Dependency on Light and Vitamin A Derivatives of the Biogenesis of 3-Hydroxyretinal and Visual Pigment in the Compound Eyes of *Drosophila melanogaster*

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ABSTRACT   When the fruitfly, *Drosophila melanogaster*, was reared on media deficient in carotenoids and retinoids, the level of 3-hydroxyretinal (the chromophore of fly rhodopsin) in the retina decreased to <1% compared with normal flies. The level of 3-hydroxyretinal increased markedly in flies that were given a diet supplemented with retinoids or carotenoids. The retinas of flies fed on all-*trans* retinoids and maintained in the dark predominantly contained the all-*trans* form of 3-hydroxyretinal, and showed no increase in the level of either the 11-*cis* isomer or the visual pigment. Subsequent illumination of the flies converted substantial amounts of all-*trans* 3-hydroxyretinal to its 11-*cis* isomer. The action spectrum of the conversion by illumination showed the optimum wavelength to be ~420 nm, which is significantly greater than the absorption maximum of free, all-*trans* 3-hydroxyretinal. Flies that were fed on carotenoids showed a rapid increase of the levels of 11-*cis* 3-hydroxyretinal and of visual pigment in the absence of light.

INTRODUCTION

Accumulating evidence indicates that the visual pigment in the compound eyes of dipteran insects contains a chromophore, 11-*cis* 3-hydroxyretinal (Vogt, 1983, 1984; Vogt and Kirschfeld, 1984; Goldsmith et al., 1986; Seki et al.,1986; Tanimura et al., 1986). However, details of the metabolism of this novel chromophore are still unclear. Schwemer (1983, 1984) has reported that visual pigment is formed upon the exposure of eyes to blue/violet light or as a result of a direct injection of 11-*cis* retinal (but not of all-*trans* retinal) into the compound eyes of the blowfly, *Calliphora erythrocephala*. He suggested that photoconversion is a necessary step in the isomerization of the chromophore and the subsequent synthesis of pigment.

Formation of visual pigment as a result of supplementing the diet with various derivatives of vitamin A has also been described in *Drosophila* (Stephenson et al., 1983). In this fly, however, neogenesis of the pigment occurs in the dark when carotenoids were given to adult flies (Isono et al., 1984). Formation of 11-*cis* 3-hydroxyretinal in the dark, presumably from carotenoids, has also been confirmed by Seki et al. (1986).

To investigate further the synthesis of fly visual pigment and the chromophore, changes in 3-hydroxyretinal content in *Drosophila* were investigated under different light conditions after the administration of various derivatives of vitamin A.

MATERIALS AND METHODS

Flies

A white-eyed strain of *Drosophila melanogaster*, homozygous for *w* (*white*, 1–1.5; Lindsley and Grill, 1968) was used throughout the study. Newly emerged adult flies were transferred to fresh vials and were maintained at 23°C under 12-h light and dark cycles until they were used for the experiments. Flies of both sexes, aged 3–7 d, were used. For microspectrophotometrical (MSP) and electrophysiological measurements, only female flies were used because of the relative ease of their manipulation.

Flies were raised on a medium based either on baker's yeast and cornmeal (a carotenoid-rich diet), or on baker's yeast exclusively (a diet deficient in carotenoids; see Table I). The yeast (*Saccharomyces cerevisiae*; Oriental Yeast Co. Ltd., Tokyo, Japan) contains neither carotenoids nor retinoids. Since cornmeal was the only ingredient that contained carotenoids, the yeast-based medium was used for the breeding and maintenance of carotenoid-deficient flies. The yeast-based medium was supplemented with 0.02% cholesterol, which is nutritionally essential for larval development (Sang, 1956).

Administration of Supplementary Retinoids and Carotenoids

Some flies were fed on a carotenoid-deficient medium supplemented with various retinoids or carotenoids. The supplements used in this study were: all-*trans* retinal, all-*trans* retinol, all-*trans* retinoic acid, all-*trans* retinyl palmitate, and all-*trans* B-carotene, (Sigma Chemical Co., St Louis, MO); 11-*cis* retinal and all-*trans* 3-dehydroretinal (from Dr. Kito, Osaka University, Japan); lutein zeaxanthin, and astaxanthin (Hoffman-La Roche & Co., Ltd., Basel, Switzerland); and xanthophyll concentrate (Tokyo Kaseikogyo Co., Ltd., Tokyo, Japan). All the commercial chemicals were of reagent grade, except for xanthopyll concentrate which was of practical grade. Supplemented media were prepared by spreading the chemicals dissolved in

TABLE 1

Ingredients for the Carotenoid-deficient and -rich Media Used in This Study

Constituents	Carotenoid-deficient medium	Carotenoid-rich medium
Agar	1 g	1 g
Corn grits	—	6 g
Dried baker's yeast	8 g	4 g
Sucrose	5 g	5 g
Cholesterol	0.02 g	—
Streptomycin	0.02 g	0.02 g
Penicillin	8,000 units	8,000 units
Tap Water	100 ml	100 ml

chloroform over the surface of the carotenoid-deficient media to generate a uniform layer of ~1 mg/cm^2. All procedures were carried out in the dark or under dim red light (Primary Red, 50% cutoff at 620 nm; Lee Filters, Andover, England).

