The Catalytic Isomerization of All-trans-retinal to 9-cis-Retinal and 13-cis-Retinal*

Sidney Futterman and Martha H. Rollins
From the Department of Ophthalmology, University of Washington School of Medicine, Seattle, Washington 98195

Summary
Nucleophiles, including dihydroflavins, dithiols, dihydrofolate, tetrahydrofolate, and dehydroascorbate, were found to promote the geometrical isomerization of all-trans-retinal to 9-cis-retinal and 13-cis-retinal. No trace of the 11-cis or 9,13-di-cis isomers could be detected among the products. If isomerization of all-trans-retinal occurred in the presence of bleached particulate rhodopsin, the photosensitive pigment isorhodopsin was produced. No tritium was incorporated into all-trans-, 9-cis-, or 13-cis-retinal when the isomerization reaction was carried out with stannous chloride and FMN in tritiated water.

Dihydroflavins, the most active agents for the production of 9-cis-retinal, functioned catalytically and were effective at concentrations as low as 1 μM. They did not catalyze the isomerization of all-trans-retinol. In the absence of catalyst, all-trans-retinal and all-trans-retinol were both resistant to thermal isomerization in the physiological range of pH and temperature.

The capacity of photoreceptor membrane preparations to promote the isomerization of all-trans-retinal to 9-cis-retinal, when they were incubated anaerobically in the presence of stannous chloride, suggests that they contain reducible endogenous flavin. The recombination of chromophore molecule with apoprotein in bleached photoreceptor membrane preparations was largely completed within about 1 min and proceeded about 20 times faster than the rate of isomerization of all-trans-retinal to 9-cis-retinal in the presence of 0.1 mM dihydro-FMN and about 80 times faster than the isomerization facilitated by the endogenous catalyst.

A convenient method has been devised for the preparation of dihydroriboflavin.

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Experimental Procedure
Materials—All-trans-, 9-cis-, and 13-cis-retinal were purchased from Eastman Kodak; riboflavin, FMN, FAD, dithiothreitol, folic acid, dihydrofolate acid, tetrahydrofolate acid, reduced DL-α-lipoic acid, 2,3-dimercaptopropanol, butylated hydroxytoluene, ascorbic acid, hydroquinone, reduced glutathione, cysteine, 2-mercaptoethanol, DPNH and TPNH from Sigma; dehydroascorbic acid from K & K Laboratories, Inc.; bovine serum albumin from Pentex; lithium borohydride from Ventrion Corp.; Nanograde petroleum ether of boiling range 30–60° from Mallinkrodt; nitrogen stated to contain approximately 2 ppm of residual oxygen from General Welding Supply Co., Inc.; frozen geometrical isomerization of all-trans-retinal to 11-cis-retinal has been thought for some time to be an important step in visual pigment regeneration in the photoreceptors of the retina during dark adaptation (3). Recently it has been suggested that an ammonium sulfate fraction obtained from frozen bovine retinal tissue, originally regarded as having "retinene (retinal) isomerase" activity for all-trans-retinal (3), may in fact be completely inert in the absence of photosomizerizing light (4). However, other studies have indicated that bleached bovine photoreceptors may utilize all-trans-retinal for the regeneration of photosensitive pigment (5–8). Under conditions of prolonged incubation during which bacterial contamination occurred, isorhodopsin formation was observed, indicating that under these conditions all-trans-retinal was converted to 9-cis-retinal (9).

The photoisomerization of isomers of retinal has been extensively studied (10, 11), but virtually nothing is known about the geometrical isomerization of all-trans-retinal in the absence of light. It is well known, however, that cis-trans isomerization is effectively catalyzed by a variety of acids, certain anions and amines (12). The iodine-catalyzed isomerization of 11-cis-retinal to all-trans-retinal has also been described (13). In addition, a limited number of enzymatic cis-trans isomerization reactions have been examined (12, 14) and in some (12, 15) the involvement of sulfhydryl groups has been implicated. The possibility of identifying a biological cis-trans isomerization catalyst that might be involved in the process of visual pigment regeneration arose from observations that the utilization of all-trans-retinal for regeneration of bleached visual pigment in crude preparations of photoreceptor outer segments could occur under anaerobic conditions (6) and that both thiols and flavins were stimulatory (16). This report describes the catalytic isomerization of all-trans-retinal.

