Biochemistry

 Volume 30, Number 3 January 22, 1991

Perspectives in Biochemistry

Membrane Phospholipids as an Energy Source in the Operation of the Visual Cycle†

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Received July 5, 1990; Revised Manuscript Received August 16, 1990

ABSTRACT: Biology depends on the coupling of the free energy of hydrolysis of phosphate esters, such as ATP, to drive processes which would otherwise be thermodynamically unfavorable. Carboxyl esters are like phosphate esters in their ability to hydrolyze with substantial negative free energies, enabling them to participate in group transfer processes as well. In particular, membrane phospholipids constitute an enormous store of potential energy that could be used to fuel energetically unfavorable processes. One such process involves the biosynthesis of 11-*cis*-retinal, the chromophore of rhodopsin, from *all-trans*-retinol (vitamin A). The difference in free energy between an all-trans retinoid and its corresponding 11-cis retinoid is approximately 4 kcal/mol. This energy is provided for in a minimally two-step process involving membrane phospholipids as the energy source. First, *all-trans*-retinol is esterified in the retinal pigment epithelium by lecithin retinol acyl transferase (LRAT) to produce an *all-trans*-retinyl ester. Second, this ester is transformed into 11-*cis*-retinol by an isomerohydrolase in a process that couples the negative free energy of hydrolysis of the acyl ester to the formation of the strained 11-*cis*-retinol.

Group transfer reactions involving ATP are at the very heart of energy metabolism in biochemistry. The concept that ATP possesses "high-energy" bonds is based on the observation that ATP is hydrolyzed to form ADP and phosphate, with a negative free energy of hydrolysis of −7.3 kcal/mol under standard conditions (Jencks, 1970). Related processes involve the hydrolysis of ATP to AMP and pyrophosphate (−7.7 kcal/mol), the hydrolysis of pyrophosphate to two phosphates (−8.0 kcal/mol), and the hydrolysis of phosphoenolpyruvate to pyruvate and phosphate (−14.8 kcal/mol) (Jencks, 1970). In addition to ATP, there are, of course, many other biologically relevant phosphate esters, including GTP, phosphoenolpyruvate, and acetyl phosphate, that can be used in group transfer reactions. All of the latter molecules have negative free energies of hydrolysis, some much larger than that of ATP itself. Although biochemistry has concentrated on the use of phosphate esters in energy utilization processes, other molecules also need to be considered as candidate high-energy compounds. The group of compounds to be focused on here is the carboxyl esters. Esters of this type are hydrolyzed with negative free energies in the −5 to −10 kcal/mol range (Jencks, 1970). For example, ethyl acetate and glycine ethyl ester are hydrolyzed under standard conditions with $\Delta G°$s = −4.7 kcal/mol and −8.4 kcal/mol, respectively (Jencks, 1970). In addition, carboxyl esters are already known to be involved in group transfer activation processes in the formation of the peptide bond (Jencks, 1970). The aminoacyl-tRNA species are carboxyl esters and are hydrolyzed with free energy changes in the −8 kcal/mol range.

In this review it will be shown how phospholipids can act as group transfer substrates by donating the acyl group at the *sn*-1 position to vitamin A to produce an *all-trans*-retinyl ester. The *all-trans*-retinyl ester is further processed with isomerization and hydrolysis to produce 11-*cis*-retinol, the precursor to 11-*cis*-retinal, the visual chromophore (Deigner et al., 1989; Trehan et al., 1990). The necessary free energy to drive the endergonic isomerization process comes from the free energy of hydrolysis of the retinyl ester (Deigner et al., 1989; Trehan et al., 1990). In this overall process, membrane phospholipids provide the ultimate source of energy needed to drive the isomerization reaction, a key component of the vertebrate visual cycle. This process reveals a new role for membrane

† The work from the author's laboratory described here was supported by U.S. Public Health Service Research Grant EY 04096 from the National Institutes of Health.

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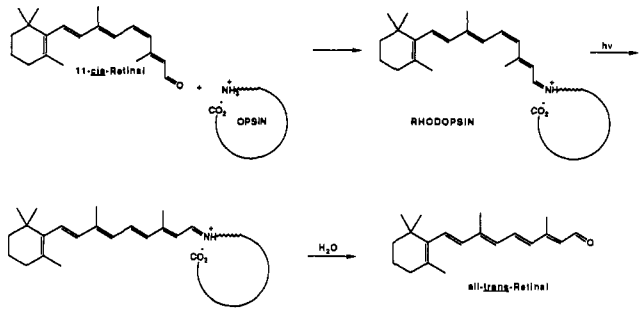

FIGURE 1: Photochemical cis to trans isomerization of rhodopsin initiates vision.

phospholipids: they may serve as an energy source.

In the following sections a brief discussion of the initial events in vision and the visual cycle is given. This is followed by a discussion of the nature of the isomerization process and the role that lecithin plays in the process. Details of the bioorganic chemistry of the processes can be found in a recently published review (Rando, 1990).

Rhodopsin and the Visual Cycle

The visual pigment rhodopsin, which is located in the rod outer segments of the retina, contains the apoprotein opsin (which is transparent to visible light) covalently linked via a protonated Schiff base to 11-*cis*-retinal (Figure 1). 11-*cis*-Retinal is a diterpene related to vitamin A (Figure 2). Vision is initiated by the photochemical cis to trans isomerization of the 11-*cis*-retinal Schiff base chromophore of rhodopsin (Wald, 1968) (Figure 1). 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 Figure 1 [as reviewed by Birge (1981)]. The isomerization reaction is largely complete by the time the first well-established intermediate, bathorhodopsin, is formed (Mathies, 1982). 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.

