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ON THE MECHANISM OF ISOMERIZATION OF OCULAR RETINOIDS BY THE CRAYFISH *PROCAMBARUS CLARKII*

RANJANA SRIVASTAVA AND TIMOTHY H. GOLDSMITH*
Department of Biology, Yale University, New Haven, CT 06520, USA

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Summary

The eyes of some crustaceans store substantial amounts of retinyl esters, with most of the retinol in the 11-*cis* configuration. Earlier work in this laboratory suggested that in lobster and crayfish the mechanism of isomerization of retinol to the 11-*cis* form involves the hydrolysis of all-*trans* retinyl esters. Although this is the same process as that occurring in the vertebrate eye, it is different from the retinal photoisomerase reaction known in other arthropods, specifically diurnal insects (Hymenoptera and probably Diptera). Using homogenates of crayfish, we have tested this proposed mechanism by inhibiting retinyl ester synthetase activity in the presence of exogenous all-*trans* retinol. Inhibition of lecithin:retinol acyl transferase with 5 μmol l^{-1} retinyl bromoacetate or 2 mmol l^{-1} phenylmethylsulfonyl fluoride blocks the formation of both all-*trans* and 11-*cis* retinyl esters as well as 11-*cis* retinol, as shown by direct assay and by the decrease in counts derived from tritiated all-*trans* retinol. The similarity of this isomerization to the mechanism in vertebrate pigment epithelium is thus an interesting example of convergent evolution in the biochemistry of visual pigments, in which the pigments themselves (the opsins) are largely conserved across phyla.

Key words: retinol isomerization, lecithin:retinol acyl transferase, crayfish, visual system, *Procambarus clarkii*.

Introduction

The means by which animals isomerize retinol or retinal to the 11-*cis* configuration for incorporation of the latter into their visual pigments is one of the most variable features of the visual pigment cycle. The first of these enzymes to be discovered was the retinal photoisomerase of cephalopod molluscs, an intrinsic membrane protein known as retinochrome (Hara and Hara, 1972; Ozaki *et al*. 1986; Hara, 1988). Certain diurnal insects also have a retinal isomerase, but in bees (*Apis mellifera*), where it is best known, it is a soluble protein (Schwemer *et al.* 1984; Smith and Goldsmith, 1991*a*,*b*). A retinal photoisomerase has also been reported in *Limulus polyphemus* (Smith *et al.* 1992), but nothing is known of its biochemistry.

A very different system is found in the pigment epithelium of vertebrate retinas, where isomerization takes place at the oxidative level of retinol (rather than retinal) by coupling the isomerization to the hydrolysis of all-*trans* retinyl palmitate (Bernstein *et al.* 1987; Law and Rando, 1988; Barry *et al.* 1989; Deigner *et al*. 1989; Cañada *et al.* 1990; Livrea *et al.* 1990).

The diurnal insects that have a retinal photoisomerase do not have reserves of retinyl esters. Vertebrate pigment epithelium, however, characteristically stores retinol as retinyl esters. Similarly, the eyes of mantis shrimps contain several hundred pmoles of 11-*cis* retinyl esters (Goldsmith and Cronin, 1993), and in macruran decapods (*Homarus, Procambarus*), there are also substantial stores of 11-*cis* retinol, principally as esters of docosahexaenoate ($C_{22:6}$) with somewhat lesser amounts of retinyl oleate ($C_{18:1}$) (Suzuki *et al.* 1988; Srivastava *et al.* 1996). Moreover, homogenates of the eyes of both *Homarus* and *Procambarus* are capable of forming 11-*cis* retinyl esters from added all-*trans* retinyl docosahexaenoate, a reaction that takes place in the dark (Srivastava *et al.* 1996). These observations suggest that some crustaceans with nocturnal or crepuscular habits not only store 11-*cis* retinyl esters but may form 11-*cis* retinol by a mechanism very similar to that employed by the vertebrate eye.

Because in vertebrate pigment epithelium the energy for isomerization is obtained by coupling to the hydrolysis of retinyl esters, if all-*trans* retinol is provided as substrate it must first be esterified before it can be isomerized to 11-*cis*. A critical test of this mechanism is to inhibit the formation of retinyl esters (Trehan *et al.* 1990).

In vertebrate pigment epithelium, coenzyme A (CoA)-dependent synthesis of retinyl esters can be demonstrated *in vitro* (Saari and Bredberg, 1988), but retinyl esters are also formed by the transfer of the fatty acid from a phospholipid by the enzyme lecithin:retinol acyl transferase (LRAT) (Barry *et al.* 1989; Saari and Bredberg, 1989). In the present experiments, we find that inhibitors of LRAT activity sharply

*e-mail: timothy.goldsmith@yale.edu.

decrease the formation of 11-*cis* retinol and 11-*cis* retinyl esters from exogenous all-*trans* retinol.