Newly eclosed, carotenoid-deficient flies were deprived of food and water for 12 h. The flies were then allowed to feed on the supplemented medium ad lib., usually for 24 h. In some experiments, flies that had been fed on the supplemented medium were irradiated with monochromatic light. They were placed in a quartz or methacrylate cuvette (Kartell UV grade, 10 × 10 × 45 mm^3) and exposed to calibrated monochromatic light from a 30-KW xenon arc lamp (Okazaki Large Spectrograph, Japan).

Microspectrophotometry

Etherized female flies were decapitated with a razor blade. The heads were mounted on the tapered edge of a thin plexiglass plate and the deep pseudopupil of the compound eye was focused under antidromic illumination (Franceschini, 1972) through an objective (Nikon Macronikkor HM-20X, focal length = 19 mm) attached to a microscope (Olympus MMSP). The aperture was reduced to a circular area that covered only the positions of the outer rhabdomeres R2, R3, and R4 (~40 µm diam). The rhodopsin (R480) of the peripheral retinular cells, R1–R6, is known to absorb maximally at 480 nm, and to be reversibly photoconverted to metarhodopsin (M580), which absorbs maximally at ~580 nm (Ostroy et al., 1974; Pak and Lidington, 1974; Harris et al., 1976; Stark and Johnson, 1980). Changes in absorbance at 580 nm by the photoconversion were monitored and measured.

For measurements, the eyes were first preadapted to an intense, monochromatic blue (460 nm; half bandwidth, 10 nm). Then the adapting light was turned off and the measuring beam of monochromatic orange (580 nm; half bandwidth, 10 nm) was turned on. The initial adapting light established a photoequilibrium with a maximum level of M580 (M580:R480, ~80%). The subsequent measuring light at 580 nm gradually photoconverted M580 back to R480, giving a new photoequilibrium at 580 nm of almost 100% R480. The difference in absorbance at the beginning and at the final plateau value was then calculated. The whole procedure usually took 2–3 min and was repeated at least twice to make sure that the photoconversion was reversible. The measurements were then averaged and the mean was regarded as an estimate of the change in absorbance for an individual fly.

Electroretinograms

An etherized female fly was immobilized and mounted with myristyl alcohol on a coverslip. A glass micropipette filled with insect saline was inserted through the cornea and the tip was placed just beneath the cornea for recording. The reference electrode was inserted into the head cavity. The fly was then dark-adapted for 1 h before recording the electroretinogram (ERG). The stimulating light was delivered from a 500-W xenon arc lamp. It was collimated, filtered, and attenuated with a set of quartz lenses, a set of quartz neutral-density filters, and an interference filter (half bandwidth, 10–15 nm). The intensity of the unattenuated light was 2 × 10^{15} photons cm$^{-2} \cdot$s^{-1}. The duration of the flash stimulus was 1 s. The amplitude of maximum negativity during the stimulation before the onset of the off-transient response was measured.

High Performance Liquid Chromatography Analysis

All the following procedures were carried out under dim red light. 100–200 live or frozen flies were chilled in liquid nitrogen. Head capsules were detached from the bodies by mechanical vibration and collected by steel meshes of appropriate size. They were then homogenized in a glass homogenizer with 100 µl of phosphate-buffer solution. In some experi-

ments, the homogenate was irradiated with an intense monochromatic orange (580 nm; half bandwidth, 10 nm) or blue light (460 nm; half bandwidth, 10 nm) for 3 min at 5°C.

The homogenates were treated with 100 μl of 1 M hydroxylamine to produce 3-hydroxy-retinaloximes, and then the oximes were extracted with 500 μl of dichloromethane and 1 ml of n-hexane. High performance liquid chromatography (HPLC) analysis was performed with a silica gel column (5 × 150 mm; particle size, 5 μm). The mobile phase was 1% isopropyl alcohol and 30% ethylacetate in n-hexane. The column was washed and eluted at a flow rate of 0.5 ml min^{-1}. Absorbance at 360 nm was monitored with an ultraviolet spectrophotometer (UVDEC 100-IV; Japan Spectroscopic Co., Ltd., Tokyo, Japan).

RESULTS

Comparisons of MSP and ERG between Flies Deficient or Rich in Carotenoid

Typical MSP recordings for a carotenoid-deficient fly and for a control fly reared on carotenoid-rich medium are shown in Fig. 1. Usually, control flies gave changes in absorbance of 0.15–0.30 with a mean and SD of 0.22 ± 0.03 ($n = 10$). No detectable (<0.003) changes in absorbance was observed for carotenoid-deficient flies.
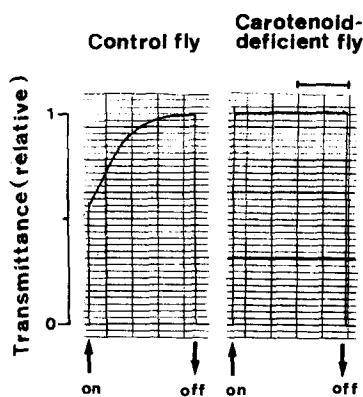

FIGURE 1. Typical example of the change in transmittance in the deep pseudopupil of a fly reared on a medium rich (left) or deficient (right) in carotenoids. The eyes were preadapted to 460 nm light. Transmittance was measured at 580 nm. Arrows indicate the beginning and end of the measurement. Scale bar, 30 s.

