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

(Received for publication, May 29, 1973)

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.

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 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 (5), may in fact be completely inert in the absence of photoisomerizing 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.

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 n-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 Ventron Corp.; Nanograde petroleum ether of boiling range 30-60° from Mallinckrodt; nitrogen stated to contain approximately 2 ppm of residual oxygen from General Welding Supply Co., Inc.; frozen...
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 1,5-Dihydroriboflavin**—The procedure was based upon observations that a variety of reducing agents including sodium dithionite readily convert riboflavin to 1,5-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, portioning between two centrifuged tubes, adding a layer of 5 ml of the phosphate buffer above the solution in each tube, centrifuging at 40,000 rpm for 25 min, removing and recovering the photoreceptor particulate remaining in suspension and adherent to the upper walls of the tubes by dilution with 40 ml of the phosphate buffer, and centrifuging at 40,000 rpm for 25 min. The photoreceptor membrane preparation was washed once with 40 ml of 154 mM KCl and stored frozen overnight.

**Preparation of Retinol**—The geometrical isomers of retinal were converted to the corresponding retinol isomers by borohydride reduction. A 2.0-ml sample of retinal was dissolved in 3 ml of ethanol petroleum ether (1:2), approximately 100 mg of LiBr, was added, and the mixture was stirred briefly. The retinol was extracted by mixing successively with 5 ml of petroleum ether 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 0-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 suspensions 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 20 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 Catalysts—**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>Geometrical isomers</th>
<th>Petroleum ether-2-octanone (V:1)</th>
<th>Petroleum ether-ether (1:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-trans-retinal</td>
<td>0.29</td>
<td>0.31</td>
</tr>
<tr>
<td>9-cis-Retinal</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>11-cis-Retinal</td>
<td>0.40</td>
<td>0.48</td>
</tr>
<tr>
<td>13-cis-Retinal</td>
<td>0.40</td>
<td>0.47</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Geometrical isomers</th>
<th>Petroleum ether-2-octanone (V:1)</th>
<th>Petroleum ether-ether (1:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.29</td>
<td>0.30</td>
</tr>
<tr>
<td>FMN + SnCl₂</td>
<td>0.280.38</td>
<td>0.470.310.39</td>
</tr>
<tr>
<td>FMN</td>
<td>0.30</td>
<td>0.26</td>
</tr>
<tr>
<td>SnCl₂</td>
<td>0.29</td>
<td>0.28</td>
</tr>
<tr>
<td>FMN + DPNH</td>
<td>0.300.41</td>
<td>0.500.330.44</td>
</tr>
<tr>
<td>FAD + SnCl₂</td>
<td>0.290.39</td>
<td>0.490.320.40</td>
</tr>
<tr>
<td>Riboflavin + SnCl₂</td>
<td>0.290.40</td>
<td>0.490.310.40</td>
</tr>
<tr>
<td>Dihydriboflavin</td>
<td>0.290.39</td>
<td>0.480.310.41</td>
</tr>
<tr>
<td>Dithiothreitol</td>
<td>0.280.39</td>
<td>0.470.320.42</td>
</tr>
<tr>
<td>Dihydrofolate</td>
<td>0.270.36</td>
<td>0.460.310.39</td>
</tr>
<tr>
<td>Dehydroascorbate</td>
<td>0.280.41</td>
<td>0.480.300.39</td>
</tr>
</tbody>
</table>

* 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 dihydroretinals including 10 μmole of either reduced ni-lipoate or 2,3-dimercaptopropanol and with 0.4 μmole of tetrahydrofuran. With monothiols, 10 μmole of either glutathione, 2-mercaptoethanol or cysteine, or 4 μg of bovine serum albumin, the component with Rp value of approximately 0.40 was barely detectable.
Similarly 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 dihydroretinal 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 13-cis-retinal and 9-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 dithiothreitol or stannous chloride and FADN 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 dihydrofolate. However, a small amount of fluorescent reaction product with the mobility of 13-cis-retinal was detected after incubation in the presence of tetrahydrofolate.

