THE ROLE OF DROSOPHILA NINAG OXIDOREDUCTASE IN VISUAL PIGMENT CHROMOPHORE BIOGENESIS

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Running title: ninag oxidoreductase and chromophore biogenesis

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We previously reported that the Drosophila ninag gene encodes an oxidoreductase involved in the biosynthesis of the (3S)-3-hydroxyretinal serving as chromophore for Rh1 rhodopsin, and that ninag mutant flies expressing Rh4 as the major opsin accumulate large amounts of a different retinoid. Here, we show that this unknown retinoid is 11-cis-3-hydroxyretinol. Reversed-phase high performance liquid chromatography coupled with a photodiode array UV/VIS absorbance detector and mass spectrometer revealed a major product eluting at a retention time, tλ, of 3.5 min with a λmax of ~324 nm and with a base peak in the mass spectrum at m/z 285. These observations are identical with those of the 3-hydroxyretinol standard. The base peak in the electrospray ionization mass spectrum arises from the loss of a water molecule from the protonated molecule at m/z 303 due to fragmentation in the ion source. These results suggest that 11-cis-3-hydroxyretinol is an intermediate required for chromophore biogenesis in Drosophila. We further show that ninag mutants fed on retinal as the sole source of vitamin A are able to synthesize 3-hydroxyretinoids. Thus, the NinaG oxidoreductase is not responsible for initial hydroxylation of the retinal ring but rather acts in a subsequent step in chromophore production. These data are used to review chromophore biosynthesis and propose that NinaG acts in the conversion of (3R)-3-hydroxyretinol to the 3S enantiomer.

Photoisomerization of a retinoid chromophore covalently linked to the apoprotein opsin underlies the light sensing ability of rhodopsins. Animals lack the ability to synthesize retinoids de novo and, therefore, derive retinoids from carotenoids present in the diet. In insects, three kinds of vitamin A derivatives, retinal, (3R)-3-hydroxyretinal, and (3S)-3-hydroxyretinal, function as chromophores (1). The Dipteran suborder Cyclorrhapha that includes Drosophila is the only suborder described so far that utilizes (3S)-3-hydroxyretinal as the Rh1 rhodopsin chromophore (2). The use of (3S)-3-hydroxyretinal occurs in visual pigments exhibiting both UV and visible light sensitivity. This led to the suggestion that UV light sensitivity is due to the unique ability of (3S)-3-hydroxyretinal to accept energy transferred from a UV-sensitizing pigment (1).

The Drosophila ninag mutant is characterized by an abnormal electroretinogram response lacking the prolonged depolarization afterpotential (PDA)1. The lack of PDA, a characteristic of a group of “ninag” genes, is a manifestation of low levels of the major opsin, Rh1, expressed in the R1-6 photoreceptor cells (3). Low Rh1 rhodopsin in ninag mutants is not due to defects in Rh1 opsin transcription, rather the ability to synthesize the Rh1 chromophore is defective. However, the ninag defect does not affect the expression of minor opsins Rh4 and Rh6 (4). Rh4 and Rh6 are expressed in the R7 and R8 photoreceptors, respectively (5,6).

The encoded NinaG protein is a member of glucose-methanol-choline (GMC) oxidoreductase family of enzymes. Related family members catalyze redox reactions on a broad spectrum of small organic substrates. ninag mutants have defects in the biogenesis of (3S)-3-hydroxyretinal and abnormally accumulate large amounts of an unknown

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retinoid (4). In this report, we identify this previously unknown retinoid as 11-cis-3-hydroxyretinol and further characterize other aspects of the retinoid metabolism in the ninaG mutant. These results allow us to propose a model for biosynthesis of the (3S)-3-hydroxyretinal chromophore and to suggest that the NinaG oxidoreductase acts in a late step in this process, likely the conversion of (3R)-3-hydroxyretinol to (3S)-3-hydroxyretinol.

