DROSOPHILA RHODOPSIN: PHOTOCHEMISTRY, EXTRACTION AND DIFFERENCES IN THE norp A^{P12} PHOTOTRANSDUCTION MUTANT*

Sanford E. Ostroy, Meegan Wilson and William L. Pak

Department of Biological Sciences, Purdue University, West Lafayette, Indiana

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SUMMARY: The visual pigments of Drosophila melanogaster eyes were studied by spectrophotometric measurements in whole eyes and digitonin extracts. The main visual pigment transition appears to be $RHODOPSIN_{480} \underset{h\nu}{\overset{h\nu}{\rightleftharpoons}} METARHODOPSIN_{580}$. Under most conditions the products are thermally stable and reversible by illumination. The norp A^{P12} phototransduction mutant appears to contain approximately one-third of the total visual pigment found in the wild type eye. The $rhodopsin_{480}$ is greatly reduced in concentration. A $rhodopsin_{420}$ may be dominant and a $metarhodopsin_{480}$ may also be present. These species may also be present in the wild type eye. The reduced visual pigment concentration of norp A^{P12} does not appear to explain the small visual receptor potentials of this mutant and one must consider a more complex visual transduction mechanism.

In the studies of visual pigments, a knowledge of the native spectra of the visual pigments and the spectral changes induced by illumination has been important in proper stimulation of the visual system and in attempting to understand the molecular and physiological operation of the photoreceptor (for reviews see 1,2). A recent approach to the study of visual systems has used visually deficient single lesion mutants of Drosophila melanogaster (3,4,5). For this species, however, no direct measurements of visual pigment absorption changes have been reported nor have many insect visual pigments been extracted in solution (6,7). In this paper, we would like to report on such extraction and measurements for the visual pigments of Drosophila melanogaster in both the wild type and the norp A^{P12} visual transduction mutant. The norp A^{P12} mutant shows abnormally small receptor amplitudes and extensive work has been done on this mutant and its alleles (3,8,9).

METHODS AND MATERIALS: The wild type (3) and norp A^{P12} phototransduction mutants (3) used in this work were genetically placed on a white eye background to avoid

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complications due to light screening pigments. The white eye was produced by a white mutation of the first chromosome in the wild type and by a combination of brown and scarlet mutations on the second and third chromosomes, respectively, in the mutant eye. These genes do not alter the ERG's of the flies except for increasing their sensitivity to light (8). Prior to use all live flies were dark adapted for at least 24 hours. We found that the observed amount of rhodopsin was sensitive to the length of time of dark adaptation and that a minimum of 24 hours of dark adaptation was necessary to yield consistent amounts. For the microspectrophotometric measurements, a single Drosophila was etherized, its head removed, and the eye was placed on the sample stage of a microscope which was part of a Cary 14 recording microspectrophotometer (10). For the digitonin extraction, the heads of 1000 dark adapted flies were removed, crushed, washed twice with water, and the visual pigment extracted by stirring for 20 minutes with a solution of 2% digitonin. In general, samples were illuminated for five minutes with a 100 watt fiber optic illuminator (Am. Optical, Type K150) containing four heat filters and broad band interference filters which transmitted blue (420-470 nm, Balzer K-2) or orange light (580-630 nm, Balzer K-5).

RESULTS: The spectral changes resulting from multiple illuminations of the wild type eye are shown in Figure 1. The first illumination with blue light results in a general absorbance decrease from 400 to 490 nm and a large increase in absorption with a peak at 580 nm (spectrum 1). A subsequent illumination with orange light results in a decreased absorption of the peak at 580 nm and general increases of absorption at 420-490 nm with a maximum at 480 nm (spectrum 2). Subsequent blue or orange illuminations give the same general results (spectra 3,4). The isosbestic for these spectral changes are in the 515-520 nm region and the 580 nm absorbance changes are approximately twice the magnitude of the absorbance changes at 400-490 nm.

