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Isomerization reactions of retinoids in the visual system

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ABSTRACT - Double-bond isomerization reactions in the retinoids provide a means to initiate and then to terminate signal transduction. An apt example is found in the visual system, where light causes the photoisomerization of the 11-cis-retinal protonated Schiff base of rhodopsin to its all-trans congener. In order for vision to proceed, the reverse thermal isomerization must take place. Because 11-cis-retinoids are approximately 4 kcal/mol higher in energy than their all-trans counterparts, an energy source is required for this conversion. It has been established that the biosynthetic pathway for 11-cis-retinoids involves the direct processing of all-trans-retinyl esters to 11-cis-retinol. In this process, the negative free energy of ester hydrolysis provides the thermodynamic driving force for the endergonic trans to cis isomerization. Since retinyl esters are themselves generated by the transfer of acyl groups from phospholipids, the energy for the isomerization reaction originates in the membrane phospholipids.

INTRODUCTION

The retinoids play important roles in cellular physiology. Their most completely understood roles are found in visual signal transduction in animals, and in energy production in halophilic bacteria . The more recent discovery of the cell growth, maintenance, and developmental roles of the retinoic acids adds a new and exciting chapter to the biology of the retinoids. The retinoids are derived from the carotenoids by oxidative processes that have not yet been characterized at the molecular level. All of the retinoids are diterpene polyene derivatives, and as such their activities are often controlled by reversible double-bond isomerization reactions involving mono-cis and all-trans isomers. In this review, the way in which double-bond isomerization reactions of retinoids are central to vision is explored. The possible relationship of insights drawn from this work to the hormonal roles of the retinoic acids is also described.

PHOTOISOMERIZATION OF RHODOPSIN AND VISUAL SIGNALING

Vision is initiated when the visual pigment rhodopsin absorbs a photon of light, leading to the cis to trans isomerization of its protonated 11-cis-retinal Schiff base chromophore (ref. 1) (Fig. 1). 11-cis-Retinal is a diterpene retinoid related to vitamin A (all-trans-retinol), and it is covalently linked to the apoprotein opsin at the latter's active-site lysine residue. The photochemical isomerization of the chromophore in rhodopsin leads to a series of spectroscopically defined intermediates, resulting in the eventual hydrolysis of the all-trans-retinyl Schiff base to produce all-trans-retinal and opsin, as shown in Fig. 1. The photoisomerization reaction has been reported to be essentially complete in only 200 femtoseconds (ref. 2) . Interestingly, noise analysis has placed an upper limit on the rate of thermal isomerization of rhodopsin in the neighborhood of several centuries, giving a signal to noise ratio in the rod of approximately 10^{23}. One of the spectroscopically defined intermediates, metarhodopsin 2, is the intermediate (R*) that transmits the information that light has been absorbed by interacting with the next molecular entity in the visual cascade, the retinal G-protein, transducin (ref.3).

