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Extrinsic Cotton Effects in Retinaldehyde Schiff's Bases†

Edward M. Johnston and Robert Zand*

ABSTRACT: Schiff's bases have been synthesized from *all-trans*-retinaldehyde and (*S*)-α-phenylethylamine, *S*-α-(1-naphthyl)-ethylamine, *R*-(+)-2,2′-dimethyl-6,6′-diaminobiphenyl, and poly-L-lysine. The Schiff's base of 11-*cis*-retinaldehyde with (*S*)-α-(1-naphthyl)ethylamine was also prepared. The absorption and circular dichroic spectra were measured for the parent compound and the protonated forms over the wavelength region of 190–700 nm. Extrinsic Cotton effects were observed in the region of the major retinaldehyde absorption band centered at about 360 nm. Protonation of the Schiff's base caused the absorption and circular dichroism band to shift to about 410 nm. The Schiff's base, N^{ϵ}-*all-trans*-retinylidene-L-lysine and *N*-*all-trans*-retinylidene-L-dipalmitoylphosphatidylethanolamine did not exhibit extrinsic Cotton effects in the region of retinaldehyde absorption. The results are pertinent to the origins of the observed Cotton effect in the visible wavelength region of rhodopsin.

The visual pigment rhodopsin exhibits a broad circular dichroic (CD) band in the region of 500 nm where the 11-*cis*-retinaldehyde component of rhodopsin has an absorption maximum. *all-trans*- and 11-*cis*-retinaldehyde are optically inactive molecules and the mechanism for the induction of the optical activity as well as the significance of the problem relative to the structure of rhodopsin is an unsolved problem.

In 1966, the CD spectrum of bovine rhodopsin was reported (Crescitelli *et al.*, 1966). The initial explanation that was considered and then rejected by these workers was that the optical activity arose from an extrinsic mechanism. The explanation advanced for the induction of optical activity into the retinaldehyde chromophore was based on the hypothesis that the binding of 11-*cis*-retinaldehyde to opsin was accompanied by a twisting of the aldehyde into an asymmetric conformation. The twisted chromophore concept was developed further (Mommaerts, 1969) by suggesting that 11-*cis*-retinaldehyde could exist in two enantiomeric forms (Figure 1). When the 11-*cis*-retinaldehyde is bound to opsin one enantiomer was thought to be preferentially stabilized and "frozen in" thereby providing the asymmetry necessary for optical activity. The concept of intrinsic optical activity in the retinaldehyde portion of rhodopsin has recently been treated more extensively (Honig *et al.*, 1973).

The extrinsic mechanism was reintroduced by Kito *et al.* (1968) to explain the observed Cotton effects in squid rhodopsin. This approach was developed further by Johnston and Zand (1972) and Waggoner and Stryer (1971). In this mechanism the optical activity may arise through a Kirkwood-type coupled oscillator mechanism (Kirkwood, 1937) so that the retinaldehyde transitions may become optically active by coupling with the transitions of nearby aromatic side chains.

The present study was undertaken with the view that spectral studies of Schiff's bases (Table I) derived from retinaldehyde and appropriate optically active amines would provide information that would help to establish the mecha-

† From the Biophysics Research Division, Institute of Science and Technology, and Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan 48105. *Received February 9, 1973.* This work includes material from a thesis submitted by E. M. J. in partial fulfillment of the requirements for the degree of Doctor of Philosophy. A preliminary report of this work has been published (Johnston and Zand, 1972). Supported in part by National Institutes of Health Grants NB-05036 GM-1355 and GM-14035.

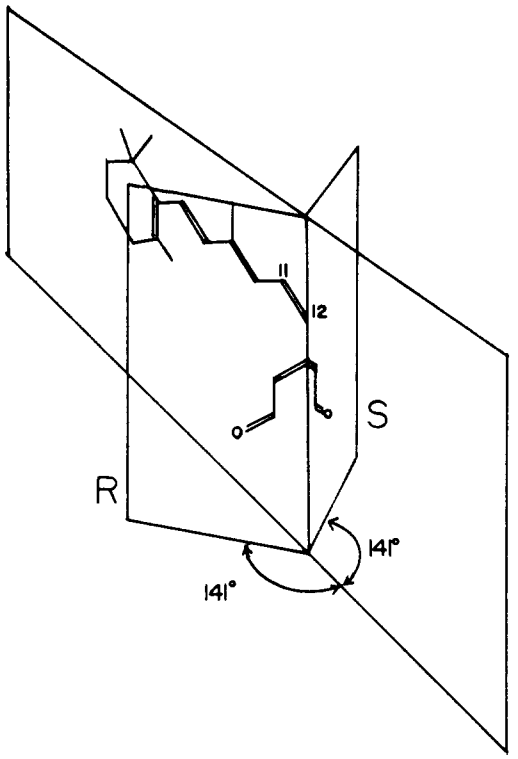

FIGURE 1: A representation of the two conformers of 11-*cis*-retinaldehyde.

nism by which optical activity was transmitted into the 11-*cis*-retinaldehyde chromophore. Additional information relating to the structure of opsin might also be provided by such spectral studies.