The difference spectra resulting from multiple illuminations of the visual mutant norp A^{P12} are shown in Figure 2. As with the wild type eye, the initial blue illumination results in production of a product with a maximum absorbance

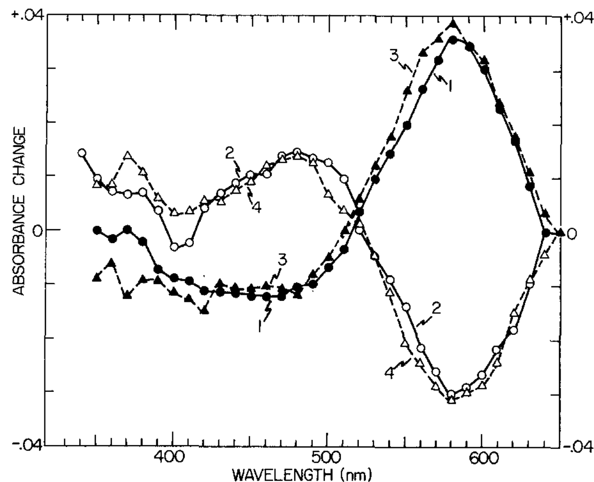


Fig. 1. Difference spectra from multiple illuminations of white eyed wild type. Spectrum 1: After 5 min. blue illumination. Same results are obtained if illuminated with 493 nm monochromatic light (B-40 Balzers Co.). Spectrum 2: Subsequent orange illumination. No spectral changes are observed if orange light used for initial illumination. Spectrum 3: Second blue illumination. Spectrum 4: Second orange illumination.

at 580 nm (spectrum 1). However, the magnitude of the 580 nm absorbance increase is only one-third of that observed in the wild type eye. Also, unlike the wild type, the absorbance decrease in the 400-490 nm region shows a distinct peak at 420 nm. Subsequent illumination in the orange region of the spectrum results in absorbance decreases at 580 nm and absorbance increases peaking at 480 nm (spectrum 2). A second illumination in the blue region shows a general absorbance decrease in the 400-490 nm region with a maximum around 420 nm, along with an absorbance increase at 580 nm (spectrum 3). Subsequent orange illumination (spectrum 4) again results in a decrease at 580 nm and an increase at 480 nm.

To illustrate the differences in visual pigments between wild type and *norp* A^{P12} eyes, the spectra 1 and 2 in Fig. 3 plots the difference in absorbance changes between wild type and *norp* A^{P12} eyes after the first and second illuminations respectively. The pigment differences illustrated in Figure 3 show absorbance maxima around 480 nm and 580 nm indicating that those are the native and light-induced pigments which are in reduced concentration in the *norp* A^{P12} visual mutant.

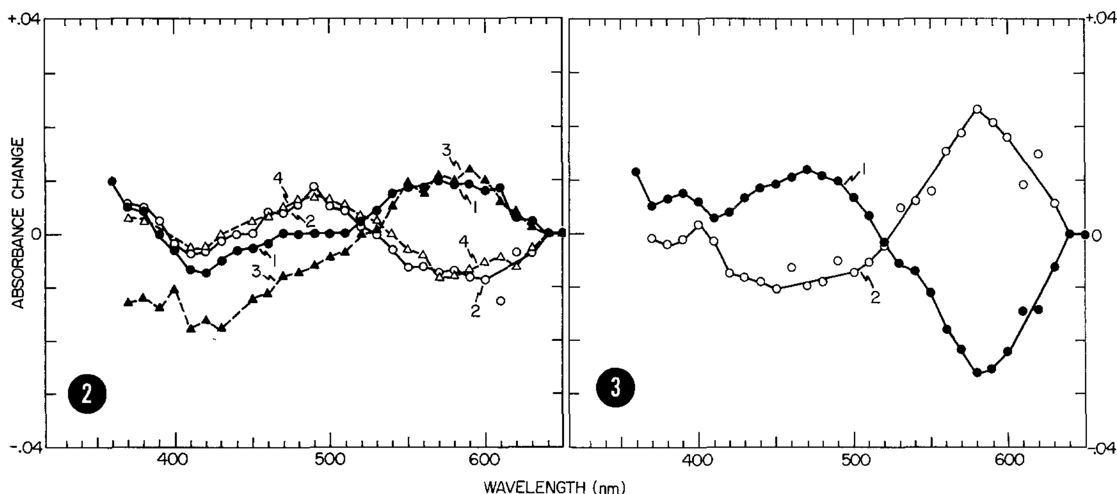


Fig. 2. Difference spectra from multiple illumination of norp A^{P12}. Spectrum 1: After initial blue illumination. Spectrum 2: Illumination with orange light. Spectrum 3: Second blue illumination. Spectrum 4: Second orange illumination.

Fig. 3. Spectral differences between wild type and norp A^{P12}. Spectrum 1: Difference between blue illuminations (Fig. 1, spectrum 1 minus Fig. 2, spectrum 1). Spectrum 2: Difference between orange illuminations (Fig. 1, spectrum 2 minus Fig. 3, spectrum 2).