The bleaching of the visual pigment, rhodopsin, by light releases all-trans-retinal from its apoprotein (1). It also has been clearly established (2) that the apoprotein can react spontaneously with 11-cis-retinal to regenerate rhodopsin. Therefore, a
bovine retinas from Hormel Co.; tritiated water from New England Nuclear Corp.; Silica Gel G from Brinkmann Instruments, Inc.; DL-α-tocopherol, and 11-cis-retinal were generously provided by Hoffmann-La Roche. The purity of stored preparations of all-trans-, 9-cis-, 11-cis-, and 13-cis-retinal was, respectively, about 100, 91, 79, and 97% as determined by spectrophotometric assay of components eluted after thin layer chromatography employing methods described in the text. When necessary, the minor contaminants were removed by preparative thin layer chromatography using the methods given in the text.

Preparation of 1,6-Dihydroriboflavin—The procedure was based upon observations that a variety of reducing agents including sodium dithionite readily convert riboflavin to 1,6-dihydroriboflavin (17, 18). Riboflavin (80 mg) was suspended in 5 ml of 0.1 M phosphate buffer, pH 7.4, sodium dithionite (120 mg) was added, the mixture was stirred for 3 min and chilled and, after brief centrifugation, the sediment was washed four times with 5 ml of cold dilute HCl, pH 4.0. The product was diluted to 5 ml using cold dilute HCl, pH 3.5, and nitrogen was bubbled through the suspension for 1 min. The preparation was assayed spectrophotometrically (19) after aerobic reoxidation to riboflavin following dilution of a portion in 0.1 M phosphate buffer, pH 7.0, and also manometrically in a Warburg apparatus. The preparation consumed approximately 0.5 mole of O₂ per mole of flavin and the yield of dihydroriboflavin was approximately 95%. The preparation could be stored under nitrogen in a refrigerator for several weeks. Solutions or reaction mixtures to which portions of the suspension of dihydroriboflavin were to be added were previously made essentially anaerobic under a stream of nitrogen and then maintained under nitrogen.

Photoreceptor Membrane Preparation—Approximately 100 frozen bovine retinas were thawed, ground in a 500-ml glass mortar and pestle in a cold room under red light (20), dispersed in 100 ml of 1.02 M sucrose solution in 67 mM phosphate buffer, pH 7.0, sedimented at 3,100 rpm and approximately 1,200 × g for 15 min to remove nuclei, diluted with 100 ml of 67 mM phosphate buffer, pH 7.0, and centrifuged at 40,000 rpm and approximately 105,000 × g for 25 min and the supernatant fluid was discarded. The sediment was subjected three times to a flotation procedure by suspension in 40 ml of the sucrose solution, after centrifugation, the upper phase was concentrated to approximately 20 pl under a stream of nitrogen and subjected to thin layer chromatography on Silica Gel G at 3° under red light.

Preparation of Retinal—The geometrical isomers of retinal were converted to the corresponding retinol isomers by borohydride reduction. A 2.0-mg sample of retinal was dissolved in 3 ml of ethanol and 5 ml of water, recovered from the upper phase by evaporating the solvent under a stream of nitrogen, dissolved in 0.2 ml of 5% Triton X-100 in ethanol, and diluted to 2 ml with water for prompt addition to reaction mixtures.