MATERIALS AND METHODS

Drosophila strains and media – The strains pRh1:Rh4; ninaG<sup>p330</sup> and pRh1:Rh4; ninaE<sup>I17</sup>, are used to allow expression of Rh4 as the major visual pigment in both ninaG and ninaG<sup>−</sup> flies (4). In the first strain, Rh1 expression is suppressed by the presence of the ninaG mutation. In the second (ninaG<sup>−</sup>) strain, Rh1 expression is eliminated by ninaE<sup>I17</sup>, a mutation in the Rh1 coding gene. Unless stated otherwise, flies were reared on a 12-hr light/12-hr dark light cycle at room temperature on standard cornmeal-molasses (referred to as vitamin A<sup>−</sup>) medium. The vitamin A-free medium was prepared as described previously (7). Flies were reared on vitamin A-free medium for one generation before retinal supplementation. Retinal supplementation was carried out by applying 100 ml of 2 mM solution of all-trans-retinal (Sigma) in ethanol onto the surface of a bottle containing 35 ml of vitamin A-free medium. Adult flies were reared on the supplemented food for 3 days prior to the retinoid extraction procedures.

Protein blot analysis – Rh4 levels were detected using anti-Rh4 antibodies (gift from Armin Huber) as previously described (8).

HPLC-UV/VIS-MS reagents and analysis – All-trans-retinal and all-trans-retinol were purchased from Sigma, and all-trans-3-hydroxyretinal was purchased from Toronto Research Chemicals. Photoisomerization of all-trans-3-hydroxyretinal to 11-cis-3-hydroxyretinol was carried out by exposing a 2 mM solution in ethanol to room light (750 lux) for 12-16 hours at room temperature (24° C). Chemical reduction of 3-hydroxyretinol to 3-hydroxyretinol was performed by adding 1 μg of anhydrous sodium borohydride to either 50 μl of a 2 mM solution of 3-hydroxyretinal in ethanol or a fly retinoid extract in 90:10 acetonitrile:water (v/v). These reactions were incubated at room temperature until effervescence had completely subsided. Retinoid extraction and HPLC-UV/VIS-MS analysis was carried out as previously described (4). For analysis of dark-reared flies, all protocols were carried out under dim red light.

RESULTS

Rh4 maturation requires chromophore in ninaG mutant flies – The NinaG oxidoreductase is involved in the synthesis of the (3S)-3-hydroxyretinal chromophore required for the expression of Rh1 rhodopsin, but not for the expression of Rh4, a minor opsin expressed in a subset of R7 cells (4). We reasoned that the Rh4 opsin must either accommodate an alternative chromophore or does not require chromophore binding for maturation. To determine if a chromophore precursor is required for Rh4 maturation, we assayed Rh4 opsin levels in pRh1:Rh4; ninaE<sup>I17</sup> flies reared on vitamin A-free media. These flies carried the pRh1:Rh4 transgene (5) allowing expression of Rh4 as major opsin in the R1-6 photoreceptor cells. The ninaE<sup>I17</sup> mutant background eliminates expression of the Rh1 opsin in the same cells. Fig. 1 shows that, while high levels of Rh4 opsin are found in flies reared on vitamin A<sup>+</sup> media, Rh4 is absent in the flies reared on the vitamin A-free media. These results demonstrate that Rh4 is dependent on the presence of a chromophore for high levels of expression and must accommodate a chromophore in the ninaG mutant that cannot be utilized by Rh1. For this reason, we sought to determine the composition of the retinoids found in ninaG flies expressing high levels of Rh4 (pRh1:Rh4; ninaG).