Figure 4 illustrates the experiment of Figs. 1-2 with digitonin extracts of the visual pigment. Only the 480 nm ⇄ 580 nm spectral changes are observed. Neither the broad 400-490 nm decrease in absorbance upon blue illumination of the wild type eye (Fig. 1 spectra 1,3) nor the $rhodopsin_{420}$ evident in the norp A^{P12} (Fig. 2 spectra 1,3) are observed. Figure 4 also shows directly the reduced concentration of the 480 nm $\underset{h\nu}{\overset{h\nu}{\rightleftharpoons}}$ 580 nm products extractable from the norp A^{P12} eye.

DISCUSSION: The spectral changes observed in the intact wild type eye (Fig. 1) and in extracted solution (Fig. 4) indicate that the main spectral transition of the Drosophila rhodopsin is 480 $\underset{h\nu}{\overset{h\nu}{\rightleftharpoons}}$ 580 nm. The 580 nm product may be designated as a metarhodopsin because illumination of a fully dark adapted eye with orange light does not cause any spectral changes indicating that the 580 nm product does not exist in the native state. Thus the main spectral transition

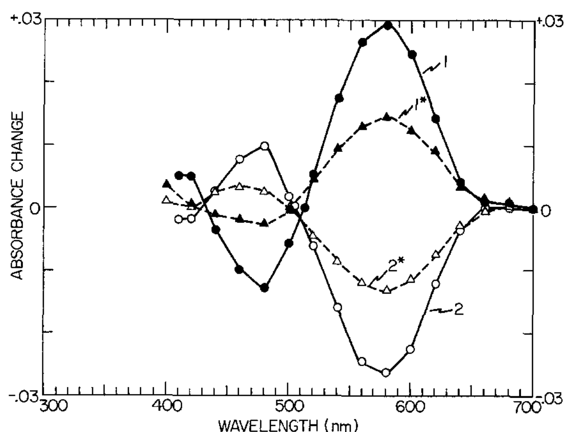
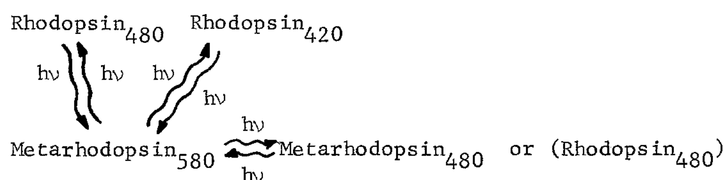


Fig. 4. Difference spectra from multiple illumination of rhodopsin in digitonin extract. Spectra 1 and 2: Wild type after five minutes of blue and orange illuminations respectively. Spectra 1* and 2*: *norp* A^{P12} after five minutes of blue and orange illuminations, respectively.

is: $\text{RHODOPSIN}_{480} \underset{h\nu}{\overset{h\nu}{\rightleftharpoons}} \text{METARHODOPSIN}_{580}$. The difference spectra, however, may give incorrect absorption maxima, if the absorption spectra of the products in the photoreversible equilibrium overlap considerably. This possibility was tested by using heat or solvent denaturation to remove one of the products from the photoreversible equilibrium. For example, the rhodopsin spectrum was obtained in a 1% Triton X-100 extract at $5^{o}C$ where no $\text{metarhodopsin}_{580}$ is observed and the rhodopsin bleaches directly to retinal_{387}. The results of these experiments showing that the correct wavelengths were obtained are presented in Fig. 5.

In the *norp* A^{P12} transduction mutant the spectra of both the excised eye (Figs. 2,3) and extracted solution (Fig. 4) indicated reduced concentration of the $\text{RHODOPSIN}_{480} \underset{h\nu}{\overset{h\nu}{\rightleftharpoons}} \text{METARHODOPSIN}_{580}$. In addition, the excised eye spectra (Fig. 2, spectra 1,3) seem to show another rhodopsin with a peak at 420 nm. For *norp* A^{P12} the data suggest the following scheme - with the rhodopsin_{480} in reduced concentration.