Use of Rhodopsin Apoprotein to Trap 9-cis- or 11-cis-Retinal—Rhodopsin contained in suspensions of the photoreceptor membrane preparation was bleached by exposure to the light from three 100-watt incandescent bulbs at a distance of 15 inches. Various supplements were then added under red light. A solution of all-trans-retinal, from which small aliquots could be added to reaction mixtures, was prepared by dissolving 5 mg in 0.5 ml of 5% Triton X-100 in ethanol and diluting to 5 ml with water. With the other geometrical isomers, 2-mg quantities and proportionately reduced volumes of solvents were used. Reaction mixtures (1 ml) were extracted by the addition of 2 ml of 2% Triton X-100 in water, clarified by centrifugation at 30,000 × g for 10 min and analyzed spectrophotometrically (21) by measuring absorbance at 500 nm before and after bleaching for 10 min as above.

Results

Geometrical Isomerization Catalyst—All-trans-retinal was completely resistant to thermal isomerization (Table I) when it was incubated at pH 7.4 for 2 hours in aqueous solution. It was

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Geometrical isomers</strong></td>
</tr>
<tr>
<td>All-trans-retinal</td>
</tr>
<tr>
<td>9-cis-Retinal</td>
</tr>
<tr>
<td>11-cis-Retinal</td>
</tr>
<tr>
<td>13-cis-Retinal</td>
</tr>
</tbody>
</table>

Supplements present as catalyst in reaction mixtures containing all-trans-retinal

| None | 0.29 | 0.30 |
| FMN + SnCl₂ | 0.30 | 0.38 |
| FMN + DPNH | 0.30 | 0.41 |
| FAD + SnCl₂ | 0.29 | 0.39 |
| Riboflavin + SnCl₂ | 0.29 | 0.40 |
| Dihydroriboflavin | 0.29 | 0.39 |
| Dihydrorotretinal | 0.28 | 0.39 |
| Dihydrofumarate | 0.27 | 0.36 |
| Dehydroascorbate | 0.28 | 0.41 |

*Other substances ineffective as catalysts were 0.4 μmole of either riboflavin, FMN, FAD or folate, or 2 μmole of DPNH, TPNH, ascorbate, hydroquinone, α-tocopherol, and butylated hydroxytoluene.

*Similar results were obtained with other diols including 10 μmole of either reduced α-lipoate or 2,3-dimercaptopropanol and with 0.4 μmole of tetrahydrurate. With monothiols, 10 μmole of either glutathione, 2-mercaptoethanol or cysteine, or 4 mg of bovine serum albumin, the component with RF value of approximately 0.40 was barely detectable.
sibly unreactive at pH 6.0 and at temperatures up to 55°. At pH 8.5, while no isomerization of all-trans-retinal could be observed at 37°, at 55° a trace of product with the mobility of 13-cis-retinal was detected.

Alone, neither the flavins, riboflavin, FMN, or FAD, nor the reducing agents capable of reducing free flavins (17, 22), DPNH, or SnCl₂, induced isomerization. However, when dihydroflavins were generated using either DPNH or SnCl₂ in the presence of either riboflavin, FMN, or FAD or when dihydrobacteriopheophytin was introduced directly into reaction mixtures, two products, in addition to unchanged all-trans-retinal, could be detected using thin layer chromatography (Table I). The products appeared to be 9-cis-retinal and 13-cis-retinal. Thiols, dehydroascorbate, dihydrofolic acid, and tetrahydrofolic acid also effectively promoted formation of the same products. However, in the case of dithiols somewhat less of the product with RF value of approximately 0.40 was formed and with monothiols barely detectable amounts of this product were present. Although the RF value for 11-cis-retinal was only a little greater than that of 9-cis-retinal in one solvent system and only a little less than that of 13-cis-retinal in the other solvent mixture, the separations were sufficient to indicate that little if any 11-cis-retinal was being produced.

When 13-cis-retinal replaced all-trans-retinal and was incubated in reaction mixtures containing either dihydrobacteriochlorophyll or stannous chloride and FMN as in Table I, it too was isomerized and products with the mobility of all-trans-retinal and 9-cis-retinal were detected. In the absence of catalyst, 13-cis-retinal, like all-trans-retinal, did not isomerize.

