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Intrinsic Optical Activity of Retinal Isomers. Implications for the Circular Dichroism Spectrum of Rhodopsin†

Barry Honig,*‡ Peter Kahn, and Thomas G. Ebrey

ABSTRACT: The source of the circular dichroism in the visible region of the rhodopsin absorption spectrum may be either an intrinsic chromophore asymmetry or one induced by its environment in rhodopsin. Since 11-*cis*-retinal is twisted about a number of bonds in its π electron system it is expected that separated conformers with opposite chirality will exhibit optical activity. Calculations of the extent of overlap of mirror image isomers show that the highly selective opsin binding site is probably incapable of binding two enantiomers of the chromophore. In addition, the calculated magnitude of the rotational strength of 11-*cis*-retinal is comparable to that of rhodopsin. Thus, the optical activity of the visual pigment may be at least partially explained in terms of a selective binding of a particular conformer by the protein. The skewed ring–chain conformation of other retinal isomers may be the source of the optical activity of visual pigments whose chromophore is not 11-*cis*-retinal.

In recent years the optical activity of rhodopsin and other visual pigments has been the subject of numerous experimental investigations (Crescitelli *et al.*, 1966; Takezaki and Kito, 1967; Kito *et al.*, 1968; Mommaerts, 1969; Horwitz and Heller, 1971; Shichi, 1971; Waggoner and Stryer, 1971; Johnston and Zand, 1972). The interpretation of these experiments has been complicated by the variety of factors that may be responsible for the observed circular dichroism of the 500- (α) and 340- (β) nm bands of rhodopsin (Figure 1). Since these electronic transitions are due to the retinylidene chromophore, their optical activity must result from either an intrinsic molecular asymmetry due to twisting, induced twisting by the protein, or to an asymmetric environment in the region of the protein near the chromophore. Although the last mechanism has been generally accepted as the source of the optical activity of rhodopsin, we feel that the importance of the intrinsic optical activity of retinal isomers has not been fully recognized (see, however, the discussion by Mommaerts,

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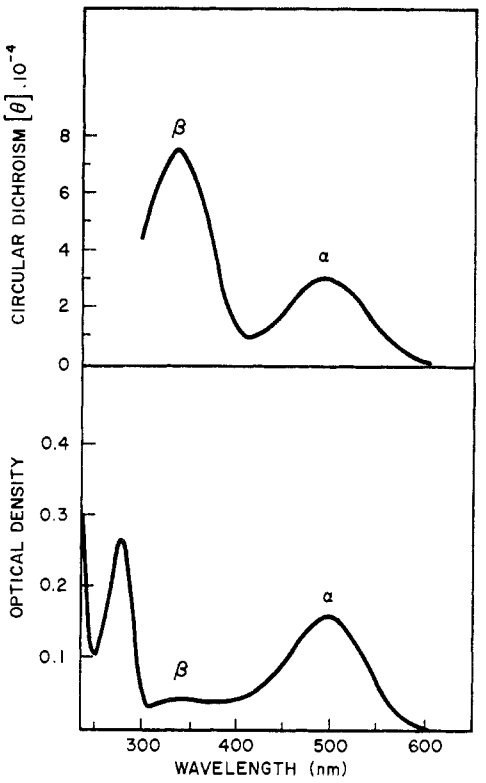

FIGURE 1: Absorption and circular dichroism spectra of cattle rhodopsin in 1.5% Ammonyx LO.