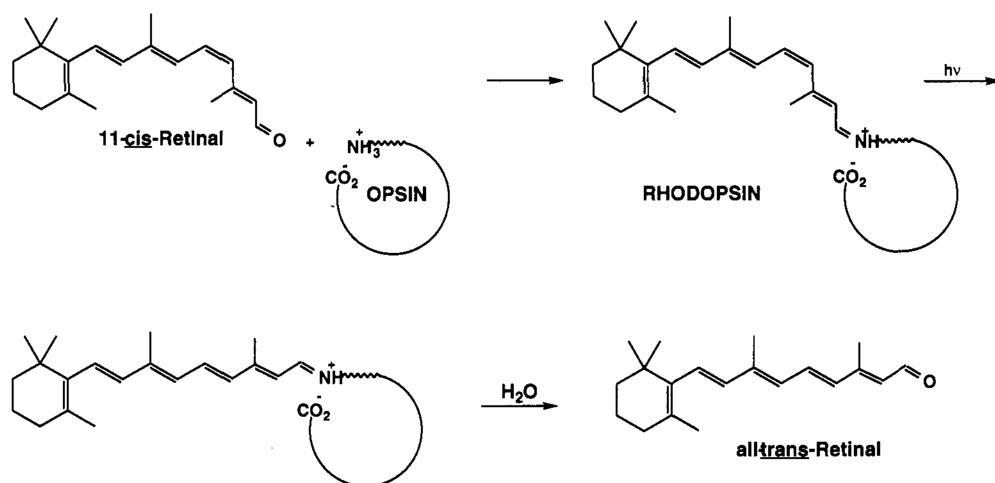

Fig. 1: The Photoisomerization of Rhodospin

ENZYMATIC REGENERATION OF 11-CIS-RETINAL AND THE VISUAL CYCLE

The fact that all-trans-retinal is liberated as a consequence of bleaching requires that a physiological mechanism be in place for the regeneration of 11-cis-retinal. The fact that vertebrate vision operates via a bleaching mechanism is presumably related to the fact that bleaching plays a role in visual adaptation (ref. 4). Visual adaptation refers to the ability of the visual system to alter its sensitivity to match the strength of the ambient signal, and this ability is importantly dependent on the amount of rhodopsin present in the rods at low light intensities. The immense sensitivity of human vision, which can operate at the single photon level (ref. 5), is partly a reflection of the exceedingly high concentration of rhodopsin in the rod outer segments; (this can be as high as 10^9 rhodopsin molecules/rod) (ref. 6). To decrease the inherent sensitivity of this system at high light intensities requires that the amount of pigment be modulated by the bleaching-regeneration cycle.

The bleaching of rhodopsin produces all-trans-retinal, and the latter must be reprocessed into 11-cis-retinal in order for vision to proceed. The presence of a retinal isomerase would appear to provide a simple solution to this problem. However, the situation is considerably more complex metabolically. The all-trans-retinal liberated by the bleaching of rhodopsin has been shown to be rapidly reduced enzymatically in the retina by specific nicotinamide linked retinol dehydrogenases to produce vitamin A. The vitamin A is then transported from the rod outer segments to the pigment epithelium, where it is esterified, largely with long chain saturated fatty acids, such as those in the palmitate and stearate series (ref. 7). For each of the three all-trans-retinoids, there is a corresponding 11-cis-retinoid, and the totality of biochemical reactions required to interconvert these six molecules comprises the classical Visual Cycle. Thus, the double-bond isomerization reaction could potentially occur with any one of three substrates and produce any one of the three corresponding products, so that there are nine possible isomerization pathways. In addition, the isomerization could occur either in the retinal pigment epithelium (the organ found at the back of the eye in contact with the retina), in the retina proper, or both in the retina and in the pigment epithelium.

In addition to the nature of the substrate for the reaction, the relative stabilities of the cis and trans-retinoids also need to be considered. When various isomers of the retinals and retinyl esters were brought into equilibrium, it was found that 11-cis-retinoids accounted for approximately 0.1% of the equilibrium mixture (ref. 8). There is a 4.1 kcal/mol difference between 11-cis-retinal and its all-trans counterpart, with similar differences being observed between the other retinoid congeners. This leads to the important question of where the energy comes from to drive the trans to cis isomerization in vivo.

The *in vitro* biosynthesis of 11-cis-retinoids from all-trans-retinol was first demonstrated with a membrane preparation from the pigment epithelium which proved capable of converting all-trans-retinol (vitamin A) to a mixture of 11-cis-retinol, 11-cis-retinal, and 11-cis-retinyl palmitate (ref. 9). All-trans-retinyl palmitate and all-trans-retinal were also generated by these membranes. The isomerase system is highly specific for all-trans-retinoids and was found largely, if not entirely, in the pigment epithelium rather than in the retina itself.