Materials and Methods

all-trans-Retinaldehyde was obtained from Eastman Kodak Co. Quantities of 11-*cis*-retinaldehyde were gifts from Paul Brown of Harvard University and from Hoffman-LaRoche Co., Nutley, N. J. The retinaldehydes were stored in the dark at −40° until needed. The amines, (−)-(*S*)-α-phenylethylamine and (−)-(*S*)-α-(1-naphthyl)ethylamine, were obtained from Norse Laboratories, Santa Barbara, Calif. Dipalmitoylphosphatidylethanolamine was obtained from Mann Research Laboratories of New York. The (+)-(*R*)-2,2′-dimethyl-6,6′-diaminobiphenyl was synthesized and resolved by the procedure reported by Meisenheimer and Höring (1927). Poly-L-lysine hydrobromide, mol wt 92,000, was obtained from Pilot Chemicals Inc., Watertown, Mass. The hydrochloride salt was prepared by exhaustive dialysis of the hydrobromide against 0.1 N HCl, followed by lyophilization. Absorption spectra were recorded on a Cary 15 spectrophotometer. CD and ORD spectra were recorded on a Jasco ORD/CD instrument incorporating the SS-20 modification.

(S)-N-all-trans-Retinylidene-α-(1-naphthyl)ethylamine. To 1 ml of dry chloroform was added 14.18 mg (0.050 mM) of *all-trans*-retinaldehyde and 11.18 mg (0.065 mM) of (*S*)-α-(1-naphthyl)ethylamine. The solution was allowed to stand overnight in the dark at room temperature. The reaction mixture was diluted 1515:1 with isooctane and absorption and CD spectra were recorded. Completion of the reaction was verified by diluting some of the original chloroform solution with more chloroform and bubbling in gaseous HCl. The 370-nm peak disappeared completely and a new peak formed

TABLE 1: Experimental Data: Dissymmetry Factors ($g = \Delta\epsilon/\epsilon$).

Amine Moiety	Solvent	Absorption λ_{max} (nm)	CD λ (nm)	CD $g \times 10^5$
A. Unprotonated Schiff's Bases between the Named Amine and *all-trans*-Retinal				
(*S*)-NEA[a]	Isooctane	360	354	+11.5
			302	0
			282	−12.7
(*S*)-NEA	$CHCl_3$	370	370	+12.1
			315	0
			293	−10.9
(*S*)-PEA	Isooctane	359	354	+4.5
			293	0
			254	−20.7
(*S*)-PEA	$CHCl_3$	369	378	+4.3
			320	0
			262	−17.5
DMAB	Isooctane	371	400	+52.2
			374	0
			346	−44.4
Poly-L-lysine (pH 11.46, α helix)	5.9% (v/v) methanol–water	335	375	+11.3
			350	0
			330	−10.6
B. Unprotonated Schiff's Base between the Named Amine and 11-*cis*-Retinal				
(*S*)-NEA	Isooctane	350	350 (not a peak; see spectrum)	+4.0
C. Protonated Schiff's Bases between the Named Amine and *all-trans*-Retinal				
			λ	$g \times 10^5$
(*S*)-NEA	$CHCl_3$	468	460	+7.5
(*S*)-PEA	$CHCl_3$	464	490	+3.3
			410	0
			273	−2.8
Poly-L-lysine (pH 2.0, U/EH)	7% (v/v) methanol–water	394	450	−9.0
			406	0
			370	+8.6
D. Amines alone				
(*S*)-NEA	Isooctane	282	293	−4.2
			286	−4.2
			269	0
		224	233	+1.8
			227	0
			208	−8.6
			200	0
(*S*)-PEA	Isooctane	258	262	+56.8
(*R*)-DMAB	Isooctane	291	300	+58.1
			294	0
			284	−91.3
PE	HFAS	218	210	+19.7

[a] Abbreviations used are: NEA, (*S*)-(−)-α-(1-naphthyl)-ethylamine; PEA, (*S*)-(−)-α-phenylethylamine; DMAB, (*R*)-(+)-2,2′-dimethyl-6,6′-diaminobiphenyl; PE, L-dipalmitoylphosphatidylethanolamine; HFAS, hexafluoroacetone sesquihydrate.

at 468 nm. The presence of unreacted retinal would have been revealed by a peak at 390 nm.