The interaction of R* with transducin results in catalysis of the exchange of GDP for GTP at the active site of transducin [as reviewed by Hofmann (1986)]. The transducin–GTP complex activates a rod outer segment phosphodiesterase specific for cGMP which can hydrolyze cGMP at the diffusion-controlled limit [as reviewed by Stryer (1986)]. This hydrolysis is thought to lower the free concentration of this effector. Since the sodium ion permeability of the rod outer segment sodium channels is gated by cGMP (Fesenko et al., 1985), hydrolysis of the latter results in the hyperpolarization of the rod outer segments. This membrane potential change is signaled to other nerve cells in the retina and communicated to the brain, where the visual image can then be constructed.

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. Otherwise, vision would have the character of a once-in-a-lifetime event. The reason why vertebrate vision operates via a bleaching mechanism is presumably related to the role that bleaching plays in visual adaptation (Weinstein et al., 1967). 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 (Hecht et al., 1937), is partly a reflection of the exceedingly high concentration of rhodopsin in the rod outer segments (which can be as high as 10^9 rhodopsin molecules/rod) (Dowling, 1987). To decrease the inherent sensitivity of this system at high light intensities requires that the amount of pigment be modulated by the bleaching–regeneration cycles.

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 (Julia et al., 1986). The vitamin A is then transported from the rod outer segments to the pigment epithelium, where it is esterified, largely to long-chain saturated fatty acid esters, such as those in the palmitate and stearate series. 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, shown in simplified form in Figure 3. 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, and it could occur either in the retinal pigment epithelium (the organ that sits at the back of the eye in contact with the retina) or in the retina proper. The identities of the isomerase substrate and its product are key issues that need to be confronted.

Energetics of the Isomerization and the Nature of the Substrate

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. This thermodynamic issue was first pointed out by Pauling (1949). Pauling argued that certain cis double

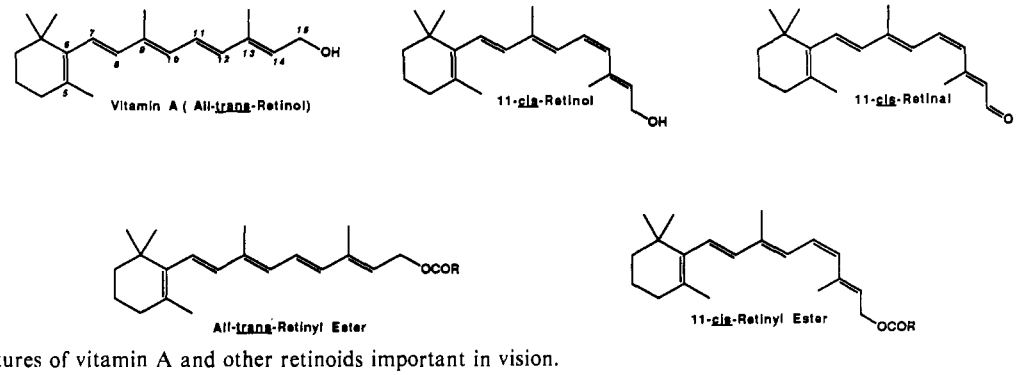

FIGURE 2: Structures of vitamin A and other retinoids important in vision.

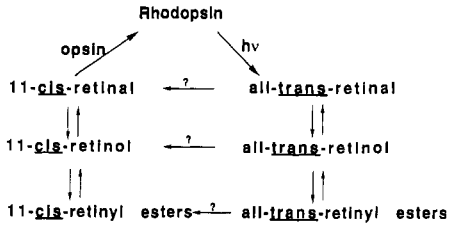

FIGURE 3: Classical visual cycle.

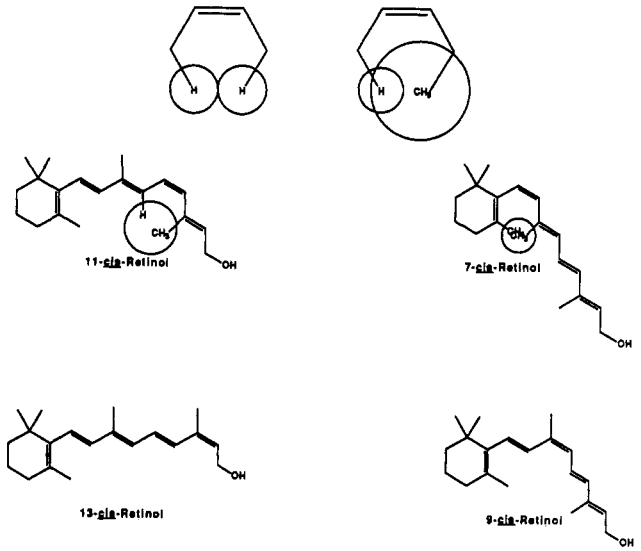

FIGURE 4: Methyl–hydrogen repulsion as a source of strain in 11-cis retinoids.

bonds of carotenoids and retinoids should be unstable with respect to their all-trans counterparts (Figure 4). The methyl groups overlap with the indicated hydrogen atoms when the polyene system as a whole remains planar. As applied to the retinals, it could be estimated that 11-*cis*-retinal should be 8–9 kcal/mol higher in energy than *all-trans*-retinal (Hubbard et al., 1968). Furthermore, although 7-*cis*-retinal would be predicted to be unstable, both 9-*cis*- and 13-*cis*-retinal, as well as 9,13-*dicis*-retinal, should be relatively stable (Figure 4).

