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Photostable Pigments Within the Membrane of Photoreceptors and Their Possible Role*

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Abstract. In the majority of ommatidia of the fly, the membrane of the central rhabdomere contains – besides the rhodopsin – a photostable pigment. Due to its selective absorption in the blue spectral range, this pigment (possibly a carotene) could modify the spectral sensitivity of the central receptor cells. It furthermore may change the fluidity of the microvillus membrane and hence affect the alignment of rhodopsin molecules. Indirect evidence for a possible role of the photostable pigment as an "antenna"-pigment for rhodopsin is discussed.

Key words: Photoreceptors – Photostable pigments – Dichroism – Antenna-pigment.

The Musca compound eye has three anatomically distinct types of photoreceptor cells in each ommatidium: *R*1–6, *R*7 and, located proximally to *R*7, the receptor *R*8. Observation of eye-cup preparations, cut at such a depth that only portions of *R*1 to *R*7 are left, shows that there are actually two different types of *R*7 present: one type has yellow rhabdomeres (7*y*) if observed in transmitted white light, the rhabdomeres of the other appear pale (7*p*) (percentage of 7*y* in flies caught outdoors: 65–75%). Both contain a photoisomerizable pigment of the type well-known for several insect species (Hamdorf et al., 1971; Stavenga et al., 1973; Pak and Lidington, 1974; Harris et al., 1976). By means of uv-light it can be shifted to a pigment absorbing maximally in the range 470–500 nm. Dichroic absorption is different in the two types of receptors. In the blue spectral range 7*p* receptors absorb maximally if the *E*-vector of linearly polarised light oscillates parallel to the microvilli, as it is expected for microvillar receptors with absorbing dipoles arranged randomly within the plane of the microvillar membrane (Moody and Parriss, 1961). The 7*y* receptors, however, absorb maximally if the *E*-vector is aligned *perpendicular* to the microvilli

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(Kirschfeld, 1969). On bleaching the isomerizable pigment with blue light after uv preadaptation, both types of receptor behave quite differently. This can be shown by permanently monitoring the dichroic transmission. From 7*p* "classical" transmission curves are measured in which the modulation of transmitted light disappears with bleaching (Fig. 1a). In 7*y*, unexpectedly, the dichroism *increases* on bleaching the photolabile pigment (Fig. 1b).

We interpret this finding by assuming that there is, in addition to the photoisomerizable pigment, a second, photostable dichroic pigment which absorbs maximally if the *E*-vector oscillates *perpendicular* to the microvilli. The absorption properties of both pigments together yield what we measure from a rhabdomere 7*y* (Fig. 1b).

The extinction spectrum of the photostable pigment as measured with linearly polarised light oscillating perpendicular to the microvilli, shows a maximum at 456 nm and two shoulders at 430 and 485 nm respectively. This indicates that the photostable pigment might be a carotene or a flavoprotein. We assume it to be a carotene because of its dichroism perpendicular to the long axis of the microvilli. This kind of dichroism is to be expected for a hydrophobic dipole (like a carotene) which orientates itself parallel to the fatty-acid chains of the phospholipids. A further argument in favour of carotene is the fact that flies (Calliphora, kindly supplied by Prof. K. Hamdorf), that have been raised on a vit. A deficient medium have almost no 7*y* rhabdomeres.

The dichroism of the photostable pigment indicates that it is part of the microvillar membrane. Such a photostable pigment could have several functional consequences:

1. Spectral sensitivities of the photoreceptors 7 and 8 may be modified by means of such a photostable pigment acting as a selective attenuator. The same is true for dichroic sensitivity: it may be increased, decreased or reversed in special spectral regions, beyond the constraint set by the dichroic ratio of the visual pigment itself.

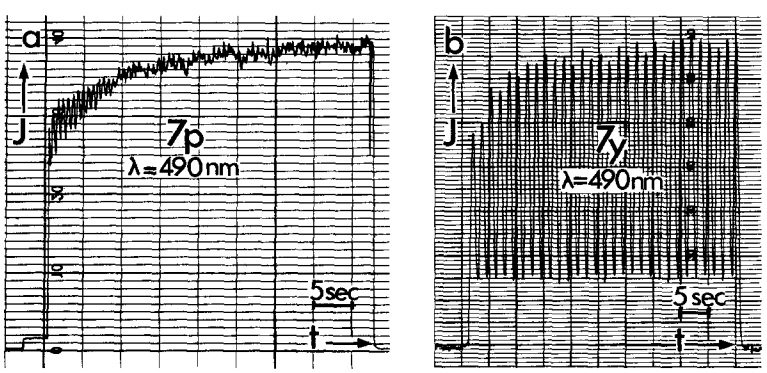

Fig. 1. Light intensities *J*, transmitted by rhabdomeres 7*p* and 7*y*. After uv-preadaptation the intensities were measured during back conversion of the photoisomerizable pigment by means of blue light into the uv-absorbing form. A polarisation filter inserted in the illuminating beam was rotated (somewhat faster in Figure a than in b) during the bleaching in order to continuously monitor dichroic transmission. Maximal absorption occurs in 7*p* if the *E*-vector of light oscillates parallel to the microvilli long axis. The dichroic ratio is nearly two (Fig. 1a). By contrast, the dichroism increases in receptors 7*y* during the conversion of the isomerizable pigment with blue light. Moreover maximal absorption occurs if the *E*-vector oscillates *perpendicular* to the microvillus long axis (Fig. 1b)

