structural changes which occur during these transitions is an area open to conjecture; however, there is no reason to expect a change in conformation of the glucose residue.

Acknowledgments. We are very grateful to Professor D. French for suggesting the problem, providing samples of CHA, and discussing the chemistry of this series of carbohydrate compounds. We thank the editor and referees whose comments on the first draft of this article were helpful in improving the presentation of the material. We are indebted to the Corn Industries Research Foundation for support of computer usage for the crystal structure analysis, and to the Health Science Computer Center of the University of Maryland School of Medicine for assistance with the condensation of our tables.

The Configuration of Random Polypeptide Chains. I. Experimental Results

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Contribution from the Department of Chemistry, Stanford University, Stanford, California. *Received March 6, 1965*

The dimensionless characteristic ratio $\langle r^2\rangle_0/n_p l_p^2$ of the measured mean square unperturbed end-to-end distance $\langle r^2\rangle_0$ to the number n_p of planar, trans peptide units multiplied by the square of the length l_p between successive α-carbons has been evaluated for four polypeptides. This ratio was deduced from intrinsic viscosities, molecular weights, and second virial coefficients. Measurements reported here on poly-β-benzyl-L-aspartate in m-cresol at 100°, on poly-L-glutamic acid in aqueous 0.3 M sodium phosphate at pH 7.85 and 37°, and on poly-L-lysine in aqueous 1.0 M sodium bromide at pH 4.54 and 37° yielded values of the characteristic ratio of 9.6, 8.8, and 8.6, respectively. From the data of Doty, Bradbury, and Holtzer for poly-γ-benzyl-L-glutamate in dichloroacetic acid at 25°, a value of 8.8 was calculated. No dependence of the unperturbed dimensions upon the solvents or amino acid side chains represented here is discernible within the estimated experimental uncertainty of ca. 10%.

The ordered configurations of synthetic polypeptides have attracted much interest in recent years.[1] In contrast, little attention has been devoted to the random coil form of the polypeptide chain. Interpretation of changes in molecular configuration accompanying protein denaturation[2-8] demands an adequate characterization of the denatured form. Denaturation usually involves disordering of the native molecule in some degree, and under certain conditions conversion to the random coil form is complete. The molecular interpretation of dimensional changes occurring in fibrous proteins[9] also requires a knowledge of the random polypeptide chain dimensions.

The dimensions of the poly(benzyl glutamate) random coil in dilute dichloroacetic acid solution have been evaluated from published data[10,11] by P. J. F.[12] and by Kurata and Stockmayer,[13] and the configuration of polysarcosine in dilute aqueous solutions has been investigated by Fessler and Ogston.[14] Only these few experimentally determined polypeptide random coil dimensions appear to be available in the literature, and theoretical investigations of polypeptide coil dimensions have been limited to free rotation treatments.[12,15] In view of the paucity of information on the configuration of random coil polypeptides, we have undertaken a systematic experimental study of the configuration of these molecules in dilute solution.

We shall focus attention in what follows on the *average unperturbed polypeptide chain dimensions* which depend only on short-range interactions within the polymer chain, *i.e.*, those determined by the covalent chemical bonding and internal rotational potentials, including interactions between near-neighbor nonbonded groups.[13,16] The unperturbed polymer chain is of particular interest inasmuch as it is amenable to theoretical treatment which permits analytical correlation of the average chain dimensions with the polymer structure. A detailed theoretical investigation of the random polypeptide chain is presented in the following paper.[17]

The unperturbed dimensions of polymer chains are subject in general to direct experimental determination in dilute solution in appropriate ideal or Θ-solvents in which the volume exclusion effect upon the coil dimensions is nullified.[13,16] Such ideal solvents are necessarily poor solvents for the polymer in question.

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In good solvents the linear dimensions of the polymer coil exceed by a factor α the unperturbed dimensions due to the excluded volume effect.[16] For sufficiently large polymer molecules the intrinsic viscosity $[\eta]$ of an unfractionated polymer sample is related to the viscosity-average molecular weight M_v and the expansion factor α by

$$[\eta] = \Phi(\langle r^2\rangle_0/M)^{3/2}M_v^{1/2}\alpha^3 \quad (1)$$

The ratio $\langle r^2\rangle_0/M$ of the mean square unperturbed distance $\langle r^2\rangle_0$ between the polymer chain ends and the molecular weight M of the chain is a constant characteristic of the polymer in question, while Φ is a universal constant with the value 2.1×10^{-3} for $[\eta]$ in dl./g. and $\langle r^2\rangle_0$ in Å.^{2}.[16] It is convenient to express the unperturbed dimensions of the polypeptide chain in terms of the dimensionless ratio $\langle r^2\rangle_0/n_p l_p^2$, where n_p is the degree of polymerization and l_p is the fixed distance of 3.80 Å. between the α-carbons of the *trans* peptide repeating units in the chain.[17] This characteristic ratio is given according to eq. 1 by

$$\langle r^2\rangle_0/n_p l_p^2 = ([\eta]/\Theta M_v^{1/2}\alpha^3)^{2/3}(M_0/l_p^2) \quad (2)$$

where M_0 is the weight of an amino acid residue. If measurements are carried out in a Θ-solvent in which $\alpha = 1$, the characteristic ratio can be calculated from the viscosity-average molecular weight and the intrinsic viscosity determined at the Θ-point. Otherwise it is necessary to rely upon a relationship between α and the second osmotic virial coefficient A_2 from polymer solution theory.[18,19] For both uncharged and charged polymers the appropriate equation is

$$A_2M/[\eta] = 188 \ln [1 + 0.886(\alpha^2 - 1)] \quad (3)$$

from which α may be evaluated if A_2, $[\eta]$, and the molecular weight are known.[20]

An estimate of the viscosity-average molecular weight, required for evaluation of $\langle r^2\rangle_0/n_p l_p^2$ from eq. 2, may be interpolated from the measured number-average molecular weight M_n and the measured weight-average molecular weight M_w for the sample. Inasmuch as M_v enters eq. 2 as $M_v^{1/3}$, small errors in the assignment of M_v have little effect on $\langle r^2\rangle_0/n_p l_p^2$.