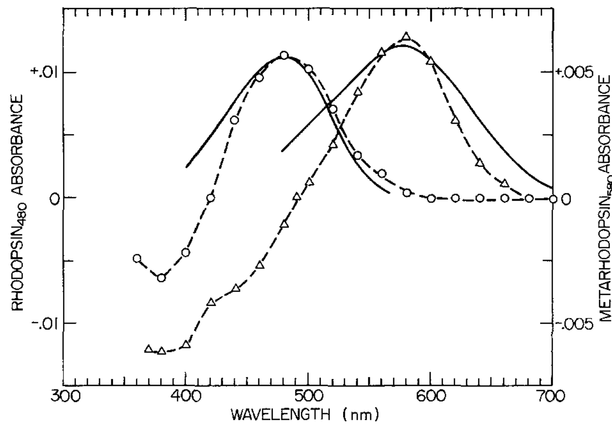


Fig. 5. Native rhodopsin$_{480}$ and metarhodopsin$_{580}$ spectra. Rhodopsin spectrum derived from 1% Triton X-100 extract at 5°C. Metarhodopsin spectrum derived from 2% digitonin extract. Sample was blue illuminated at 26°C at which temperature the metarhodopsin$_{580}$ thermally decays. Observed metarhodopsin$_{580}$ spectrum is difference between sample spectrum taken one minute after end of illumination minus sample spectrum taken 44 minutes later. Solid lines are the Dartnall nomograms (11) for visual pigment peaking at 480 nm and 580 nm respectively.

In the wild type eye, a broad and flat spectral change is observed on blue illumination (Fig. 1, Spectra 1,3) and differences between wild type and norp A^{P12} (Fig. 3) indicate peaks at only 480 and 580 nm. Thus the rhodopsin$_{420}$ observed in the norp A^{P12} may also be present in the wild type eye. The lack of rhodopsin$_{420}$ in the extracted solution for either wild type or norp A^{P12} suggests that it cannot be extracted by the present technique. Finally, we have proposed the presence of a metarhodopsin$_{480}$ for norp A^{P12}, because the metarhodopsin$_{580}$ goes over to a product peaking at 480 nm on orange illumination (Fig. 2, spectra 2,4). The same argument also applies to the wild type eye (Fig. 1, spectra 2,4). However, it is not possible to separate metarhodopsin$_{480}$ from rhodopsin$_{480}$ on spectral criteria alone.

It is reasonable to ask whether the reduced pigment concentration observed in the norp A^{P12} transduction mutant can alone explain the absent or greatly reduced receptor potential of this mutant (0-2 mv compared to 15 mv for wild type). If one assumes that the Drosophila photoreceptor is a single photon

detector as are the vertebrate rods (12,13) and the housefly photoreceptors (14), the reduced pigment concentration is not a sufficient explanation. The remaining visual pigment should be adequate to give receptor potentials of normal amplitudes. To explain the results, one must consider a more complex visual transduction mechanism in which the reduced pigment concentration can be a secondary effect of the unknown primary phototransduction block or where the reduced concentration affects certain "reaction center" rhodopsins which are more tightly coupled to the transduction process.

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1. Abrahamson, E. W. and Ostroy, S. E. (1967) Prog. Biophys. Mol. Biol. 17: 179-215.
2. Bridges, C. D. B. (1967) in Comp. Biochem. 27:31-77, Eds. M. Flokin and H. Stotz, Elsevier Pub. Co., N. Y.
3. Pak, W. L., Grossfield, J. and White, N. V. (1969) Nature 222:351-354.
4. Hotta, Y. and Benzer, S. (1970) Proc. Nat. Acad. Sci. 67:1156-1163.
5. Heisenberg, M. (1971) Drosophila Inform. Serv. 46:68.
6. Goldsmith, T. H. (1958) Proc. Nat. Acad. Sci. 44:123-126.
7. Paulsen, R. and Schwemer, J. (1973) Eur. J. Biochem. 40:577-583.
8. Alawi, A. A., Jennings, V., Grossfield, J. and Pak, W. L. (1972) in The Visual System, Ed. G. B. Arden, Plunum Publishing Corp. N. Y., pp. 1-19.
9. Ostroy, S. E. and Pak, W. L. (1973) Nat. New Biol. 221:120-121.
10. Strehler, B. L., Konigsbert, I. R. and Kelley, J. E. T. (1963) Exp. Cell Res. 32:232-241.
11. Dartnall, H. J. A. (1953) Brit. Med. Bull. 9:24-30.
12. Hecht, S., Schlaer, S. and Pirenne, M. H. (1942) J. Gen. Physiol. 25:819-840.
13. Fain, G. L. and Dowling, J. E. (1973) Science 180:1178-1181.
14. Reichardt, W. E. (1965) Symp. Quant. Biol. 30:505-515.