Since all-trans-retinol is converted into all-trans-retinal, all-trans-retinyl palmitate, and the three 11-cis congeners by the pigment epithelium membrane preparation described, it cannot be assumed that all-trans-retinol is the substrate for the isomerase system or that 11-cis-retinol is the direct product of the isomerase action. Experiments to follow the fate of specifically isotopically labeled substrates and the use of specific ester synthetase inhibitors, such as all-trans-retinyl-α-bromoacetate (RBA), allowed for a demonstration of the actual isomerization pathway and showed the obligate intermediacy of retinyl esters in 11-cis-retinoid formation (ref. 10). RBA is a potent affinity-labeling agent of lecithin retinol acyl transferase (LRAT) (ref. 11), the enzyme responsible for retinyl ester biosynthesis in the pigment epithelium (ref. 11). This novel enzyme specifically transfers acyl groups from the *sn*-1 of lecithin to vitamin A and analogs (refs. 12-14). When pigment epithelial membranes are treated with RBA, neither retinyl esters nor 11-cis-retinoids are generated, and all-trans-retinyl esters are directly processed to 11-cis-retinol (ref. 10). These experiments leave little doubt that it is all-trans-retinyl ester and not all-trans-retinol that is processed to 11-cis-retinol, and that retinyl ester formation is an obligate part of the isomerase pathway.

Why might the linkage of ester synthesis to isomerization be of interest? As mentioned before, esters are 'high energy' compounds and are hydrolyzed with free energies of hydrolysis in the -5 kcal/mol range. If the free energy of hydrolysis of an ester could be coupled to the isomerization process, more than enough energy would be provided to drive the latter process. As applied here, the energies sumto yield a mechanism by which ester hydrolysis could be linked to isomerization (refs. 15-17). Here, the substrate is an all-trans-retinyl ester and the product is 11-cis-retinol. As predicted by this mechanism, labeling experiments show that the original oxygen of the vitamin A is lost during isomerization. In addition, inversion of the absolute configuration of the prochiral CH_2OH also accompanies isomerization (ref. 15). If ester hydrolysis occurs simultaneously with isomerization, then the enzyme catalyzing the isomerization reaction is an isomerohydrolase rather than a simple isomerase, because the substrate and product of enzymatic reaction are not isomers of one another.

In the scheme shown above, LRAT has an energy transducing function, and may be a member of a new class of enzymes. Hence it has been studied in some detail. The kinetic mechanism was clearly demonstrated to be ordered ping pong bi bi (ref. 18). In this case, the lecithin binds to the enzyme first and an acyl group is transferred from the *sn*-1 position of the phospholipid to an active-site residue. Following the desorption of the 2-acyl lysophospholipid, vitamin A binds at the active-site and the acyl group is transferred to it. The identity of the active-site amino acid to which the acyl group is transferred remains to be determined, but the fact that the enzyme is

catalytically inactivated by sulfhydryl and serine-directed reagents suggests an active-site serine or cysteine residue. As previously mentioned, the enzyme is affinity labeled by RBA (ref. 11). By use of ^{3}H-RBA, a labeled protein of approximately 24kD has been identified as a candidate for the holoenzyme or a subunit of it (ref. 11). The finding that the retinyl esters are produced by using lecithin as an acyl donor shows that lecithin is being used in a group transfer reaction in much the same way that ATP would be. Therefore the energy to drive the endergonic cis to trans isomerization process in the visual cycle originates in the membrane itself. Previously, lecithin was not thought to play a role in the visual cycle, and retinyl esters were thought to be important only as innocuous storage forms of the retinols. Our current understanding of the visual cycle is shown in Fig. 2. Upon bleaching,