Materials and methods

Preparation of homogenates

Crayfish (*Procambarus clarkii* Girard) (Carolina Biological Supply Company, Burlington, NC, USA) were kept in a shallow-water aquarium at room temperature (range 20–22 °C) and fed dry dog food for periods of up to 2 weeks prior to use. The animals were immobilized by placing them on crushed ice for 10–20 min, the eyestalks were removed by cutting at their base, the eyes were bisected, and the soft tissue was removed. The pigmented tissue was severed from the optic nerve and homogenized manually (Wheaton tissue grinder with hollow glass pestle) in 0.1 mol l^{-1} phosphate buffer (pH 7.4) containing 2 mmol l^{-1} dithiothreitol, using a volume of 0.5 ml per eye. This crude homogenate was used for the assays.

Assay for isomerase activity

For substrate, [11,12-^{3}H(N)]-all-*trans* retinol (Dupont NEN Research Products, Boston, MA, USA) was diluted to a specific activity of 8.88×10^6 Bq μmol^{-1} using unlabeled all-*trans* retinol (Sigma, St Louis, MO, USA). For each reaction mixture, 200–300 pmol of this retinol in hexane was added to 1 ml of homogenate, using 100 μl of bovine serum albumin (100 mg ml^{-1}) as carrier. The reaction mixture was incubated for 0–120 min in the dark at 22 °C, and the reaction was stopped by the addition of an equal volume of chilled ethanol. In control tubes, the homogenate was added after the addition of ethanol and the tubes were kept for 2 h at either 0 or 22 °C. There was no difference in the levels of either retinol or retinyl esters between these controls. In previous experiments involving retinyl esters as substrates and (in the case of lobster) higher levels of endogenous esters, there was no consistent evidence for loss of retinoid during 2 h of incubation (Srivastava *et al.* 1996).

Retinol and its esters were extracted into hexane and separated by high-performance liquid chromatography (HPLC). 1 ml samples of the eluate were collected and radioactivity was measured in a liquid scintillation counter (Beckman Instruments LS 8000, Columbia, MD, USA). The counts that entered 11-*cis* retinyl esters were more than two orders of magnitude lower than the tritium label found in 11-*cis* retinol; reported changes of 20–40 cts min^{-1} are relative to base values of 100–200 cts min^{-1} in individual ester fractions.

High-performance liquid chromatography

Isomers of retinol and retinyl esters were separated using a normal-phase silica column (Rainin Microsorb, Woburn, MA, USA) and isocratic elution at a flow rate of 1 ml min^{-1}. The mobile phase was 9 % dioxane in hexane for free retinol. Under these conditions, the esters elute close to the front; they were recovered as a single peak and separated using 0.35 % dioxane in hexane as the mobile phase. The absorbance of the eluate was measured at 328 nm and the system was quantified using peak integration software (Perkin Elmer Nelson Systems, Cupertino, CA, USA). The fluorescence of the eluate was also monitored. See Srivastava *et al.* (1996) for further details and for the identification of the fatty acid moieties of the retinyl esters.

Use of inhibitors

Isomerization of retinol was examined in the presence of known inhibitors of retinol esterification: all-*trans* α-retinyl bromoacetate (RBA), phenylmethylsulfonyl fluoride (PMSF) and progesterone. Retinol, PMSF and progesterone were obtained from Sigma (St Louis, MO, USA); RBA was synthesized by reacting retinol with bromoacetylbromide (Aldrich, Milwaukee, WI, USA) in the presence of pyridine (Gawinowicz and Goodman, 1982).

In these studies, 1 ml of eye homogenate was preincubated with several concentrations of inhibitor. RBA was preincubated with the homogenate for 10 min at 30 °C, while PMSF and progesterone were preincubated for 10 min and 6 min respectively at 25 °C, before the addition of ^{3}H-labeled all-*trans* retinol. After 2 h of incubation, retinol and retinyl esters were extracted and the geometric isomers were assayed as described above.

Results

*Formation of 11-*cis *retinol*

When homogenized crayfish eyes were incubated at 22 °C with all-*trans* retinol (150–200 pmol eye^{-1}), there was a several-fold increase in the concentration of the 11-*cis* isomer over 2 h (Fig. 1). By comparison, there was much smaller change in the level of 13-*cis*, the isomer most likely to form non-enzymatically from the all-*trans* substrate. Because there were traces of the 13-*cis* isomer in the unlabeled all-*trans* retinol with which the tritiated compound was diluted to form the stock solution, 1–2 pmol of 13-*cis* retinol had probably been added to the reaction mixture with the substrate. There

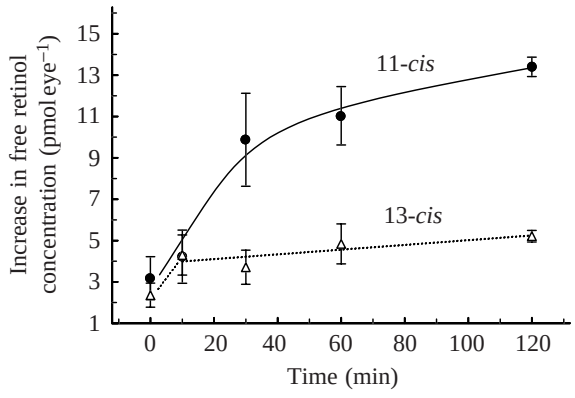

Fig. 1. Increases in 11-*cis* and 13-*cis* retinol levels in homogenates of two crayfish eyes to which had been added 100–150 pmol eye^{-1} of all-*trans* retinol with bovine serum albumin (BSA) as carrier. Values are means ± S.E.M. for 4–6 experiments. As described in the text, some unlabeled 13-*cis* retinol was probably added with the substrate.

