PARTIAL MOLAR ROTATORY POWERS AND OPTICAL ACTIVITY IN
PROTEINS AND POLYPEPTIDES, II. THE ALPHA-HELIX
OF POLY-L-ALANINE

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Because of the number and diversity of well-characterized molecular systems containing the peptide moiety, this chromophore provides a unique opportunity to develop an empirical expression for rotational strength. Once determined, an empirical equation would find wide application to important biological problems as well as to the determination of configuration and conformation of organic molecules in general. With these goals in mind it has recently been proposed¹ that the rotational strength, R , of a transition, e.g., the $n - \pi^*$ transition of the peptide chromophore, be written in general as the sum of products of geometric factors, G_n^m , and partial molar rotatory powers, $[P_n^m]$, i.e.,

$$R = \frac{1}{N} \sum_{n=1}^N \sum_k \sum_l \sum_m G_n^m(k)_l [P_n^m(k)_l], \quad (1)$$

where the initial summation is over the identical chromophores which contain the transition; the second summation is over the different types of vicinal groups in the molecule which contribute to the optical activity, e.g., the methyl groups and peptide groups in poly-L-alanine; the third summation numbers the identical vicinal groups, e.g., the methyl group of residue $n + 1$, etc.; and the fourth summation allows that the physical properties of a perturbing vicinal group may have different geometric factors and that there may be terms of opposing sign with different distance dependencies. Once the proper geometric factors and partial molar rotatory powers are determined for a vicinal moiety, then it should be possible to place the vicinal group in any position relative to the peptide chromophore and calculate its contribution to the rotational strength of the peptide $n - \pi^*$ transition; that is, in principle one can calculate, for example, the expected ellipticity for a given polypeptide conformation.

The requirement of two terms in the summation over m has been determined, and it has been shown that these terms differ in sign and in distance dependence but in such a self-consistent empirical analysis one term cannot have an octant dependence and the other term a quadrant dependence.¹ Due to the large values of rotational strength obtained when an r^{-6} distance dependence is taken for the short-range term and fit to the L-5-methyl pyrrolid-2-one and L-3-methyl-2,5-diketopiperazine data, this distance dependence is discounted. Accordingly, the short-range term was taken as r^{-5} and the long-range term was variously considered with an r^{-4} or r^{-3} distance dependence.

In the preceding paper in this series,² values were calculated for the rotational strength of the peptide $n - \pi^*$ transition in the fully extended chain and the anti-parallel β -pleated sheet conformations of poly-L-alanine. In this communication, calculated values for the α -helix of poly-L-alanine are reported. At this

stage acceptable values are obtained for glycyl-L-alanyl-glycinate Cu (II), for the fully extended chain, for the cross β -structure, and for the α -helix when the following explicit expression is used:

$$R = \frac{1}{N} \sum_{n=1}^N \sum_k \sum_l \left\{ \frac{\gamma_x(k)_l \gamma_y(k)_l}{r^5(k)_l} [P_n^1(k)_l] + \frac{\gamma_x(k)_l \gamma_y(k)_l}{r^3(k)_l} [P_n^2(k)_l] \right\}, \quad (2)$$

where $r(k)_l$ is the distance, for example, of the l th methyl group from the peptide oxygen of interest in poly-L-alanine and γ_x and γ_y denote the direction cosines, x/r and y/r , respectively.

The successes in calculating the rotational strength of the peptide $n - \pi^*$ transition for the above-mentioned cases increase the possibility that the correlations are meaningful and not simply fortuitous. Accordingly, chain-length effects are calculated and related to proposed conformations of the antibiotic gramicidin S. In addition, the left-handed helix of poly-L-alanine and the 3_{10} helix are briefly considered.