When the retinals and retinyl esters were brought into equilibrium with either iodine or trifluoroacetic acid from the various isomers, it was found that 11-cis retinoids accounted for approximately 0.1% of the equilibrium mixture (Rando & Chang, 1983). The same results are obtained when the retinols are brought into equilibrium with iodine. 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. Since the destabilizing interaction involves intramolecular steric repulsion betweeen the C10 H and the methyl group at C13 (or the C13 vinyl moiety when the molecule is in the 12-s-cis form), the nature of the substitution at C15 does not have a marked effect on the equilibrium position. Thus, Pauling's brilliant structural hypothesis is qualitatively correct and leads to the important question of where the energy comes from to drive the trans to cis isomerization in vivo.

This energy cannot come from light, because the regeneration process in vertebrates occurs in the dark. Furthermore, the rates of rhodopsin regeneration in dim light are independent of the wavelength of light that the animal is being exposed to (Rushton, 1957). These two facts support the idea that the physiologically relevant trans to cis isomerization phenomenon is purely thermal in nature. Although this presents chemical difficulties, there are clear teleological advantages in the process being a thermal enzymatic one. Most importantly, 11-*cis*-retinal is most needed at low light intensities, where photosynthesis would be low. Moreover, an enzymatic regeneration process will be independent of the wavelength and intensity of the ambient light, and an enzymatic process can be subjected to efficient control mechanisms unavailable in the photochemical process.

In order to begin to address the issue of the energy source, it was important first to gain some understanding of the nature of the isomerase substrate. It turns out that these issues are inextricably linked. To approach the issue of substrate, an in vivo double-labeling experiment diagrammed in Figure 5 was performed (Bernstein & Rando, 1986). The idea behind this experiment is straightforward. A racemic mixture of [15(*S*)-^{3}H]- and [15(*R*)-^{3}H]-*all-trans*-retinol plus [15-^{14}C]-*all-trans*-retinol was administered to either rats or frogs (Bernstein & Rando, 1986). If isomerization occurs at the aldehyde level of oxidation, than half of the ^{3}H should be lost in the initially formed 11-*cis*-retinol (ester), whereas ^{3}H should be retained if isomerization occurs at the alcohol level of oxidation.

The double-label experiments, as performed on the rat, produced unequivocal results (Bernstein & Rando, 1986). Approximately 70% of the ^{3}H of the starting material was retained in the initially formed 11-*cis*-retinyl esters, and this number could be increased to greater than 80% by the inclusion of 4-methylpyrazole, a well-characterized inhibitor of the retinol dehydrogenases (Bernstein & Rando, 1986). After

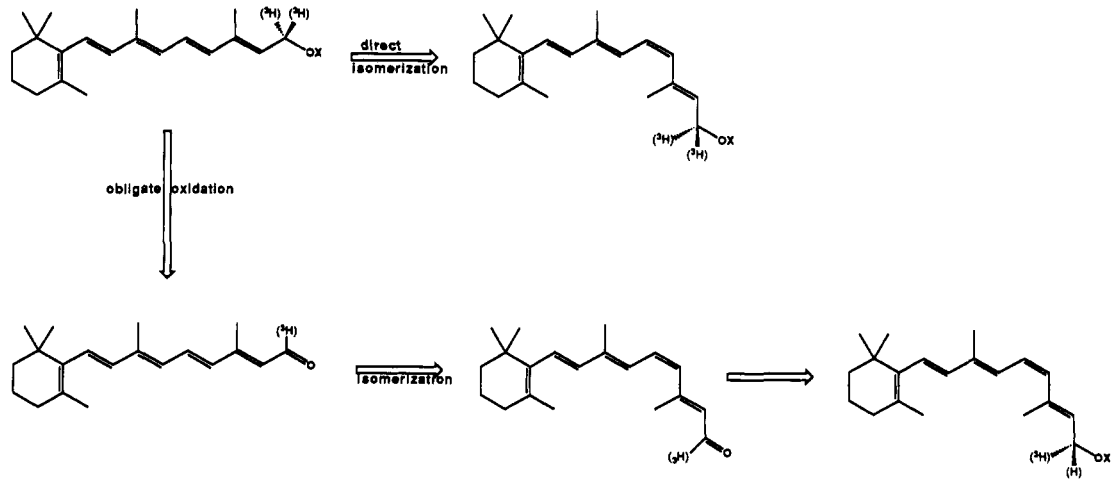

FIGURE 5: Double-label experiment designed to determine if isomerization occurs at the alcohol or aldehyde oxidation state.

a long period of incubation (24 h), the ^3H loss for all of the retinoids was approximately 50%, a result that shows that only stereospecific loss of ^3H occurred. Thus, isomerization must occur at the alcohol level of oxidation; i.e., *all-trans*-retinal cannot be the substrate. Although these experiments still leave both ester and alcohol as possible substrates, they favor the former, because under all conditions the recovered *all-trans*-retinol had lost 50% of its ^3H and hence could not easily have served as an isomerase substrate, given that the products retained substantially greater than 50% ^3H. The *all-trans*-retinyl esters, on the other hand, retained approximately 90% of their ^3H and hence could be isomerase substrates.