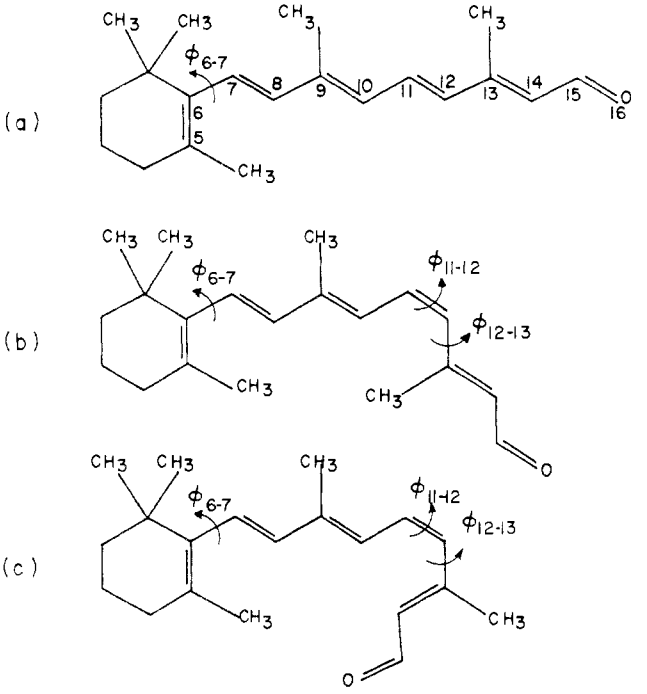

FIGURE 2: (a) 6-*s-cis-all-trans*-retinal; (b) 6-*s-cis*,12-*s-cis*,11-*cis*-retinal; (c) 6-*s-cis*,12-*s-trans*,11-*cis*-retinal.

1969). In this paper we consider the possibility that at least part of the circular dichroism of the visual pigments is due to the skewed conformation of 11-*cis*-retinal and of the other retinal isomers. Our argument is based upon the known conformation of these molecules, on a theoretical calculation of their rotational strengths and on the expected nature of the rhodopsin binding site.

Theoretical considerations (Nash, 1969; Honig and Karplus, 1971), nuclear magnetic resonance (nmr) evidence (Honig *et al.*, 1971), and crystallographic evidence (Bart and MacGillavry *et al.*, 1968; Gilardi *et al.*, 1971) have shown that due to steric hindrance, retinal isomers are twisted about a number of bonds in their π electron system (Figure 2). As is the case for almost all related molecules, the β-ionone ring is twisted out of the plane of the side chain in an approximate s-cis conformation. The torsional angle about C-6–C-7 ranges between 110 and 150° (Honig *et al.*, 1971) (the planar s-cis conformation is defined to be 180°). Additional twisting is possible in the chain itself. In 11-*cis*-retinal the chain is planar only from C-6 to C-13 with the last three atoms twisted out of the plane of the preceding segment. The torsional angle about C-12–C-13 is about 140° (s-cis) in the crystal (Gilardi *et al.*, 1971) but s-cis and s-trans (less than 90°) conformations are probably in thermal equilibrium in solution at room temperature (Honig and Karplus, 1971). Other retinal isomers have essentially planar side chains extending from C-6 to the carbonyl group.

Regardless of their exact conformation it is clear that retinal isomers should exhibit the optical activity characteristic of other twisted butadiene-like systems. However, no retinal isomer, including 11-cis, shows optical activity (Crescitelli *et al.*, 1968). The reason for this is that the twisting can either be right or left handed; in solution there is no energetic reason to favor one over the other, and thus both left- and right-handed forms (enantiomers) will be present in equal numbers, probably in thermal equilibrium. Were it possible to separate these forms one would expect to measure optical activity for all retinal isomers as a result of their skewed ring–chain conformation. An additional contribution to the optical activity would be present in 11-*cis*-retinal due to its twisting about C-12–C-13.

At least three hypotheses can be proposed to explain the optical activity of rhodopsin. Perhaps the most commonly accepted model is one where an asymmetric protein environment interacts with a nonoptically active chromophore through a dipole–dipole interaction (Crescitelli *et al.*, 1966; Waggoner and Stryer, 1971; Johnston and Zand, 1972). In rhodopsin, the two interaction dipoles would be the retinal chromophore and some transition of the opsin, probably of an amino acid side chain.

Another hypothesis is that opsin will combine with only one enantiomeric form of retinal to form a visual pigment (Mommaerts, 1969), Since the enantiomer will be optically active, this would be the source of rhodopsin's optical activity. This mechanism must be distinguished from a second related one in which the protein actually twists the chromophore into a dissymmetric conformation, thereby inducing optical activity.

If we examine the properties of the rhodopsin binding site, it would appear unlikely that two conformational enantiomers of opposite helical sense of a chromophore could be bound in equal amounts. The opsin binding site is highly selective and will only couple with the 11-cis and 9-cis isomers of retinal (Hubbard and Wald, 1952). Other isomers, such as 13-cis or all-trans, cannot form pigments with opsin, presumably because of steric limitations.

Besides 11-*cis*- and 9-*cis*-retinal, a few chemically modified analog of these isomers will also form pigments with opsin.