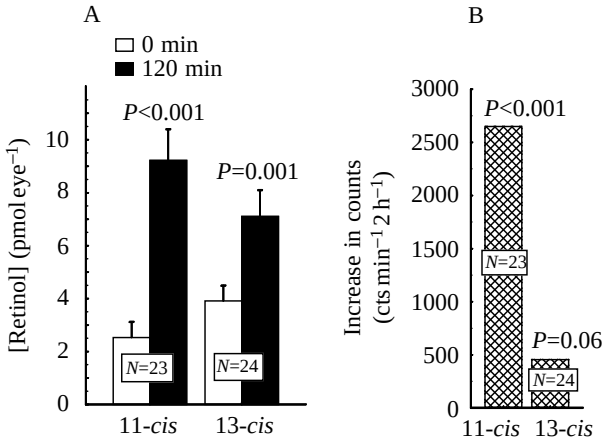

Fig. 2. (A) Increases in 11-*cis* and 13-*cis* retinol levels in extracts of crayfish supplemented with all-*trans* retinol and incubated in the dark for 2 h. The several-fold increase in 11-*cis* retinol level was significant (paired *t*-test, $P<0.001$, values are means + S.E.M., $N=23$). There is little 13-*cis* retinol in crayfish eyes; the levels present in these experiments were therefore introduced with the 200–300 pmol of all-*trans* substrate. There was an increase in levels during the 2 h incubation period ($P<0.001$, $N=24$), but less, proportionally, than the increase in 11-*cis* retinol. (B) Increases in counts in 11- and 13-*cis* retinol during the 2 h incubation with tritiated all-*trans* retinol. Significance (paired *t*-tests) and numbers of repetitions are shown as in A.

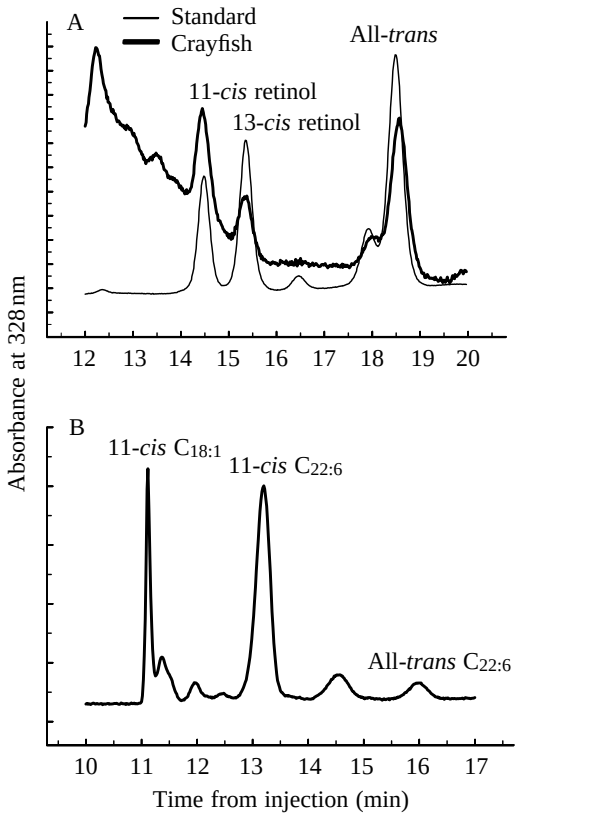

Fig. 3. (A) Separation by HPLC of the isomers of intrinsic free retinol of crayfish (heavy trace) compared with a standard (light trace). The former is shown at a 10-fold higher gain. The mobile phase was 9 % dioxane in hexane. The molar absorbance of 11-*cis* retinol is smaller than that of the other two isomers and, although the eyes contain several pmoles of the 11-*cis* form, they characteristically have less than 1 pmol of 13-*cis* retinol. (B) HPLC of the principal isomers of retinyl esters of crayfish. The mobile phase was 0.35 % dioxane in hexane. For identification of the fatty acid moieties, see Srivastava *et al.* (1996).

was little increase in 13-*cis* retinol level during the 2 h incubation, which is why the 13-*cis* retinol that is recovered at 2 h has such a low specific activity relative to the 11-*cis* isomer.