Protonated (S)-N-all-trans-Retinylidene-α-(1-naphthyl)ethylamine. The reaction was carried out as described for the preparation of the unprotonated form. It was then diluted with chloroform to a concentration suitable for spectral measurements. To this chloroform solution was added several drops of chloroform previously treated with dry gaseous HCl. The resulting solution was used immediately to obtain absorption and CD spectra.

(S)-N-all-trans-Retinylidene-α-phenylethylamine. To 1 ml of chloroform was added 14.27 mg (0.050 mM) of *all-trans*-retinaldehyde and 9.40 mg (0.078 mM) of (*S*)-α-phenylethylamine. The solution was allowed to react overnight in the dark at room temperature. The reaction mixture was diluted 758:1 with isooctane and the resulting solution was used immediately for absorption and CD spectra.

Protonated (S)-N-all-trans-Retinylidene-α-phenylethylamine. The reaction was carried out as for the unprotonated form. The solution was then diluted appropriately with chloroform. To this chloroform solution was now added 1 or 2 drops of HCl-treated chloroform and the absorption and CD spectra were recorded.

(R)-N-all-trans-Retinylidene-2,2'-dimethyl-6,6'-diaminobiphenyl. To 1 ml of carbon tetrachloride was added 14.21 mg (0.050 mM) of *all-trans*-retinal and 21.09 mg (0.099 mM) of (*R*)-2,2'dimethyl-6,6'-diaminobiphenyl. The mixture was allowed to react overnight in the dark at room temperature. It was then diluted 2800:1 with isooctane. Addition of several drops of HCl-treated chloroform shifted the absorption maximum from 386 to 495 nm. No detectable peak was present between 350 and 400 nm.

(S)-N-11-cis-Retinylidene-α-(1-naphthyl)ethylamine. To 1 ml of chloroform was added 7.16 mg (0.025 mM) of 11-*cis*-retinaldehyde and 5.35 mg (0.031 mM) of (*S*)-α-(1-naphthyl)-ethylamine. The mixture was allowed to react for 1 hr in the dark at room temperature and then diluted 500:1 with isooctane. Complete reaction was determined by the addition of HCl-saturated chloroform. This resulted in the disappearance of the 366-nm peak and the appearance of a new peak at 467 nm.

N-all-trans-Retinylidene-L-dipalmitoylphosphatidylethanolamine. Chloroform and absolute ethanol were dried with Linde 3A molecule sieve and then mixed to form a 2:1 (v/v) chloroform–ethanol solvent mixture. To 10 ml of 2:1 chloroform–ethanol was added 8.18 mg (0.029 mM) of *all-trans*-retinaldehyde. To 1.99 ml of 2:1 chloroform–ethanol was added 4.82 mg (0.0073 mM) of L-dipalmitoylphosphatidylethanolamine. To the phosphatidylethanolamine solution was now added 1 ml of the retinaldehyde stock solution containing 0.0029 mM retinaldehyde and 10 μl of triethylamine to give a final volume of 3 ml. The mixture was allowed to react overnight in the dark at room temperature. An aliquot of this solution was removed, diluted with 14 volumes of chloroform, and the absorption spectrum was obtained. Completeness of reaction was ascertained by the absence of a band at 390 nm and by the addition of acidified chloroform.

N^{ϵ}-all-trans-Retinylidene-L-lysine. The procedure utilized was that of Akhtar *et al.* (1968). To 50.08 mg (0.18 mM) of *all-trans*-retinaldehyde in 10 ml of methanol was added 1 ml of a distilled water solution containing 35.74 mg (0.20 mM) of L-lysine monohydrochloride and 7.3 mg of triethylamine. The mixture was allowed to react in the dark at room temperature for 6 hr, after which time a sample was withdrawn and diluted 50:1 with methanol. The diluted solution was utilized for absorption and CD spectra and to check for completeness of reaction as previously indicated.