3-hydroxyretinol accumulates in ninaG mutant flies – In agreement with previous HPLC studies on the ninaG mutant (4), Fig. 2A shows that pRh1:Rh4; ninaG flies (solid trace) accumulate a major retinoid component eluting at 3.5 min when compared to pRh1:Rh4; ninaE<sup>I17</sup> flies (outlined trace). Other prominent chromatographic peaks eluted at 3.8 and 4.1 min in both sets of flies. In order to identify these components, we compared retention times,
UV/VIS absorbance spectra, and mass spectra of known retinoid standards to the corresponding data from these suspected retinoids. The results obtained from retinoid standards are summarized in Table 1. HPLC-UV/VIS-MS analysis of all-trans-3-hydroxyretinal standard yielded a major chromatographic peak with $t_r = 3.8$ min (Fig. 2D, solid trace), $\lambda_{max}$ of ~374 nm (Fig. 2E), and a base peak in the ESI mass spectrum at m/z 301 (data not shown), which corresponds to an ion representing the protonated intact molecule.

Light treatment is known to cause photoisomerization of of all-trans-retinal to cis isomers (9,10). Photoisomerization of all-trans-3-hydroxyretinal followed by HPLC-UV/VIS-MS analysis (Fig. 2D, outlined trace), revealed an increase in the relative abundance of the component with $t_r = 4.1$ min, which also had a $\lambda_{max}$ of ~374 nm (Fig. 2F) and a base peak in the ESI mass spectrum at m/z 301 (data not shown). This component was also detected as a major peak in wild type flies known to contain 11-cis-3-hydroxyretinal as the major retinoid, and as a minor peak in the all-trans-3-hydroxyretinal standard. These observations support the assignment of the component eluting at 3.8 min as all-trans-3-hydroxyretinal and the component eluting at 4.1 min contains its geometric isomer 11-cis-3-hydroxyretinal. Likely the 4.1 min peak obtained from the photoisomerized all-trans-3-hydroxyretinal contains other cis isomers, but other isomers are not a major component of biological samples (1,11).

HPLC-UV/VIS-MS results for the chromatographic peak with $t_r = 3.5$ min revealed a $\lambda_{max}$ of ~324 nm (Fig. 2B) compared to the $\lambda_{max}$ of ~374 nm for the 3-hydroxyretinal isomers (Fig. 2C: pRh1:Rh4; ninaG extract; Fig. 2E: all-trans-3-hydroxyretinal standard; Fig. 2F: 11-cis-3-hydroxyretinal standard) and a base peak in the ESI mass spectrum at m/z 285 (Fig. 3A). It has been previously reported that the $\lambda_{max}$ of retinoids with a terminal alcohol is ~324 nm while the $\lambda_{max}$ of retinoids with a terminal aldehyde is ~374 nm (12). Indeed, this is consistent with our observations of $\lambda_{max}$ of ~324 nm for a retinol standard and for $\lambda_{max}$ of ~374 nm for both 3-hydroxyretinal isomers and all-trans-retinal (3-hydroxyretinal isomers shown in Fig. 1E and 1F and data not shown for all-trans-retinal and all-trans-retinol). Furthermore, it was observed that under identical reversed-phase HPLC conditions, the retinol form of a retinoid had a shorter retention time than the corresponding retinal, as expected due to polarity differences, for example, in our HPLC experiments, $t_r = 11.4$ min for all-trans-retinol and $t_r = 13.2$ min for all-trans-retinal (data not shown). Thus, the component with $\lambda_{max}$ of ~324 nm and $t_r = 3.5$ min, which elutes prior to $t_r = 3.8$ min of all-trans-3-hydroxyretinal, could be 3-hydroxyretinol. However, the ESI mass spectrum showed a base peak of m/z 285 with only a low relative abundance (<10%) peak observed at the expected protonated molecule at m/z 303 (Fig. 3A). The observed base peak corresponds to the loss of water from the protonated molecule. Water loss during LC/MS of retinol has been reported previously (13). We also observed that the retinoid standard generated a base peak at m/z 269, which corresponds to a loss of water from the expected protonated molecule at m/z 287 (Fig. 3B).

