Mechanism of Isomerization of 11-*cis*-Retinal in Lipid Dispersions by Aromatic Amines†

Brian S. Fulton and Robert R. Rando*
Department of Pharmacology, Harvard Medical School, Boston, Massachusetts 02115
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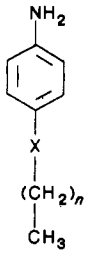
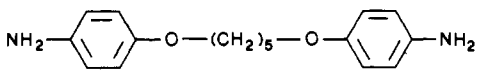
ABSTRACT: It has previously been shown that retinotoxic, primary aromatic amines catalyze the isomerization of 11-*cis*-retinal to its all-trans congener after Schiff base formation [Bernstein, P. S., Fulton, B. S., & Rando, R. R. (1986) *Biochemistry 25*, 3370–3377]. This process led to the short-circuiting of the visual cycle and the observed retinotoxicity when it occurred in vivo. The catalysis was also observed to occur in vitro in phosphatidylcholine-based vesicles but not in hydrocarbon solutions. The rate of isomerization of an aromatic amine Schiff base of 11-*cis*-retinal in the phospholipid vesicles was typically 10^3-fold more rapid than in hydrocarbon solutions. In this article, the mechanistic basis of this apparently membrane-specific catalysis is described. It was found that the rate enhancement effect observed was independent of the lipid used. Moreover, a bilayer structure was not important because rate enhancements were also observed in micelles. The rapid isomerization rates observed in lipid dispersions appear not be free radical initiated because free radical quenching agents, such as α-tocopherol and β-carotene, had little effect on the isomerization rates. It was further found that aliphatic amines, such as *n*-dodecylamine, could be substituted for the aromatic amines in phospholipid. Finally, and most importantly, it was found that the isomerization of the aromatic amine retinal Schiff bases in phospholipid vesicles was acid-catalyzed. It is concluded that the rate enhancements observed for the isomerization of 11-*cis*-retinal–aromatic amine Schiff bases in lipid dispersions over that in hydrocarbon solvents are due to the occurrence of acid–base catalysis in the former.

Aromatic amines are retinotoxic and function by causing the depletion of all 11-*cis*-retinoids in the retina and pigmented epithelium (Bernstein & Rando, 1985; Bernstein et al., 1986b). Structure–activity studies show that virtually any primary aromatic amine of the structural type **1** causes the depletion



1, $n = 1$–5; X = O, CH_2 (substitution can also be meta)

of 11-*cis*-retinoids in the eye (Bernstein et al., 1986a). In addition, the antischistosomal drug 1,5-bis(*p*-aminophenoxy)pentane (DAPP) (**2**) is also a powerful retinotoxic drug



2, DAPP

that functions physiologically in a way identical with the other aromatic amines (Bernstein & Rando, 1985).

The relatively broad structural requirements for an active retinotoxic drug suggest a general chemical role for these molecules in vivo rather than a specific drug–receptor-mediated event (Bernstein et al., 1986b). In vivo structure–activity studies show that a primary amine is required for activity. Hence, Schiff base formation between the amine and a retinal was suggested to be important in the mechanism of action of these drugs (Bernstein et al., 1986a). This possibility was further supported by the detection of the appropriate Schiff bases formed between different retinotoxic aromatic amines and retinal in the eyes of animals injected intraperitoneally with the drugs (Bernstein et al., 1986a). Furthermore, it was found that several aromatic amines, when combined with 11-*cis*-retinal in PC MLV,[1] catalyzed the thermodynamically downhill isomerization of 11-*cis*-retinal to its all-trans congener (Bernstein et al., 1986b). In further studies it was found that only those aromatic amines capable of Schiff base formation with retinal were isomerization catalysts (Bernstein et al., 1986a).

It had been proposed that aromatic amines short-circuit the visual cycle by catalyzing the isomerization of 11-*cis*-retinal to its all-trans congener in vivo, effectively depleting the endogenous retinoid crucial for the visual process (Bernstein et al., 1986b). 11-*cis*-Retinal is bound via a Schiff base to the active site lysine of opsin, forming rhodospin (Wald, 1968). When rhodopsin interacts with light, the 11-*cis*-retinal Schiff base chromophore is isomerized to its all-trans congener, which is liberated as *all-trans*-retinal (Wald, 1968). In order for vision to proceed, 11-*cis*-retinal must be resynthesized in the eye and maintained for a long enough time for combination with opsin to occur. The retinotoxic aromatic amines rapidly isomerize the stores of 11-*cis*-retinal, thus preventing pigment regeneration from occurring.