Biosynthesis of 11-Cis Retinoids in Vitro

The in vitro biosynthesis of 11-cis retinoids from *all-trans*-retinol was first demonstrated with a 600*g* supernatant membrane preparation from the amphibian pigment epithelium which proved capable of converting vitamin A to a mixture of 11-*cis*-retinol, 11-*cis*-retinal, and 11-*cis*-retinyl palmitate (Bernstein et al., 1987a). *all-trans*-Retinyl palmitate and *all-trans*-retinal were also generated by these membranes. The specificity of the isomerase system toward particular retinol isomers is considerable. In washed pigment epithelium membranes, added 9-*cis*- and 13-*cis*-retinol are efficiently esterified; however, neither is isomerized to a significant extent (Bernstein et al., 1987b). Thus, the isomerase system appears to be highly specific for all-trans-retinoids. As would be expected of a biologically significant process, the membrane-bound 11-cis retinoid biosynthetic activity proved to be heat sensitive (Bernstein et al., 1987a). Intact protein and membrane are required, because activity was abolished by treatment with proteinase K or phospholipase C and by heat (Bernstein et al., 1987b). The process is saturable with *all-trans*-retinol, and with crude bovine pigment epithelial membranes it has an apparent V_{max} of approximately 1 nmol h^{-1} (mg of protein)$^{-1}$ and a K_M of approximately 0.5 μM. These values can account for the known rates of 11-cis retinoid biosynthesis in vivo. Recently, it has been demonstrated that added *all-trans*-retinol is processed into 11-cis retinoids by intact pigment epithelium cultures (Das & Gouras, 1988; Flannery et al., 1988).

Since labeled *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. The possibility that *all-trans*-retinal is the isomerization substrate was easily ruled out. In the case of the bovine system, isolated pigment epithelium membranes will not cause *all-trans*-retinal to be isomerized to 11-*cis*-retinal (Fulton & Rando, 1987). Furthermore, [15-^3H,15-^{14}C]-*all-trans*-retinol is processed to 11-*cis*-retinol by the native frog and bovine pigment epithelium membranes with complete retention of the tritium label (Berstein et al., 1987b). This result is completely consistent with the in vivo variant of the double-label experiment described above (Bernstein et al., 1986) and rules out the possibility that free *all-trans*-retinal is the isomerase substrate, since tritium would be lost if the retinol were oxidized before isomerization. Further experimentation was required to decide between the retinyl esters and retinols as the putative substrates and products of the isomerase.

An intriguing relationship was found to exist between endogenous retinyl ester synthesis and isomerization (Fulton & Rando, 1987). For example, chemically diverse reagents, such as ethanol, hydroxylamine, phenylmethanesulfonyl fluoride, and *p*-(hydroxymercuri)benzoate, all appear to inhibit both activities in a roughly parallel manner (Fulton & Rando, 1987). The biochemical basis of this relationship was uncovered with *all-trans*-retinyl α-bromoacetate as a specific inhibitor of retinyl ester synthesis (Trehan et al., 1990). In the pigment epithelial system, *all-trans*-retinyl α-bromoacetate does not inactivate the synthetase but rather is an exceedingly potent inhibitor of it, operating in the micromolar range. When pigment epithelium membranes are pretreated with this molecule and then with *all-trans*-retinol, there is virtually no (<5%) conversion of the vitamin to retinyl esters, and no 11-cis retinoids are generated. However, when the membranes were first treated with *all-trans*-retinol, producing largely *all-trans*-retinyl esters, and then were treated with inhibitor, 11-*cis*-retinol production proceeded close to control levels (i.e., with no added inhibitor). 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. Had a direct *all-trans*-retinol to 11-*cis*-retinol isomerization occurred, then pretreatment of the membranes with *all-trans*-retinyl α-bromoacetate should have actually enhanced the isomerization, as less substrate would have been lost to ester formation. Furthermore, it was also found that, in the presence of inhibitor, *all-trans*-retinyl esters were processed into 11-*cis*-retinol without the formation of 11-*cis*-retinyl esters. This experiment eliminates an ester–ester isomerization route from consideration. Therefore, the actual isomerization reaction in the retinal pigment epithelium converts the *all-trans*-retinyl ester directly into 11-*cis*-retinol (Deigner et al., 1989; Trehan et al., 1990). Although they are exceedingly hydrophobic and difficult to work with, it has been found that added *all-trans*-retinyl esters can also serve as substrates for the isomerase under appropriate conditions (Deigner et al., 1990).

Retinyl Ester Hydrolysis Drives the Isomerization Process

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 (Jencks, 1970). 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. The free energy of any complex chemical reaction can be calculated if it can be formally broken down into simpler chemical reactions whose free energy changes are known. As applied here, the energies sum as shown in Figure 6 to yield a possible mechanism by which ester hydrolysis could be linked to isomerization (Deigner et al., 1989). Here, the substrate would be an *all-trans*-retinyl ester; the product, 11-*cis*-retinol. The enzyme catalyzing the isomerization reaction is actually an isomerohydrolase rather than a simple isomerase.

The mechanism shown in Figure 6 makes at least two experimentally testable predictions. The first is that C–O bond cleavage must accompany the isomerization process. Second, the prochiral centers of the substrate and product are connected by an achiral intermediate, making it likely that there will be stereochemical consequences at C15 during isomerization.