The α -Helix.—In the present calculations on the α -helix, as in the previous calculations on the antiparallel β -pleated sheet conformation,² poly-L-alanine is treated as consisting of three vicinal moieties—the β -methyl, the α CH, and the CONH groups. Partial molar rotatory powers for the methyl group are calculated for assumed geometric factors from the model compounds—L-5-methyl pyrrolid-2-one and L-3-methyl-2,5-diketopiperazine. The partial molar rotatory powers and associated geometric factor are then used to calculate the contribution of the β -methyl moiety to the rotational strength of the peptide $n - \pi^*$ transition. The vicinal peptide moiety is treated as a single, center-of-mass point with the partial molar rotatory powers for an associated geometric factor being calculated from L-pyrrolid-2-one-5-carboxylic acid amide and L-2,5-diketopiperazine-3-acetamide. The α CH moiety is assumed to be the same as a methyl moiety. This approximation was made with the expectation that the moiety would finally be parametrized in terms of a methyl moiety in order to obtain a correct value. However, the straightforward calculation gave such good correlation with experimental data that no parametrization was necessary. This may derive from the fact that there are two opposing effects which tend to cancel. The α CH moiety has two less hydrogen atoms, which should decrease the negative rotational strength compared to a methyl moiety in a positive quadrant or octant. In addition, the α CH moiety resides between two peptide groups which are aromatic in character. Accordingly, one expects an inductive withdrawal of electrons causing the α CH group to be more electropositive and thereby increasing the negative rotational strength when compared to a methyl moiety in a positive quadrant or octant. This may be seen by studying the data on the pyrrolidone series.¹

The most refined values for the geometry of poly-L-alanine in the α -helix have recently been reported by Arnott and Dover.³ From those data the transformation matrix⁴ was calculated to be

$$A = \begin{bmatrix} 0.849 & -0.518 & 0.104 \\ 0.488 & 0.844 & 0.222 \\ -0.202 & -0.138 & 0.970 \end{bmatrix}. \quad (3)$$

The rotational strength of the $n - \pi^*$ transition of helical poly-L-alanine in trifluoroethanol is reported to be -24×10^{-40} on the basis of simultaneous resolution of absorption and circular dichroism data.⁵ The partial molar rotatory powers and associated geometric factors were given in the previous paper in this series.² Table 1 contains a summary of the calculated values. It is seen that a quadrant angular dependence in connection with r^{-5} and r^{-3} distance dependencies gives the value which most closely approaches the experimental value of -24×10^{-40} . These are the same angular and distance dependencies which adequately described the antiparallel β -pleated sheet conformation. In the β -conformation the orientation of the peptide moiety alternates, whereas in the α -helix the acyl oxygen points toward the carboxyl end of the helix and is always displaced in the negative z direction with respect to a center-of-mass point for the amide. From the series of pyrrolidones it can be argued that the sign of the partial molar rotatory power is determined by the more electronegative oxygen.¹ When correction is made for this regular deviation, the quadrant, r^{-5} , r^{-3} calculation gives a rotational strength of -20×10^{-40} . The values in parentheses in Tables 1 and 2 contain this correction.

A significant point brought to light by the values calculated in Table 1 is the contribution of the β -methyl group. If the octant rule were operative, the major negative rotational strength would derive from the β -methyl perturbation, whereas if a quadrant rule were correct, the β -methyl group would make a smaller, positive contribution to the rotational strength. Because of this, we have studied the ordered copolymers (L-ala-L-ala-L-ala-gly)_n and (L-ala-L-ala-gly)_n. If the octant rule were correct, removal of the methyl group, as achieved by glycine replacement of alanine, would cause a decrease in rotational strength of the $n - \pi^*$ transition; however, if the system were adequately described by a quadrant rule, the rotational strength of the $n - \pi^*$ transition would increase. By normalizing on the magnitude of the second negative band and by going to very low temperatures (-30°C) to achieve a fully ordered state, it appears that the ellipticity of the first negative band for (L-ala-L-ala-L-ala-gly)_n is enhanced relative to the second band when compared to that of poly-L-alanine. This is what is to be expected for a quadrant rule. The magnitude of the effect correlates with the lower value calculated for $R(\alpha\text{CH})$ with the use of Pauling's coordinates (see below).

The residue contributions to the rotational strength of the $n - \pi^*$ transition in residue n are given in Table 2 for a quadrant, r^{-5} , r^{-3} dependence. With these

TABLE 1. α -Helix (chain length of 21).

