

Theoretical Calculation of the Circular Dichroism of Unordered Polypeptide Chains

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Synopsis

A calculation has been done of the circular dichroism (CD) spectrum of an unordered polypeptide chain. This has been based on a Boltzmann averaging over a dipeptide conformational CD map. This is shown to be valid by comparing the CD spectra of 28-mer oligopeptides with those generated by summing dipeptide CD spectra. The calculated CD spectrum of an unordered polypeptide chain is found to agree with the assignment proposed by Tiffany and Krimm from experimental studies.

INTRODUCTION

A true understanding of the circular dichroism (CD) spectra of polypeptides and proteins must rest ultimately on the ability to correlate the observed spectrum with that calculated for a relevant structural model from a valid theory. A satisfactory start in this direction has been made in recent years for regular structures such as the α -helix^{1,2} and the β conformation,²⁻⁵ as well as for certain dipeptides.^{6,7} The situation is less clear for the case of unordered polypeptide chain structures.

The situation with respect to unordered chains is complicated for two reasons. First, the validity of calculations for determining the CD of unordered polypeptide chains⁸⁻¹⁰ may still be questionable. Second, and probably more important, there continues to be disagreement over the experimental CD spectrum which is to be taken as representative of an unordered chain. Although the spectra of charged poly-L-glutamic acid or charged poly-L-lysine have been assumed¹¹ to be that of a "random coil," it has been pointed out¹² that such structures are not valid models because of the likelihood that the side chain charges stabilize a preferred extended helical conformation of the backbone. The geometry of this structure was determined by conformational energy calculations¹³ to be close to a three-fold lefthanded helix. This structural assignment of the observed CD spectrum of charged chains has since been supported by spectral studies of uncharged chains known to have an analogous conformation.¹⁴ The CD spectrum of an unordered polypeptide chain was, partly on this basis, associated with that of poly-L-proline in concentrated aqueous CaCl₂ solu-

tion,¹⁵ an assignment which was strongly supported by an extended study of a variety of synthetic polypeptides, fibrous proteins, and globular proteins.¹⁶ The latter CD spectrum, in contrast to that of the extended helix, has no positive band near 220 nm but decreases monotonically from a value of $\Delta\epsilon = 0$ near 240 nm to a negative band centered near 200 nm (depending on the system) and of variable intensity ($\Delta\epsilon \cong -1$ to -4). Subsequent detailed experimental investigations¹⁷ tend to confirm this assignment.

In order to shed further light on this problem we have done a theoretical calculation of the CD spectrum of an unordered polypeptide chain. This calculation differs in several details from those previously done,⁸⁻¹⁰ and we believe represents an improvement over them.

METHOD OF CALCULATION

Our calculation is based on the monopole approximation to the far ultraviolet transitions of the peptide group, a method which seems to have at least a qualitative reliability.^{1,6,18,21} This calculation uses an SCF wave function for the peptide monomer ground state and the $n\pi^*$ and $\pi\pi^*$ transitions. The polypeptide wave function is constructed from this monomer wave function by configuration interaction.

The $n\pi^*$ and $\pi\pi^*$ transitions are both included in the secular determinant rather than treating the $\pi\pi^*$ by an exciton calculation and then adding in the $n\pi^*$ as a perturbation. By including both transitions the calculation becomes more difficult numerically, but it is conceptually simpler and seems to lead to more reliable results.¹ The $n\sigma^*$, $n'\pi^*$, and NV_2 transitions are very poorly understood, and they have not been included. Of course, if the $n\sigma^*$ transition lies close to the $\pi\pi^*$ transition, for which there may be some evidence,²² then it should be taken into account. Since the assignment of the $n\sigma^*$ transition is so uncertain, however, we feel that its inclusion would be essentially arbitrary at this time.