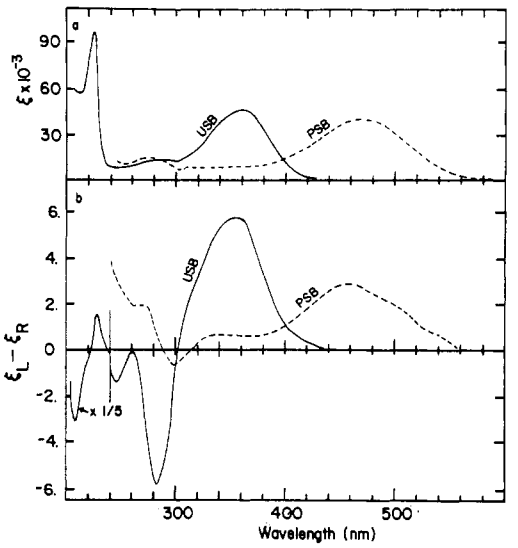

FIGURE 2: (a) The absorption spectra of the protonated and unprotonated forms of (*S*)-*N-all-trans*-retinylidene α-(1-naphthyl)ethylamine in isooctane solution. (b) The CD spectra of the protonated and unprotonated Schiff's base. These spectra have been corrected by subtracting out the CD contribution from 30% excess amine.

N^{ϵ}-all-trans-Retinylidene-poly-L-lysine. To a solution of 6.17 mg (0.037 mM lysine residues) of poly-L-lysine in 0.5 ml of distilled water and 40 μl of NaOH was added 0.5 ml of methanol containing 0.1945 mg of *all-trans*-retinaldehyde (0.00068 mM). The mole ratio of lysine residues to retinal molecules was thus 54:1. The mixture was allowed to react overnight in the dark at room temperature. An aliquot of this solution was diluted by the addition of 7.4 volumes of water and the pH was adjusted to 11.46 by the addition of 1 N sodium hydroxide. Absorption and CD spectra were recorded on this solution. To convert the poly-L-lysine to the β conformation the sample was heated to 50° by passing an ethylene glycol–water mixture from a circulating bath through the water jacket of the cell. After about 20 min no further change in the CD at 224 nm was observed and the complete CD spectrum was then recorded.

Protonated N^{ϵ}-all-trans-Retinylidene-poly-L-lysine. The Schiff's base was prepared as described for the unprotonated α-helical form. Next, the pH was adjusted to 2.0 by the addition of concentrated HCl. Absorption and CD spectra were obtained on the resulting solution.

Results and Discussion

The CD spectrum of the Schiff's base formed from *all-trans*-retinaldehyde and (*S*)-α-(1-naphthyl)ethylamine is shown in Figure 2. Also shown is the CD spectrum for the protonated form of this compound and for comparison the CD and absorption spectra of the free amine shown in Figure 3. The presence of a Cotton effect at 354 nm with a rotational strength of +0.27 DBM is noteworthy. *all-trans*-Retinal absorbs at 368 nm in isooctane and is optically inactive. The amine by itself has no circular dichroism in the 360-nm region; hence the observed band is a consequence of the formation of the Schiff's base. This Cotton effect at 354 nm can be assigned to the retinylidene band which occurs at 360 nm in the Schiff's base and at 368 nm in free *all-trans*-retinaldehyde. Moving through the CD spectrum we observe a negative Cotton effect

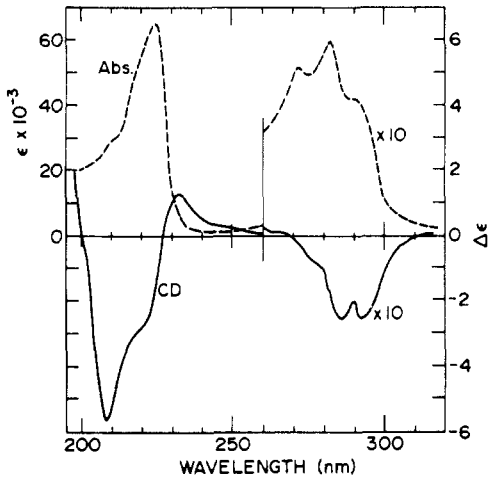

FIGURE 3: The absorption and circular dichroism spectra of (*S*)-α-(1-napthyl)ethylamine in isooctane solution.

at 282 nm having a rotational strength of −0.116 DBM. This band at 282 nm is about 20 times larger than that of the free amine, although it coincides with the ^1L_a transition of the amine. The origin of this band can be shown to arise from the interaction of the 282-nm transition of the naphthyl group with the 360-nm transition of the retinylidene group. The interaction is such that the 282-nm transition acquires a negative value for *R* and the 360-nm transition acquires a positive value for *R*. Below 250 nm, the free amine has strong optical activity and must be subtracted out from the CD spectrum of the Schiff's base to find the new Cotton effects induced by Schiff's base formation. When this is done, induced peaks are found at 246, 227, and 208 nm. The R_{246} = −0.027 DBM, R_{227} = +0.062 DBM, and R_{208} = −0.120 DBM. The band at 246 nm corresponds to no obvious absorption or CD band in the free naphthylethylamine. The Cotton effect at 227 nm very likely corresponds to the 224 nm ^1B_b transition that is present in the parent amine.