The catalysis of the isomerization of 11-*cis*-retinal by aromatic amines in PC MLV is quite profound. At 65 °C in *n*-heptane, the first-order rate of isomerization of 11-*cis*-retinal

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* Author to whom correspondence should be addressed.

[1] Abbreviations: BHT, 2,6-di-*tert*-butyl-4-methylphenol; DAPP, 1,5-bis(*p*-aminophenoxy)pentane; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; HPLC, high-performance liquid chromatography; MLV, multilamellar vesicles; PC, egg phosphatidylcholine; PE, egg phosphatidylethanolamine; SDS, sodium dodecyl sulfate.

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is 2.4×10^{-6} s^{-1} (Lukton & Rando, 1984a). Under the same conditions, the isomerization rate of the *p*-(*n*-hexyloxy)aniline Schiff base is even slower, at 1.5×10^{-6} s^{-1} (Bernstein et al., 1986a). However, the latter Schiff base is isomerized at a rate of 1.4×10^{-4} s^{-1} at 37 °C in PC membranes, a factor of approximately 10^3 faster than the extrapolated rate at the same temperature in *n*-heptane (Bernstein et al., 1986a). The studies described here were designed to probe the mechanistic basis of this apparently membrane-dependent isomerization rate enhancement.

Materials and Methods

Chemicals. All chemicals were purchased from Aldrich, Sigma, or Fluka unless otherwise mentioned. All lipids were purchased from Avanti Biochemicals and stored at −80 °C under argon unless otherwise noted. The phospholipids gave one spot by thin-layer chromatography (silica gel 60, 65:25:4 chloroform–methanol–water, sulfuric acid spray), and egg phosphatidylcholine was ninhydrin-negative. Cholesterol, *n*-dodecylamine, and *p*-(*n*-hexyloxy)aniline were recrystallized from warm methanol, warm ethanol, and warm 50% ethanol, respectively. Diethyl ether was distilled from sodium metal/benzophenone, and heptane was distilled from lithium aluminum hydride.

Multilamellar Vesicle Preparation and Kinetic Measurements. All procedures were conducted under dim red light with samples kept on ice. All Schiff bases were preformed and checked for Schiff base formation as previously described (Lukton & Rando, 1984b). To a solution of Schiff base or 11-*cis*-retinal in heptane was added the appropriate lipid in chloroform as well as other reagents such as radical inhibitors, and the solvents were removed in vacuo to give a thin film. The film was dried under reduced pressure for 10 min. To the film was added 40 mM phosphate buffer (pH 7.35) to give a final concentration of 10 μM in retinoid and 250 μM in lipid. The suspension was vortexed for 1 min and divided into two equal portions for duplicate runs. When the lipid was dimyristoyl- or dipalmitoylphosphatidylcholine, the suspension was incubated at 37 or 46 °C, respectively, before vortexing. The liposome suspensions were then equilibrated in septum-capped culture tubes at 37 °C for 10 min.

At appropriate intervals, aliquots were removed, and the reaction was quenched with 1 M hydroxylamine (pH 6.5) in a test tube on ice. The retinal oximes were extracted with an equal volume of heptane and the geometric isomers analyzed by HPLC with detection at 350 nm by standard procedures (Groenendijk et al., 1980a). After 24 h at 37 °C, less than 0.5% 11-*cis*-retinal Schiff base remained. Starting with *all-trans*-retinal Schiff bases gave no detectable amounts of the 11-cis congener.

The isomeric ratios were measured from the *syn*-oxime peak areas, after correction for the relative extinction coefficients. The peak areas were integrated by use of an Apple IIe–Anadata Chromcard II system interfaced with a Waters Lambda-Max Model 480 LC spectrophotometer.