These are 9-desmethyl-, 13-desmethyl-, 9,13-desmethyl-, 5,6-dihydro-, and 5,6-epoxyretinal (Nelson *et al.*, 1970; Blatz *et al.*, 1969, 1970). On the other hand several other molecules with slightly more extensive modification of the retinal structure will not couple with opsin. They include compounds in which the chain has been made longer, shorter, or the β-ionine ring removed (Blatz *et al.*, 1970). Thus it appears that anything more than minor changes in the retinal structure will preclude pigment formation.

Consistent with this picture of a restrictive binding site are a number of experiments which indicate that at least parts of the chromophore are shielded and are not freely accessible to substances that react with it in free solution. Wald and Hubbard (1960) found that the enzyme lipoxidase which will attack retinal in solution cannot attack the retinal of rhodopsin. Retinal is covalently bound *via* a Schiff's base linkage to the ε-amino group of a lysine residue of the opsin. The reagents hydroxlamine and sodium borohydride which should be able to attack this Schiff's base cannot do so until the rhodopsin structure is changed by bleaching (Wald and Brown, 1951; Bownds and Wald, 1965). All this suggests that the chromophore is somewhat protected from the external aqueous environment, probably by being in a fairly hydrophobic region of the opsin which would prevent even small molecules from reaction with it. This is not unreasonable since retinal itself is a hydrophobic molecule and might be expected to be surrounded by hydrophobic amino acids.

All of this evidence, taken together, seems to argue for a highly restrictive and selective binding site for retinal. In the next section we show that for all possible conformations of 11-*cis*-retinal the ability of both enantiomers to form pigments is hard to reconcile with the evidence that rhodopsin has a selective binding site. We then present calculations of the rotational strength of retinal isomers and show that strong intrinsic optical activity of these molecules would be expected. We do not attempt to interpret the circular dichroism (CD) spectrum of rhodopsin on the basis of these calculations since the relationship of the electronic structure of the chromophore of rhodopsin to that of retinal is unclear. On the other hand, the magnitude of the rotational strength that we obtain is sufficiently large to require a strong intrinsic chromophore component in the CD spectrum of rhodopsin if a particular conformer is selectively bound. In the discussion we consider the merits of the three models for the optical activity of rhodopsin in light of the available experimental evidence.

Results

Three-Dimensional Orientation of Retinal Isomers. It is most convenient to discuss the orientation of retinal isomers in terms of the plane defined by the side-chain atoms (*i.e.*, C-6 to O-16 in Figure 2). For a molecule twisted about a particular bond there will always be two equivalent mirror image isomers corresponding to a displacement to either side of the plane. For example in the case of *all-trans*-retinal there are two possibilities for the twisted ring–chain conformation as shown in Figure 3b. Of course, the molecule is optically active in either conformation.

Since 11-*cis*-retinal is twisted about two bonds, there are a number of rotational isomers to consider. As seen in Figure 3c–h there are six pairs of enantiomers corresponding to s-cis and s-trans conformations about C-6–C-7 and C-12–C-13. There are six rather than four conformations because the oxygen may be either on the same side (3e,f) or the opposite side (3g,h) of the plane as is C-5. Either possibility will lead to a different screw sense and thus to a different rotational strength.