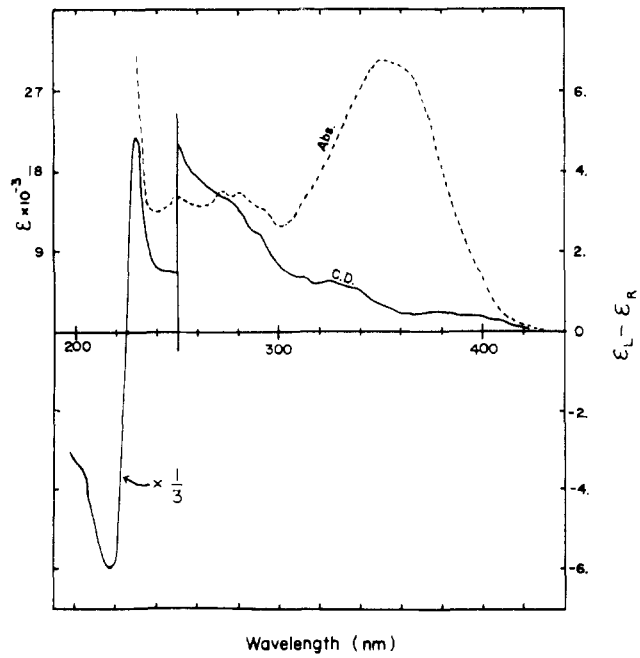

FIGURE 4: The absorption and CD spectra of (*S*)-*N*-11-*cis*-retinylidene-α-(1-naphthyl)ethylamine in isooctane solution. The solution contains a 23% excess of amine over the Schiff's base.

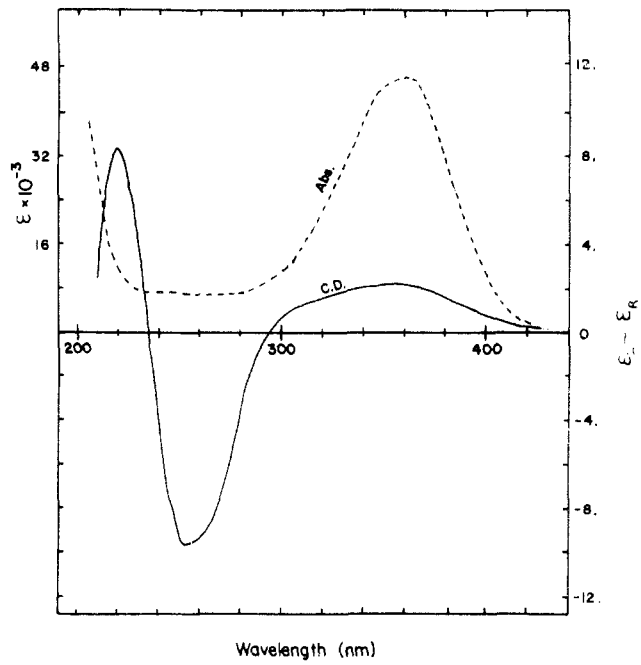

FIGURE 5: The absorption and CD spectra of (*S*)-*N*-*all-trans*-retinylidene-α-phenylethylamine in isooctane solution. The solution contains a 55% excess of amine over the Schiff's base.

Experimentally it was not possible to measure spectra below 200 nm, but additional information may be obtained about that spectral region based on the following argument. Klevens and Platt (1949) reported that the ^1B_a transition of naphthalene was located at 167 nm. If the (*S*)-α-(1-naphthyl)-ethylamine also absorbs at this wavelength and if this is the only other optically active transition in the spectrum, then according to the Kuhn sum rule (Kuhn, 1930), it must have R_{169} = −0.067 DBM. The use of the Kuhn sum rule in the absence of more definitive assignments of the possible transitions below 200 nm, places the above calculation on less firm ground than normally desirable. However, in the absence of these firm assignments, it is legitimate to carry out the calculation using the Klevens and Platt assignment in order to indicate where the remaining rotational strength of the system is located and potentially assignable.