The first-order rate constants were calculated from plots of percent 11-*cis*-*syn*-oxime of the total syn-oximes vs. time on a semilog graph. A best fit line was determined by linear regression analysis. It has been noticed that there is some variation in measured rate constants, depending on the batch of PC used. All the kinetic studies reported in this article with PC MLV were performed with a single batch of highly pure PC. In certain experiments, where noted, aged or oxidized PC was used with strikingly different kinetic results. Thus, PC was stored at −80 °C under argon with aged or oxidized PC being stored at −20 °C under air.

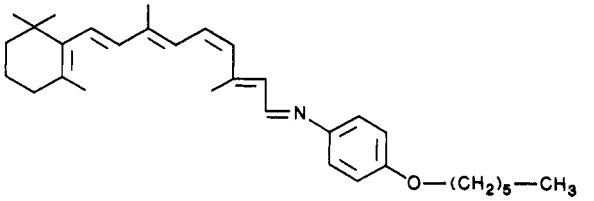
Isomerizations in Micelles. Isomerizations in Triton X-100 were conducted as above except that retinoid in 500 μL of absolute ethanol was added to 300 μM Triton X-100 to give 12 μM in retinoid. Isomerizations in SDS were conducted by adding a 3.0 mM solution of SDS (phosphate buffer, pH 7.35) to a film of retinoid in a round-bottom flask, yielding a final concentration (after vortexing) of 10 μM in retinoid. Assays were then performed as above.

Analysis of Egg PC MLV and Schiff Base Hydrolysis. The relative extent of oxidation of egg PC was qualitatively determined by two methods. The presence of peroxides in the chloroform stock solutions of egg PC was determined colorimetrically (Gordon & Ford, 1972) by adding 0.5 μmol of the PC to a crystal of potassium iodide in 20 μL of glacial acetic acid. The oxidized PC solution turned yellow, indicating a low peroxide content, while the fresh PC solution remained colorless. The second method was based on previously published methodology (Klein, 1970). To a quartz cell containing 1 mL of absolute ethanol was added 10 μL of a 1.53 mM solution of PC in phosphate buffer (pH 7.25), to give a final concentration of 15.3 μM. The absorption spectrum was then recorded from 400 to 200 nm. The PC stored at −20 °C under air showed significant absorption at 235 and 270–280 nm, where polyene hydroperoxides absorb light, whereas fresh PC did not.

Extent of Schiff base hydrolysis was determined by reverse-phase HPLC on a Waters liquid chromatograph Model 440, with 100% methanol at 1 mL/min as elutant and a Merck 5-μm silica LiChrosorb RP-18 RT column (250 × 4 mm column), with detection at 405 nm. A 25-μL sample was removed from a 10 μM MLV suspension and diluted with 75 μL of HPLC-grade methanol. The total sample was then injected. The results are recorded in Figure 1.

Results

Effects of Lipid on Isomerization of 11-cis-Retinal–p-(n-Hexyloxy)aniline Schiff Base (**3**). In the studies reported here, Schiff bases of *p*-(*n*-hexyloxy)aniline were investigated as representative of the class of retinotoxic aromatic amine 11-*cis*-retinal conjugates **3** that undergo facile isomerization



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when incorporated into PC-based vesicles. Initial experiments were conducted to determine the possible role of the lipid environment in this catalysis (Table I). As can be seen in Table I, virtually any lipid will catalyze the isomerization of **3**, as compared to the rate in hydrocarbon solvents, although phospholipids containing unsaturated fatty acids appeared to be somewhat more effective than saturated phospholipids. The bulk state of the lipid matrix did not appear to be important, as evidenced by the results in DMPC and DPPC at temperatures above and below their main phase transitions. Moreover, a bilayer structure was not required, as shown by the fact that catalysis also occurred in Triton X-100 and SDS micelles.

It should be noted however that rate differences were observed between PC and oxidized PC (Materials and Methods) when **3** was formed from 11-*cis*-retinal and a small excess of *p*-(*n*-hexyloxy)aniline. The basis of the difference between aged, oxidized PC and fresh PC with respect to their abilities