Reaction mixtures like those of Table I, but containing all-trans-retinal in place of all-trans-retinal were incubated at 37° for two hours, extracted and examined by thin layer chromatography on Silica Gel G (23) at room temperature using as solvent mixture petroleum ether-2-methyl-2-hepten-6-one (11:2). Under these conditions no evidence for the formation of any isomerization product was obtained in the presence of dihydroflavins, thiols, dehydroascorbate or dihydrofolic acid. However, a small amount of fluorescent reaction product with the mobility of 13-cis-retinal was detected after incubation in the presence of tetrahydrofolic acid.

A comparison of the time course of formation of products from all-trans-retinal at pH 6.8 and 7.4 in the presence of either dihydro-FMN or dithiothreitol (Fig. 1) supported impressions that 13-cis-retinal was the major product, that more 9-cis-retinal was obtained in the presence of a dihydroflavin than with a dithiol, and that only about 25% of the retinal was present as products as equilibrium was approached.

Identification of Products—To ensure that even if only a small quantity of 11-cis-retinal were present on thin layer plates between the major products, it would not escape detection, zones of Silica Gel G containing each of the reaction products obtained in the presence of either dithiothreitol or dihydrobacteriochlorophyll were scraped from thin layer chromatographic plates so that all of the intermediate region between the products was included and then were eluted using ethanol, and absorption spectra were obtained (Fig. 2). The spectrum of the product of lower mobility was indistinguishable from that of commercial 9-cis-retinal eluted from Silica Gel G following chromatography. Similarly, the spectrum of the reaction product of higher mobility was identical with that of authentic 13-cis-retinal. Had significant amounts of either 11-cis-retinal or 9,13-di-cis-retinal (24) been present in the reaction mixtures, the spectra of the reaction products would have deviated markedly from those of the standard solutions of 9-cis- or 13-cis-retinal at wave lengths shorter than 300 nm.

Further evidence that 11-cis-retinal was not among the reaction products was obtained by carrying out the isomerization reaction in the presence of bleached rhodopsin. It was assumed that any 11-cis-retinal formed, perhaps as an unstable intermediate in the
presence of the catalyst, might be trapped instantaneously by combination with the visual pigment apoprotein. When sufficiently large amounts of photosensitive pigment were regenerated to assure high absorbancies, it was possible to distinguish clearly rhodopsin with $\lambda_{\text{max}}$ at about 500 nm (21) formed from 13-cis-retinal and isorhodopsin with $\lambda_{\text{max}}$ at 487 nm (21) produced from 9-cis-retinal. Under these conditions no regeneration of pigment occurred if reaction mixtures contained only bleached visual pigment and excess all-trans-retinal (Fig. 5). When either dihydro-FMN or dithiothreitol was present in reaction mixtures in addition to all-trans-retinal, the bulk of the apoprotein was converted to photosensitive pigment with the spectral properties of isorhodopsin (Fig. 3B). There was no spectral evidence for the presence of any significant amount of rhodopsin in addition to isorhodopsin. It should be noted that 13-cis-retinal does not react with visual pigment apoprotein to form a photosensitive pigment (21).

Although it had been observed that SnCl$_2$ did not catalyze the isomerization of all-trans-retinal, SnCl$_2$ was capable of reducing an endogenous component of the photoreceptor membrane preparation, which in its reduced state was capable of isomerizing added all-trans-retinal. Under these conditions, the bulk of the bleached pigment was again converted to isorhodopsin (Fig. 3B). Isorhodopsin was also the exclusive product formed when bleached rhodopsin and all trans-retinal were incubated with either SnCl$_2$ alone or SnCl$_2$ and FMN at pH values of 6.8 to 7.5. The photoreceptor membrane preparations contained some bleached rhodopsin but were substantially free from mitochondrial contamination as judged by the inability of these preparations to utilize added succinate as the reducing agent in contrast to crude preparations of photoreceptors (16).