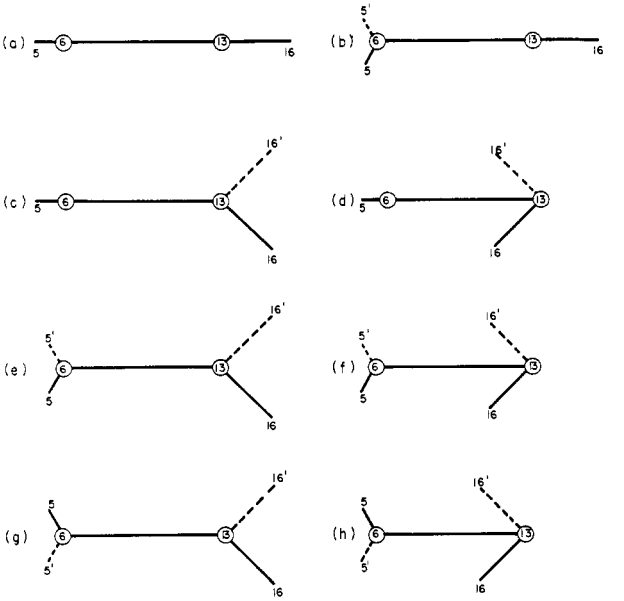

FIGURE 3: Schematic diagram of the enantiomers of retinal isomers viewed in the plane of the C-6–C-13 atoms. Twisting of the molecule is represented by the linear segments above and below the C-6–C-13 line. Numbers represent the positions of the appropriate atoms. Dotted lines are the fragments of one enantiomer obtained by reflecting the other enantiomer (solid lines) through the C-5–C-13 plane. Figures a–h correspond to the conformations defined in Table I. (a) all-trans,6-s-trans-, (b) all-trans,6-s-cis; (c) 11-cis,6-s-trans,12-s-cis (same side); (d) 11-cis,6-s-trans,12-s-cis (same side); (e) 11-cis, 6-s-cis,12-s-trans (opposite side); (f) 11-cis,6-s-cis,12-s-cis (opposite side).

The conformations illustrated in Figure 3 include all possibilities that are plausible in light of theoretical and experimental studies on retinal isomers. Conformations 3b and 3h correspond respectively to the known crystal structures of *all-trans*- and 11-*cis*-retinal. Some of the other conformations are probably present for retinal in solution since there is a great deal of flexibility about single bonds (Honig and Karplus, 1971). Since the conformation of 11-*cis*-retinal when bound to the protein is unknown, we include all possible conformations in our calculations.

All of the calculations in this paper are performed on an idealized molecule with single and double bond lengths of 1.46 and 1.35 Å, respectively. Torsional angles are taken to be 0° for a bond in a trans configuration. Since the only significant deviations from planarity result from twisting about C-6–C-7 and C-12–C-13, the various conformations may be represented by the values of the dihedral angles ϕ_{6-7} and ϕ_{12-13} (see Figure 2). ϕ_{11-12} is respectively 0 or 180° for *all-trans*- or 11-*cis*-retinal. The angles for the conformations represented in Figure 3 are given in Table I. The values of 60 and 130° for the dihedral angles of C-12–C-13 correspond respectively to the minimum energy s-trans and s-cis conformations calculated by Honig and Karplus (1971). Similarly 0 and 135° have been chosen as the s-trans and s-cis dihedral angles for C-6–C-7. The values for crystalline 11-*cis*-retinal are 141° for C-12–C-13 and 140° for C-6–C-7 (Gilardi *et al.*, 1971). However since crystal packing forces have a significant effect on these angles and since no experimental values for the 12-s-trans conformation are available we have used the theoretical rather than crystal values in this work. We should emphasize here

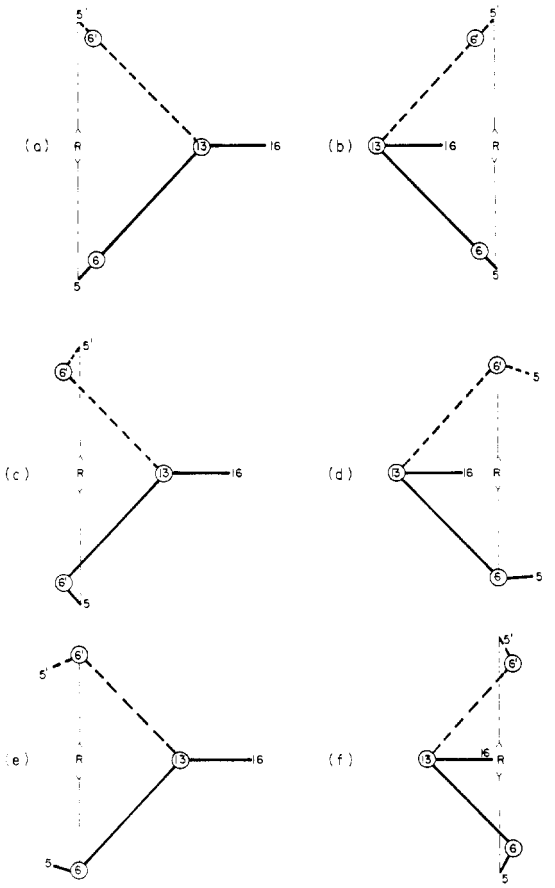

FIGURE 4: Schematic diagram of the enantiomers of retinal isomers viewed in the plane of the C-13–O-16 atoms. Atoms C-13–O-16 are superimposed for each pair. R represents the maximum distance between equivalent atoms for each pair.

that the qualitative arguments presented below do not depend on the exact value of the dihedral angle that is used.