Table I: Thermal Isomerization of 11-*cis*-Retinal and **3** in Various Lipids

retinoid	T (°C)	lipid added	k_1 ($\times 10^{-4}$ s^{-1})
11-*cis*-retinal	37	*n*-hexane	<0.001
11-*cis*-retinal	37	PC	0.065
11-*cis*-retinal	37	PC (oxidized)	0.063
11-*cis*-retinal	37	dimyristoyl-PC	0.065
11-*cis*-retinal	37	dipalmitoyl-PC	0.093
11-*cis*-retinal	37	dioleoyl-PC	0.084
11-*cis*-retinal	37	dilinoleoyl-PC	0.077
11-*cis*-retinal	37	triton X-100	0.034
11-*cis*-retinal	37	sodium dodecyl sulfate	0.07
3	37	PC	1.6[a]
3 (1 equiv of amine)	37	PC (oxidized)	0.2
3 (5 equiv of amine)	37	PC (oxidized)	2.0
3	37	dimyristoyl-PC	0.62
2	20	dimyristoyl-PC	0.31[b]
3	37	dipalmitoyl-PC	0.56
3	43	dipalmitoyl-PC	0.99
3	37	dioleoyl-PC	1.6
3	37	dilinoleoyl-PC	2.5[b]
3	37	Triton X-100	0.13
3	37	sodium dodecyl sulfate	1.27

[a] The isomerization followed first-order kinetics for at least four half-lives at which time a 92% decrease in initial percent 11-*cis*-retinoid had occurred. [b] Rate constants based on initial rate measurements.

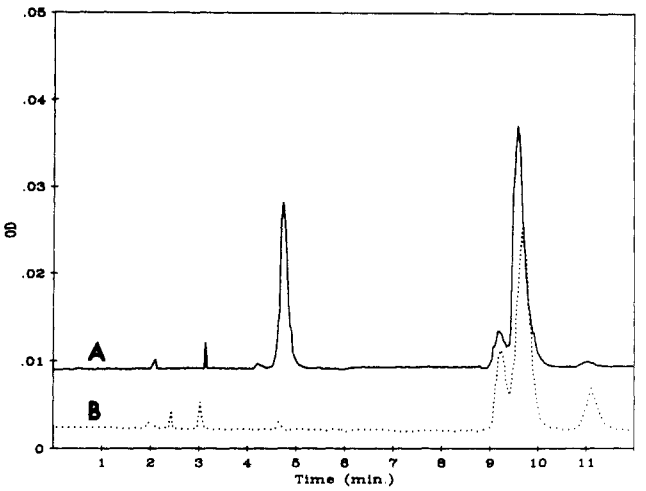

FIGURE 1: Reverse-phase HPLC analysis of Schiff base hydrolysis in oxidized PC liposomes. The extent of Schiff base hydrolysis was determined as described under Materials and Methods. HPLC trace A is of a 1:1 mixture of (*n*-hexyloxy)aniline to 11-*cis*-retinal, and trace B is of a 5:1 mixture. Coinjection of 11-*cis*-retinal or **3** coeluted with the peaks at 4.8 and 9.8 min, respectively. Detection was at 405 nm, with separation being performed on a Merck RP-18 LiChrosorb column eluted with 100% methanol at 1 mL/min.

to catalyze the isomerization of **3** was further explored. First of all, it was found that addition of excess aromatic amine to **3** in PC MLV did not substantially enhance the rate of isomerization of **3** (Bernstein et al., 1986a), whereas the opposite was true in oxidized PC. The maximum rate of isomerization observed for **3** in oxidized PC was only obtained after the addition of about 5 equiv of aromatic amine to 11-*cis*-retinal in oxidized PC. This suggested the possibility that hydrolysis of **3** had occurred in oxidized PC. To study this possibility, the amount of **3** in oxidized PC vesicles was determined by reverse-phase HPLC when 1 and 5 equiv of aromtic amine were added. This study showed that hydrolysis of the Schiff base to 11-*cis*-retinal had indeed occurred when only 1 equiv of amine was added (Figure 1A). When 5 equiv of aromatic amine was used, hydrolysis was not observed because the excess amine re-formed the Schiff base (Figure 1B). Very little, if any, hydrolysis of **3** in fresh PC was found in separate experiments.