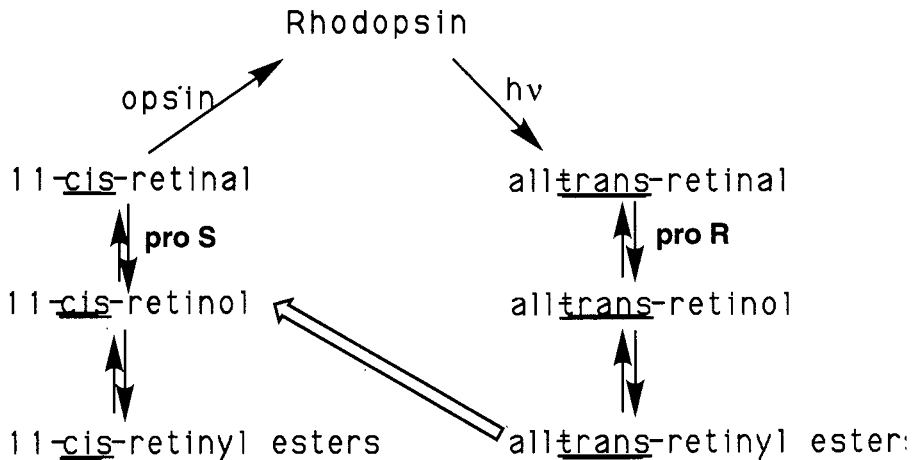

Fig. 2: The Visual Cycle

the all-trans-retinal is reduced by a pro-R specific dehydrogenase in the rod outer segments to produce all-trans-retinol. The all-trans-retinol travels to the pigment epithelium, probably bound as a complex with the interphotoreceptor retinoid binding protein (IRBP) (refs. 19-21). The all-trans-retinol is esterified via LRAT action in the pigment epithelium and the retinyl ester is processed to 11-cis-retinol. Oxidation of the 11-cis-retinol to 11-cis-retinal occurs by means of a pro-S specific dehydrogenase (ref. 16) followed by the delivery of IRBP-bound 11-cis-retinal to the photoreceptors, thus completing the visual cycle (ref. 22).

ISOMERIZATION OF RETINOIC ACIDS

All-trans-retinoic acid is known to possess important hormonal and developmental activities which are manifest through RAR nuclear receptors. Recently two groups have observed that isomers of retinoic acid other than the all-trans isomer possess biological activities (refs. 23, 24). Specifically, 9-cis-retinoic acid has been shown to be the favored ligand for the RXR receptor (refs. 23, 24). In fact it appears likely that signaling through the RXR receptors may have much more widespread physiological impact than signaling through the RAR receptors. Whatever the physiological link may be between the two different receptor types, it is of interest to inquire about the possible metabolic relationship between all-trans-retinoic acid and its 9-cis congener.

It is reasonable to think that what we have learned about isomerization processes in the visual system may be applicable to the problem of 9-cis-retinoic acid biosynthesis. The problem of the biosynthesis of 9-cis-retinoids may be much simpler than that encountered in the formation of 11-cis-retinoids, because 9-cis-retinoids do not suffer

from intramolecular steric interactions that render them highly unstable relative to their all-trans counterparts. Hence, an energy yielding reaction is not required for the formation of 9-cis-retinoic acid, which comprises approximately 15% of an equilibrium mixture.

A simple mechanism involves the direct isomerization of all-trans-retinoic acid to 9-cis-retinoic acid. In fact, a process in which all-trans-retinoic acid is isomerized to a mixture of 9-cis-retinoic acid and 13-cis-retinoic has already been reported to occur in cells in culture (ref. 24). We have demonstrated that bovine liver membranes can non-stereospecifically isomerize all-trans-retinoic acid into 9-cis-retinoic acid and 13-cis-retinoic acid. However, the isomerization process cannot be saturated. Moreover, the isomerization of all-trans-retinoic acid to the congeneric mono-cis isomers also occurs in certain buffers in the presence of bovine serum albumin. The spontaneous formation of 9-cis isomers in the retinoid series is highly unusual, and suggests that isomerization mechanism(s) are possible in the retinoic acid series that are not available in the retinal and retinol series. It is entirely possible that this kind of mechanism operates in cells and is physiologically important. A major drawback of any equilibrium process of this type is that it cannot spatially separate the all-trans and 9-cis-retinoic acids. A process analogous to what was described above in the biosynthesis of 11-cis-retinoids which does not suffer from this drawback is interesting to consider. The processing of an all-trans-retinyl ester to 9-cis-retinol would provide a one-way reaction to the latter (oxidation of 9-cis-retinol to 9-cis-retinoic acid is readily feasible via retinol oxidases). An energy-linked mechanism of this type would allow for the physiologically irreversible conversion of an all-trans-retinoid into a 9-cis-retinoid and allow for the spatial separation of the activities of these retinoids.

Finally, it should be mentioned that 9-cis-retinoic acid might arise directly from a dietary 9-cis-β-carotene, obviating the need for an isomerase. Carotenoids in the 9-cis series are well-known and have been observed to be present in human serum (ref. 25).

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