**Time Course of Formation of 9-cis-Retinal and Reaction of Chromophore Molecule with Apoprotein—**After bleaching, photoreceptor membrane preparations that are subsequently incubated in the dark with excess all-trans-retinal show no tendency to regenerate any pigment in 4 hours (Fig. 4). In contrast, the final step in visual pigment regeneration, the recombination of the apoprotein with suitable chromophore molecule, either 9-cis-retinal to form isorhodopsin or 11-cis-retinal to regenerate rhodopsin, is virtually complete within 1 or 2 min. The rate of isomerization of all-trans-retinal to 9-cis-retinal in the presence of 0.1 mM dihydro-FMN was only about one twentieth the rate of recombination, but about 4 times faster than the rate of isomerization in the presence of either 10 mM dithiothreitol or SnCl$_2$-reduced endogenous catalyst (Fig. 4). The substantial lag period observed using dithiothreitol suggested that the formation of 9-cis-retinal was in this instance attributable primarily to reduction of what was assumed to be an endogenous flavin in the photoreceptor membrane preparation and not to direct catalysis of isomerization by dithiothreitol. The reduction of flavins by dithiols has been studied (25).

In the presence of equal amounts of the dihydro forms of flavins, similar rates of isomerization of all-trans-retinal were obtained with dihydro-FMN and dihydro-FAD (Fig. 5) and a slightly higher rate with dihydroriboflavin. The results suggested that the reaction of SnCl$_2$ with flavin to produce the catalytically active reduced form proceeded a little more rapidly with riboflavin than with FMN or FAD, but far more rapidly than the reductive activation of the endogenous component of the photoreceptor membrane preparation. In each case the spectrum of the regenerated pigment was that of isorhodopsin.

**Effective Concentration of Catalyst—**With various agents other than dihydroriboflavins, appreciable quantities of 9-cis-retinal were not produced in reaction mixtures within 30 min and millimolar concentrations of these agents were required to obtain appreciable isorhodopsin during prolonged incubation of reaction mixtures (Fig. 6). In contrast, dihydroriboflavins were effective at concentrations as low as 1 $\mu$M. Dihydroriboflavin was somewhat more active than either dihydro-FMN or dihydro-FAD (Fig. 6).

To obtain evidence that dihydroriboflavins were promoting the geometrical isomerization of all-trans-retinal by functioning as catalysts, the molar ratio of product formed to dihydroriboflavin added was examined (Table II). The presence of a catalytic amount of dihydroriboflavin was capable of facilitating the production of a more than 10-fold larger quantity of 9-cis-retinal that was trapped and measured as isorhodopsin. In addition, the equilibrium established between all-trans-retinal, 9-cis-retinal, and 13-cis-retinal (Table II) in the presence of dihydroriboflavin was identical over a 10-fold range of flavin concentration.

**pH Dependence—**The isomerization of all-trans-retinal was carried out in the presence of bleached rhodopsin so that 9-cis-retinal formation could be assessed by measuring the isorhodopsin produced. In the absence of a catalyst, little isorhodopsin was formed during prolonged incubation of bleached rhodopsin with all-trans-retinal (Fig. 7). Optimal conversion of all-trans-retinal to 9-cis-retinal occurred in the region of pH 7.0 to 7.4 when dihydroriboflavin was added directly to reaction mixtures or when the dihydroriboflavin was generated in reaction mixtures by reduction of FMN with SnCl$_2$. A similar pH dependence was observed for the endogenous catalyst activated by reduction with SnCl$_2$. When the time of incubation with dihydroriboflavin was increased to 2 hours, the region of maximal formation of isorhodopsin...
was extended to include lower pH values. The recombination of apoprotein with either 9-cis-retinal or 11-cis-retinal was effective over a wide region below pH 7.5 but not at high pH values.