Table II: Effect of Free Radical Traps on Rates of Isomerization of **3** in PC-Based MLV

retinoid	T (°C)	phospholipid	compound added[a]	k_1 ($\times 10^{-4}$ s^{-1})
3	37	PC MLV		1.6
3	37	PC MLV	α-tocopherol	2.6
3	37	PC MLV	α-tocopherol acetate	1.2
3	37	PC MLV	β-carotene	0.86
3	37	PC MLV	BHT	0.87
3	37	PC MLV	cholesterol	0.67
3	37	PC (oxidized) MLV + 5 equiv of amine		2.0
3	37	PC (oxidized) MLV + 5 equiv of amine	α-tocopherol	2.0

[a] A 10-fold excess of all radical inhibitors was used while a 5-fold excess of cholesterol was used.

Table III: Thermal Isomerization of 11-*cis*-Retinal by Aliphatic Amines[a] and Lipids

amine (equiv)	T (°C)	lipid added	k^1 ($\times 10^{-4}$ s^{-1})
	37	PC	0.065
n-dodecylamine (0.5)	37	PC	0.49
n-dodecylamine (1.0)	37	PC	0.69
n-dodecylamine (5.0)	37	PC	2.35
n-octylamine (5.0)	37	PC	0.14
PE (0.5)	37	PC	1.47
PE (1.0)	37	PC	1.94
PE (5.0)	37	PC	1.74
PE + *p*-(*n*-hexyloxy)aniline (1:1)	37	PC	1.20
N-methyl-PE (1.0)	37	PC	2.57
N,*N*-dimethyl-PE (1.0)	37	PC	0.037
PS[b] (5)	37	PC	0.72
PS (5) + *p*-(*n*-hexyloxy)aniline (2.0)	37	PC	2.0

[a] Schiff bases from *n*-dodecylamine and *n*-octylamine were preformed as described under Materials and Methods. [b] PS = bovine brain phosphatidylserine.

Free Radical Quenchers and Isomerization of **3** *in PC MLV.* A possible mechanism for the enhanced isomerization of **3** in PC vesicles over that in hydrocarbon solvents involves a free radical initiated mechanism. To explore this possibility, the effects of well-established lipid-soluble free radical quenchers α-tocopherol, β-carotene, and BHT on the rate of isomerization of **3** were studied. As can be seen in Table II, none of these quenchers led to substantial effects on the basal rate of isomerization of **3**. The slight rate depressions observed with β-carotene and BHT may simply be due to a lipid perturbation effect, since cholesterol also produced a small negative effect on the rate (Table II).

Isomerization of 11-cis-Retinal by Aliphatic Amines in the Presence of Phospholipids. Given that aromatic amines markedly catalyzed the rates of retinal isomerization in phospholipid MLV, it was of interest to determine what effect aliphatic amines might have. The effects of a variety of aliphatic amines on the isomerization rates are given in Table III. These results show that aliphatic amines are about as potent as aromatic amines in their ability to catalyze the isomerization of 11-*cis*-retinal in MLV. It has been previously shown that PE can catalyze this isomerization, and these results are confirmed here (Groenendijk et al., 1980b). It can also be inferred from Table III that Schiff base formation between the retinal and the amine is crucial for catalysis, a

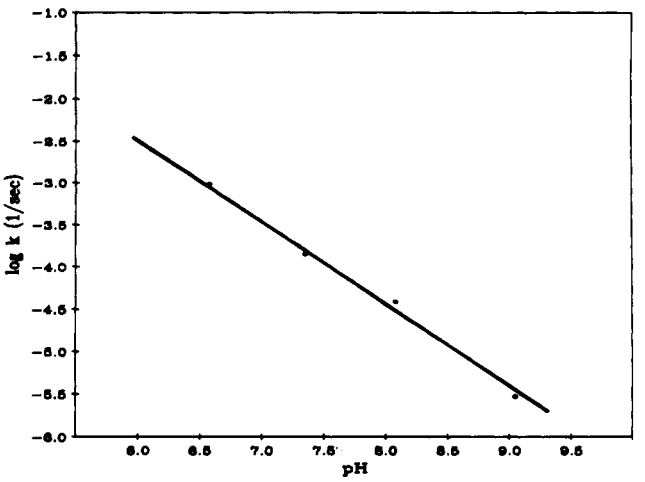

FIGURE 2: Effect of pH on rate of isomerization of **3** in PC MLV. The first-order rate constants for the isomerization of **3** in PC MLV were determined at pH 6.55 (40 mM phosphate), 7.35 (40 mM phosphate), 8.09 (40 mM phosphate), and 9.06 (40 mM borate buffer). The rate constant determined at pH 6.55 was based on initial rate measurements.

point already clear with the aromatic amines (Bernstein et al., 1986a).