The prediction of C–O bond cleavage was readily testable by studying the fate of the ^{18}O of [15-^{18}O]-*all-trans*-retinol after enzyme-catalyzed isomerization in $H_2{}^{16}O$-containing buffer by field desorption mass spectrometry (Deigner et al., 1989). This experiment demonstrated unequivocally that the 11-*cis*-retinol product formed contained only ^{16}O (Deigner et al., 1989). Hence, complete cleavage and replacement of the

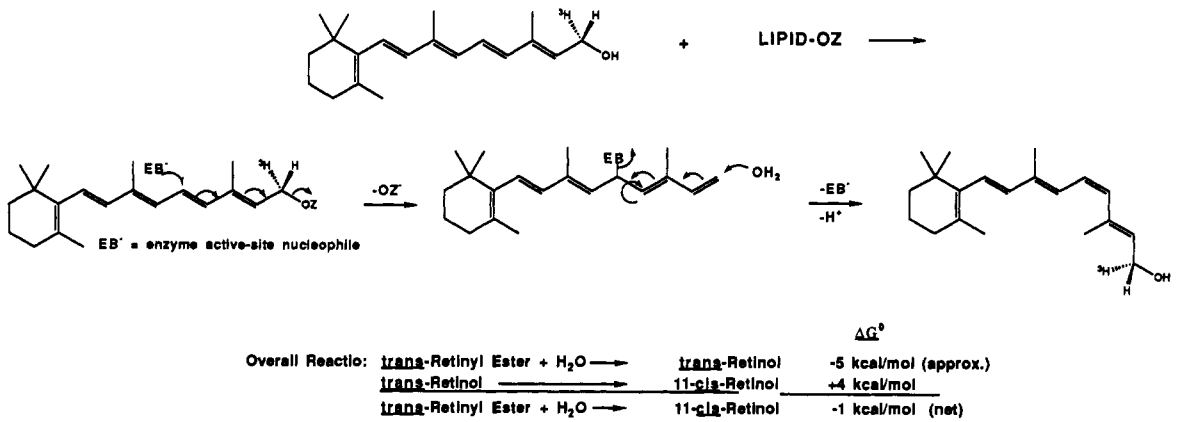

FIGURE 6: A coupled reaction can drive the thermodynamically unfavorable isomerization reaction. OZ refers to a hydrophobic acyl moiety.

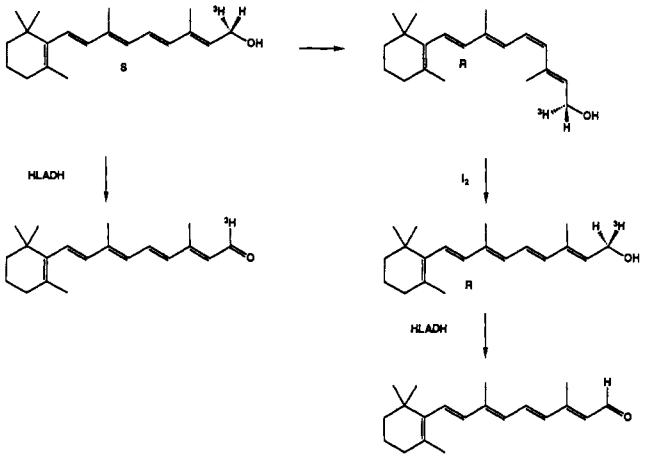

FIGURE 7: The isomerization reaction occurs by inversion of stereochemistry at the C15 prochiral center of vitamin A

C–O bond accompanies isomerization.

The ^{18}O release experiments are consistent with the type of mechanism shown in Figure 6. It was of interest to determine whether stereochemical retention or inversion occurs at C15 as a consequence of the isomerization reaction (Figure 7). To these ends, stereochemical experiments were performed with chirally labeled [15(*R*)-^{3}H]-[15(*S*)-^{3}H]-*all-trans*-retinol (Law & Rando, 1988). The labeled retinols, along with [15-^{14}C]-*all-trans*-retinol, were incubated with either amphibian or bovine pigment epithelial membranes (Law & Rando, 1988). At the end of the incubation period, the retinols and retinyl esters were collected and analyzed. The analyses of the isolated retinols and retinyl esters were performed as indicated in Figure 7. The enzymatically formed 11-*cis*-retinol was isomerized with I_2, which is, of course, without effect on the stereochemistry at C15, to produce *all-trans*-retinol. Then, by use of horse liver alcohol dehydrogenase (HLADH), it could readily be decided if inversion or retention of absolute configuration had occurred (Law & Rando, 1988). Within experimental error, complete inversion of configuration had occurred as a consequence of isomerization in both the bovine and amphibian cases (Law & Rando, 1988). The stereochemical inversion result is readily understandable in terms of the mechanism shown in Figure 6 and supports this mechanistic interpretation.

In Figure 6 an S_N2' mechanism is shown. However, no direct evidence for this kind of mechanism has been obtained, and other mechanistic possibilities must be considered (Figure 8). Several distinct mechanisms are possible, as long as they postulate intermediates higher in energy than 11-*cis*-retinol. Three other categories of mechanisms are shown in Figure 8. Carbonium ion mechanisms (A) are certainly possible, given the polyene nature of the retinols. C–H bond abstraction mechanisms (B) are also attractive candidates. Here the most likely candidate involves C–H cleavage at the C9 methyl group as C–H abstraction at the C13 methyl would not affect the C11–C12 double bond and abstraction at the C5 methyl or at the cyclohexyl ring would produce very stable intermediates. The last category shown in Figure 8 (C) involves σ-tropic rearrangements, in which the ester moiety "walks" down the polyene backbone. Although we cannot at present distinguish among the different mechanisms, structure–activity studies with various retinoids bear on this issue (Law et al., 1988; Cañada et al., 1990). In Figure 9 are shown a series of dihydroretinoids, demethylretinoids, and vitamin A_2. All of the retinoids shown are initially processed to *all-trans*-retinyl esters prior to any possible isomerization reaction. None of the dihydroretinoids is isomerized, demonstrating that an intact polyene chain is critical for activity (Law et al., 1988). These observations are perhaps most consistent with a carbonium-type mechanism shown in Figure 8. Furthermore, the dehydroretinoid vitamin A_2 was isomerized after esterification. This is interesting because vitamin A_2 is an important visual chromophore in early amphibian development (Law et al., 1988).