Distance dependence	Octant Angular Dependence					
	r^{-5}, r^{-3}			r^{-5}, r^{-4}		
Moiety	βC	αC	CON	βC	αC	CON
Rotational strength $\times 10^{40}$	-47.6	1.7	-7.4	-59.5	-2.6	-7.7
Total $\times 10^{40}$		-53.3			-69.8	
Distance dependence	Quadrant Angular Dependence					
	r^{-5}, r^{-3}			r^{-5}, r^{-4}		
Moiety	βC	αC	CON	βC	αC	CON
Rotational strength $\times 10^{40}$	10.2	-35.3	9.0(5)	22.9	-27.4	8.7
Total $\times 10^{40}$		-16.1 (-20)			4.2	

TABLE 2. α -Helical poly-L-alanine,* quadrant r^{-5} , r^{-3} .

Residue number	βC	αC	CON	Residue R
$n - 7$	-0.2	-0.2	0	-0.4
$n - 6$	0	0	0	0
$n - 5$	0.4	0.2	-0.4	0.2
$n - 4$	-0.3	0.5	0.1 (0.2)	0.3 (0.4)
$n - 3$	0.5	-0.1	0	0.4
$n - 2$	-2.1	-2.8	2.0 (2.6)	-2.9 (-2.3)
$n - 1$	9.3	7.4	-2.2 (-2.4)	14.5 (14.3)
n	-19.6	0	0	-19.6
$n + 1$	-0.9	0	-3.8 (-3.5)	-4.7 (-4.3)
$n + 2$	-20.2	-42.3	8.0 (4.7)	-54.5 (-57.8)
$n + 3$	39.1	14.6	0.4 (0.2)	54.1 (53.9)
$n + 4$	20.5	1.6	1.3 (0.9)	23.4 (23.0)
$n + 5$	-11.7	-8.1	2.3 (1.7)	-17.5 (-18.1)
$n + 6$	-2.4	-3.8	0.6 (0.4)	-5.6 (-5.8)
$n + 7$	0	-0.3	0.1	-0.2
$n + 8$	0	-0.2	0.2 (0.1)	0 (-0.1)
$n + 9$	-1.5	-1.0	0.2	-2.3
$n + 10$	-0.2	-0.3	0.1	-0.4
$n + 11$	0	-0.1	0	-0.1
$n + 12$	-0.2	-0.2	0.1	-0.3
$n + 13$	-0.3	-0.2	0	-0.5
Total	10.2	-35.3	9.0 (5)	-16.1 (-20)

* All values have been multiplied by 10^{40} .

values, a chain-length dependence may be calculated (see Table 3). A maximum in magnitude of rotational strength is seen at three to four residues and the chain-length effect is essentially complete by six residues. This is in contrast to the octant rule calculation, which exhibits a minimum in absolute rotational strength of 10 to 11×10^{-40} at two to three residues with increasingly larger negative values up to 20 residues. The chain-length effect reported in Table 3 is in marked contrast to the calculated chain-length effects for cross β -structures.² A cross β -structure of two chains with ten residues each calculates to have a mean residue rotational strength, R , of -15.4×10^{-40} , whereas a value of $+14.1 \times 10^{-40}$ is obtained for two chains with five residues each. These chain-length effects have two significant implications. (1) Resolution of the circular dichroism curves for complex globular protein into per cent α , β , and random-coil conformations based on circular dichroism curves of fully ordered and large-molecular-weight model polypeptides would be suspect in cases where short chain lengths are involved. (2) Should these chain-length effects be correct, they would afford a distinction between possible conformations of small naturally occurring polypeptides such as gramicidin S and oxytocin.

Optical rotatory dispersion and circular dichroism studies on gramicidin S indicate large negative mean residue rotations and ellipticities in the peptide wavelength range,⁶⁻⁸ which, if used in the usual manner to calculate helical contents, would indicate a 100 per cent helical structure. As gramicidin S is a cyclic decapeptide, it cannot be completely helical. Conformations based on minimization of peptide energy have been proposed by Liquori *et al.*⁹ and by Vanderkooi *et al.*¹⁰ Liquori proposed a structure with two helical segments of four residues each, joined by two D-phenylalanine residues. Vanderkooi *et al.* report ψ and ϕ

angles¹¹ for six of the ten residues which more nearly approach those of the anti-parallel β -pleated sheet. Without here carrying out a detailed calculation, it is apparent that the Liquori structure would agree with and even support the chain-length effects herein calculated for the α -helix, whereas that of Vanderkooi *et al.* appears to be in disagreement. By the calculated chain-length effects a structure of ten peptide moieties containing two helical segments of four residues each would be expected to exhibit mean residue rotations and ellipticities near 233 m μ and 220 m μ , respectively, which approximate those of the completely helical model polypeptides.