The secular determinant of the polypeptide wave function is estimated by a monopole approximation where point charges are placed on or near atoms of the chain. The magnitudes and positions of the monopoles are estimated from the SCF wave function for the monomer. We have selected monopoles which optimize the agreement between observed and calculated CD spectra of a known polypeptide chain structure, *viz.*, the righthanded α -helix. These monopoles combine features of those due to Woody¹ and those due to Bayley et al.⁶ Our monopoles differ from Woody's by having $n\pi^*$ charges on the carbon as well as on the oxygen of the carbonyl group, and by not splitting the amide ground state monopoles in two. The first difference seems justified from the results of recent SCF calculations,²⁰ the second was necessary in order to prevent unusual proximity of monopoles in the α -helix. We have, however, assumed the $\pi\pi^*$ monopoles to be split, there being theoretical justification for this since they describe a transition localized in the pi electrons only. The monopoles which we used (for a poly-L-alanine chain) are given in Table I. They are the same as

TABLE I
Monopoles and Parameters Used in the Calculation

Peptide Ground-State Charges ¹⁸				
Center	Charge (10^{-10} esu)			
Carbon	2.04			
Oxygen	-1.87			
Nitrogen	-1.7112			
Hydrogen	1.27			
α -Carbon	0.6382			
α -Hydrogen	-0.367			
β -Carbon	1.101			
β -Hydrogen	-1.101			

Peptide $\pi\pi^*$ Monopoles ^a			
Center	Distance (\AA)		Charge (10^{-10} esu)
Carbon	1.0806		0.41
Oxygen	0.7737		0.45
Nitrogen	0.9813		-0.86

Peptide $n\pi^*$ Monopoles ^b				
Center	Location (\AA)			Charge (10^{-10} esu)
	X	Y	Z	
Carbon	0.6076	0.6076	0	-0.5277
	-0.6076	0.6076	0	0.5277
	-0.6076	-0.6076	0	-0.5277
	0.6076	-0.6076	0	0.5277
Oxygen	0.6076	0.6076	0	-0.3518
	-0.6076	0.6076	0	0.3518
	-0.6076	-0.6076	0	-0.3518
	0.6076	-0.6076	0	0.3518

Transitions	
Wavelength:	$\pi\pi^*$, 190 nm; $n\pi^*$ 212 nm
Bandwidth:	$\pi\pi^*$ 12 nm; $n\pi^*$, 10 nm

^a Equal charges are placed above and below the plane of the peptide group, centered over the C, O, and N atoms. The $\pi\pi^*$ electric transition dipole moment is 3.05 D,²⁰ forming an angle of 9° toward the C atom from the N-O line.¹⁸

^b Quadrupole symmetry is taken around the C and O atoms. Coordinates are in the standard coordinate system, with Z along the C to O line and X in the plane of the peptide group.¹⁸ The $n\pi^*$ magnetic transition dipole moment is 1.1506 BM,²⁰ pointing from O to C.

those of Bayley et al., except that we have added peptide ground-state monopoles¹⁸ and we have used semiempirical screening coefficients.¹

The CD spectrum of a 20-monomer righthanded α -helix of poly-L-alanine was calculated using the monopoles and parameters given in Table I. This spectrum is compared with the experimental spectrum²³ in Figure 1. The agreement is seen to be satisfactory. It should be noted that in this calculation the $n\pi^*$ transition, which on the basis of a study of simple amides in solution¹⁹ would be at 212 nm, has been taken to be at 220 nm.

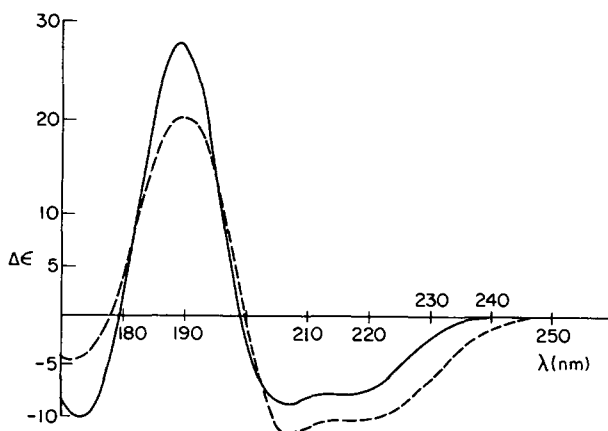


Fig. 1. CD spectrum of the righthanded poly-*L*-alanine α -helix: —, calculated for $\varphi = -47^\circ$ $\psi = -57^\circ$, using monopoles and parameters of Table I; ---, experimentally observed.²²

This is thought to be a consequence of the shielding of the carbonyl group in the α -helix from the water solvent.¹⁹ Since no such shielding is expected for the case of the unordered chain, we have placed the $n\pi^*$ transition at 212 nm in these calculations.

