The Calculated Rotatory Properties of Random-Coil Poly-L-alanine

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Received September 3, 1969

ABSTRACT: The rotatory properties of poly-L-alanine in the randomly coiled conformation are calculated from existing theories. The calculated rotational strengths of the various electronic transitions in the amide group are averaged over all conformations accessible to the random coil. Two different descriptions of the electronic structure of the amide group are employed, and the results they yield are compared with circular dichroism data from the literature. Both electronic descriptions of the amide group lead to semiquantitative agreement with experiment. However, it is not possible to render a definite judgment regarding the preferability of one description over the other.

Any study of the conformationally dependent properties of polypeptides by optical rotatory dispersion (ORD) or circular dichroism (CD) requires a knowledge of the optical activity of each of the various polypeptide conformations, *e.g.*, α-helix, 3_{10}-helix, β structures, and random coils. The ORD and CD spectra of a variety of polypeptides encompassing each of their basic conformations have appeared in the recent literature.[1-14] Several theoretical investigations[15-18] have attempted with some success to calculate the optical activity of polypeptides in their rigid conformations, *i.e.*, α- and 3_{10}-helices,[15-16] β structures,[17] and poly-L-proline I- and II-helices.[18] These calculations have enabled the various experimental ORD and CD bands to be assigned to specific electronic transitions in the amide group. However, the rotatory properties of randomly coiling polypeptides have not as yet been treated theoretically, although Bayley, Nielson, and Schellman[19] have calculated the rotatory properties of dipeptides as a function of the rotation angles φ and ψ about the α-carbon atom.

Calculation of the rotatory properties of polypeptides presupposes an accurate description of the electronic structure of the amide group. Recently Basch, Robin, and Kuebler[20] have published the results of extensive Gaussian-type orbital self-consistent field (GTO–SCF) calculations of the electronic structure of the amide group. It is believed their calculations provide a foundation, which is firmer than results[15-18] heretofore based on molecular orbitals appropriate for formaldehyde,[21] for obtaining the charge distributions, the magnetic transition moments, and the excited state electric dipole moments necessary in the evaluation of the rotatory properties of polypeptides.

It was the purpose of the present investigation to calculate the optical rotatory strengths R of the various amide electronic transitions per residue of random-coil poly-L-alanine. A secondary objective was to compare the calculated results based on the description of the electronic structure of the amide group as given by Basch, *et al.*,[20] to those obtained from the electronic structure adopted in previous investigations.[16]

Theory and Method of Calculation[22,23]

The intrinsic optical activity of the asymmetric α-carbon atom in the peptide residue is ignored, *i.e.*, only the contribution made by the electronically asymmetric environment of the α-carbon atom to the rotational strengths of the amide group electronic transitions is considered. Each residue is assumed to have well-defined electronic eigenstates which are perturbed by their interactions with the field of neighboring residues. The eigenstates among different residues are weakly coupled, and the states within a given residue are mixed as a result of these interactions. The coupling among the residues of degenerate eigenstates is treated by Tinoco's extension[23] of the exciton theory.[24] In addition, the exchange or transfer of

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TABLE I
THE CALCULATED ROTATIONAL STRENGTHS OF THE ELECTRONIC TRANSITIONS IN RANDOM-COIL POLY-L-ALANINE

Transition	Wavelength λ, mμ		Rotational strength R, D-BM	
	Woody and Tinoco[a]	Basch, *et al.*[b]	Woody and Tinoco[a]	Basch, *et al.*[b]
$n\pi^*$	218	218	1.59 (1.43)[c]	0.283
NV_1	197	197	−0.268 (−1.58)[c]	−6.94
$n'\pi^*$	150		−2.73	
$n\sigma^*$	150		−2.46	
NV_2	125	135	−1.38 (−1.89)[c]	−4.17
NV_1 (exciton)	184, 213	160, 256	±0.00735	±0.00735
Polarizable groups	100	100	5.21 (2.10)[c]	10.8

[a] See ref 16. [b] See ref 20. [c] The rotational strength evaluated assuming that the contributions made by the $n'\pi^*$ and $n\sigma^*$ transitions are negligible.

electrons among the residues is ignored. The equations necessary to the calculation of the rotational strength R of each electronic transition have been described in detail elsewhere[16,22,23] by Tinoco, *et al.*, and are not reproduced here.