**Absence of Tritium Incorporation into Retinal during Isomerization**—An attempt was made to observe the transfer of tritium during isomerization of all-trans-retinal incubated in the presence of FMN and SnCl₂ or SnCl₂ alone in tritiated water with bleached rhodopsin present to trap 9-cis-retinal as it was formed (Table III). Some tritium was retained in extracts of the preparation incubated in the absence of isomerization catalyst, but no increase in incorporation was observed under the conditions in which geometrical isomerization occurred. The presence of catalyst did not affect the recovery of tritium in the extract containing the

**Evidence that dihydroflavins function as catalysts**

Reaction mixtures in Experiment 1, containing bleached photoreceptor membrane preparation in 0.9 ml of 0.11 M phosphate buffer, pH 7.4, 352 nmoles of all-trans-retinal, and, as indicated, 4.9 nmoles of dihydroriboflavin, were incubated in a final volume of 1.0 ml under nitrogen for 4 hours in the dark at 37º with shaking, extracted with Triton X-100, and assayed as described in the text.

Reaction mixtures in Experiment 2, containing 352 nmoles of all-trans-retinal, 0.9 ml of 0.11 M phosphate buffer, pH 7.4, 10 nmoles of SnCl₂ and FMN, as indicated, were incubated in a final volume of 1.0 ml under nitrogen in the dark with shaking for 16 hours and extracted. The extracts were subjected to thin layer chromatography and the remaining all-trans-retinal and reaction products, 9-cis-retinal and 13-cis-retinal, were eluted into ethanol and analyzed spectrophotometrically.

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**FIG. 4** (left). Time course of catalytic isomerization and formation of isorhodopsin. Photoreceptor membrane preparations were bleached, supplemented, incubated, and extracted with Triton X-100 under the conditions given in the legend of Fig. 3, except that reaction mixtures containing either 11-cis-retinal or 9-cis-retinal were not incubated under nitrogen. ———, the initial decrease in visual pigment during bleaching; O, regeneration of rhodopsin after addition of 11-cis-retinal; formation of isorhodopsin after addition of 9-cis-retinal (●) or all-trans-retinal together with either SnCl₂ and FMN (○), SnCl₂ alone (△), or dithiothreitol (□); ■, lack of regeneration in the presence of all-trans-retinal alone.

**FIG. 5** (right). Comparison of time course of production of 9-cis-retinal in the presence of dihydroriboflavin, dihydro-FMN, and dihydro-FAD. Reaction mixtures (1.0 ml) containing 352 nmoles of all-trans-retinal, 5 nmoles of SnCl₂, bleached photoreceptor membrane preparation, 0.7 ml of 0.01 M phosphate buffer, pH 7.4, and either 1 nmole of riboflavin (●), FMN (○), or FAD (△) or without added flavin (●) were incubated under nitrogen in the dark at 37º with shaking, extracted with Triton X-100 and analyzed as described in the text.

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**TABLE II**

*Evidence that dihydroflavins function as catalysts*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Dihydroflavin or dihydro FMN</th>
<th>Isorhodopsin present*</th>
<th>Difference</th>
<th>Ratio of product to dihydroflavin</th>
<th>Per cent of all-trans-retinal converted to products</th>
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<tr>
<td>1</td>
<td>None</td>
<td>57</td>
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<td></td>
<td>4.9</td>
<td>125</td>
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<td>0</td>
<td>5</td>
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</tbody>
</table>

*Samples also contained residual rhodopsin.

* Approximately 14.4% of the retinal was present as 9-cis-retinal after incubation in the presence of dihydro-FMN.
oxime of retinal. The 9-cis-retinal generated either in the presence of dihydro-FMN or in the presence of the endogenous catalyst did not become labeled with tritium. In other experiments all-trans-retinal was incubated as above but in the absence of bleached rhodopsin. The retinal was extracted and the all-trans, 9-cis, and 13-cis isomers were separated by thin layer chromatography as described in the legend of Table I and counted. No tritium was present in any of the isomers of retinal.