For a given conformation we wish to calculate the dimensions of a binding site capable of containing both enantiomers. One way of doing this is to superimpose identical fragments of each enantiomer and calculate the distance between equivalent atoms on either side of the mirror plane. If both enantiomers are to be bound, the binding site must be large enough to accommodate the largest distance obtained. In Figure 3 we have superimposed the largest fragment for each pair but this is unrealistic when one considers the fact that the chromophore

TABLE I: Torsional Angles of Conformations in Figure 2.

Conformation[a]	ϕ_{6-7}	ϕ_{11-12}	ϕ_{12-13}
a	0	0	0
b	135	0	0
c	0	180	60
d	0	180	130
e	135	180	60
f	135	180	130
g	−135	180	60
h	−135	180	130

[a] Torsional angles for the mirror image are $\phi'_{ij} = -\phi_{ij}$.

TABLE II: Maximum Distance between Equivalent Atoms in Enantiomers of 11-*cis*-Retinal as Shown in Figure 3.

Conformaton	R (Å)
a	12.1
b	12.5
c	13.8
d	12.0
e	14.6
f	13.0

is covalently bound to the protein at position 16. Therefore it seems more reasonable to superimpose the carbonyl ends of each enantiomer and calculate distances between equivalent atoms further along the chain. This has been done in Figure 4. Clearly, the ring atoms of the molecule will be moved the greatest distance from their equivalent atoms in the other enantiomeric form.

In Table II we have calculated the distance between the furthest apart equivalent atoms in the enantiomeric pairs of each of the six 11-*cis*-retinal conformations of Table I and Figure 4. Coordinates of the idealized molecule given above were used in the calculation. It is striking that no two enantiomers may be superimposed to within 10 Å.

This result, however, is based on a rather restrictive assumption about the flexibility of the attachment site. It may be that the lysine can rotate about one or more of its single bonds so as to facilitate an optimum fit of the chromophore. To see how this degree of freedom affects the results of Table II, we kept one enantiomeric form of the chromophore rigid and rotated the other about the two single bonds in the lysine that are adjacent to the Schiff's base linkage so as to minimize the distance between equivalent atoms. In this case our calculations showed that it is impossible to superimpose equivalent chromophores to less than 5 Å. That is, the largest distance between equivalent atoms of mirror image enantiomers for the conformations in Figure 4a–f was 5 Å or more if we allow rotation about the lysine, and at least 10 Å if we do not.

The large size of these numbers, in view of the severe restrictions of the retinal binding site, suggest that it is unlikely that both enantiomers of the chromophore of rhodopsin are bound. Since our calculations included all likely candidates for the conformation of retinal in rhodopsin, they are not dependent upon an assumption as to what the conformation actually is. The presence of any two enantiomers in equal quantities would imply the rather unlikely conclusion that a change in the spatial orientation of the lysine and the rather bulky chromophore makes little or no difference to the energy of the system. Thus it seems probable that the protein performs a separation of enantiomers and as a result induces optical activity in rhodopsin. In the next section we estimate the magnitude of the effect by calculating the rotational strengths of the various skewed conformations of 11-*cis*-retinal.

Circular Dichroism Calculations. Our method of calculating rotational strengths is identical to that given by Eyring and coworkers (Cheong *et al.*, 1970) and will only be outlined here. It is assumed that the observed spectral features are due primarily to $\pi \rightarrow \pi^*$ excitations and thus only the π electron system of the molecule is considered (*i.e.*, C-5 to O-16).

TABLE III: Energy, Oscillator Strength, and Rotational Strength of First Excited Singlet of *all-trans*- and 11-*cis*-Retinal.

Conformation[a]	E (eV)	f	R (DM)
a	3.60	1.32	0
b	3.66	1.21	1.04
c	3.75	0.81	0.71
d	3.85	1.20	0.64
e	3.81	0.53	−0.10
f	3.94	0.85	0.01
g	3.81	0.58	3.27
h	3.95	0.90	3.25

[a] See Figure 2 and Table I.

TABLE IV: Energy, Oscillator Strength, and Rotational Strength of First Excited State of 9-*cis*-Retinal.