In the treatment[25] of the rotatory properties of the α- and 3_{10}-helix of poly-L-alanine conducted by Woody and Tinoco,[16] five distinct electronic transitions in the amide group were considered; $n\pi^*$ (λ = 210 mμ), NV_1 (λ = 190 mμ), $n'\pi^*$ (λ = 165 mμ), $n\sigma^*$ (λ = 150 mμ), and NV_2 (λ = 125 mμ). All higher energy transitions were lumped together as a polarizability contribution at λ = 100 mμ. From the analysis of the amide group electronic spectra in the condensed phase conducted by Basch, *et al.*,[20] it was concluded that the $n\pi^*$, NV_1, and NV_2 transitions at λ = 218, 197, and 135 mμ, respectively, together with the polarizability contribution at λ = 100 mμ give an adequate representation of the electronic structure of a peptide residue. All magnetic transition moments, all partial charges in the ground and excited states, all excited state dipole moments and transition moments between excited states, and the NV_2 transition moment were taken from the calculated results of Basch, *et al.*[20] The dielectric constant appropriate[26] for the short-range internal electric field in a polypeptide (ε = 3.5) was used in calculating all potential energies of interaction. Structural parameters used by Brant and Flory[26] in their study of the configurational characteristics of random-coil polypeptides were adopted here. Values of all other parameters involved[22,23] in the present calculations were taken from Woody and Tinoco.[16]

The interactions of a single residue with both of its nearest neighbors were accounted for, and the exciton contribution to the rotational strength of the NV_1 transition was evaluated[23] for a dipeptide. All interactions and their resulting contributions were averaged over those conformationally accessible portions of the potential energy map appropriate for randomly coiled polypeptides as calculated by Brant, Miller, and Flory.[27] The rotational strength R of each transition was calculated at 10° intervals in the rotation angles[28] φ and ψ about the α-carbon atom. The following ranges in φ and ψ, which correspond[27] to conformational potential energies $E(\varphi,\psi)$ of not more than 5 kcal/mol above the minimum calculated energy, were considered: $(-10 \leq \varphi \leq 140,\ 220 \leq \psi \leq 10)$, $(-10 \leq \varphi \leq 140,\ 110 \leq \psi \leq 150)$, and $(230 \leq \psi \leq 250,\ 220 \leq \psi \leq 10)$. The rotational strengths calculated at each pair of (φ,ψ) were multiplied by the appropriate statistical weight W and summed to obtain their statistical mechanical averages. These

$$W = \exp[-E(\varphi,\psi)/RT]/\sum_{\varphi,\psi} \exp[-E(\varphi,\psi)/RT] \quad (1)$$

calculations were repeated for the electronic description of the amide group previously employed by Woody and Tinoco.[16]

Calculated Results and Their Comparison with Experiment[14]

The calculated rotational strengths of each electronic transition based on the Woody and Tinoco[16] and Basch, *et al.*,[20] descriptions of the amide group are presented in Table I. If the band shapes are assumed to be Gaussian[29] then eq 2 can be used to construct the predicted CD spectrum from the calculated rotational strengths, where $[\theta_i^0] = 0.875 \times 10^4(\nu_i/\Theta_i)R_i$. The

$$[\theta_i] = [\theta_i^0] \exp[-(\nu_i - \nu)^2/\Theta_i^2] \quad (2)$$

rotational strength of the transition R_i is in Debye–Bohr magnetons, Θ_i is the half-band width (Θ = 0.085 for $n\pi^*$ and 0.108 for all other transitions),[13,14] and ν_i is the frequency of the transition. Conversely, the experimental rotational strengths of the $n\pi^*$ and NV_1 transitions in random-coil poly-L-alanine can be approximated according to eq 2 from the data[30] on

(25) Since completion of the present work, a paper by R. A. Woody has appeared in the literature (*J. Chem. Phys.*, **49**, 4797 (1968)) describing improvements made in the calculation of the rotational strength of the $n\pi^*$ transition in polypeptides.