In order to determine conclusively if the unknown retinoid found at high levels in Rh4; ninaG is 3-hydroxyretinol, a 3-hydroxyretinol standard was synthesized by chemically reducing 3-hydroxyretinal with sodium borohydride (2). HPLC-UV/VIS-MS experiments performed on the 3-hydroxyretinol products revealed a major component eluting at 3.2 min and a minor component eluting at 3.5 min (Fig. 2G). Both components have a $\lambda_{max}$ of ~324 nm (Fig. 2H, f) and produce an ESI mass spectrum with a base peak at m/z 285 and a low (<10%) relative abundance peak at m/z 303 (Fig. 3C). Given that the starting material was predominately all-trans-3-hydroxyretinal having a small amount of 11-cis-3-hydroxyretinal, the reduced product was expected to consist mostly of all-trans-3-hydroxyretinol with a small amount of 11-cis-3-hydroxyretinol. Therefore, the agreement between the HPLC-UV/VIS-MS results from the 3-hydroxyretinol standard and those from the retinoid extracts of the pRh1:Rh4; ninaG flies show that 11-cis-3-hydroxyretinol is the product accumulating in these flies.

ninaG flies synthesize hydroxyretinoids – The presence of large amounts of 11-cis-3-hydroxyretinol in ninaG mutants suggested that the NinaG oxidoreductase does not act in the
hydroxylation of the retinoid ring. However, given the possibility that these hydroxyretinoids can be generated directly from centric cleavage of xanthophylls obtained from the diet, we sought evidence of conversion of retinoids to hydroxylated retinoids in the ninaG mutant. For this reason, we analyzed the retinoid extracts from ph1:Rh4; ninaG flies reared with all-trans-retinal as the sole source of vitamin A. The flies were reared under dark condition to minimize isomerization of all-trans-retinal to 11-cis-retinal. Fig. 4A shows that these flies produce both all-trans-3-hydroxyretinal, eluting at 3.8 min with λmax ~374 (Fig. 4B), and all-trans-3-hydroxyretinol, eluting at 3.2 min with λmax ~324 (Fig. 4C). Thus, the ninaG flies are capable of hydroxylation of the retinoid ring, and excludes the possibility that the NinaG oxidoreductase is required for this reaction.

DISCUSSION

We have compared results of HPLC-UV/VIS-MS experiments of retinoid extracts and retinoid standards to identify 3-hydroxyretinol as the retinoid accumulating in Rh4; ninaG flies. These results establish that the major retinoid found in the ninaG retina is not an aldehyde and therefore cannot form the Schiff-base linkage needed to serve as the chromophore for Rh4 or other visual pigments. For this reason, it is likely that 3-hydroxyretinol is an intermediate compound accumulating as a result of the defect in the (3S)-3-hydroxyretinal synthesis pathway caused by the ninaG mutation. Vertebrates are known to use a retinol species as an intermediate in the retinoid biogenesis. In the vertebrate visual system, the all-trans-retinal arising from the photobleaching of rhodopsin must first be converted to all-trans-retinol before 11-cis-retinal is regenerated (14,15).

We propose the pathway outlined in Fig. 5 to account for the current information regarding (3S)-3-hydroxyretinal biosynthesis in Drosophila. Xanthophylls, such as lutein and zeaxanthin that are hydroxylated at C3 position of the retinoid ring, and β-carotene, are the major dietary source of chromophore precursors (11,16). All naturally occurring xanthophylls are in the 3R, 3′R configuration thereby ruling out the possibility of obtaining retinoid species in (3S) configuration by direct cleavage of such xanthophylls by an oxygenase. Further, the ninaB-encoded oxygenase only catalyzes cleavage of β-carotene and not these xanthophylls (17). The NinaB enzyme is essential for formation of chromophore from both xanthophylls and β-carotene. These considerations suggest that xanthophylls are first converted to β-carotene before being acted on by the NinaB oxygenase.

The production of retinal by the NinaB oxygenase occurs outside the retina (18). It is not known if retinal itself is transported to the retina or is converted to retinol before transport. Both possibilities are shown in Fig. 5. These retinoids appear to be processed in retinal pigment cells because PINTA, a retinoid binding protein essential for chromophore production, acts in these cells (19). In vitro binding assays shows that PINTA is capable of interacting with both retinol and retinal, with a stronger binding affinity for the retinol.