The demethylated series shown in Figure 9 are of interest with respect to possible C–H abstraction mechanisms. The esters formed from 5-demethyl-*all-trans*-retinol and 13-demethyl-*all-trans*-retinol are isomerized, eliminating possible C–H bond abstraction mechanisms at C3, C5, and C13 (Figure 9) (Cañada et al., 1990). Interestingly, 9-demethyl-*all-trans*-retinol (Figure 9) was not isomerized after esterification, which leaves open the possibility that C–H abstraction at C9 is important (Cañada et al., 1990). However, this mechanism is rendered highly unlikely, because, when the isomerization reaction was carried out with unlabeled retinoids in D_2O, no deuterium incorporation into the generated 11-cis retinoids was found (Cañada et al., 1990). Moreover, isomerization of 9,9,9-trideuterio-*all-trans*-retinol, after esterification, proceeded at the same rate as that of *all-trans*-retinol, after esterification, requiring that if C–H abstraction occurs, it is not rate limiting (Cañada et al., 1990). These results, when taken together, render it unlikely that a C–H abstraction mechanism is important in isomerase action.

LRAT AS THE ENERGY TRANSDUCER

Irrespective of the precise isomerization mechanism, the results described above are completely in accord with the mechanism shown in Figure 6, in which the free energy of hydrolysis of the *all-trans*-retinyl esters drives the isomerization process. It was of substantial interest then to determine how

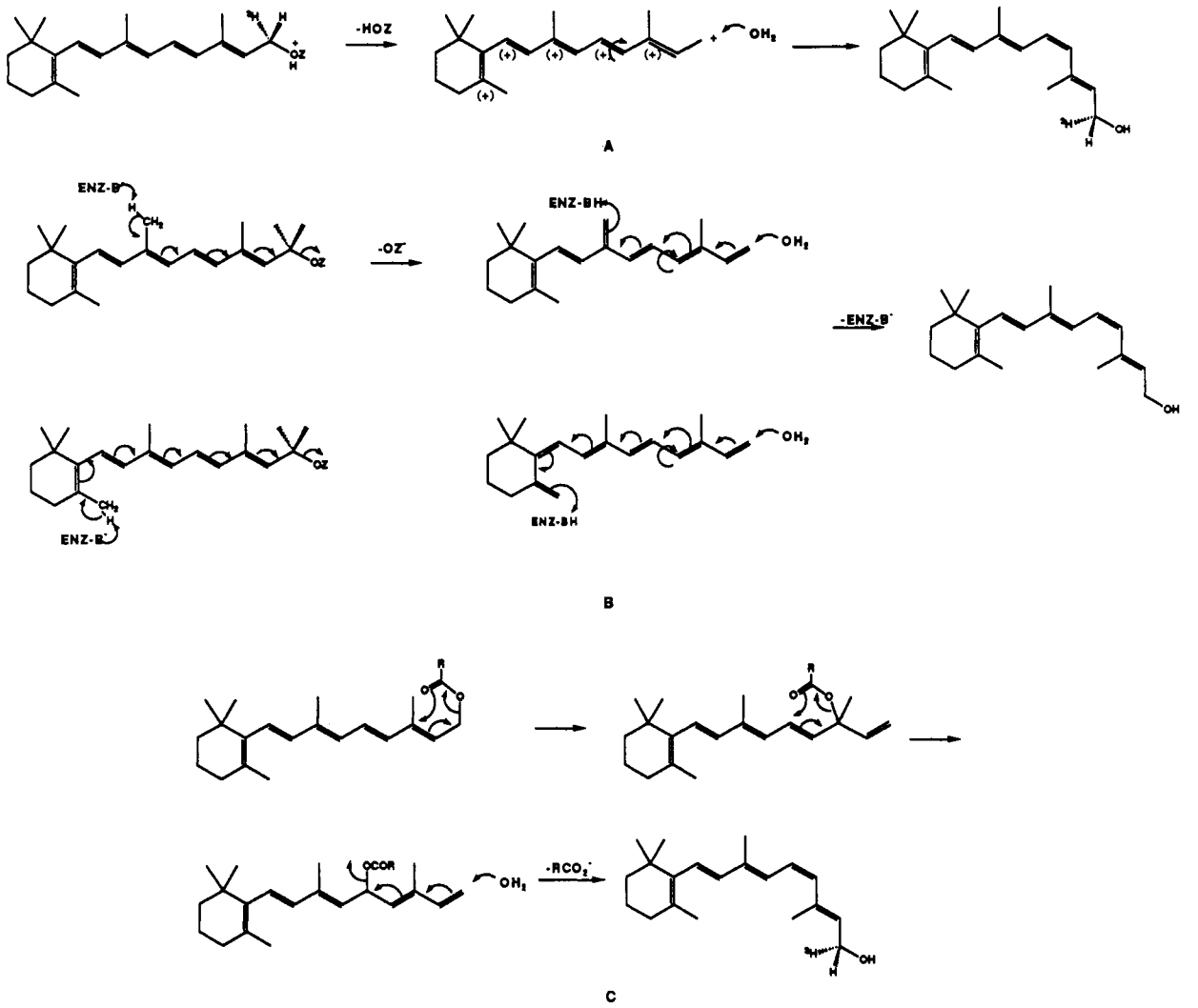

FIGURE 8: Possible isomerase mechanisms.