The results of 24 experiments are summarized in Fig. 2, where the open bars indicate the amount of *cis* isomer present immediately after adding substrate and the filled bars the amount 2 h later. The 2–3 pmol eye^{-1} of 11-*cis* retinol initially present was endogenous retinol (Fig. 3A). Extracts of eyes contain less 13-*cis* retinol (<1 pmol eye^{-1}), however, and the 3–4 pmol of 13-*cis* isomer was added with the much larger amount of all-*trans* substrate. The increases in levels of both 11- and 13-*cis* retinol in Fig. 2A are highly significant ($P<0.001$, paired *t*-tests).

The corresponding increase in tritium activity associated with the two *cis* isomers is shown in Fig. 2B. That the all-*trans* substrate is the source of the 11-*cis* retinol is confirmed by a highly significant increase in counts in the latter ($P<0.001$). There was a much smaller increase in activity of the 13-*cis* isomer.

*Formation of 11-*cis *retinyl esters*

Crayfish eyes contain retinyl esters of (principally) two fatty acids (Fig. 3B), docosahexaenoate ($C_{22:6}$) and oleate ($C_{18:1}$) (Srivastava *et al.* 1996). The amounts are several times larger than the amounts of free retinol present (Fig. 4, open bars), and in both esters the retinyl moiety is largely in the 11-*cis* configuration, with smaller amounts of the all-*trans* form. Following incubation with all-*trans* retinol, there are small

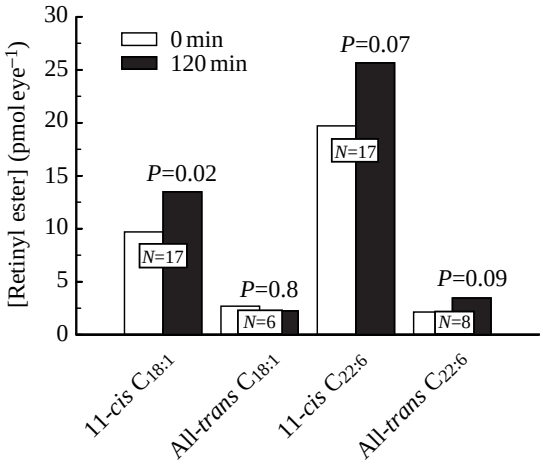

Fig. 4. Average levels of isomers of retinyl esters in homogenates of crayfish eyes before and after a 2 h incubation with all-*trans* retinol. The significance of changes is given by the *P* value above each pair of bars (paired *t*-test); *N* is the number of replicates.

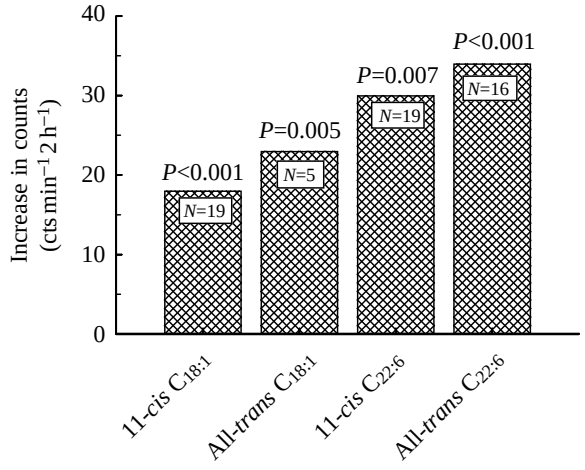

Fig. 5. Increases in tritium label in the isomers of retinyl ester during a 2 h incubation. The experiments shown are the same as in Fig. 4. The significance of changes (120 min incubation minus 0 min control) is given by the *P* value above each bar (paired *t*-test); *N* is the number of replicates. As described in Materials and methods, unlike free 11-*cis* retinol, only a very small amount of label appeared in 11-*cis* esters.

increases in levels of the 11-*cis* retinyl esters (Fig. 4) and significant increases in activity in all four isomers (Fig. 5).

Inhibition of isomerization by retinyl bromoacetate (RBA)

If in crayfish eyes the isomerization of all-*trans* retinol to the 11-*cis* configuration involves the hydrolysis of all-*trans* retinyl esters, the results described in Figs 1, 2, 4 and 5 imply the presence of the following sequence of reactions:

$$\text{all-}trans\text{ retinol} \xrightarrow{\text{ester synthetase}} \text{all-}trans\text{ retinyl ester}$$

$$\text{all-}trans\text{ retinyl ester} \xrightarrow{\text{hydrolysis and isomerization}} \text{11-}cis\text{ retinol}$$

$$\text{11-}cis\text{ retinol} \xrightarrow{\text{ester synthetase}} \text{11-}cis\text{ retinyl ester}.$$

In other words, the appearance of 11-*cis* retinol requires a sequence of two reactions, and the formation of 11-*cis* retinyl esters a third. Retinyl bromoacetate (RBA) is a potent inhibitor of lecithin:retinol acyl transferase (LRAT) (Trehan *et al.* 1990). If LRAT is responsible for retinyl ester synthetase activity in crayfish, RBA provides an important test of the hypothesis that isomerization of retinol requires all-*trans* retinyl ester as an intermediate, for RBA should block the formation of both 11-*cis* retinyl esters and 11-*cis* retinol. However, if 13-*cis* retinol forms non-enzymatically, any increase in its level should be independent of ester synthetase activity.