Acid Catalysis of Isomerization of **3** *in PC MLV.* Since the isomerization of aromatic and aliphatic amine retinal Schiff bases is acid–base-catalyzed in organic solvents (Lukton & Rando, 1984a, 1984b), it is important to determine if the same holds true for the isomerization of **3** in PC MLV. An important question here is whether the expected low pK_A of the protonated form of **3** would render this a moot point. The pK_A of the protonated form of *n*-dodecylamine–*all-trans*-retinal Schiff base in Triton X-100 micelles has been measured to be 5.21 (DePont et al., 1970). Using the same system, we measured the pK_A of the protonated form of **3** to be between 2 and 3. The instability of the Schiff bases at low pH did not permit an accurate determination here. Nevertheless, first-order constants for the isomerization of **3** in PC MLV at various pHs was measured (Figure 2). As can been seen, a clear effect of acid concentrations between pHs of 6–9 was observed, with the rates increasing in a linear fashion with the concentration of acid. This means that the probable reason for the enhancement of the isomerization rate of **3** in aqueous lipid dispersions over hydrocarbon solvents is the possibility of acid–base catalysis in the former.

Discussion

The purpose of the study reported here is to determine why molecules such as **3** undergo facile isomerization in PC vesicles but not in organic solvents such as *n*-hepane. The study began by probing the possible role of lipid in this phenomena. The Schiff base **3** was incorporated into MLV and micelles of varying lipid type, and its rate of isomerization was studied (Table I). It is apparent that a specific effect of lipid is absent here. In addition to egg PC, dioleoyl-PC and dilinoleoyl-PC also enhanced the isomerization rate, all to essentially the same degree. The isomerization rates were about 3-fold slower in saturated phospholipid, DMPC or DPPC, but again, a rate acceleration over that in hydrocarbon solvents was clearly observed (Table I). It was also noted that the isomerization rates were not strongly dependent on the presumptive bulk state of the saturated phospholipids. The rates in the two saturated phospholipids were quite similar to each other both above and below their main phase transition temperatures (24 °C for DMPC and 41 °C for DPPC) (Stillwell et al., 1982). Moreover, the enhanced isomerization rate was also observed in SDS and Triton X-100 micelles, although more strongly in the former perhaps due to a surface charge effect. Interestingly though, a significant difference was observed between freshly opened PC and that stored at −20 °C in air (Table I), and this could be related to the extent of oxidation of the latter.

The possibility of a free radical mediated isomerization process was entertained. This is especially attractive with aromatic amine Schiff bases because of the enhanced stability of the free radical intermediate brought about by the aromatic ring (Bernstein et al., 1986b). However, none of the lipid-soluble free radical quenchers tried had any marked effects on the isomerization rates (Table II). The small effects observed with β-carotene and BHT could be accounted for by general lipid perturbation effects. Similar small effects were also observed with cholesterol and α-tocopherol acetate, two molecules incapable of being radical quenchers. These results in aggregate are totally inconsistent with a mechanism requiring the intermediacy of a free radical species sufficiently long lived to be trapped. In addition, these results also eliminate the possibility that a radical initiator capable of being trapped might be involved in the isomerization process.

The fact that aromatic amine Schiff bases of 11-*cis*-retinal are not isomerized by a free radical initiated process would suggest that aromatic amines would not have a particular catalytic advantage over ther aliphatic counterparts. Indeed, we found that hydrophobic aliphatic amines such as *n*-octylamine and *n*-dodecylamine also catalyze the isomerization of 11-*cis*-retinal when incorporated into PC MLV (Table III), in contrast to previous studies (Groenendijk et al., 1980b). However, it is likely that if oxidized PC was used in these previously published studies, the Schiff bases formed between *n*-octadecylamine and retinal would have been hydrolyzed, resulting in an elimination of the rate enhancement observed here. As expected from previously published studies, PE catalyzed the isomerization of 11-*cis*-retinal in PC MLV (Groenendijk et al., 1980b). Interestingly, PE appeared to be more effective than *n*-dodecylamine, a result possibly due to intramolecular acid–base catalysis (Groenendijk et al., 1980b). It was also found here that *N*-methyl-PE was active but *N*,*N*-dimethyl-PE was not. This result was expected, given that Schiff base formation between retinal and the amine is crucial for catalysis (Lukton & Rando, 1984b).