Absorbance could be affected by factors other than the concentration of the visual pigment. In carotenoid-deficient flies the axial length of the rhabdomere was not affected, while the cross-sectional area of the rhabdomeres was reduced to 50–70% of control values as a result of a reduction in the number and axial length of microvilli (our unpublished observations). These variations cause an apparent decrease in the changes in absorbance. However, since the fraction of light passing through nonrhabdomeric tissue is still low ($^1/_{10}$–$^1/_{20}$ of that for the rhabdomeric tissue), the reduction in the changes in absorbance should be mostly due to the decrease in the concentration of visual pigment (for a detailed discussion see Johnson and Pak, 1986).

The ERG response in the two groups of flies was then compared. Fig. 2 shows some examples of the intensity-response relationship to 581-nm orange light. Except for near-threshold (<3 mV) or near-saturating stimuli, the response usually increased linearly with logarithmic increases in the intensity of light, over a range of more than 3 log-units. There was no statistical difference in the slope of the curves for the carotenoid-deficient flies and the control flies (means ± SD values were

5.5 ± 0.6 [n = 10] and 5.7 ± 0.6 [n = 10] mV/log intensity, respectively). The sensitivity of the response, however, revealed a noticeable difference between the flies. The carotenoid-deficient flies required a mean intensity of light 110-fold higher than for the control flies to evoke the same magnitude of ERG response. A similar difference in the sensitivity was also obtained by stimulation with 479-nm blue light (about 120-fold difference, n = 5 and 6 for the control and the carotenoid-deficient flies, respectively).

Levels of 3-Hydroxyretinal in Flies Deficient or Rich in Carotenoid

When the chromophore of visual pigment was subjected to analysis by HPLC, four major elution peaks were seen for control flies, which corresponded to the syn
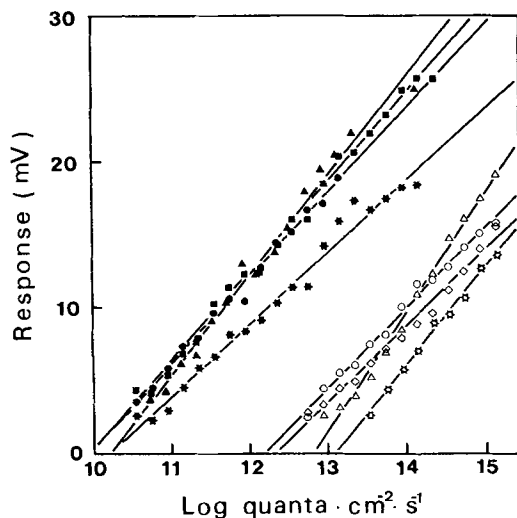

FIGURE 2. Examples showing the intensity-response relationship of ERG to monochromatic light of 581 nm for carotenoid-rich (filled symbols) and carotenoid-deficient flies (open symbols). Measurements from different individuals are indicated by different symbols. Least-square lines are superimposed for each individual.

oximes of 11-*cis* and all-*trans* 3-hydroxyretinal and their antioxime isomers (Fig. 3 A). The total amount of 3-hydroxyretinal was estimated to be 25.6 ± 2.5 pmol/mg of head (mean ± SD), or ~1.28 pmol/eye. Oximes prepared from the same amounts of homogenates of the heads of carotenoid-deficient flies gave no detectable peaks (Fig. 3 B). To quantitate the amount of 3-hydroxyretinal in the carotenoid-deficient fly, about seven times more than the amount of material used to analyze carotenoid-rich flies was used for the analysis of oximes in carotenoid-deficient flies (Fig. 3, C and D; note the difference in scales). The average amount of 3-hydroxyretinal in carotenoid-deficient flies was 0.15 ± 0.06 pmol/mg of head, or ~0.008 pmol/eye. Thus, the carotenoid-deficient flies contained only 0.6% of the amount of 3-hydroxyretinal found in the controls.

Irradiation of the homogenate of carotenoid-deficient flies with 460 nm (Fig. 3 D), or 581 nm after 460-nm light (Fig. 3 C), before the formation of oximes, produced reversible changes of the 11-*cis* and all-*trans* isomers. Thus, most of the residual 3-hydroxyretinal found in carotenoid-deficient flies should be the chromophore of the pigment, as it is in the control flies (cf., Tanimura et al., 1986). It should also be noted that the approximately hundredfold reduction in the amount of the chromophore was comparable to the reduction in the ERG sensitivity.
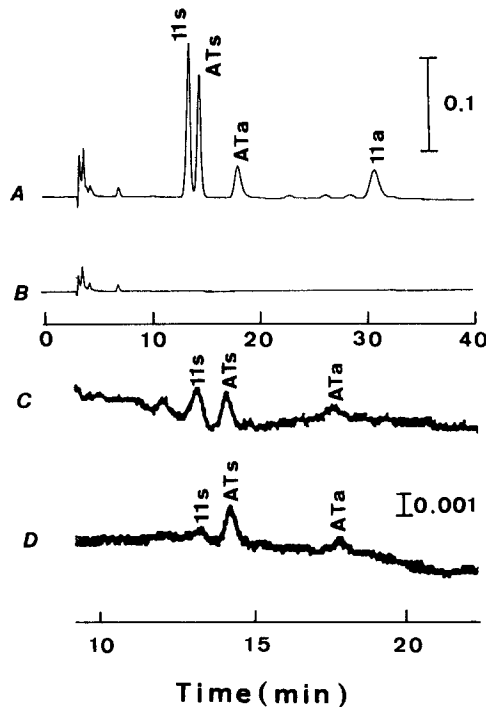
FIGURE 3. Analysis of isomers of 3-hydroxyretinal by HPLC for the homogenates of heads of carotenoid-rich (A) and carotenoid-deficient (B, C, and D) flies. 20 mg head homogenate was analyzed in A and B. Elution peaks of the *syn* isomers 11-*cis* (11s) and all-*trans* (ATs) 3-hydroxyretinaloxime, and the *anti* isomers all-*trans* (ATa) and 11-*cis* (11a) 3-hydroxyretinaloxime, are indicated. (C) and (D) HPLC analysis using 140 mg of homogenates of heads of carotenoid-deficient flies monitored at a higher sensitivity of the ultraviolet detector. The homogenates were irradiated with an intense 460-nm light, and then with 581-nm light (C) or just with 460-nm light alone (D) before the formation of oximes. Same scales for A and B, C and D.