2. The fluidity (crytallinity) of a photoreceptor membrane might be modified by molecules like a carotene, fitting between the phospholipids, thereby modifing and stabilizing the arrangement of the absorbing dipoles of the visual pigment. This might also modify the dichroic ratio of the visual pigment.

3. The photostable pigment might act as an "antenna"-pigment, i.e. a photostable accessory pigment that absorbs light and transfers the energy to the effector pigment. An "antenna"-function of the photostable pigment in receptors 7 is suggested by the following result.

The isomerizable pigment can be bleached with blue light, linearly polarised with the *E*-vector either parallel or perpendicular to the long axis of the microvilli. The time constant for bleaching with the *E*-vector perpendicular is longer by a factor of 2–3 than that for bleaching with the *E*-vector parallel to the microvilli. A factor two, however, is already expected from the dichroic ratio of 2 of the isomerizable pigment according to the Moody and Parriss (1961)-model. A still higher factor would be expected if the absorbing dipoles were better aligned parallel to the microvilli. In addition the photostable pigment has a pronounced screening effect, which is larger for light oscillating perpendicular to the microvilli (see Fig. 1b). This too would increase the ratio of the time constants for bleaching the isomerizable pigment with polarised light. It is not yet possible to give a quantitative estimate of this increase, however, as we have no conclusive data on the absolute absorption of the photostable pigment within the rhabdomeres 7*y*, nor on the waveguide properties of these special rhabdomeres: both affect the estimate. If the measured factor of the bleaching time constants is actually smaller than expected, we have to consider that the photostable pigment may act as an "antenna"-pigment. This function of carotene in fly receptors then would be similar to the effect carotene has in photosynthesis, where it transfers energy to the chlorophyll. Such a photostable "antenna"-pigment could obviously modify spectral and dichroic sensitivities of a photoreceptor. Furthermore it may increase the total number of quanta caught by a receptor, compared with the situation where only visual pigment is present.

According to electrophysiological measurements on Drosophila, receptors No. 7 are uv-sensitive receptors (Harris et al., 1976). This means that the uv-pigment is the rhodopsin. The energy transfer discussed here would thus occur between the photostable pigment and *metarhodopsin*. An energy transfer between the photostable pigment and also the rhodopsin cannot be excluded.

We have more direct evidence for an antenna-pigment function in fly photoreceptors 1–6. Here the suspected antenna-pigment is not a carotene but a pigment, absorbing predominantly in the ultraviolet spectral range. It causes the second, relatively high sensitivity peak in these photoreceptors. This conslusion is based on absolute extinction spectra, on difference spectra, on an analysis of dichroism in the uv and visible as well as on electrophysiological data of normal flies and flies raised on Vit. A deficient media (unpublished experiments performed in collaboration with B. Minke). This conclusion is similar to that suggested by Chance (1964), for the uv-sensitivity of the *Limulus* lateral eye. However, instead of assuming an energy transfer from the photostable pigment to rhodopsin by means of fluorescence, we expect a more direct interaction like, for example, the induced resonance transfer of energy worked out by Förster (1951).

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Kirschfeld's Lecture

Clayton's Remarks:

If you want to calculate the probability of energy transfer from the carotene to the rhodopsin you need to know the distance between the molecules, the relative orientations (which one can presumably guess at) and also the amount of overlap between the fluorescence of the carotene excitation donor and absorption by the rhodopsin acceptor. This raises a problem, because the carotene in vitro does not show fluorescence. With such an efficient thermal quenching mechanism, how can it be such a good energy transferring pigment? The answer could be that in the membrane it is held rigidly and this rigidity eliminates the pathways for thermal deexcitation. It would then follow that if you could prepare a mutant either of a photosynthetic tissue that lacks a chlorophyll excitation acceptor, or, of a fly lacking the rhodopsin excitation acceptor but in which the carotene is still constrained as it was in the wild type, then fluorescence should be shown. In the absence of such an experiment, you are only able to guess at the fluorescence spectrum of the carotene to compute the hypothetical overlap. There is a simple thermodynamic argument for transfer between heterogenous types of molecules, the so called Förster transfer. The argument rests on the assumption that the natural lifetime is long enough to allow thermal equilibration among the vibrational state populations in the excited as well as in the ground states. Following an argument of detailed thermodynamic balance, you can show that the fluorescence spectrum is then simply the product of the absorption spectrum and the Planck Radiation Distribution Law for a black body at room temperature.