{mol}^{-1}$.
k^2, the orientation factor; assumed to be $^2/_3$, the value for fast isotropic motion. This assumption seems not to be critical with respect to the intended conclusion, since smaller values lead to even shorter distances and larger values require a specific orientation of the two chromophores indicative of a specific attachment.
γ_F, the fluorescence quantum yield in the absence of energy transfer; 0.033 from the β-lactoglobulin-retinol-complex (Fugate and Song 1980) was used. The assumption of γ_F probably introduces the largest uncertainty in the estimate of a maximal possible R. For example, if the sensitizing pigment had the high γ_F = 0.25 (parinaric acid in solid phospholipid layers, Sklar et al. 1979), the above calculated R has to be corrected by a factor 1.4.
n, the refractive index; for microvillus membrane n = 1.4 was used.
2. The transfer efficiency is almost constant over the observed absorptance range at 490 nm (95% to 55%). The constancy over this range which corresponds to a decrease in visual pigment concentration by a factor of four suggests a fixed distance between the two chromophores.
3. The fine structure in the UV-region can be explained if a retinyl compound which exhibits usually no fine structure is bound to a protein (Hemley and Kohler 1977; Schreckenbach et al. 1977; Fugate and Song 1980). However, since there are also retinyl compounds, e.g., retro-retinol, which exhibit an intrinsic fine structure, this argument is not fully conclusive (for a more detailed discussion see Franceschini 1982 or Kirschfeld et al. 1983).
4. The central receptor 7y exhibits a high UV sensitivity which is also probably due to a sensitizing pigment (Hardie and Kirschfeld 1983). Besides the sensitizing pigment and the rhodopsin ($\lambda_{max} \approx 430$ nm) the rhabdomeric membrane contains a carotenoid absorbing in the same spectral range as the

rhodopsin (Kirschfeld et al. 1978). Although the carotenoid is present in a molar ratio of about 20 : 1 with respect to the rhodopsin (McIntyre and Kirschfeld 1981) apparently it is not a very potent competitor in quenching the sensitizing pigment as indicated by the high UV sensitivity. This fact may be explained easily by assuming a special spatial arrangement between visual and sensitizing pigment.

Finally we make an estimate of the molar ratio between sensitizing pigment and visual pigment. On the one hand the possible candidates for the sensitizing pigment have a molar extinction coefficient of similar magnitude as rhodopsin ($\approx 4{,}000\ m^2 \cdot mol^{-1}$). On the other hand, relative UV spectral sensitivities of up to 1.5 can be measured (e.g., Guo 1980) in receptors with moderate visual pigment content [according to Eq. (2) corresponding to less than 50% absorptance at 490 nm] or, a relative UV photosensitivity of 1.6 (*Calliphora* rhodopsin, Minke and Kirschfeld 1979). In view of the measured transfer efficiency this makes a ratio of two molecules of sensitizing pigment per molecule visual pigment most likely.

Appendix

The polarisation sensitivity (PS) in the UV is given by:

$$PS(359) = \frac{E_{R_\perp}(359)\Delta_R + \gamma_S E_S(359)}{E_{R_\perp}(359) + \gamma_S E_S(359)} \tag{A1}$$

where γ_S is the quantum efficiency of energy transfer, if the following conditions in a photoreceptor are fulfilled: 1) low extinction by the sensitizing pigment (E_S) and visual pigment (E_R); 2) a constant dichroism due to the rhodopsin $\Delta_R(\lambda) = \Delta_R = E_{R\parallel}/E_{R\perp}$, and no dichroism due to the sensitizing pigment $\Delta_S = 1$; 3) no extinction by the sensitizing pigment in the visible $E_S\ (490) = 0$.

The relative [$S\ (490) = 1$] spectral sensitivity for unpolarised light, is given by

$$S(359) = \frac{E_{R_\perp}(359)(1 + \Delta_R)/2 + \gamma_S E_S(359)}{E_{R_\perp}(490) \cdot (1 + \Delta_R)/2} \tag{A2}$$

or, after rearrangement,

$$\gamma_S E_S(359) = [S(359)E_{R_\perp}(490) - E_{R_\perp}(359)](1 + \Delta_R)/2$$

Substituting $\gamma_S E_S(359)$ in (A1) leads to

$$PS(359) = [S(359) + D]/[S(359) - D]\ ;$$

where

$$D = [E_{R_\perp}(359)/E_{R_\perp}(490)]\ (\Delta_R - 1)/(\Delta_R + 1) = a(\Delta_R - 1)/(\Delta_R + 1)$$

with $a = E_R(359)/E_R(490)$, the relative extinction coefficient of rhodopsin.

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