APPLICATION TO UNORDERED POLYPEPTIDE CHAIN

Since the monopoles and parameters of Table I account very well for the CD spectrum of the α -helix, we have adopted them for the calculation of the unordered chain. The CD of an unordered chain was taken to be a Boltzmann distribution over the conformational energy map of a dipeptide, the CD of the dipeptides as a function of φ and ψ being calculated according to the methods of the previous section. In order to establish the validity of this approach, the CD spectra of several arbitrary oligopeptide structures were calculated and compared with spectra computed as a sum of dipeptide contributions. The results of these preliminary calculations will now be discussed.

The calculations of the dipeptide CD spectra were done at 10° intervals in φ and ψ for the entire range of φ, ψ angles. It is of interest to note that the resulting spectra fall into a relatively small number of types, thus permitting a plot of a dipeptide conformational CD map. We have classified these spectra according to the following scheme, which is illustrated by specific examples in Figure 2.

Type 1: A net negative CD at wavelengths greater than 215 nm followed by a positive CD with a maximum at a wavelength greater than 187 nm.

Type 2: Similar to type 1 except that the positive maximum occurs at wavelengths shorter than 187 nm.

Type 3: The CD is essentially zero at all wavelengths.

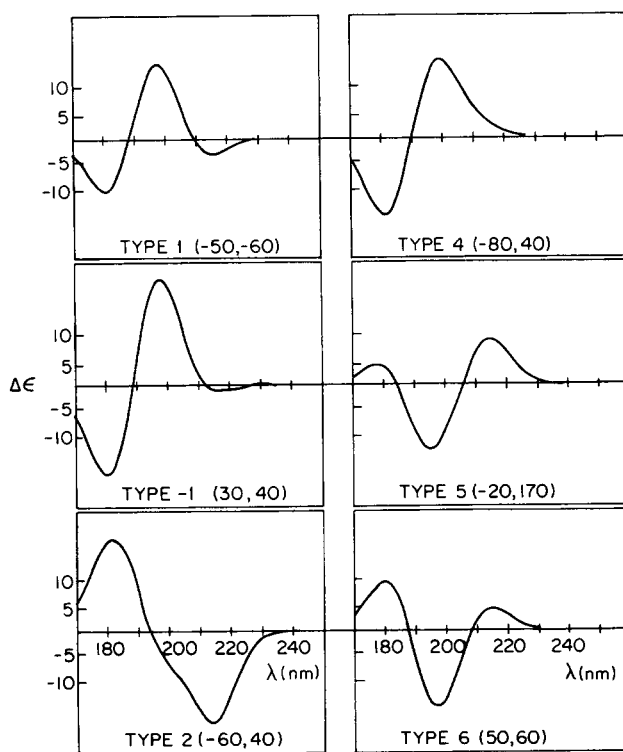


Fig. 2. Examples of types of dipeptide CD spectra classified in the text. The numbers in parentheses are the (ϕ, ψ) values used for the examples.

Type 4: A positive CD at wavelengths greater than 215 nm followed by a negative CD with a minimum at a wavelength shorter than 185 nm.

Type 5: Similar to type 4 except that the minimum occurs between 185 and 198 nm.

Type 6: Similar to type 4 except that the minimum occurs at wavelengths greater than 198 nm.

A conformational CD map based on this classification is given in Figure 3. Some ϕ, ψ points do not quite fit into the above classification in that small contributions are present in the 230–250 nm region. These have been designated with a minus sign in Figure 3, indicating that there is a small band in this region which is positive for types 1 and 2 and negative for types 4, 5, and 6. An example of type -1 CD spectrum is given in Figure 2.

In order to test the proposal that the CD of a polypeptide chain with an arbitrary sequence of ϕ, ψ angles can be adequately represented by a sum of the corresponding dipeptide CD spectra, calculations were made on oligopeptides containing twenty-eight residues. Then, using the same set of twenty-seven ϕ, ψ angles, the average CD based on dipeptide spectra were determined. Many such calculations were done, but the results are