The hydroxylation of retinal to form 3-hydroxyretinal has been studied by Seki et al. (11). These studies implicated a role of a cytochrome P-450 monooxygenase in the hydroxylation of the retinal ring. Notably, this reaction exclusively produced the 3R enantiomer, as shown in Fig. 5. A second important observation made by Seki et al. (11) was that the hydroxylation reaction produced a retinoid with λmax ~330, thereby suggesting the formation of (3R)-3-hydroxyretinol. It is not clear if (3R)-3-hydroxyretinol is formed directly by the P-450 monooxygenase or is the product of a subsequent reaction involving a retinal dehydrogenase. There are three short chain dehydrogenases with enriched transcripts within retinal tissues with the potential to catalyze this type of reaction (20).

Most insect visual pigments use the (3R)-3-hydroxyretinal enantiomer as the visual pigment chromophore. Only members of the higher Diptera, the Cyclorapha, have been shown so far to synthesize and use the (3S)-3-hydroxyretinal enantiomer as chromophore (1). In this report, we have shown that 3-hydroxyretinoids are formed in the ninaG mutant, and infer that these are exclusively the (3R) enantiomer because they are not capable of
serving as chromophore for Rh1 rhodopsin. These results allow us to assign a role of the NinaG oxidoreductase in the biochemical pathway responsible for conversion of (3R)-enantiomer to the (3S)-enantiomer. The (3R) isomer is used by Rh4 rhodopsin, at least in the ninaG mutant. It is not known if Rh4, and other minor Drosophila visual pigments, use the (3R) enantiomer in wild type (ninaG+) flies.

Given the proposal that ninaG mutant is defective in the production of (3S)-3-hydroxyretinal from the (3R) enantiomer, the NinaG oxidoreductase could be responsible for two different reactions. First, NinaG could act in the conversion of the (3R)-3-hydroxyretinoid (alcohol or aldehyde form) to the corresponding (3S)-3-hydroxyretinoid. Streptomyces cholesterol oxidase, a GMC oxidoreductase, is responsible for the oxidation of a hydroxyl group on a six member carbon ring of cholesterol and the subsequent rearrangement of a double bond within the cholesterol rings (21). The second possibility is that the NinaG enzyme acts in the oxidation of (3S)-3-hydroxyretinol to (3S)-3-hydroxyretinal. This assumes that only the alcohol form, (3R)-3-hydroxyretinol, is converted to (3S)-3-hydroxyretinol by an uncharacterized enzyme. Then NinaG would be responsible for the subsequent step, that is, the oxidation of (3S)-3-hydroxyretinol to (3S)-3-hydroxyretinal. However, short chain dehydrogenases typically catalyze retinol – retinal inter-conversions and therefore involvement of NinaG in this reaction seems unlikely.

The model presented in Fig. 5 also explains the production of some 3-hydroxyretinal, postulated to be exclusively the 3R isomer, in ninaG flies. This isomer cannot be used by Rh1 rhodopsin, and hence the low Rh1 content is observed in ninaG mutants. On the other hand, Rh4 and another visual pigment, Rh6, are produced in the ninaG mutant because they are able to use the 3R isomer.