Fig. 6A shows the increase in 11-*cis* retinol formed from all-*trans* retinol substrate after 2 h as a function of the concentration of RBA present. Less 11-*cis* retinol appeared as the concentration of RBA increased. Fig. 6B shows that 5 μmol l^{-1} RBA significantly decreased (P<0.01, paired *t*-test for six experiments) the amount of tritium label that appeared in 11-*cis* retinol.

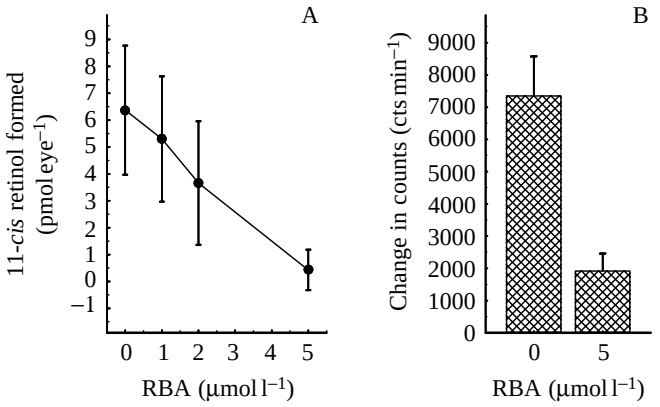

Fig. 6. (A) Effect of the concentration of retinyl bromoacetate (RBA) on the amount of 11-*cis* retinol formed during a 2 h incubation with all-*trans* retinol substrate. In the presence of 5 μmol l^{-1} RBA, there is little or no net increase in 11-*cis* retinol levels. Values are means ± S.E.M., N=5–9. (B) Change in radioactivity of tritiated retinol during 2 h in the presence of 0 and 5 μmol l^{-1} RBA. Values are means + S.E.M., N=7–9.

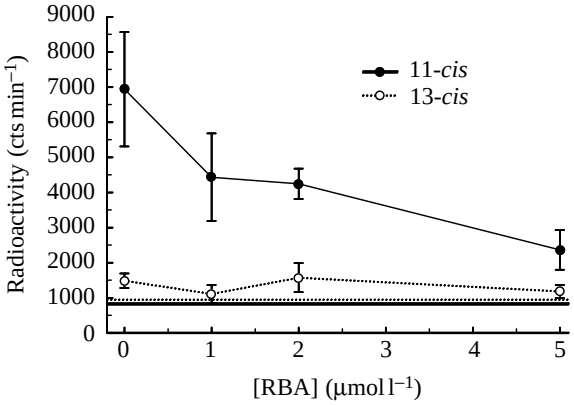

Fig. 7. Appearance of tritium label into 11- and 13-*cis* retinol during a 2 h incubation as a function of the concentration of RBA. The horizontal lines are the average radioactivity levels at the start of the incubation. The small increase in activity over 2 h for 13-*cis* retinol is independent of the presence of RBA and is probably non-enzymatic. Values are means ± S.E.M., N=4–7.

Fig. 7 shows the counts in 11- and 13-*cis* retinol after 2 h of incubation at three concentrations of RBA. The two horizontal lines (at <1000 cts min^{-1}) show the average activity associated with each isomer at the start of incubation and immediately following the addition of substrate. As expected from the data of Fig. 6, increasing the RBA concentration diminished the appearance of counts in 11-*cis* retinol but had no effect on the much smaller activity that accumulated in 13-*cis* retinol during a 2 h incubation.

RBA also inhibited the formation of 11-*cis* retinyl esters. Fig. 8A shows the decrease in post-incubation levels of $C_{22:6}$ and $C_{18:1}$ esters with increasing RBA concentration. The pre-incubation levels are shown for comparison by the horizontal lines. In the presence of 2 and 5 μmol l^{-1} RBA, the 11-*cis* $C_{22:6}$

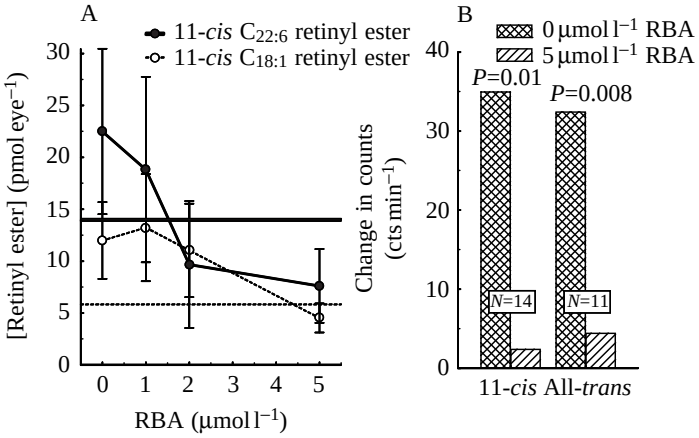

Fig. 8. (A) The effect of RBA concentration on the amount of 11-*cis* retinyl ester after a 2 h incubation. Horizontal lines represent the average levels of each ester present at the start of the incubation. RBA inhibited the formation of 11-*cis* retinyl ester, and (particularly for $C_{22:6}$) there was a net hydrolysis of ester at higher concentrations of RBA. Values are means ± S.E.M., *N*=4–8. (B) Inhibition of the appearance of tritium in 11-*cis* retinyl esters by 5 μmol l^{-1} RBA. Pooled data for $C_{22:6}$ and $C_{18:1}$ esters. Inhibition was significant (paired *t*-tests).