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(30) Myer's data[14] were obtained in salt-free aqueous systems where the polyelectrolyte polypeptide random coils are highly extended, while the conformational energy calculations of Brant and Flory[26] were based on the unperturbed polypeptide chain. Unfortunately it does not appear to be possible to obtain optical activity data on unperturbed random coil polypeptides. Those solvents which dissolve polypeptides as random coils are necessarily good solvents and not Θ solvents where the polypeptide random coil is unperturbed. Thus, it must be hoped that the charged polypeptide random coil in Myer's salt free aqueous systems are not expanded far enough from the unperturbed Θ condition to vitiate the comparison made in the present work.

randomly coiled poly-L-lysine and poly-L-glutamic acid reported by Myer[14] if the CD bands at 218 and 197 mμ are attributed solely to these two transitions, respectively. The results are $R_{n\pi^*} \approx 0.15$ D–BM and $R_{NV_1} \approx -1.26$ D–BM.

If only the $n\pi^*$, NV_1, and NV_2 transitions are considered in the Woody and Tinoco[16] description of the amide group, then the calculated rotational strength of the NV_1 transition agrees quite well with Myer's[14] data. On the other hand, the same electronic description[16] of the amide group overestimates $R_{n\pi^*}$ by a factor of 10. The electronic description of the amide group given by Basch, *et al.*,[20] leads to a calculated $R_{n\pi^*}$ in fair agreement with experiment, while the calculated magnitude of the NV_1 rotational strength is almost six times that obtained from Myer's[14] experimental CD spectra. The magnitude of these discrepancies is similar to those encountered in previous[15–18] optical activity calculations performed on polypeptides in their rigid conformations.

Although both descriptions of the electronic structure of the amide group predict the correct signs for the rotational strengths of the $n\pi^*$ and NV_1 transitions, neither leads to the correct magnitudes for both transitions. Schellman, *et al.*,[19] calculated[31] the rotational strenghts of the $n\pi^*$ and NV_1 transitions for a dipeptide and found that $|R_{NV_1}|$ should be at least an order of magnitude greater than $|R_{n\pi^*}|$. This finding is borne out by the calculations based on the Basch, *et al.*,[20] description and by experiment,[14] while the electronic parameters given by Woody and Tinoco[16] lead to $|R_{NV_1}| \gtrsim |R_{n\pi^*}|$. Since the GTO–SCF calculations of Basch, *et al.*,[20] yield an energy for the NV_1 transition which is 3 eV higher than observed experimentally, one should probably not be too surprised by the disparity between the calculated and experimental values of R_{NV_1}.

Neither description of the electronic structure of the amide group leads to a completely satisfactory prediction of the rotatory properties of randomly coiled poly-L-alanine, thereby precluding an unambiguous selection between the two. In addition, the validity of the present adaptation of the Tinoco method[22,23] for calculating rotational strengths cannot be assessed further from the results presented here.

Acknowledgments. It is a pleasure for the author to acknowledge Drs. H. Basch and M. B. Robin for making available the details of their GTO–SCF calculations on the amide group and for many helpful discussions. Professor D. A. Brant is also gratefully acknowledged for providing the calculated conformational potential energies of randomly coiling polypeptides.

(31) They assumed that the $n\pi^*$ and NV_1 transitions adequately describe the electronic structure of the amide group and used the Woody and Tinoco[16] set of electronic parameters after significant modification.

An Instrument for Measuring Retardation Times of Deoxyribonucleic Acid Solutions

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ABSTRACT: A new instrument is described which measures retardation times for solutions of very large macromolecules in a creep–recovery type of experiment. Retardation times are closely related to relaxation times, and therefore should be useful parameters for determining molecular weights of macromolecules. Results of measurements on solutions of T2 bacteriophage DNA are presented. The molecular weights calculated by assuming that the DNA molecules are random coils agree well with the accepted value.

The "relaxation time" of a chain macromolecule is an interesting quantity that can be related to the molecular size. Theory[2] predicts that there are a number of relaxation times, τ_k, for a chain molecule given by a formula of the form

$$\tau_k = \alpha_k M\eta[\eta]/RT \qquad (1)$$

where M is the molecular weight of the molecule, η is the solvent viscosity, $[\eta]$ is the intrinsic viscosity of the macromolecule, and α_k is a constant that depends on the index k and also to some extent on the structure of the chain. This formula has been verified experimentally in a number of cases (for a review, see Ferry[3]). The longest relaxation time, τ_1, is usually the most prominent in measured properties such as viscosity or flow birefringence.

Solutions of large deoxyribonucleic acid (DNA) molecules show pronounced relaxation effects.[4,5] In particular, viscoelasticity, especially as manifested by the elastic recovery of the solutions from mechanical stress, is a familiar phenomenon to those who work with such solutions. D. S. T. noticed that this effect

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