We have proposed here that the accumulation of 3-hydroxyretinol in ninaG mutant flies is not because it serves as an alternative chromophore, but rather because it is an intermediate in the chromophore biosynthetic pathway or a byproduct of the NinaG defect. An additional observation is that 3-hydroxyretinol is present in greater amounts in ninaG mutants than in wild type. We propose that the accumulation of 3-hydroxyretinol occurs because it is a precursor in chromophore biosynthesis. The model for chromophore biogenesis in Fig. 5 shows NinaG acting in the conversion of (3R)-3-hydroxyretinol to (3S)-3-hydroxyretinol. Likely this reaction requires an oxidized intermediate of the carbon ring that is then reduced to generate (3S)-3-hydroxyretinoid. NinaG, a GMC oxidoreductase, would act in one or both of these reactions. Therefore ninaG mutants lacking this enzyme, accumulate (3R)-3-hydroxyretinol. An alternative possibility is that (3R)-3-hydroxyretinol is the retinoid converted to the (3S) isomer and in the absence of this reaction a side reaction converts (3R)-3-hydroxyretinal to (3R)-3-hydroxyretinol and results in the accumulation of (3R)-3-hydroxyretinol.

A higher level of 3-hydroxyretinol was observed in ninaG flies expressing Rh4 than in ninaG flies expressing Rh1 or no visual pigment (4). The reason for this is not known. One possibility is that photoreceptor cells maintain membrane structures in greater quantity when visual pigments are synthesized and transported to the rhabdomeric membranes, thereby perhaps greater capacity for biogenesis or storage of this retinoid precursor (22,23). The discovery of the ninaG oxidoreductase has provided a useful probe into the biosynthesis of visual pigment chromophores. The Drosophila visual system offers a much promise for further insights, including investigation of the role of (3R)- and (3S)-3-hydroxyretinal enantiomers as chromophores in the function of specific visual pigments.

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FOOTNOTES
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1 Abbreviations: PDA, prolonged depolarizing afterpotential; nina, neither inactivation nor afterpotential; (GMC) oxidoreductase, glucose-methanol-choline oxidoreductase; HPLC, high performance liquid chromatography; UV/VIS, ultraviolet-visible absorption spectroscopy; MS, mass spectrometry; ESI, electrospray ionization; (m/z), mass-to-charge ratio; t, retention time; λ_max, absorbance maxima; PINTA, PDA is not apparent.
FIGURE LEGENDS

Figure 1: Immunoblot analysis of Rh4 rhodopsin in the presence and absence of dietary vitamin A. Protein blot shows Rh4 rhodopsin is depleted in head homogenate of pRh1:Rh4 flies raised on vitamin A-free (vitamin A⁺) medium. Head homogenate of pRh1:Rh4 flies raised on standard cornmeal-molasses medium (vitamin A⁺) is the control.

Figure 2: HPLC-UV/VIS profile of retinoids extracted from pRh1:Rh4; ninaG and HPLC-UV/VIS profiles of 3-hydroxyretinol and 3-hydroxyretinol standards. (A) Elution profiles of pRh1:Rh4; ninaG (solid trace) and pRh1:Rh4; ninaE/I17 (outlined trace) at 324 nm. Note the increase in the retinoid eluting at 3.5 min in pRh1:Rh4; ninaG flies. (B, C) UV/VIS absorbance profiles of the 3.5 and 4.1 min peaks from Rh4; ninaG. The 3.5 min peak has λmax ~324 nm (B) and the 4.1 min peak has a λmax ~374 nm (C). D. Elution profiles of all-trans-3-hydroxyretinol standard before (solid trace) and after (outlined trace) light exposure at 374 nm. Note the increase in 11-cis-3-hydroxyretinal fraction eluting at 4.1 min after light exposure. UV/VIS absorbance profiles of all-trans-3-hydroxyretinol (E) and 11-cis-3-hydroxyretinol (F) shows λmax ~374 nm for both isomers. (G) Elution profile of 3-hydroxyretinol standard obtained after reduction of 3-hydroxyretinol standard by sodium borohydride. Two peaks, eluting at 3.2 and 3.5 min, represent all-trans-3-hydroxyretinol and 11-cis-3-hydroxyretinol, respectively. (H, I) UV/VIS absorbance profiles of all-trans-3-hydroxyretinol (H) and 11-cis-3-hydroxyretinol (I) shows λmax ~324 nm for both isomers. These data allow assignment of 3.5, 3.8, and 4.1 min peaks in pRh1:Rh4; ninaG as 11-cis-3-hydroxyretinol, all-trans-3-hydroxyretinol and 11-cis-3-hydroxyretinal, respectively.