The mechanism by which aliphatic amines catalyze the isomerization of 11-*cis*-retinal has been considered previously (Lukton & Rando, 1984b). Schiff base formation by itself does not cause significant rate enhancements; acid–base catalysis is required as well (Lukton & Rando, 1984b). Protonation of the Schiff base nitrogen is followed by nucleophillic attack at the double bond to be isomerized, which results in catalysis of the isomerization process.

The fact that hydrophobic aliphatic amines can catalyze the isomerization of 11-*cis*-retinal when membrane bound at about the same rate as the aromatic amines suggests that the mechanisms for the isomerization processes may be the same. However, in considering the possible mechanisms for the isomerization process, careful attention must be paid to the pK_A's of the protonated alphatic vs. aromatic amine Schiff bases. Whereas the protonated aliphatic amine–retinal Schiff bases have pK_A's of approximately 5, their aromatic counterparts have pK_A's in the neighborhood of 2–3. Nevertheless, it was important to determine if the isomerization of **3** in PC was sensitive to pH. Indeed, the isomerization rates were clearly dependent on the pH, with the rates strongly increasing as the pH was lowered (Figure 2). This means that the re-

action is acid and probably acid–base catalyzed by analogy with studies on the aliphatic amine–retinal Schiff bases. The fact that the slope of the pH vs. rate profile is approximately 1 is consistent with the idea that a single proton is involved during catalysis. This latter result is not unexpected, since only a single proton is required for protonation of the Schiff base. However, how does one reconcile the apparent disparity between the pK_A's of the protonated aliphatic and aromatic amine Schiff bases of retinal and their similar isomerization rates? For example, at pH 7.0 only approximately one part in 10^4 of the aromatic amine Schiff base would be protonated whereas one part in 10^2 of the aliphatic amine Schiff base would be protonated, assuming that the bulk pH and the effective pH at the membrane–buffer interface is the same. Studies on the pK_A's of membrane-bound protonated PE show that its pK_A is within approximately 1 unit of those measured for aliphatic amines in solutions (Tsui et al., 1986). The apparent paradox can be resolved when the reactivities toward isomerization of the aliphatic and aromatic amine Schiff bases are considered. Aromatic amine–retinal Schiff bases are isomerized 10^2–10^3 more rapidly than their aliphatic amine counterparts when equally protonated in chloroform solutions (Lukton & Rando, 1984b). What this factor might be in PC MLV is not known, although it is likely to be greater than that in chloroform solutions. Therefore, the decreased propensity of the aromatic amine Schiff bases to be protonated is counterbalanced by their increased reactivity toward isomerization when protonated.

An important objective of the exercise described here is to understand why aromatic amines in particular are retinotoxic. As shown here, both aliphatic and aromatic amines are capable of catalyzing the exothermic isomerization of 11-*cis*-retinal when incorporated into PC MLV, and hence, in principle, both should be retinotoxic. However, aliphatic amines are not retinotoxic and will not deplete 11-*cis*-retinoid pools in vivo (Bernstein et al., 1986a). Only aromatic amines were active in vivo. It is likely that the key here lies in the low pK_A's of protonated aromatic amines, which are in the 4–5 range. These low pK_A's ensure that the amines readily penetrate the blood–retina barrier, which is similar to the blood–brain barrier in excluding charged species. Protonated aliphatic amines, of course, have pK_A's in the 9–10 range. The additional fact that aliphatic amines are likely to be readily metabolized by the peripheral amine oxidases is probably also important here.

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Registry No. **2**, 2391-56-2; **3**, 105519-34-4; DMPC, 13699-48-4; DPPC, 2644-64-6; dioleoyl-PC, 10015-85-7; dilinoleoyl-PC, 6542-05-8; $NH_2(CH_2)_{11}CH_3$, 124-22-1; $NH_2(CH_2)_7CH_3$, 111-86-4; $NaO_3SO(CH_2)_{11}CH_3$, 151-21-3; Triton X-100, 9002-93-1; 11-*cis*-retinal, 564-87-4.

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