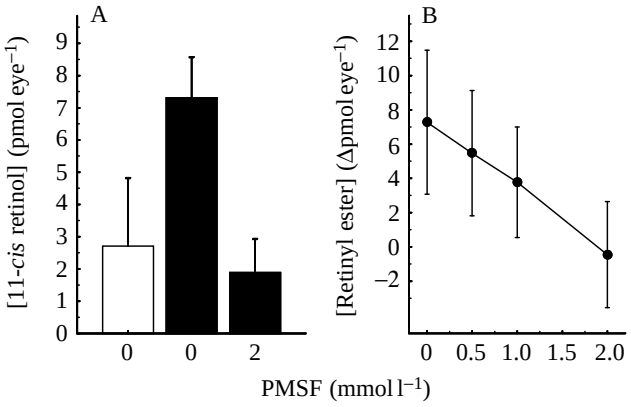

Fig. 9. (A) Inhibition of the formation of 11-*cis* retinol during a 2 h incubation in the presence of 2 mmol l^{-1} PMSF (*P*=0.05, values are means + S.E.M., *N*=4). The open bar gives values before the 2 h incubation and the filled bars give values after the incubation. (B) Effect of PMSF concentration on the increase in 11-*cis* retinyl ester levels during a 2 h incubation of homogenates containing all-*trans* retinol. Values are means ± S.E.M., *N*=11–13 experiments.

ester level fell below the 14 pmol eye^{-1} originally present, signifying a net hydrolysis of ester.

Net accumulations of counts in *cis* and *trans* retinyl esters are shown in Fig. 8B. RBA (5 μmol l^{-1}) caused highly significant decreases in both cases. As outlined above, the all-*trans* retinyl esters are the presumptive precursors for 11-*cis* retinol.

Effects on isomerization of other agents

Phenylmethylsulfonyl fluoride (PMSF) is an inhibitor of LRAT but not of CoA-dependent esterification of retinol (Ong *et al.* 1988; Randolph *et al.* 1991). In homogenates of crayfish eyes, PMSF inhibited the formation of 11-*cis* retinol from all-*trans* retinol substrate (Fig. 9A). The formation of 11-*cis* retinyl esters also appeared to be inhibited (Fig. 9B).

Progesterone is an inhibitor of acetyl CoA:retinol acyltransferase (Ross, 1982; Yost *et al.* 1988). In the present study, progesterone had no significant effect on the isomerization of exogenous retinol (data not shown).

Discussion

In the hypothesized mechanism of isomerization of retinoids, all-*trans* retinyl esters should form as intermediates in the isomerization process. The relatively small amounts of all-*trans* retinyl ester do not increase significantly in the presence of endogenous all-*trans* retinol, whereas the larger amounts of 11-*cis* retinyl esters do increase (Fig. 4). All-*trans* retinyl esters do form, however, as shown by their increasing levels of radioactivity (Fig. 5). The simplest interpretation is that, during the 2 h period of incubation, retinol is passing through small and relatively constant pools of all-*trans* retinyl ester, consistent with an isomerization mechanism for retinol that employs all-*trans* retinyl esters as an intermediate.

Inhibition of LRAT blocked the accumulation of tritium in all-*trans* retinyl esters (Fig. 8B) as well as the formation of both 11-*cis* retinol and 11-*cis* retinyl esters. This is consistent both with the proposed mechanism of isomerization coupled to the hydrolysis of all-*trans* retinyl esters and with the corollary conclusion that LRAT provides the major path for the formation of all-*trans* retinyl esters in crayfish eyes. LRAT may also form 11-*cis* esters, but the present data cannot confirm this directly because blocking the formation of 11-*cis* retinol should prevent the formation of 11-*cis* retinyl esters by any path. Less extensive experiments with progesterone, an inhibitor of CoA-dependent esterification, provided no evidence for activity of this alternative pathway.

Although crayfish eyes contain much more retinyl ester than free retinol, roughly equivalent amounts of 11-*cis* retinol and 11-*cis* retinyl ester were formed from exogenous all-*trans* retinol (compare Figs 2A and 4). This result is consistent with the hypothesized pathway, in which all-*trans* retinol must be isomerized to the 11-*cis* configuration before it is esterified for storage. There is a complication, however. In the presence of RBA (2–5 μmol l^{-1}), there was a net loss of $C_{22:6}$ retinyl ester (Fig. 8A). This indicates that in these homogenates the level of ester was determined both by its rate of formation from exogenous retinol and by a hydrolysis that is probably prevented *in vivo*.