Torsional Angles 6–7	12–13	E (eV)	f	R (DM)
135	0	3.63	1.01	1.31
−0	60	3.75	1.17	1.76
−0	130	3.71	0.87	1.82
−135	60	3.94	0.79	0.78
−135	130	3.78	0.55	0.51
135	60	3.95	0.87	3.75
135	130	3.78	0.60	4.13

Wave functions for the molecule were obtained from an SCF–CI calculation in the Pariser–Parr–Pople scheme. The dependence of the wave functions on the torsional angle ϕ is introduced through the expression for the resonance integral (Roos and Skancke, 1967)

$$\beta = \beta(r) \cos |\phi| \quad (1)$$

$$\beta(r) = -2.43 \times 3.21(r - 1.397) \quad (2)$$

where values of the ionization potential W_{ii} and one-center repulsion integrals γ_{ii} suggested by Dewar and Morita (1969) were used. Two center repulsion integrals were obtained from the Ohno relationship (Ohno, 1964)

$$\gamma_{ij} = e^2/(\rho_{ij} + r^2_{ij})^{1/2} \quad (3)$$

where

$$\rho_{ij} = 2e^2/(\gamma_{ii} + \gamma_{jj}) \quad (4)$$

and r_{ij} is the interatomic distance. The Mataga relationship (Mataga and Nishimoto, 1957) for γ_{ij} was also tested but led to qualitatively similar results.

The rotational strength is given by the expression

$$R_{ij} = (\hbar^3 e^2/2m^2c^2E)\nabla_{ij} \cdot (r\nabla)_{ij} \quad (5)$$

where ∇_{ij} is the dipole velocity matrix element between states i and j and $(r \times \nabla)$ is the angular momentum operator. The dipole velocity rather than the dipole length expression was used in the calculations to insure origin independence. E is the excitation energy taken here to be that obtained from the configuration interaction (CI) calculation. This expression may be written in terms of expanded CI wave functions as derived by Cheong *et al.* (1970). The matrix elements are calculated by breaking them down into terms involving both local bond moments and group moments (Caldwell and Eyring, 1971).

The results for the excitation energies, oscillator strengths, and rotational strengths for the first excited singlet state of *all-trans*- and 11-*cis*-retinal are reported in Table III. It is clear that both the sign and magnitude of the rotational strength is strongly dependent upon the conformation of the molecule. We first note that the rotational strength of skewed *all-trans*-retinal (Figure 3b) is rather large and is of the order of magnitude expected for skewed dienes (Moscowitz *et al.*, 1961; Charney, 1965; Cheong *et al.*, 1970). The rotational strength increases by about a factor of three when the side-chain oxygen is twisted away from the ring (Figure 3g,h) and is very small when it appears on the same side of the mirror plane as the ring (Figure 3e,f).

We have also calculated the rotational strength of 9-*cis*-retinal since it is known that its pigment, isorhodopsin, has comparable optical activity to that of rhodopsin. As is seen from the first row in Table IV 9-*cis*-retinal with a planar side chain has considerable optical activity due to the skewed ring–chain conformation. The magnitude of the rotational strength obtained is consistent with the strong optical activity of isorhodopsin. In addition to this contribution we believe it plausible to assume that further twisting of the side chain takes place for all retinal analogs that form pigments since the natural chromophore, 11-*cis*-retinal, is nonplanar. This protein induced twisting should occur about single bonds which have shallow rotational potentials. For comparison's sake we have calculated the rotational strength of 9-*cis*-retinal with torsional angles about C-12–C-13 identical with those used in Table III for 11-*cis*-retinal. The results as seen in Table IV are similar to those obtained for the 11-cis isomer.

In order to estimate the extent to which our results are dependent upon parameters, we have carried out the calculations by using the Mataga relationship for the two-center integrals and by varying the ionization potential and one-center repulsion integrals of the carbonyl group. All results were qualitatively similar to those presented in Table III, but the Mataga relationship reduced the magnitude of the rotational strength by about a factor of 2.

It is clear that the results may not be assigned any precise quantitative meaning since it is well known that calculations of this type often overestimate oscillator strengths by a factor of 2 or 3. Similar uncertainties are expected for rotational strengths as well. On the other hand, the orders of magnitude obtained tend to be reliable. Thus the large rotational strengths we have calculated indicate that separated enantiomers of retinal isomers should exhibit optical activity. In all cases the magnitude and relative intensities of the excited states are rather insensitive to moderate ($\pm 20°$) changes in values of the torsional angles.