Perhaps the most fundamental statement concerning these calculations on polypeptides is that while long chains of α - and of β -structures give experimental rotational strengths for the $n - \pi^*$ transition which are negative and of similar magnitude, it is to be expected that they exhibit very different values for short chain lengths. In this connection it should be mentioned that if the α CH moiety does have a greater absolute contribution to the rotational strength than a methyl group, then other combinations of angular and distance dependence may approach the experimental values for the α - and β -conformations. Irrespective of this, basic differences in chain-length effects of the α - and β -structures can be expected to obtain. The most fundamental statement of this approach is that $R_n(\text{CH}_3)_n$ for the α helix is about -20×10^{-40} , whereas $R_n(\text{CH}_3)_n$ for a β -structure is about $+20 \times 10^{-40}$.

Pauling Coordinates and the Left-Handed Helix.—It is of interest to utilize the Pauling coordinates¹² for two reasons. (1) They offer a variation on the α -helix (the tilt of the amide moiety with respect to the helix axis is different than that in the Arnott and Dover coordinates) which allows one to determine the sensitivity of the calculated values to small changes in coordinates. (2) They provide consistent values for consideration of the left-handed helix of poly-L-alanine. Using the following transformation matrix,⁴

$$A = \begin{bmatrix} 0.835 & -0.552 & 0.003 \\ 0.535 & 0.809 & -0.243 \\ 0.132 & 0.205 & 0.970 \end{bmatrix}, \quad (4)$$

one obtains substantially the same results; that is, instead of -53 , -70 , -16 , and 4 of Table 1, values of -43 , -58 , -12 , and -1 , respectively, are calculated. Thus small variations in the coordinates of the α -helix may not be expected to have large effects. It may also be noted relative to the (L-ala-L-ala-L-ala-gly)_n results and the octant versus quadrant question (see above) that the rotational strength contribution of the β -methyl moiety calculates to be 4×10^{-40} when Pauling's coordinates are used as opposed to 10×10^{-40} when the Arnott and Dover coordinates are used.

The mirror image of the right-handed helix of poly-L-alanine is the left-handed helix of poly-D-alanine. These enantiomers give equal and opposite rotational strengths. Accordingly, it is to be expected that the left-handed helix of poly-L-alanine would not have the exact equal and opposite relationship, but rather that only the contributions of the α CH and peptide vicinal groups would relate in this way. The net contribution of the β -methyl would not.

TABLE 3. Chain-length effect on the rotational strength of the peptide $n - \pi^*$ transition of poly-L-alanine in the α -helical conformation.

Residue rotational strength $\times 10^{40}$	Number of Residues in a Helical Segment								
	1	2	3	4	5	6	7	8	35
R_1	-19.6	-23.9	-81.7	-27.8	-4.8	-22.9	-28.7	-28.9	
R_2		-5.3	-9.6	-67.4	-13.5	9.5	-8.6	-14.4	
R_3			-7.6	-11.9	-69.7	-15.8	7.2	-10.9	
R_4				-7.2	-11.5	-69.3	-15.4	7.6	
R_5					-6.8	-11.1	-68.9	-15.0	
R_6						-6.6	-10.9	-68.7	
R_7							-6.6	-10.9	
R_8								-7.0	
Mean residue rotational strength $\times 10^{40}$	-19.6	-14.6	-33.0	-28.6	-21.3	-19.4	-18.8	-18.5	-20

The calculated values give a net rotational strength of the β -methyl which is such that the total rotational strength is nearer zero, -6.6×10^{-40} for a quadrant rule and 1×10^{-40} for an octant rule using r^{-5} and r^{-3} distance dependencies. A polypeptide containing L-amino acids which is thought to be stable in the left-handed helical conformation is poly- β -methyl-L-aspartate.¹³⁻¹⁵ This polymer had $R_{n-\pi^*}$ of approximately 16×10^{-40} (see ref. 1). Therefore, within the framework of this analysis, in order for poly- β -methyl-L-aspartate to be in the left-handed helix, the ester group must contribute a large positive rotational strength.