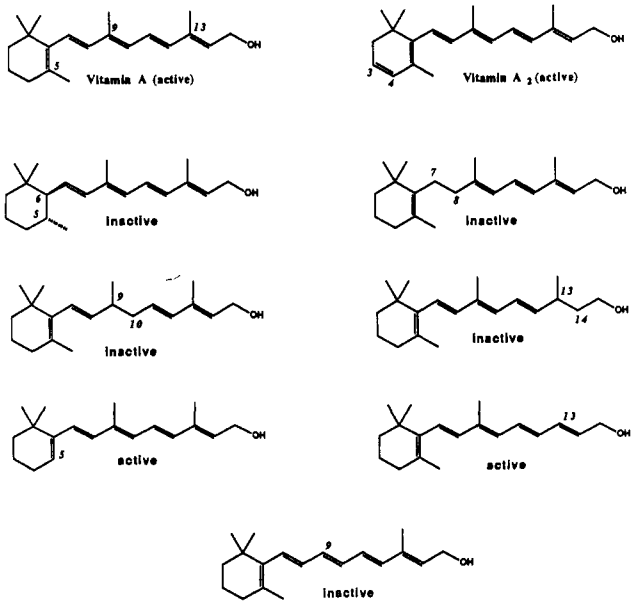

FIGURE 9: Structure–activity studies on the isomerase.

retinyl esters are formed in the pigment epithelial membranes. As previously mentioned, PE membranes are capable of producing retinyl esters from added retinols in the absence of added acylating agents, such as fatty acyl coenzyme A derivatives. This result, first discovered by Krinsky (1958),

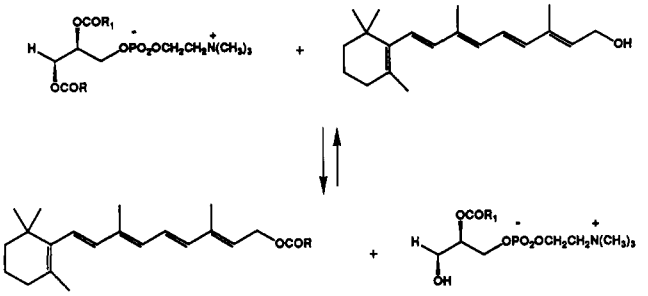

FIGURE 10: Transesterification of retinol with phosphatidylcholine (lecithin) catalyzed by the enzyme LRAT.

suggests that the membranes themselves contain the acyl donors. Solubilization and partial purification of the retinyl ester synthetase readily revealed the source of the acyl donor. It was discovered that the acyl donor for retinyl ester synthesis is the membrane constituent lecithin (phosphatidylcholine) and that the transfer occurs from the 1-position of the phospholipid to the retinol, producing a retinyl ester and a 2-acyl lysophospholipid (Figure 10) (Barry et al., 1989). Hence, the enzyme is a lecithin retinol acyl transferase (LRAT). Similar conclusions were drawn by Saari and Bredberg (1989), working with crude membrane fractions. Thus, the retinyl ester synthetase operates by a transesterification route similar to that found in the liver (MacDonald & Ong, 1988). The LRAT found in the retinal pigment epithelium strongly prefers lecithin as substrate over other phospholipids, such as phos-

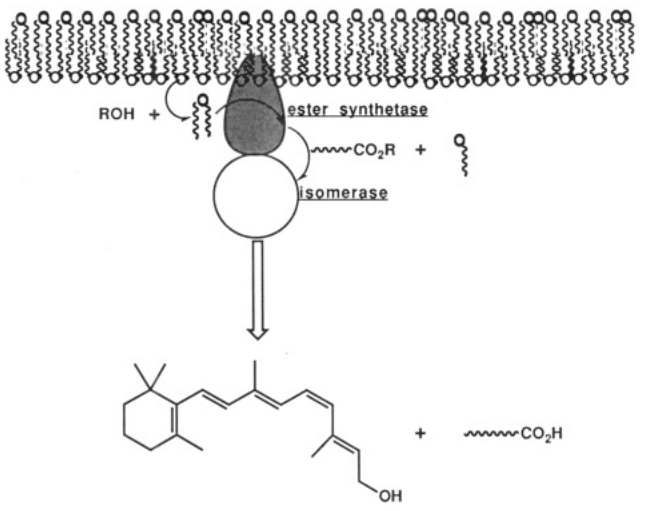

FIGURE 11: Biosynthesis of 11-*cis*-retinol showing the involvement of membrane phospholipids. The LRAT and isomerase enzymes are shown to be associated here for the sake of simplicity, although no direct evidence bears on this issue.

phatidylethanolamine or phosphatidylserine (Cañada et al., 1990). Moreover, vitamin A like compounds are strongly preferred as substrates over other hydrophobic alcohols, including 1-dodecanol and cholesterol (Cañada et al., 1990). However, within the vitamin A series, little preference is found with respect to stereochemistry or backbone substitution pattern (Cañada et al., 1990). It should be noted that this enzyme appears to be, in some ways, similar to lecithin cholesterol acyl transferase (LCAT) (Jauhiainen et al., 1988). This latter soluble enzyme transfers an acyl group from the 2-position of lecithin to cholesterol to produce cholesterol esters (Jauhiainen et al., 1988). Both enzymes are irreversibly inhibited by similar group-specific reagents such as the sulfhydryl alkylating agents, including the arsenicals, and serine acylating agents, such as diisopropyl fluorophosphate and phenylmethanesulfonylfluoride. The purification of LRAT will enable us to determine how deep the similarities of LCAT are.