The formation of the Schiff's base from (*S*)-α-(1-naphthyl)-ethylamine and 11-*cis*-retinaldehyde gives rise to a CD spectrum (Figure 4) that is very different from the one exhibited by the Schiff's base of the all-trans isomer. Whereas the all-trans compound has a positive Cotton effect at 354 nm, the 11-cis compound exhibits a CD spectrum that is rather amorphous in the region of 300–400 nm with no defined Cotton effect at 354 nm. This is clear evidence that no isomerization of the 11-*cis* to *all-trans*-retinylidene group occurred during the scan. An explanation for the amorphous appearance of the CD spectrum based on cis → trans isomerization seems unlikely since such an isomerization would convert a sufficient fraction of the molecules to the all-trans form to produce a Cotton effect at 354 nm. After eight spectra were traced of the same solution a new peak did appear at 380 nm. A well-defined positive peak is present at 280 nm and a negative peak at 218 nm.

The lack of a distinct Cotton effect at 354 nm in the 11-cis compound may be a reflection of a lack of conformational rigidity that is present in such a simple analog of rhodopsin. The CPK model suggests that free rotation is possible at the C_{12}–C_{13} single bond of the 11-cis isomer. Studies by Nash

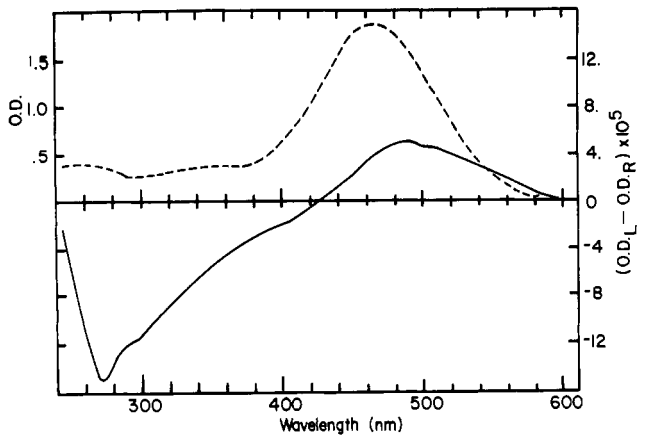

FIGURE 6: The absorption and CD spectra of the protonated form of (*S*)-*N*-*all-trans*-retinylidene-α-phenylethylamine in chloroform solution. The solution contains a 55% excess of amine hydrochloride over the Schiff's base hydrochloride.

(1969), Honig *et al.* (1971), and Honig and Karplus (1971) have been interpreted by them as showing that the *S*-cis and *S*-trans conformers about the C_{12}–C_{13} bond are probably in thermal equilibrium in solution at room temperature. This would imply the presence of a very low-energy barrier to rotation under these conditions and supports our observations made with CPK models. If this rotation is in fact free, the observed CD spectrum would be an average over a wide range of relative orientations for the retinylidene transition dipole moment and the naphthyl transition dipole moment so that the observed CD spectrum would exhibit an amorphous character.

Protonation of the 11-cis Schiff's base gave rise to variable and nonreproducible CD spectra. In Figures 5 and 6 the CD and absorption spectra for the unprotonated and protonated forms of (*S*)-*N*-*all-trans*-retinylidene-α-phenylethylamine are shown. The Schiff's base derived from (*S*)-phenylethylamine and *all-trans*-retinaldehyde shows the typical major band at 359 nm in the absorption spectrum and a 354-nm band in the CD spectrum. The 254-nm negative Cotton effect is assigned to the ^1L_b transition of the phenyl moiety. The ^1L_b negative Cotton effect induces an equal positive Cotton effect in the retinal. The phenyl group also acquires a second induced positive band at 219 nm that is not assigned at this time. It may be the ^1L_a band of the phenyl group which would be expected near 203 nm. The degenerate ^1B_a and ^1B_b bands are reported by Salem (1966) to lie in the region of 180 nm much too far from 219 nm to be the likely transitions responsible. When this Schiff's base is protonated the λ_{max} in the absorption spectrum is shifted to 464 nm while the λ_{max} in the CD spectrum is shifted to 490 nm.

For the Schiff's base derived from *all-trans*-retinaldehyde and (*R*)-2,2′-dimethyl-6,6′-diaminobiphenyl in which it is possible that 2 mol of aldehyde are coupled to 1 mol of amine, we observe the induction of a double Cotton effect (Figure 7). There is a crossover in the CD at 374 nm which is close to the absorption maximum of 371 nm, and this is flanked by a positive Cotton effect at 400 nm and a negative Cotton effect at 346 nm.