The 3_{10} Helix and Lysozyme.—Recent X-ray data on the enzyme lysozyme indicate that in addition to α -helix there are short segments of threefold or 3_{10} helix as well as antiparallel β -pleated sheet.¹⁶ Particularly since there are no model polypeptides known to exist in the threefold helical conformation, it is of interest to gain some information about the contributions of these segments to the total rotational strength of the $n - \pi^*$ transition of lysozyme. From the coordinates of Donohue¹⁷ the transformation matrix⁴ is calculated to be

$$A = \begin{bmatrix} 0.687 & -0.711 & 0.148 \\ 0.637 & 0.688 & 0.347 \\ -0.349 & -0.144 & 0.926 \end{bmatrix}$$

With calculations as for the α - and β -structure, a much larger negative rotational strength (-90×10^{-40}) is obtained for the long-chain threefold helix when quadrant, r^{-5} and r^{-3} dependencies are used. Since such large rotational strengths are seldom observed, the calculated value is suspect. The magnitude, however, is derived in part from the summation of many contributions of negative sign from groups which are at substantial distances. In the calculations thus far we have neglected the presence of groups which occur between the peptide oxygen and the perturbing vicinal moiety being considered. By so doing we have assumed an effective dielectric constant to be the same as in the model compounds in which the distances were 5 Å or less. As a result, one anticipates

an overestimate of groups at greater distances. These considerations were of little significance in the α -helix or the 2×10 cross β -structure, but are important in the 3_{10} helix and the essentially infinite antiparallel β -pleated sheet.² Even so, it is to be expected that the 3_{10} helix results in substantially larger negative values than the α -helix and it is possible to understand the lysozyme data within this framework.

In the usual calculation of α -helical content for lysozyme one obtains a value near 40 per cent which approximates that of the total helical content as determined by the X-ray structure. Yet it was mentioned above that by the present calculations, a 2×5 cross β -structure would give a positive rotational strength. As lysozyme contains such a structure this approach would indicate a much lower estimate of helical content. Compensation for the 2×5 cross β -structure is found in conformations which give larger negative values for the rotational strength of the $n - \pi^*$ transition than the α -helix. Such structures could be the segments of threefold helix. Lysozyme has three and six residue segments with coordinates close to those of the threefold helix.¹⁶ The mean residue rotational strength for these segments, considering backbone and β -methyl moieties, would be approximately -40×10^{-40} and -50×10^{-40} , respectively. Thus consideration of the 3_{10} helix as well as the other conformations in lysozyme yields a consistent picture. It should be emphasized that other moieties in the side chains could, if properly located, have large effects on a final value; however, one intuitively expects much cancellation of contributions from moieties other than those considered here because of the size of the enzyme and the variability in side-chain positions of moieties other than the β -methyl.

Summary.—The concept of partial molar rotatory powers has been used to calculate the rotational strength of the $n - \pi^*$ transition of poly-L-alanine in the right-handed α -helical conformation. Left-handed α -helix and right-handed 3_{10} helix of poly-L-alanine were also discussed. It was found that a quadrant rule employing two oppositely signed terms with r^{-5} and r^{-3} distance dependencies gave satisfactory values. Chain-length effects were presented and discussed with respect to data on gramicidin S.

The most fundamental point to be derived from these calculations is the difference in chain-length effects for the β -pleated sheet and α -helix conformations. This derives from the contribution of the methyl group on the same residue, that is, $R_n(\text{CH}_3)_n$ is about 20×10^{-40} for the β -conformation and about -20×10^{-40} for the α -helix, yet both the larger structures have negative rotational strengths of similar magnitude. This point is of particular significance in the conformational analysis of small, but also of large, polypeptides and proteins. A second point which may be expected to obtain regardless of the approximations is the difference in contribution of the βCH_3 moiety depending on whether an octant or quadrant rule is operative.

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⁴ The transformation starts with a right-handed rectangular coordinate system centered on a peptide oxygen with the z axis parallel to the helix axis and effects the series of rotations which places the z axis along the C—O bond and makes the yz plane coincident with the peptide plane.

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