Changes in the Amount of Visual Pigment and Isomers of 3-Hydroxyretinal after the Administration of Retinoids

The time course of the increase in the change in absorbance of the deep pseudopupil when adult, carotenoid-deficient flies were fed retinoids or carotenoids is shown in Fig. 4. Flies were transferred to a vial that contained a supplement for the first day (at time zero), and then transferred back to carotenoid-deficient medium for the subsequent days. Flies that were given all-*trans* retinal are indicated by circles. Note that no significant increase in the concentration of pigment was observed for the first 2 d in the dark (filled circles). An earlier experiment showed that, even after 7 d, the pigment concentration remains very low when flies are kept continuously in
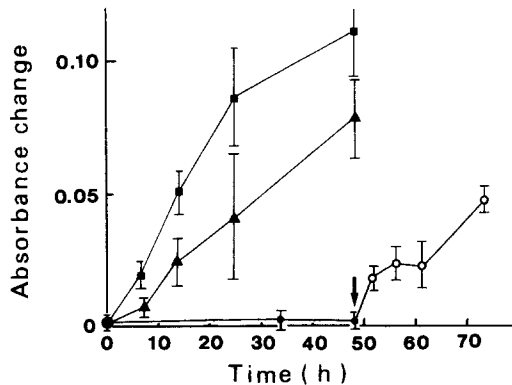

FIGURE 4. Time course of the increase in the change in absorbance in flies given supplements of all-*trans* retinal (circles), B-carotene (triangles), or xanthophyll (squares). (Arrow) The onset of illumination for flies given all-*trans* retinal and kept in the dark. (Open circles) Measurements after the onset of illumination. (Vertical bars) SD of the measurements from different individuals (n = 4 or 5).

the dark after they have been given all-*trans* retinal. On the third day, flies were exposed to a fluorescent light (open circles). After exposure to light, a significant amount of pigment was detectable within 4 h. The amount of the pigment continued to increase until, after 24 h, the change in absorbance was 0.045 ± 0.024 (mean ± SD), about one-fourth the value of that for the control flies.

A similar light-dependent increase in levels of the pigment was observed when the following supplements were used in the experiment just described: all-*trans* retinol, all-*trans* 3 -hydroxyretinal, and all-*trans* retinyl palmitate. Supplementation with all-*trans* 3-dehydroretinal (vitamin A_2 aldehyde) or all-*trans* retinoic acid, however, resulted in no detectable increase in the amount of pigment, regardless of the lighting conditions.
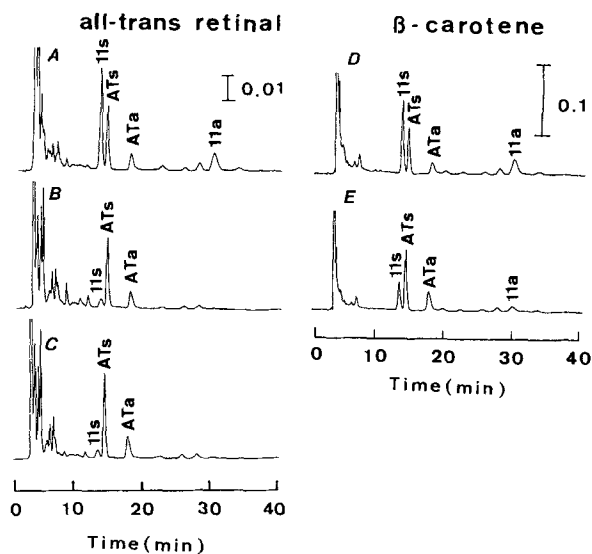

FIGURE 5. Analyses by HPLC of the heads of flies given various supplements and prepared under different light conditions. 20 mg of heads were analyzed. The abbreviations for the eluted peaks are the same as in Fig. 3. *A*, *B*, and *C* correspond to flies given all-*trans* retinal; *D* and *E* to flies given *B*-carotene. The flies given all-*trans* retinal were maintained either in the dark (*B*, *C*) or under 12-h light/12-h dark cycles (*A*). The flies given *B*-carotene were maintained in the dark. The homogenates in *C* and *E* were irradiated with light at 581 and 460 nm, respectively, before the formation of oximes. Other homogenates were prepared without irradiation. Same scales for *A*, *B*, and *C*; and for *D* and *E*.