The finding that the retinyl esters are produced with lecithin as an acyl donor shows that this membrane constituent is being used in a group transfer reaction much like ATP. Therefore, the energy to drive the endergonic cis to trans isomerization process in the visual cycle originates in the membrane itself. A scheme for the use of membrane phospholipids in this process is shown in Figure 11. In this figure, the lecithin and the retinyl esters play a dynamic role in the operation of the visual cycle. 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. The idea that the retinyl esters play a mere passive role as a storage form of retinol must be discarded and replaced with a vision of them in a critical dynamic role as the precursors of the 11-cis retinoid chromophores.

On the basis of the results discussed above, our current understanding of the visual cycle is shown in Figure 12. Upon bleaching, the *all-trans*-retinal is reduced by a *pro-R*-specific dehydrogenase in the rod outer segments to produce *all-trans*-retinol (Law et al., 1988). The *all-trans*-retinol travels to the pigment epithelium, probably bound as a complex with the interphotoreceptor retinoid binding protein (IRBP) (Adler & Evans, 1985; Chader et al., 1983). The *all-trans*-retinol is esterified via LRAT action in the pigment epithelium, and the retinyl ester is processed to 11-*cis*-retinol by the isomerase (Bernstein et al., 1987). Oxidation of the 11-*cis*-retinol to 11-*cis*-retinal occurs by means of a *pro-S*-specific dehydrogenase (Law et al., 1988). The free 11-*cis*-retinal(ol) is probably found complexed to cellular retinal(ol) binding protein (CRALBP) (Saari et al., 1984; Bok, 1985). Finally, the 11-*cis*-retinal is delivered to the rod outer segments, probably complexed to IRBP, producing rhodopsin and thus completing the visual cycle (Jones et al., 1989).

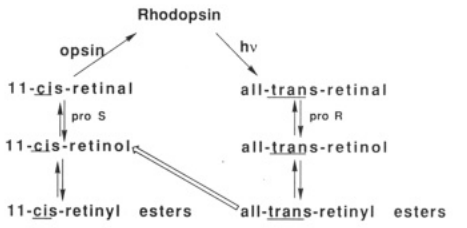

FIGURE 12: Current model of the visual cycle.

Conclusions

The studies described above demonstrate that, at least in the retinal pigment epithelium, membrane phospholipids are utilized in group transfer reactions to provide energy to drive an otherwise thermodynamically unfavorable process—in this case the thermodynamically uphill isomerization of all-trans-retinoids to 11-cis retinoids. Simple solutions to the problem, such as that entailed in the retinal isomerase conjecture, could not be and are not correct because they do not provide a mechanism for energy transduction. Unlike retinol, retinal does not contain a group that can be activated by a group transfer reaction, and hence, retinal cannot be activated in a thermodynamically fruitful way. Many intriguing questions remain to be solved. With respect to the isomerization process, it will be important to determine what the actual mechanism of the isomerase is. Several mechanistic possibilities have been considered. Which if any of these possibilities apply will have to be determined. Moreover, the exact relationship that holds between the ester synthetase and the isomerase needs to be further elucidated, as does the relationship among the remaining enzymes of the visual cycle. The isomerohydrolase and LRAT will have to be purified and sequenced to determine their possible relationships to other known enzymes. Knowing the primary sequences of these enzymes will be important in determining whether any of the important diseases of vision might find an etiology in mutations of these enzymes.

The lecithin-dependent energy transduction mechanism could be of general interest as a new mechanism of energy transduction in membranes, with phospholipids being used as an energy source. The mechanism also establishes a hitherto unsuspected role for membranes. An important issue to address further is how general this mechanism might be. It would be surprising if membrane phospholipids were only used as an energy source in this one instance. In particular, it will be interesting to determine whether acyl transferase enzymes, such as LRAT, are widely distributed in various tissues and whether these transfer reactions are involved in energy utilization. Since LRAT strongly prefers phosphatidylcholine as a substrate, it will be of interest to determine if this particular phospholipid is turned over more rapidly than the others in various cell types. Rapid phosphatidylcholine turnover would be predicted as a necessary outcome of it being metabolically active as an energy source.

On a more speculative level, it is interesting to consider the possibility that phospholipids were the original energy sources during evolution and that the process occurring in the visual cycle is a remnant of this. There are several attractive features about the concept of phospholipids being utilized as primordial

energy sources. First, a membrane would be initially required anyway before a primitive cell could form. Second, hydrophobic membrane-forming materials containing ester groups could be spontaneously generated without the expenditure of energy in an appropriately and randomly generated hydrophobic environment. This is because it is principally 55 M H_2O that causes the hydrolysis of acyl esters to be exothermic. In a hydrophobic environment, acyl esters will form spontaneously from alcohols and carboxylic acids (Pernas et al., 1990). The spontaneous generation of phosphoryl esters, such as those found in ATP, is not so simple, because the thermodynamically favored hydrolysis of these esters is importantly dependent on intrinsic chemical factors, as well as on the high concentrations of H_2O. Of course, once the phosphoryl esters were formed, they would be expected to be the favored currency of the cell over phospholipids, because of their increased solubility in H_2O, in addition to their greater chemical versatility in group transfer processes.

ACKNOWLEDGMENTS

I gratefully acknowledge the important contributions of Dr. Robert Barry, Dr. Paul S. Bernstein, Dr. Francisco J. Cañada, Dr. Andrew Chang, Dr. Peter Deigner, Dr. Brian S. Fulton, Dr. Wing C. Law, Dr. Achla Trehan, and Mr. John Lichtman to the research described here.

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