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 insure that even if only a small quantity of 11-cis-retinal were present, it would not escape detection, reaction mixtures of all-trans-retinal, either dihydroretinal or 13-cis-retinal at wave lengths shorter than 300 nm. 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 suffi-
ciently large amounts of photosensitive pigment were regenerated 
to assure high absorbancies, it was possible to distinguish clearly 
rhodopsin with $A_{max}$ at about 500 nm (21) formed from 11-cis-
retinal and isorhodopsin with $A_{max}$ at 487 nm (21) produced from 
9-cis-retinal. Under these conditions no regeneration of pigment 
occurred if reaction mixtures contained only bleached visual 
membrane preparation and no added catalyst, isorhodopsin synthetized 
during incubation with 9-cis-retinal (22). Isorhodopsin produced 
by incubating bleached photoreceptor membrane preparation with 9-cis-retinal (22), pigment present after incubation with all-trans-retinal (22), rhodopsin present after incubation with 11-cis-retinal (22), and 
isorhodopsin synthesized during incubation with 9-cis-retinal (22). B, isorhodopsin produced by incubating bleached photo-
receptor membrane preparation with 9-cis-retinal (22), pigment present after incubation with all-trans-retinal (22), and 
isorhodopsin synthesized during incubation with 9-cis-retinal (22).

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 7.8.
The photoreceptor membrane preparations contained some 
bleached rhodopsin but were substantially free from mitochondrial 
contamination as judged by the inability of these prepara-
tions 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, photore-
ceptor 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 rho-
dopsin, 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 isom-
erization 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 sug-
gested 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 concen-
trations as low as 1 pm. 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 11-cis-retinal (Table II) in the presence of dihydro-
FMN was identical over a 10-fold range of flavin concentration.

**pH Dependence**—The isomerization of all-trans-retinal was 
conducted 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 presence of pH 7.0 to 7.4 when dihy-
droriboflavin 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

---

**Fig. 3.** Synthesis of isorhodopsin from apoprotein of rhodopsin 
and product of catalytic isomerization of all-trans-retinal to presence 
of either dihydro-FMN, dithiothreitol, or reduced endogenous 
component of photoreceptor membrane preparation. Reaction mixtures 
containing photoreceptor membrane preparation in 0.7 
ml of 0.11 m phosphate buffer, pH 7.4, were exposed to bleaching 
light, supplemented with 352 nmoles of 9-cis-retinal, 11-cis-retinal, 
or all-trans-retinal and, as indicated, 10 nmoles of dithiothreitol, 
1 nmoles of SnCl$_2$, or 0.1 nmoles of FMN in a final volume of 1.0 ml. 
Incubated under nitrogen for 4 hours in the dark at 37°C with shak-
ing and extracted with Triton X-100 and spectra were determined 
before and after bleaching as described in the text. A, rhodopsin 
in unbleached preparation (□), residual pigment after bleaching 
(○), pigment present after incubation with all-trans-retinal (●), 
rhodopsin present after incubation with 11-cis-retinal (□), 
and isorhodopsin synthesized during incubation with 9-cis-retinal (□).  
B, isorhodopsin produced by incubating bleached photoreceptor 
membrane preparation with 9-cis-retinal (○), pigment present after incubation with all-trans-retinal (○), and isorhodopsin synthesized 
during incubation with 9-cis-retinal (○).
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
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 the endogenous catalyst. 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 (18), but such a process is not likely to be of biological significance. The rapidity of the recombination of chromophore molecule with apoprotein 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 dihydroflavine concentration on the equilibrium established in the geometrical isomerization of all-trans-retinal and the ability of a small quantity of added dihydroflavine to promote the formation of a much larger quantity of 9-cis-retinal indicated that the role of the dihydroflavine 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 stannous 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.
may be necessary, while the dihydroflavin interacts with the carbonyl group in order for isomerization to occur specifically at the trans-Δ^{13} 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:

\[
\text{dihydroflavin} \rightarrow 2 \text{all-trans-retinal} \rightarrow 0 \text{-cis-retinal} + 13 \text{-cis-retinal}
\]

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
Sidney Futterman and Martha H. Rollins


Access the most updated version of this article at [http://www.jbc.org/content/248/22/7773](http://www.jbc.org/content/248/22/7773)

Alerts:
- When this article is cited
- When a correction for this article is posted

[Click here](http://www.jbc.org/content/248/22/7773.full.html#ref-list-1) to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/248/22/7773.full.html#ref-list-1](http://www.jbc.org/content/248/22/7773.full.html#ref-list-1)