Discussion

There are at least two clearly resolved circular dichroism bands due to the chromophore of rhodopsin; both are positive in sign. The α-CD band at about 490 nm and the β-CD band at about 335 nm correspond respectively to the α and β

absorption bands. There is probably also a third chromophore band corresponding to the third excited state of the chromophore (T. Ebrey and T. Yoshizawa, in preparation). The absolute magnitudes of the CD bands depend on both the species of opsin and the environment of the rhodopsin. In digitonin, the α band of squid rhodopsin has twice the rotational strength of cattle rhodopsin (Kito *et al.*, 1968); cattle rhodopsin undissolved in a detergent has twice the rotational strength of cattle rhodopsin in digitonin.

A second variation found in the circular dichroism spectrum of rhodopsin is in the ratio of the α:β bands. Considering only cattle rhodopsin, this ratio can vary from about 2 for rhodopsin undissolved in a detergent (Shichi, 1971) to 0.3 for rhodopsin in a detergent-like digitonin (Takezaki and Kito, 1967).

Besides the visual pigments themselves, the optical activity of several of the intermediates of bleaching have also been studied. Two important results can be briefly stated. For cattle rhodopsin, the α-CD band of several of the intermediates, lumirhodopsin (T. Ebrey and T. Yoshizawa, in preparation), metarhodopsin I, and metarhodopsin II (Waggoner and Stryer, 1971), are as large as that of rhodopsin. Secondly, the β band of these intermediates is quite small (T. Ebrey and T. Yoshizawa, in preparation; also Waggoner and Stryer, 1971. Figure 3).

We now wish to consider the possible origins of the circular dichroism of rhodopsin. In the introduction we reviewed evidence from isomeric specificity experiments, artificial pigment experiments and chemical reactivity experiments which strongly suggest that retinal's binding site to opsin is severely restricted. Thus it is highly probable that only one enantiomer of the chromophore of rhodopsin will be able to bind to the opsin. Rhodopsin could then become optically active by selecting one form of an inherently dissymmetric chromophore. Our calculations (Table III) show that for the longest wavelength transition of 11-*cis*-retinal, rotational strengths as large as that of the α band of rhodopsin (0.52 DM) can easily be obtained. This is true even for the planar-chained isomers, such as 9-cis and all-trans (Tables III and IV), as long as only one enantiomer of the twisted ring–chain bond is present. Thus even for isorhodopsin and the presumed all-trans chromophores of the intermediates of rhodopsin such as lumirhodopsin and metarhodopsin I, optical activity could still arise from this intrinsic twisting. In addition one would expect induced twisting of 9-*cis*-retinal about the 12–13 single bond so as to mimic as much as possible the conformation of 11-*cis*-retinal. For these reasons, the fact that isorhodopsin and metarhodopsin I exhibit optical activity does not argue strongly against the intrinsic mechanism for any visual pigment.

The other possible contribution to rhodopsin's CD spectrum that has been quantified is the rotational strength due to the interaction between the dipoles of two different absorption bands. In this case the rotational strength in Debye magnetons is given by

$$R_{ij} = 3.4 \times 10^{54} \frac{G_{ij} D_i D_j \nu_i \nu_j}{r^2(\nu_i^2 - \nu_j^2)} \qquad (6)$$

Where G_{ij} is the orientation factor, r is the distance between the two dipoles, D_i and D_j are the respective dipole strengths of the retinal chromophore and the opsin, and ν_i and ν_j are the respective frequencies of these absorption bands. Waggoner and Stryer (1971) calculated the value of R_{ij} for the 500-nm transition. From the absorption spectrum of rhodopsin it is known that $D_i = 74 \times 10^{-36}$ cgs units and $\nu_i = 20{,}000\ \text{cm}^{-1}$. It is not known what the interacting dipole from the opsin is. Waggoner and Stryer made the plausible assumption that it was one of the ultraviolet transitions of an amino acid with $D_i = 20 \times 10^{-36}$ cgs units and $\nu_i = 50{,}000\ \text{cm}^{-1}$. Then making the additional assumption that the orientation between the two interacting dipoles is optimal, they calculated that the value of $r = 4$ Å would give $R_{ij} = 0.75$ for the 500-nm rhodopsin band. This compares with the experimental values of $R_{ij} = 0.5$ for cattle rhodopsin and 1.0 for squid rhodopsin, both in digitonin. The difficulty with this model is that it cannot easily explain the optical activity of the β band, where the experimental value for squid is about $R = 2$. Since the dipole strength of this transition is one-fifth that of the α band, eq 6 would predict a rotational strength of only one-sixth the experimental value, even assuming a maximum orientation factor.