Figure 3: Positive ion mode ESI mass spectra of 3.5 min chromatographic peak, retinol standard, and 3-hydroxyretinol standard. (A) ESI mass spectrum of the 3.5 min chromatographic peak from pRh1:Rh4; ninaG extracts. (B, C) ESI mass spectra of retinol and 3-hydroxyretinol standards. The ESI mass spectra show base peaks that are due to the loss of a water molecule from the intact protonated molecule. All ESI mass spectra shown were background subtracted.

Figure 4: HPLC-UV/VIS profile of retinoids extracted from pRh1:Rh4; ninaG flies reared on retinal. (A) Retinoid composition of pRh1:Rh4; ninaG flies reared in dark on vitamin A-free medium supplemented only with retinal. Elution profile is shown at 324 nm. (B, C) UV/VIS absorbance profiles of the prominent peaks eluting at 3.8 and 3.2 min show λmax of ~374 and ~324 nm, respectively. These data establish the ability of ninaG flies to synthesize 3-hydroxyretinol and 3-hydroxyretinal.

Figure 5: Biogenesis pathway of (3S)-3-hydroxyretinal. β-carotene, obtained directly from diet or indirectly from dietary xanthophylls, is converted to retinal by the ninaB-encoded β-carotene oxygenase outside of the retina (17,18). Retinal could interconvert with retinol at this point, and 3-hydroxyretinol could interconvert with 3-hydroxyretinol at later steps, by action of a short chain dehydrogenase. Retinal or retinol is transported to the retinal pigment cells, where the recently described PINTA retinoid binding protein (19) acts in chromophore maturation. A cytochrome P450 monoxygenase type enzyme (11) generates (3R)-3-hydroxyretinal from retinal, or alternatively (3R)-3-hydroxyretinol from retinol. If (3R)-3-hydroxyretinal is formed, it is reduced to (3R)-3-hydroxyretinol by action of a short chain dehydrogenase. (3R)-3-hydroxyretinol accumulates in ninaG mutants, due to requirement of NinaG oxidoreductase in isomerization of (3R)-3-hydroxyretinol to (3S)-3-hydroxyretinol. Also in ninaG mutants, Rh4 accepts (3R)-3-hydroxyretinol as the chromophore. An alternative role (marked with *) for NinaG, oxidation of (3S)-3-hydroxyretinol to (3S)-3-hydroxyretinal, is discussed in the text. The model shows that only (3S)-3-hydroxyretinal is able to serve as Rh1 chromophore, whereas (3R)-3-hydroxyretinal is able to serve as Rh4 chromophore in the ninaG mutant. The possibility that Rh4 uses (3R)-3-hydroxyretinal, when available in ninaG⁻ flies, is also indicated.
| Retinoid               | $t_r$ (min) | $\lambda_{\text{max}}$ (nm) | Observed ESI-MS base peak (m/z) |
|------------------------|-------------|-------------------------------|----------------------------------|
| Retinal                | 13.2        | ~374                          | 285                              |
| 11-cis-3-hydroxyretinal| 4.1         | ~374                          | 301                              |
| All-trans-3-hydroxyretinal| 3.8       | ~374                          | 301                              |
| Retinol                | 11.4        | ~324                          | 269                              |
| 11-cis-3-hydroxyretinol| 3.2         | ~324                          | 285                              |
| All-trans-3-hydroxyretinol| 3.2          | ~324                          | 285                              |

Table 1: Retention times ($t_r$), $\lambda_{\text{max}}$, and observed ESI-MS base peak m/z values for the retinoid standards obtained by HLPC-UV/VIS-MS.
Figure 1.
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ninaG oxidoreductase
Figure 2.
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Figure 5.
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ninaG
The role of Drosophila ninaG oxidoreductase in visual pigment chromophore biogenesis
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