**DISCUSSION**

Direct thermal isomerization without catalysis has been reported in the case of 11-cis-retinal (13), but such a process is not likely to be of biological significance. The rapidity of the recombination of chromophore molecule with apo-protein to regenerate rhodopsin insures that accumulation of free 11-cis-retinal will not occur during dark adaptation. Both all-trans-retinal and all-trans-retinol were resistant to direct thermal isomerization at physiological pH and temperatures in the absence of an appropriate catalyst.

A number of biologically important compounds including thiols, dihydroflavins, dihydrofolate, tetrahydrofolate, and dehydroascorbate were found to promote the conversion of all-trans-retinal to reaction products separable using thin layer chromatography. Spectral studies indicated that these reaction products were 9-cis-retinal and 13-cis-retinal. Among the apparent catalysts, only the dihydroflavins were active at sufficiently low concentrations to be of possible importance in the physiological isomerization reaction associated with visual pigment regeneration during dark adaptation.

The lack of influence of dihydroflavin concentration on the equilibrium established in the geometrical isomerization of all-trans-retinal and the ability of a small quantity of added dihydroflavin to promote the formation of a much larger quantity of 9-cis-retinal indicated that the role of the dihydroflavin in the reaction was that of catalyst. As dihydroflavins did not catalyze isomerization of all-trans-retinol, it is not likely that all-trans-retinol is a substrate for the isomerization mechanism associated with the visual cycle.

There are several possible explanations for the observation that photoreceptor membrane preparations, although containing a component that after reduction by sodium chloride was capable of catalyzing the geometrical isomerization of all-trans-retinal, regenerated isorhodopsin rather than rhodopsin. The freezing and thawing of the photoreceptor organelle before preparation of the photoreceptor membrane particulate may have drastically altered both the conformation and catalytic properties of the endogenous catalyst. The biologically important catalyst may have been among the soluble protein components lost from the photoreceptor organelle during preparation of the photoreceptor membrane particulate. Another and perhaps more likely possibility is that the immobilization of the polyene chain of all-trans-retinal in an appropriately rigid hydrophobic environment.

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**Table III**

Absence of tritium incorporation into 9-cis-retinal during catalytic isomerization of all-trans-retinal in tritiated water

<table>
<thead>
<tr>
<th>Supplements</th>
<th>Tritium recovered</th>
<th>Isorhodopsin present</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10.9, 12.8, 9.4</td>
<td>29.4, 27.3, 29.6</td>
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<tr>
<td>SnCl₂ + FMN</td>
<td>12.1, 12.5, 13.9</td>
<td>110.3, 108.9, 112.6</td>
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<tr>
<td>None</td>
<td>31.6, 30.2, 34.1</td>
<td>34.7, 30.5, 35.3</td>
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<tr>
<td>SnCl₂</td>
<td>29.8, 28.3, 30.2</td>
<td>111.6, 106.1, 114.2</td>
</tr>
</tbody>
</table>

* The specific activity of the water in reaction mixtures was approximately 7 cpm per n mole.
* Samples also contained residual rhodopsin.
may be necessary, while the dihydroflavin interacts with the carbonyl group in order for isomerization to occur specifically at the trans-$\Delta^{11}$ double bond.

The absence of exchange of tritium between tritiated water and the protons attached to the carbon atoms of the double bond of all trans-retinal undergoing isomerization eliminates carbonium ion or carbanion mechanisms in which such an exchange should occur. It suggests nucleophilic catalysis by dihydroflavins by a mechanism similar to that presented for catalysis of geometrical isomerization by secondary amines (12). Presumably methyl group steric hindrance accounts for the absence of 11-cis-retinal among the isomerization products. The isomerization that has been studied can be represented by the following equation:

$$\text{dihydroflavin} \quad 2 \text{all-trans-retinal} \quad 7 \text{-cis-retinal} \quad 13 \text{-cis-retinal}$$

This reaction should as yet be viewed only as a possible model for the physiological isomerization mechanism occurring in the retina.

REFERENCES
The Catalytic Isomerization of All-trans-retinal to 9-cis-Retinal and 13-cis-Retinal
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