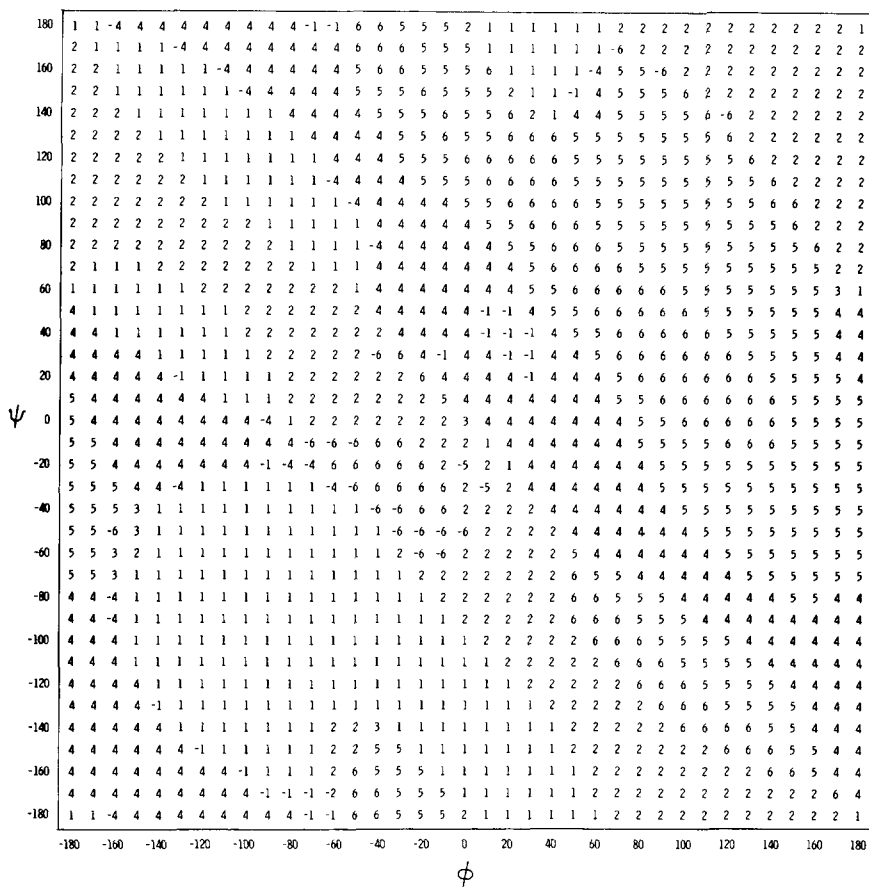


Fig. 3. Dipeptide conformational CD map. Numbers at each φ, ψ represented spectral types, and are described in text.

typified by the three examples given in Figures 4a, b, and c. In the upper lefthand corner of each figure is a series of points representing the φ, ψ angles used for that particular oligopeptide. (One specific sequence of φ, ψ is shown, but an examination was made of other sequences and these generally give the same type curve.) The points are restricted to the region of the map represented by $-180 \leq \varphi \leq 0, 0 \leq \psi \leq 180$, since this is the region most relevant to the unordered chain. In all cases the dipeptide sum spectrum is a reasonable approximation to that of the oligopeptide. The following comparisons are to be noted: (1) the maxima and minima of the oligopeptide in the region of about 170 to 200 nm are at shorter wavelengths than for the dipeptide spectrum; (2) in both the dipeptide and oligopeptide spectra, the long wavelength negative band (which is a summation of $\pi\pi^*$ and $n\pi^*$ rotational strengths) is centered near the $n\pi^*$ transition (212 nm in this case); (3) the amplitudes of the oligopeptide bands in the region of 190 to 250 nm are generally larger than those in the comparable dipeptide spectra. Despite these differences, it appears that the