A second objection might also be raised. Previous measurements by us (Ebrey and Honig, 1972) indicate the extinction of the β band decreases only about 10% upon isomerization, yet the CD of this band decreases by 80% in going to lumirhodopsin (T. Ebrey and T. Yoshizawa, in preparation). It is difficult to explain this change with eq 6 since the CD of the α band remains large in lumirhodopsin suggesting that r has not changed greatly.

On the other hand, we do not believe that these considerations exclude the possibility of the dipole–dipole mechanism contributing to the optical activity of rhodopsin. Furthermore, it is difficult to calculate the specific contribution of the intrinsic mechanism to the optical activity of both the α and β bands. This must await a more complete characterization of the electronic states of the chromophore when bound in rhodopsin and the spectroscopic assignment of these bands. An attempt to correlate the calculated oscillator and rotational strengths of protonated Schiff's bases with the absorption and circular dichroism spectrum of rhodopsin is presently under way.

To summarize, our calculations show that separated enantiomers of retinal isomers should have optical activity comparable to that of rhodopsin. We have further argued that it is improbable that any pair of conformers of 11-*cis*-retinal of opposite chirality is bound to the protein in equal numbers. Although this implies a strong intrinsic chromophore component in the optical activity of the visual pigments, it is not yet possible to establish the specific contribution of this mechanism to their CD spectra.

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Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Oxytocin, Related Oligopeptides, and Selected Analogs†

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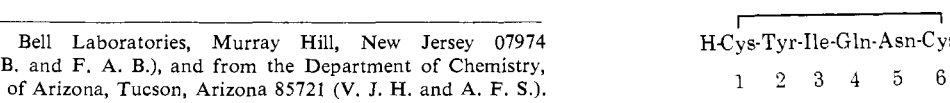
ABSTRACT: The C-13 nuclear magnetic resonance (nmr) spectra of oxytocin and several analogs in dimethyl sulfoxide solution have been studied. Assignments of all 43 carbon resonances have been made by reference to the spectra of the linear precursors and through the use of partially deuterated peptides and oxytocin derivatives. The closing of the disulfide bond yielding oxytocin from the linear nonapeptide precursor results in a number of significant chemical shift changes, particularly of the α and β carbons, which cannot at present be interpreted in detail. Significant chemical shift differences are observed for some oxytocin analogs which may be related to conformational changes.

In the past few years there has been a marked upsurge of interest in the conformations of cyclic polypeptides. Their study by proton nuclear magnetic resonanse (nmr) has been particularly rewarding and has been recently reviewed (Urry and Ohnishi, 1970; Bovey *et al.*, 1972; Hassall and Thomas, 1971; Thomas, 1973). Very recently, ^{13}C nmr has been applied to this problem. Investigations of gramicidin S (Gibbons *et al.*, 1970), of valinomycin and its K^+ complex (Ohnishi *et al.*, 1972; Patel, 1973a), of antamanide and its Na^+ complex (Patel, 1973b,c), and of oxytocin (Smith *et al.*, 1972; Deslauriers *et al.*, 1972; Bovey, 1972) have been reported. As yet, ^{13}C nmr data, *i.e.*, chemical shifts and in some studies T_1 values, cannot be as directly interpreted in terms of conformation as ^1H nmr data. One of the most useful and unambiguous conformation correlations is that of the chemical shift of the γ carbon of proline with the conformation, cis or trans, of the preceding peptide bond (Dorman and Bovey, 1973; Thomas and Williams, 1972; Wüthrich *et al.*, 1972; Deslauriers *et al.*, 1972). As we shall see, other marked variations in carbon shielding can occur which seem to be primarily related to alterations of conformation.

In this paper, we present assignments for the ^{13}C spectra of the neurohypophyseal hormone, oxytocin, its analogs, [7-D-proline]-oxytocin and [4-glycine]-oxytocin, and deamino-oxytocin, as well as for seven precursors of oxytocin. These assignments are presented in more detail and with more supporting evidence than in the preliminary report (Bovey, 1972). They differ at some points from those of Deslauriers *et al.* (1972) for oxytocin.

Oxytocin is a nonapeptide with a hexapeptide cyclic portion closed by a disulfide bond and a tripeptide linear segment terminated by a carboxamide group.



Various aspects of the conformation of oxytocin and related peptides have been studied by proton magnetic resonance (pmr) spectroscopy (Johnson *et al.*, 1969; Urry *et al.*, 1970;

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