Typical examples of HPLC analysis for flies that were supplemented with all-*trans* retinal are shown in Fig. 5 *A–C*. Flies were fed on the supplemented medium for the first day, and then transferred back to the carotenoid-deficient medium to be maintained either (*A*) under 12-h light/12-h dark cycles or (*B*) in the dark for 2 d. A considerable amount of 3-hydroxyretinal was found to be synthesized in these flies. The all-*trans* isomer was predominant in the flies kept in the dark, with very low amounts of 11-*cis*, whereas the flies kept under light-dark cycles contained considerable amounts of the 11-*cis* isomer. Substantial amounts of the 11-*cis* were photoisomerized into the all-*trans* form by irradiation of the homogenate with intense 460-nm light, before the formation of oximes, which suggests that the 11-*cis* originates from the chromophore. However, the all-*trans* 3-hydroxyretinal in the retina

of the flies that had been maintained in the dark was not photoisomerized into the 11-*cis* by irradiation of the homogenate with intense 580-nm light (Fig. 5 *C*).

These data provide strong evidence against the possibility that the all-*trans* 3-hydroxyretinal in the retina of flies maintained in the dark originates from meta-rhodopsin M580. This is compatible with the previous observation that the concentration of pigment remained very low as long as the flies were kept in the dark. It seems, therefore, that the all-*trans* isomer formed in the dark is either in a free state or is bound to a protein other than the visual pigment.

Unlike the case with all-*trans* retinoids, when carotenoid-deficient flies were given 11-*cis* retinal, the pigment was synthesized in the absence of light, as has been reported in the blowfly (Schwemer, 1983). The homogenate of the heads of *Drosophila*, given 11-*cis* retinal and kept in the dark for 2 d, contained amounts of 11-*cis* 3-hydroxyretinal comparable to those in the control flies. Therefore it is suggested that 3-hydroxylation can occur directly from 11-*cis* retinal, as is the case for all-*trans* retinal.

Localization of All-Trans 3-Hydroxyretinal

Localization of the nonpigment, all-*trans* 3-hydroxyretinal was investigated with eye mutants of *Drosophila*. No 3-hydroxyretinal was detectable in the head of an eyeless mutant *sine oculis* (Lindsley and Grell, 1968) that fed on a diet supplemented with all-*trans* retinal. The precursor was also absent from preparations of the thorax and abdomen of normal flies. This result confirms the fact that 3-hydroxyretinal is found only in the retina; any 3-hydroxyretinal in other tissues and organs being below the level of detection.

Flies carrying another mutation, *ora* (Koenig and Merriam, 1977; O'Tousa et al., 1986), which induces the degeneration of rhabdomeres in R1-R6 cells at the late pupal stage but leaves the cytoplasm almost intact, contained amounts of all-*trans* 3-hydroxyretinal similar to those in normal flies. Therefore it is suggested that all-*trans* 3-hydroxyretinal is probably located in the cytoplasmic, nonrhabdomeral regions of the photoreceptor cells, or in the pigment cells.

Photoisomerization In Vivo of All-Trans 3-Hydroxyretinal

Flies that were fed a diet supplemented with all-*trans* retinal in the dark were irradiated with monochromatic light and immediately frozen in liquid nitrogen for HPLC analysis. Fig. 6 compares the elution profiles from flies irradiated by light at four different wavelengths with equal numbers of photons (3×10^{13} photons $cm^{-2} \cdot s^{-1}$ for 60 min). Note that 11-*cis* 3-hydroxyretinal was formed as a result of irradiation and that the reaction was more efficient at ~400–450 nm than at longer or shorter wavelengths. Since the increase in the amount of the 11-*cis* isomer was accompanied by a comparable decrease in the amount of the all-*trans* isomer (the molecular extinction coefficient of 11-*cis* 3-hydroxyretinal is about one half of that of the all-*trans* isomer), the photoreaction was considered to be the isomerization from all-*trans* to 11-*cis* 3-hydroxyretinal.

Action spectra of the formation of 11-*cis* isomer at three different intensities are shown in Fig. 7. At lower intensities, the maximum efficiency of the isomerization occurs in violet light, at ~420 nm. The action spectrum becomes broader at higher

intensities and differs from that of the photoconversion of metarhodopsin to rhodopsin, which has a peak at ~580 nm. Free all-*trans* hydroxyretinal in organic solvents absorbs maximally in the near-ultraviolet range, at ~385 nm (Mayer and Santer, 1980). It is, therefore, apparent that all-*trans* 3-hydroxyretinal in the fly retina exists neither as the chromophore of the pigment nor in the free state.

Changes in the Amount of Visual Pigment and Isomers of 3-Hydroxyretinal after the Administration of Carotenoids

Carotenoid-deficient flies were fed B-carotene or xanthophyll supplements and were maintained in the dark. The time course of the increase in the change in
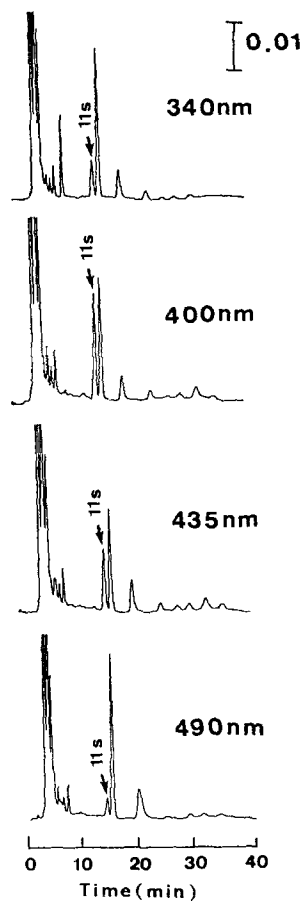