For the macromolecular system N^{ϵ}-*all-trans*-retinylidene-poly-L-lysine, in which the ratio of retinaldehyde molecules to L-lysine residues was kept at about 1:54, an interesting biphasic spectrum was found as shown in Figure 8. In the α-helical conformation a peak is present at 375 nm, a crossover at 350 nm, and a trough at 330 nm. When the α-helical

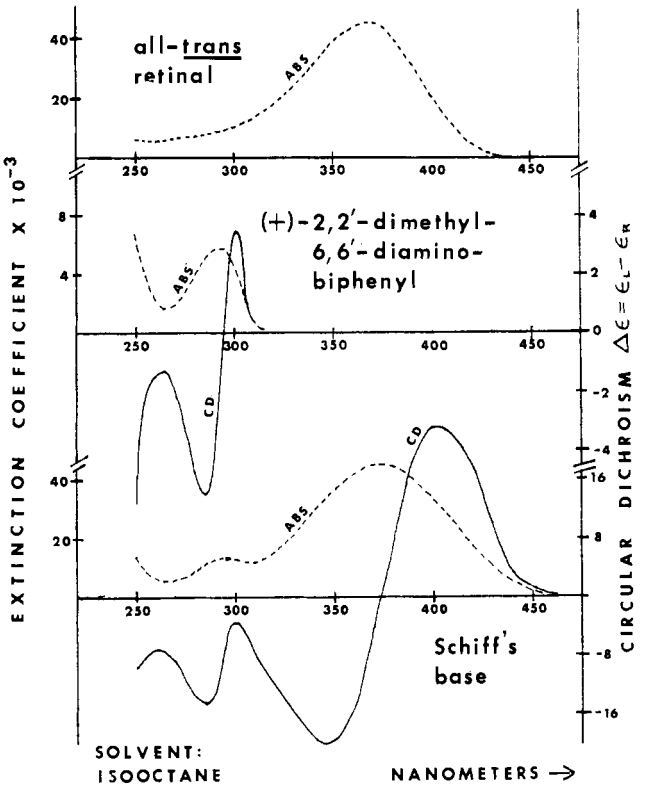

FIGURE 7: The absorption spectra of *all-trans*-retinaldehyde-(*R*)-(+)-2,2′-dimethyl-6,6′-diaminobiphenyl and the Schiff's base formed from them. The CD spectrum is also shown. All spectra are in isooctane solution and those of the Schiff's base contain a 99%, excess of the free amine.

form is converted to the β form by heating, the circular dichroism was not abolished. The CD curves resemble the original α-helical spectrum decreased in intensity by 40%. When the pH of the solution is adjusted to 2.0, the poly-L-lysine converts to a form designated as unordered-extended helix (Dearborn and Wetlaufer, 1970), the CD spectrum is markedly changed (Figure 9). The biphasic form is retained but now a negative Cotton effect is present at 450 nm, a crossover occurs at 406 nm, and a positive Cotton effect is now centered at 370 nm.

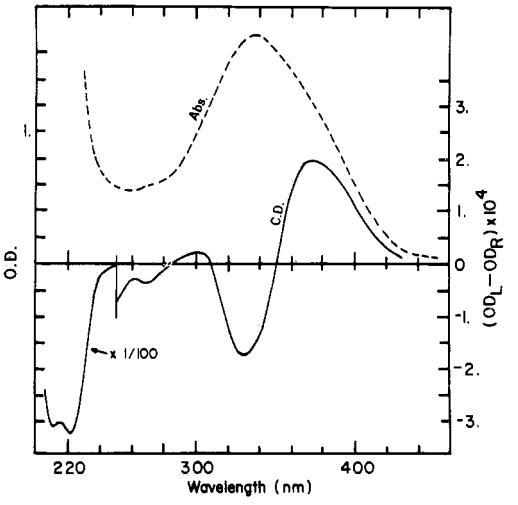

FIGURE 8: The absorption and CD spectra of N^{ϵ}-*all-trans*-retinylidene-poly-L-lysine in 5.9% (v/v) methanol–water solution. The pH is 11.46, the poly-L-lysine conformation is α helical and there is a 60:1 molar excess of lysine residues over retinylidene residues.