When retinol is added to homogenates of the eye as all-*trans* retinyl docosahexaenoate, 11-*cis* retinyl esters of both $C_{22:6}$ and $C_{18:1}$ are formed (Srivastava *et al.* 1996). The isomerization presumably occurs in association with hydrolysis, and the 11-*cis* esters should form secondarily. In the present work, the substrate was all-*trans* retinol, which was neither esterified nor isomerized when the transesterification enzyme (LRAT) was inhibited.

Approximately 15 pmol per eye of 11-*cis* retinoid (alcohol plus esters) are made in 2 h under the conditions of these experiments. This quantity is approximately 15 % of the retinal present in the eye. Although the reaction may be more efficient *in vivo*, there is little reason to believe that much faster rates are necessary. First, there are endogenous stores of 11-*cis* retinyl esters amounting to approximately 30 pmol per eye in these experiments, but the levels increase when the animals are kept cold (Suzuki *et al.* 1988) or in darkness (R. Srivastava and T. H. Goldsmith, unpublished data). Second, in arthropod metarhodopsin, all-*trans* retinal is not exchanged for the 11-*cis* form to regenerate visual pigment. Either metarhodopsin is photoisomerized to rhodopsin or the entire rhodopsin molecule is replaced in a process that involves *de novo* synthesis of opsin (reviewed by Schwemer, 1986). Hafner and Bok (1977) measured the appearance of tritiated leucine in crayfish rhabdoms and showed that opsin replacement takes place over a time span of hours or days. Similarly, Cronin and Goldsmith (1984) found that, following intense adapting exposures, recovery of crayfish visual pigment required several days in darkness.

In the insects that have been studied (see references in the Introduction), retinal is isomerized by a mechanism that uses the energy of absorbed light. What adaptive processes might be responsible for two such different isomerization processes in arthropods? The soluble photoisomerase of insects occurs in strongly diurnal species (*Apis mellifera*, *Calliphora erythrocephala*), and in *Apis* retinoid reserves consist of retinal bound to the photoisomerase. Retinol is also present, but it remains bound to protein and does not become esterified in significant amounts. This is an isomerization system that works well where there is ample light, but it may serve poorly where the ambient light level is not dependable. Crayfish and lobster have superposition optics, which is an anatomical adaptation for low light conditions and where a photoisomerase may be an inefficient system for regenerating chromophore. Their alternative of coupling isomerization to hydrolysis of esters ultimately derives its energy from metabolism and, as it operates in darkness, may well be more suitable for nocturnal or crepuscular conditions or for life in murky waters. A useful feature of retinyl esters is that they are readily stored in lipid vesicles.

There is, however, another difference to consider in making this comparison: the arthropods known to have the retinal photoisomerase are insects, whereas the species depending on ester hydrolysis are crustaceans. There are, of course, crustaceans with apposition eyes and more diurnal habits, and there are nocturnal insects with superposition eyes. A wider comparative search should reveal whether, within the arthropods, these two systems for retinoid isomerization reflect phylogenetic inertia or physiological adaptations to ecological conditions.

Cronin and Goldsmith (1984) found that, in crayfish, visual pigment recovery time was shortened if the last adapting light flash was blue rather than orange. Whether this signifies the presence of a supplementary photoisomerase, however, is not clear. We have been unable to find evidence for a photoisomerase *in vitro*. In flies, metarhodopsin is removed from the membrane faster than rhodopsin and, as a consequence, opsin is removed faster in the presence of green light (which does not activate the photoisomerase) than in darkness (Schwemer, 1986). Although this observation cannot account for the effect of blue light on the rate at which crayfish rhabdoms are restored, it indicates that the relative amounts of rhodopsin and metarhodopsin in the photoreceptor membranes can affect the rate of recovery. This is clearly a matter that requires further exploration.

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References

BARRY, R. J., CAÑADA, F. J. AND RANDO, R. R. (1989). Solubilization and partial purification of retinyl ester synthetase and retinoid isomerase from bovine ocular pigment epithelium. *J. biol. Chem.* **264**, 9231–9238.

BERNSTEIN, P. S., LAW, W. C. AND RANDO, R. R. (1987). Biochemical characterization of the retinoid isomerase system of the eye. *J. biol. Chem.* **262**, 16848–16857.

CAÑADA, F. J., LAW, W. C., RANDO, R. R., YAMANOTO, T., DERGUINI, F. AND NAKANISHI, K. (1990). Substrate specificities and mechanism in the enzymatic processing of vitamin A into 11-*cis*-retinol. *Biochemistry* **29**, 9690–9697.

CRONIN, T. W. AND GOLDSMITH, T. H. (1984). Dark regeneration of rhodopsin in crayfish photoreceptors. *J. gen. Physiol.* **84**, 63–81.

DEIGNER, P. S., LAW, W. C., CAÑADA, F. J. AND RANDO, R. R. (1989). Membranes as the energy source in the endergonic transformation of vitamin A to 11-*cis*-retinol. *Science* **244**, 968–971.