FIGURE 6. Analyses by HPLC of heads of flies given all-*trans* retinal in the dark and irradiated in vivo with monochromatic light of different wavelengths at an intensity of 3 x 10^{13} photons $cm^{-2} \cdot s^{-1}$ for 60 min. 20 mg of samples of heads were analyzed in each case. Abbreviations for the eluted peaks are the same as in Fig. 3.

absorbance of the deep pseudopupil is shown in Fig. 4 (triangles and squares). A measurable change in absorbance was already recognized within 3 h. The change continued to increase until it reached a level comparable to that found in the control flies. Supplementation with xanthophyll resulted in a slightly faster increase in levels of the pigment than with B-carotene. In both cases it should be noted that synthesis of the pigment was initiated in the absence of light soon after the administration of carotenoids.

Similar light-independent increases in the amount of pigment were also observed with other xanthophylls, lutein (*B,E*-carotene-3,3'-diol) and zeaxanthin (*B,B*-carotene-3,3'-diol). Supplementation with astaxanthin (3,3'-dihydroxy-*B,B*-carotene-4,4'dione), an oxygenated compound of xanthophyll, were ineffective and gave no increase in the change in absorbance even after irradiation with light.

Fig. 5, *D* and *E* show HPLC profiles for flies given *B*-carotene and maintained in the dark for 24 h. Samples were prepared either (*D*) without illumination or (*E*) with illumination of the homogenate at 460 nm before the formation of oximes. Note that most of the 3-hydroxyretinal is in the 11-*cis* form in Fig. 5 *D*. Since illumination at 460 nm isomerizes the 11-*cis* to the all-*trans* isomer (Fig. 5 *E*), the 11-*cis* isomer must be present mainly as the chromophore of the pigment. The formation of 11-*cis* 3-hydroxyretinal in the dark was also observed when diets were supplemented with the xanthophylls lutein and zeaxanthin, but not with astaxanthin; neither 11-*cis* nor all-*trans* 3-hydroxyretinal was observed when flies were given astaxanthin.
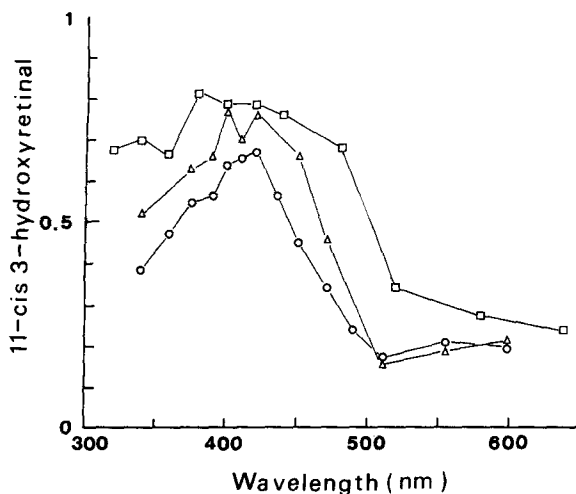

FIGURE 7. Action spectra of the photoisomerization in vivo of all-trans 3-hydroxyretinal to the 11-*cis* isomer. The ordinate shows the proportion of 3-hydroxyretinal that occurred as the 11-*cis* isomer. Light intensities: 3×10^{13} (circles), 8×10^{13} (triangles), and 6×10^{15} (squares) photons $cm^{-2} \cdot s^{-1}$.

DISCUSSION

Hydroxylation in the 3-Position

According to Vogt (1984), insects belonging to the orders Diptera and Lepidoptera have visual pigments (xanthopsins) with the chromophore 3-hydroxyretinal. When these insects are dependent on diets with nonhydroxyretinoids or carotenoids as sources of vitamin A, hydroxylation in the 3(3') position should occur to generate the prosthetic group of the pigment. In addition, the 11-*cis* configuration is required for the synthesis *de novo* of the visual pigment of the fly (Schwemer, 1983). This is probably the case in most other animals as well. Supplementing carotenoid-deficient flies with all-*trans* retinal has allowed us to dissociate the two processes and has demonstrated that only hydroxylation takes place in the absence of light. In addition to all-*trans* retinoids, 11-*cis* retinal and nonhydroxycarotene were also hydroxylated in the 3(3') position in the fly retina, which is in accordance with the

results of Vogt (1984). Therefore, a wide range of retinoid and carotenoid precursors can be used as the substrate for the reaction.

Among the supplements that were not accepted as precursors to the chromophore, both 3-dehydroretinal and astaxanthin (*B,B*-carotene, 3-3'-diol, 4,4'-dioxy) have structural modifications at the 3 and 4 positions. Steric hindrance could possibly block hydroxylation at the 3 position of these compounds. Since flies given these compounds did not form any MSP-detectable pigment, fly opsin may not accept these retinoids, or they may be degraded before they can bind to opsin. Degradation of the chemicals before they were ingested by the fly might be noted, however, that in the case of all-*trans* retinal, the half-life was between 5 and 10 h on the medium at room temperature in the dark.