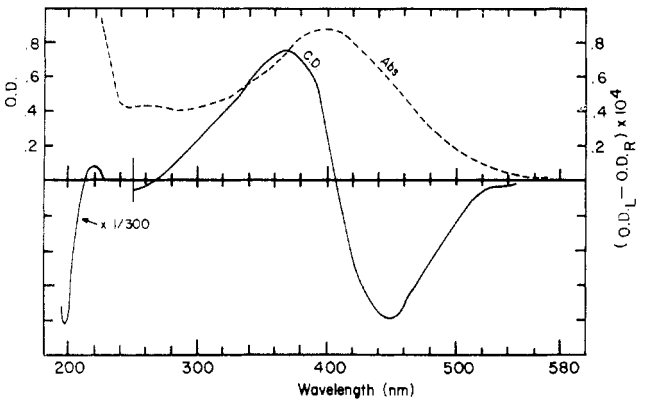

FIGURE 9: The absorption and CD spectra of the protonated form of N^{ϵ}-*all-trans*-retinylidene-poly-L-lysine in 7% (v/v) methanol–water solution. The pH is 2.0, the poly-L-lysine conformation is unordered-extended helix. There is also a 60:1 molar excess of lysine residues over retinylidene residues.

The appearance of biphasic Cotton effects in the spectra of *N-all-trans*-retinylidene-2,2′-dimethyl-6,6′-diaminobiphenyl and N^{ϵ}-*all-trans*-retinylidene-poly-L-lysine unprotonated and protonated might be explicable on the basis of retinal–retinal interactions. The amine component of these Schiff's bases have two or more amino groups available for reaction with molecules of retinaldehyde. If two retinaldehyde molecules react at adjacent sites that are asymmetrically arranged, they can induce optical activity in each other. Alternatively if two retinaldehydes interact intermolecularly they can also induce optical activity in each other in these systems. If one of the two retinaldehyde acquires a positive rotational strength, the other must acquire a negative one in order to keep the sum of the rotational strengths zero for the whole spectrum. In general, these two Cotton effects will occur at slightly different wavelengths, so they will produce a peak–trough spectrum like that observed rather than cancelling out.

It is possible that the circular dichroism induced in each retinaldehyde is greater than would appear from the spectrum. Such a situation is possible when two approximately Gaussian curves of opposite sign are slightly offset and then added. Under this circumstance they will tend to cancel and produce a crossover in the region where they overlap while their maxima will be somewhat diminished in intensity. The positive and negative Cotton effects at 400 and 376 nm, respectively, are separated by a crossover point at 374 nm. This value is sufficiently close to the absorption maximum of 371 nm as to make the tentative explanation attractive.

In the N^{ϵ}-*all-trans*-retinylidene-poly-L-lysine CD spectra we again observe a crossover centered near the peak of the absorption maximum flanked by a biphasic Cotton effect. Three explanations can be offered for the biphasic effect in these systems. One possibility is that the retinaldehydes are binding to adjacent ϵ-amino groups and are held in an asymmetric configuration by the helical structure of the polymer backbone. A second possibility is that the CD is induced merely by the asymmetric electric field due to the helix. The third explanation that would also account for the observed CD spectrum is that the polymer, though predominantly helical, may also contain segments that are folded. Thus a retinal attached to residue X may be adjacent to a retinal at residue Y even though in the completely extended form these residues would be separated by appreciable distances. Since the circular dichroism is not abolished when the α-helical structure is converted to the β structure, it would appear that adjacent retinal sites remain unchanged in the α and β transition. The inversion of Cotton effects that occurs when the pH is adjusted to 2.0 eludes a definitive explanation at this time. The backbone conformation at this pH is the so called unordered-extended helix conformation.

The Schiff's base *N-all-trans*-retinylidene-L-dipalmitoylphosphatidylethanolamine showed no extrinsic Cotton effect in the region of the retinaldehyde absorption maximum. That the Schiff's base was formed could be readily demonstrated by the red shift of the main absorption peak on protonation. A comparable situation was present in the CD spectrum of N^{ϵ}-*all-trans*-retinylidene-L-lysine. No optical activity could be demonstrated in the region of 380 nm.

The absence of extrinsic Cotton effects in the compounds N^{ϵ}-retinylidene-L-lysine and *N*-retinylidene-L-dipalmitoylphosphatidylethanolamine is perhaps not surprising. The L-lysine component of *N*-retinylidene-L-lysine and the L-phosphatidylethanolamine group have no strong transition that could interact with the retinylidene to induce optical activity. Moreover, since the retinal is attached to the ϵ-amino group in one instance and the ethanolamine group in the other, it is quite remote from the carbonyl group of these molecules, which is the only double-bonded unit in the molecules. The single bonds in the side chain of lysine and ethanolamine confer conformational flexibility on the whole system, bending to average out any small CD that might have been induced.

The results of this study using Schiff's bases of retinaldehyde and small, optically active aromatic amines indicate that the induction of optical activity in the retinaldehyde moiety does not require the imposition of conformational asymmetry on the backbone of the retinaldehyde, as has been proposed for rhodopsin.

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