GAWINOWICZ, M. A. AND GOODMAN, D. S. (1982). Retinoid affinity label for the binding site of retinol-binding protein. *Biochemistry* **21**, 1899–1905.

GOLDSMITH, T. H. AND CRONIN, T. W. (1993). The retinoids of seven species of mantis shrimp. *Vis. Neurosci.* **10**, 915–920.

HAFNER, G. S. AND BOK, D. (1977). The distribution of ^{3}H-leucine labeled protein in the retinula cells of crayfish retina. *J. comp. Neurol.* **174**, 397–416.

HARA, T. (1988). Visual photoreception of the squid: Photopigment regeneration. In *Molecular Physiology of Retinal Proteins*, Yamada Conference XXI (ed. T. Hara), pp. 305–310. Osaka, Japan: Yamada Science Foundation.

HARA, T. AND HARA, R. (1972). Isomerization of retinal catalyzed by retinochrome in the light. *Nature New Biol.* **242**, 39–43.

LAW, W. C. AND RANDO, R. R. (1988). Stereochemical inversion at C-15 accompanies the enzymatic isomerization of all-*trans* to 11-*cis*-retinoids. *Biochemistry* **27**, 4147–4152.

LIVREA, M. A., TESORIERE, L. AND BONGIORNO, A. (1990). All-*trans* to 11-*cis* retinol isomerization in nuclear membrane fraction from bovine retinal pigment epithelium. *Exp. Eye Res.* **52**, 451–459.

ONG, D. E., MACDONALD, P. N. AND GUBITOSI, A. M. (1988). Esterification of retinol in rat liver. *J. biol. Chem.* **263**, 5789–5796.

OZAKI, K., TERAKITA, A., HARA, R. AND HARA, T. (1986). Rhodopsin and retinochrome in the retina of a marine gastropod, *Conomulex luhuanus*. *Vision Res.* **26**, 691–705.

RANDOLPH, R. K., WINKLER, K. E. AND ROSS, A. C. (1991). Fatty acyl

Co-A-dependent and -independent retinol esterification by rat liver and lactating mammary gland microsomes. *Archs Biochem. Biophys.* **288**, 500–508.

ROSS, A. C. (1982). Retinol esterification by rat liver microsomes. Evidence for a fatty acid coenzymeA:retinol acyl transferase. *J. biol. Chem.* **257**, 2453–2459.

SAARI, J. C. AND BREDBERG, D. L. (1988). CoA- and non-CoA-dependent retinol esterification in retinal pigment epithelium. *J. biol. Chem.* **263**, 8084–8090.

SAARI, J. C. AND BREDBERG, D. L. (1989). Lecithin:retinol acyltransferase in retinal pigment epithelial microsomes. *J. biol. Chem.* **264**, 8636–8640.

SCHWEMER, J. (1986). Turnover of photoreceptor membrane and visual pigment in invertebrates. In *The Molecular Mechanism of Photoreception*, Dahlem Konferenzen (ed. S. Stieve), pp. 303–326. Berlin, Heidelberg, New York: Springer-Verlag.

SCHWEMER, J., PEPE, I. M., PAULSEN, R. AND CUGNOLI, C. (1984). Light-induced *trans–cis* isomerization of retinal by a protein from honeybee retina. *J. comp. Physiol.* A **154**, 549–554.

SMITH, W. C., FRIEDMAN, M. AND GOLDSMITH, T. H. (1992). Retinoids in the lateral eye of *Limulus*: Evidence for a retinal photoisomerase. *Vis. Neurosci.* **8**, 329–336.

SMITH, W. C. AND GOLDSMITH, T. H. (1991*a*). Cellular localization of retinal photoisomerase in the compound eye of the honeybee (*Apis mellifera*). *Vis. Neurosci.* **7**, 237–249.

SMITH, W. C. AND GOLDSMITH, T. H. (1991*b*). The role of retinal photoisomerase in the visual cycle of the honeybee. *J. gen. Physiol.* **97**, 143–165.

SRIVASTAVA, R., LAU, D. AND GOLDSMITH, T. H. (1996). Formation and storage of 11-*cis* retinol in the eyes of lobster (*Homarus*) and crayfish (*Procambarus*). *Vis. Neurosci.* **13**, 215–222.

SUZUKI, T., MAEDA, Y., TOH, Y. AND EGUCHI, E. (1988). Retinyl and 3-dehydroretinyl esters in the crayfish retina. *Vision Res.* **28**, 1061–1070.

TREHAN, A., CAÑADA, F. J. AND RANDO, R. R. (1990). Inhibitors of retinyl ester formation also prevent the biosynthesis of 11-*cis*-retinol. *Biochemistry* **29**, 309–312.

YOST, R. W., HARRISON, E. H. AND ROSS, A. C. (1988). Esterification by rat liver microsomes of retinol bound to cellular retinol-binding protein. *J. biol. Chem.* **263**, 18693–18701.