All-Trans 3-Hydroxyretinal as a Precursor

All-*trans* 3-hydroxyretinal, in the eyes of the flies given all-*trans* retinoids, remained stable in the retina and could readily be isomerized by light to the 11-*cis* isomer. The amount of isomerized 3-hydroxyretinal is comparable to the amount of pigment synthesized thereafter in the retina. Therefore, the stable 3-hydroxyretinal can be regarded as a major precursor of the chromophore. To summarize the main features of the photoisomerization, it is, firstly, highly sensitive and efficient: ~70% of the all-*trans* precursor can be photoisomerized by irradition of the fly with 410–430 nm light, at $<10^{14}$ photons $cm^{-2} \cdot s^{-1}$ for 1 h. Secondly, the formation of photoproduct, the 11-*cis* isomer, is highly specific. Finally, the peak of the action spectrum of the photoisomerization shifts to a wavelength some 30–40 nm longer than the absorption maximum of the free all-*trans* 3-hydroxyretinal (386 nm in chloroform; Mayer and Santer, 1980). It is strongly suggested that the precursor, 3-hydroxyretinal, is bound to a specific binding protein, as is the case for the retinal-based pigment systems, such as the retinochrome in the cephalopod retina (Hara and Hara, 1972) or retinal-binding proteins in the honeybee retina (Pepe et al., 1976). It should be noted that the photoisomerization of 3-hydroxyretinal is consistent with the result in the blowfly where renewal of visual pigment is mediated by blue/violet light (Schwemer, 1984).

The presence of the precursor 3-hydroxyretinal in the mutant *ora*, suggests that the protein-precursor complex is localized in a nonrhabdomeral cytoplasmic site in the retina. Since *ora* contains very low amounts of the visual pigment in the peripheral retinular cells, studies using this mutant should facilitate the further characterization and the isolation of the putative 3-hydroxyretinal–binding protein in the fly.

Light-independent Pathway of Carotenoid Precursors

When flies were given various carotenoids, the whole process of increasing the levels of 11-*cis* 3-hydroxyretinal and of visual pigment in the retina was able to proceed in total darkness. To convert carotenoids ultimately to 11-*cis* 3-hydroxyretinal, it is necessary for the fly to have a mechanism for the cleavage of carotenoids in addition to mechanisms for 3-hydroxylation and isomerization. If the cleavage of carotenoids occurs before the isomerization, then irradiation by light should be required for the isomerization, as it is required in the retinoid pathway. Therefore, there must be

another mechanism to account for the isomerization of carotenoids into the 11-*cis* form. One such mechanism might involve the isomerization at the 11 (11′) position, either before cleavage or simultaneously with it.

Although the levels of carotenoids were not analyzed in the present study, the presence of carotenoids has been recognized in the dipteran retinae (Kirschfeld et al., 1983; Vogt, 1984). Such carotenoids are regarded as potential, light-independent precursors of the chromophore. Most species of *Drosophila* are herbivorous and depend on diets that contain carotenoids as sources of the chromophore. Therefore, under natural conditions, the photo-independent isomerization is probably a more important pathway for the synthesis of the chromophore than is the photoisomerization of all-*trans* retinoids. In the blowfly *Calliphora erythrocephala*, however, Schwemer (1984) has shown that the synthesis of pigment depends solely on the light-dependent pathway, a result that suggests that the photo-independent pathway is absent or is not functioning. Further studies are necessary if we are to fully understand the mechanism of isomerization in the dark.

The photo-independent mechanism may also be involved in the slow recovery of the pigment in the dark, from metarhodopsin to rhodopsin, which has been postulated to occur in the fly and in other animals (the octopus *Eledone moschata*, Schwemer, 1969; the lobster *Hoarus americana*, Goldsmith and Bruno, 1973; the fly *Drosophila melanogaster*, Pak and Lidington, 1974; the moth *Galleria mellonella*, Goldman et al., 1975; the butterfly *Aglais urticae*, Stavenga, 1975; the butterfly *Apodemia mormo*, Bernard, 1979).

REFERENCES

Bernard, G. D. 1979. Red-absorbing visual pigment of butterflies. *Science.* 203:1125–1127.

Franceschini, N. 1972. Pupil and pseudopupil in the compound eye of *Drosophila. In* Information Processing in the Visual Systems of Arthropods. R. Wehner, editor. Springer-Verlag. Berlin/Heidelberg/New York. 75–82.

Goldman, L. J., S. N. Barnes, and T. H. Goldsmith. 1975. Microspectrophotometry of rhodopsin and metarhodopsin in the moth *Galleria. Journal of General Physiology.* 66:383–404.

Goldsmith, T. H. 1958. The visual system of the honeybee. *Proceedings of the National Academy of Sciences.* 44:123–126.

Goldsmith, T. H., and M. S. Bruno. 1973. Behavior of rhodopsin and metarhodopsin in isolated rhabdoms of crabs and lobster. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag, Berlin/Heiderberg/New York. 147–153.

Goldsmith, T. H., B. C. Marks, and G. D. Bernard. 1986. Separation and identification of geometric isomers of 3-hydroxyretioids and occurrence in the eyes of insects. *Vision Research.* 26:1763–1769.

Hamdorf, K., R. Paulsen, and J. Schwemer. 1973. Photoregeneration and sensitivity control of photoreceptors of invertebrates. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag, Berlin/Heidelberg/New York. 155–166.