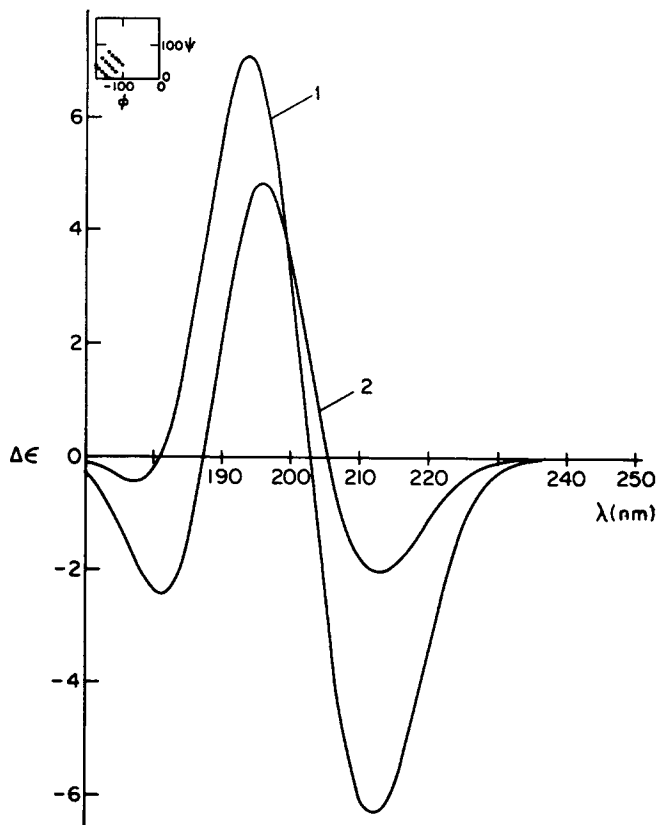
dipeptide CD can be satisfactorily used to represent the spectrum of an unordered chain. If we do so, we may expect that the actual chain will have its $\pi\pi^*$ peak at a shorter wavelength and that its band may be somewhat more intense.

RESULTS AND DISCUSSION

Having shown that the CD of a non-regular chain is reasonably well represented by a sum of dipeptide contributions, we have evaluated the CD of an unordered polypeptide chain from

$$\bar{R}(\lambda) = \frac{\sum_i R_i(\lambda) \exp(-E_i/RT)}{\sum_i \exp(-E_i/RT)} \quad (1)$$

In this expression: i represents a φ, ψ pair, with the summation being taken at 10° intervals over the range $-180 \leq \varphi \leq 0, 0 \leq \psi \leq 180$ (a calculation



(a)

Fig. 4. (continued)

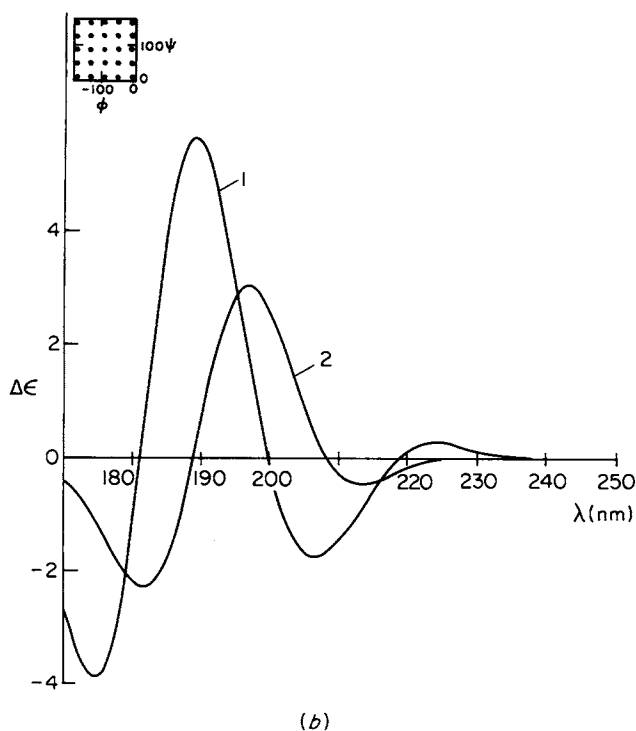


Fig. 4. (continued)

with $-180 \leq \varphi, \psi \leq 180$ gave essentially the same result, which is reasonable since most of the low energy conformations are concentrated in the former angular range); $R_i(\lambda)$ is the circular dichroism as a function of wavelength for a particular dipeptide, which is shown in Figure 3 and given in detail elsewhere;²¹ E_i is the energy of the dipeptide²⁴ (the results are similar²¹ if previously published energies²⁵ are used); $R = 1.986 \times 10^{-3}$ kcal/deg-mole; and $T = 288^\circ\text{K}$. The result of such a calculation is shown in Figure 5.