Hara, T., and R. Hara. 1972. Cephalopod retinochrome. *In* Handbook of Sensory Physiology. Vol. VII/1. Photochemistry of Vision. H. J. A. Dartnall, editor. Springer-Verlag, Berlin/Heidelberg/New York. 720–746.

Harris, W. A., W. S. Stark, and J. A. Walker. 1976. Genetic dissection of the photoreceptor system in the compound eye of *Drosophila melanogaster*. *Journal of Physiology*. 256:415–439.

Isono, K., Y. Tsukahara, and T. Tanimura. 1984. Rhodopsin synthesis in the compound eye by supplement of retinoids and carotenoids to vitamin A-deficient fly. *Zoological Science*. 1:861. (Abstr.)

Johnson, E. C., and W. L. Pak. 1986. Electrophysiological study of *Drosophila* rhodopsin mutants. *Journal of General Physiology*. 88:651–673.

Kirschfeld, K., R. Feiler, R. Hardie, K. Vogt, and N. Franceschini. 1983. The sensitizing pigment in fly photoreceptors: properties and candidates. *Biophysics of Structure and Mechanism*. 10:81–92.

Koenig, J., and J. E. Merriam. 1977. Autosomal ERG mutuants. *Drosophila Information Service*. 52:50–51.

Lindsley, D. L., and E. H. Grell. 1968. Genetic variations of *Drosophila melanogaster*. Carnegie Institution of Washington, Washington DC. 232 and 266–267.

Mayer, H., and J. M. Santer. 1980. Synthese von Optisch Aktiven, Naturlichen Carotinoiden und Strukturell Verwanden Naturprodukten. VII. Synthese von (3*R*)-3-Hydroxyretinol, (3*R*)-3-Hydroxyretinal und (3*R*)-3-Hydroxyretinsaure. *Helvetica Chimica Acta*. 63:1467–1472.

Ostroy, S. E., M. Wilson, and W. L. Pak. 1974. *Drosophila* rhodopsin: photochemistry, extraction and differences in the *norp* A^{P12} phototransduction mutant. *Biochemical and Biophysical Research Communications*. 59:960–966.

O'Tousa, J. E., D. S. Leonard, and W. L. Pak. 1986. Morphological defects in *ora* photoreceptors caused by mutation in R1-6 opsin gene of *Drosophila*. *In* Cold Spring Harbor Meeting on Molecular Neurobiology of *Drosophila* Abstracts. Cold Spring Harbor, New York. 71. (Abstr.)

Pak, W. L., and K. J. Lidington. 1974. Fast electrical potential from a long-lived long-wavelength photoproduct of fly visual pigment. *Journal of General Physiology*. 63:740–756.

Pepe, I. M., A. Perrelet, and F. Baumann. 1976. Isolation by polyacrylamide gel electrophoresis of a light-sensitive vitamin A–protein complex from the retina of the honeybee drone. *Vision Research*. 16:905–908.

Sang, J. H. 1956. The quantitative nutritional requirements of *Drosophila melanogaster*. *Journal of Experimental Biology*. 33:45–72.

Schwemer, J. 1969. Der Sehfarbstoff von *Eledone moschata* und seine Umsetzungen in der lebenden Netzhaut. *Zeitschrift für Vergleichende Physiologie*. 62:121–152.

Schwemer, J. 1983. Pathways of visual pigment regeneration in fly photoreceptor cells. *Biophysics of Structure and Mechanism*. 9:287–298.

Schwemer, J. 1984. Renewal of visual pigment in photoreceptors of the blowfly. *Journal of Comparative Physiology A*. 154:535–547.

Seki, T., S. Fujishita, M. Ito, N. Matsuoka, C. Kobayashi, and K. Tsukida. 1986. A fly, *Drosophila melanogaster*, forms 11-*cis* 3-hydroxyretinal in the dark. *Vision Research*. 26:255–258.

Stark, W. S., and M. A. Johnson. 1980. Microspectrophotometry of *Drosophila* visual pigments: determinations of conversion efficiency in R1-6 receptors. *Journal of Comparative Physiology*. 140:275–286.

Stavenga, D. G. 1975. Dark regeneration of invertebrate visual pigments. *In* Photoreceptor Optics. A. W. Synder and R. Menzel, editors. Springer-Verlag, Berlin/Heidelberg/New York. 290–295.

Stephenson, R. S., J. O'Tousa, N. J. Scavarda, L. L. Randall, and W. L. Pak. 1983. *Drosophila* mutants with reduced rhodopsin content. *In* The Biology of Photoreception. D. J. Cosens and D. Vince-Price, editors. Cambridge University Press, Cambridge, England. 477–501.

Tanimura, T., K. Isono, and Y. Tsukahara. 1986. 3-hydroxyretinal as a chromophore of *Drosophila melanogaster* visual pigment analyzed by high-pressure liquid chromatography. *Photochemistry and Photobiology.* 43:225–228.

Vogt, K. 1983. Is the fly visual pigment a rhodopsin? *Zeitschrift für Natürforschung, Section C.* 38c:329–333.

Vogt, K. 1984. The chromophore of the visual pigment in some insect orders. *Zeitschrift für Naturforschung, Section C.* 39c:196–197.

Vogt, K., and K. Kirschfeld. 1984. Chemical identity of the chromophores of fly visual pigment. *Naturwissenschaften.* 71:211–213.