Our calculated CD spectrum of an unordered polypeptide chain, shown in Figure 5, disagrees with previous calculations^{8,9} in not exhibiting any long wavelength positive band. On the other hand, this result is in agreement with detailed experimental studies^{16,17} characterizing this spectrum. The calculated negative band, at about 213 nm, is at a somewhat longer wavelength than that observed.¹⁶ It must be remembered however, that this is directly related to the location of the $n\pi^*$ transition, about which there may be some uncertainty for such structures. In addition, there is evidence¹⁷ that the negative peak moves to longer wavelengths in the presence of certain salts. These spectra also show that a crossover to positive $\Delta\epsilon$ exists at shorter wavelengths, as predicted in Figure 5. In view of the fact that the calculated magnitude of the 213-nm band is also

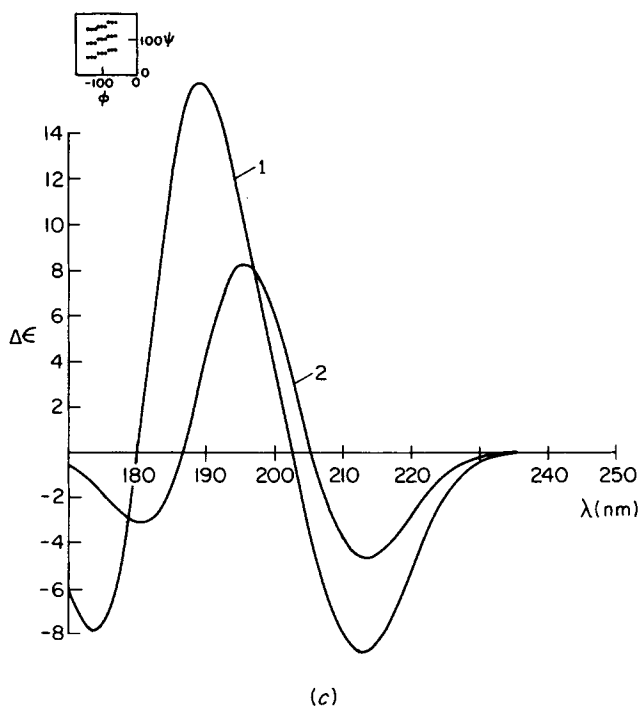


Fig. 4. Calculated CD spectra of: (1) oligopeptide of twenty-eight residues, (2) the sum of dipeptides with the same twenty-seven pairs of ϕ, ψ angles. The ϕ, ψ angles used are shown in the upper left hand corner of each figure. a), b), and c) represent different sets of ϕ, ψ angles.

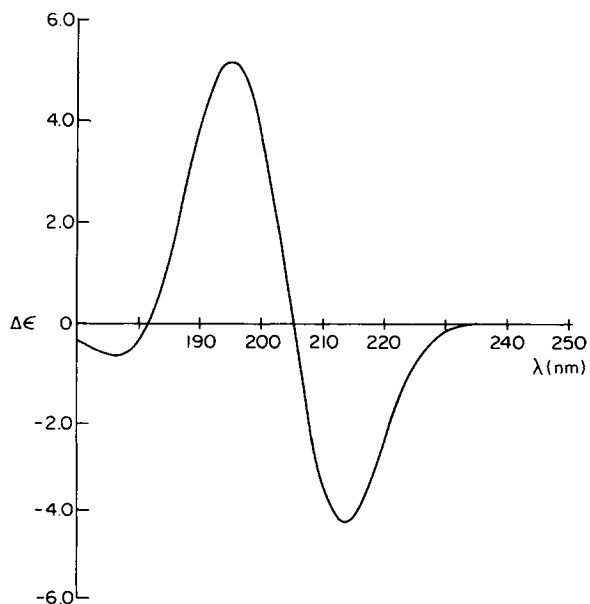


Fig. 5. Calculated CD spectrum of an unordered polypeptide chain.

in reasonable agreement with that observed for the negative peak,¹⁶ we feel that the present calculation makes plausible the revised assignments^{15,16} for the CD spectrum of an unordered polypeptide chain.

It has been stressed before,¹⁶ but it is perhaps worth emphasizing again, that there is probably no single unordered polypeptide chain structure. Different non-regular systems probably sample the φ, ψ space in different ways, depending on such factors as interaction with solvent, side chain steric and electrostatic interactions, etc. Our model of the unordered chain is based on a dipeptide energy map in which such factors are minimal. An examination of the CD spectra of a wide variety of unordered polypeptides^{16,17} indicates that the spectrum of Figure 5 is more closely approached the greater is the tendency toward randomization of order in the system. In this sense, the above calculated spectrum could be considered as that of a truly random chain, which is approached more or less closely by real polypeptide systems.

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