Systems Investigated

The tendency of polypeptides to form ordered structures[21] limits the choice of polymer–solvent systems in which the polypeptide random coil dimensions may be investigated in dilute solution. Moreover, those few solvents which dissolve polypeptides as random coils are of necessity good solvents; attempts to approach Θ-solvent conditions seem invariably to result in precipitation of crystalline polymer or transition of the molecule from the random coil to a soluble helical configuration. Thus, conditions for direct experimental determination of polypeptide unperturbed dimensions appear unattainable, and it becomes necessary to measure coil dimensions in good solvents and to correct the resulting values for perturbation by solvent–polymer interaction through the use of polymer solution theory as related above.

Poly-L-glutamic acid (PLGA) and poly-L-lysine (PLL), both in aqueous solutions, and poly-β-benzyl-L-aspartate (PBLA) in *m*-cresol solution have been chosen for the present studies. Each system offers the possibility of achieving experimental conditions under which the necessary corrections may be applied with confidence. The greater the deviation of the actual polymer dimensions from the unperturbed dimensions, the greater is the latitude of error in corrections based on polymer solution theory. We have consequently chosen conditions for the above systems in which the expansion factor α is not much greater than unity.

Poly-L-glutamic acid in aqueous solution at basic pH[22] and poly-L-lysine in aqueous solution at acidic pH[23] exist as random coils, presumably because the high density of charge on the polymers under these conditions prevents formation of the helices characteristic of the respective uncharged molecules. By addition of sufficient amounts of appropriate electrolytes, the electrostatic expansion of the chains may be reduced. We have accordingly made measurements on PLGA in aqueous 0.3 *M* sodium phosphate buffer at pH 7.85 and 37° and on PLL in aqueous 1.0 *M* sodium bromide at pH 4.54 and 37°. Search for a Θ-solvent for PLGA was unsuccessful. This involved the quest for conditions leading to liquid–liquid phase separation in aqueous salt solutions of the polymer, using various salts at concentrations covering wide ranges.

Poly-β-benzyl-L-aspartate is reported on the basis of optical rotatory dispersion measurements to undergo a transition from helix to coil in *m*-cresol in the neighborhood of 40° as the temperature is raised.[24] We find that the reduced specific viscosity η_{sp}/c of a 0.2% solution of PBLA (number-average molecular weight = 187,000) in *m*-cresol decreases more than fivefold as the temperature is raised from 20 to 70°. Near 70° an abrupt change occurs in the slope of η_{sp}/c *vs.* T, and η_{sp}/c varies only a few per cent between 70 and 130°. We have chosen accordingly to measure the unperturbed dimensions of PBLA in *m*-cresol at 100°. This temperature should be safely above the helix–coil transition region and yet low enough to ensure a value of α not much greater than unity.

Certain strongly acidic solvents, *e.g.*, dichloroacetic acid[21] and some fluoro ketone hydrates,[25] dissolve polypeptides in the random coil form. The possibility of specific effects upon the unperturbed coil dimensions due to strong solvation of the polymer in these solvents[26] makes them relatively unattractive choices as solvents for studies of polypeptide unperturbed dimensions. We have nevertheless calculated the unperturbed dimensions of poly-γ-benzyl-L-glutamate (PBLG) in dichloroacetic acid at 25° from data in the literature[10] for comparison with our experimental results.

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Table I

Polymer	M_0	Solvent	Temp., °C.	$[\eta]$, dl./g.	$M_n \times 10^{-3}$ osmotic	$A_2 \times 10^4$, cm.^{3} mole/g.^{2}	$M_v \times 10^{-3}$ est.	α	$\frac{\langle r^2\rangle_0}{n_p l_p^2}$	$\frac{\langle r^2\rangle_0}{\langle r^2\rangle_{0,f}}$
PBLA	205	*m*-Cresol	100	0.915 (±0.6%)	187 (±5.3%)	3.37 (±4.1%)	187	1.22 (±2.0%)	9.6 (±10%)	5.0 (±10%)
Na-PLGA	151	Water, 0.3 *M* sodium phosphate, pH 7.85	37	1.316 (±0.5%)	68.5 (±2.9%)	19.4 (±1.0%)	114	1.34 (±1.4%)	8.8 (±10%)	4.6 (±10%)
PLL-HBr	209	Water, 1.0 *M* sodium bromide, pH 4.54	37	0.767 (±0.4%)	115 (±3.5%)	6.46 (±3.9%)	115	1.33 (±2.3%)	8.6 (±10%)	4.5 (±10%)
PBLG[a]	219	Dichloroacetic acid	25	1.84	336	7.5	336	1.51	8.8	4.6

[a] Data from light scattering and intrinsic viscosity measurements reported in ref. 10.

Experimental Procedures and Results

All polymer samples were purchased from Pilot Chemical Co., Watertown, Mass. The PBLA was from Lot A-13, the PLGA was from Lot G-52S, and the PLL was from Lot L-34. Reagent grade sodium phosphate and sodium bromide were used. Both solvents, *m*-cresol and water, were twice distilled immediately before use. The polymer designation and the molecular weight of its repeat unit under the conditions of the experiment are given in the first two columns of Table I. Under the experimental conditions poly-L-glutamic acid exists in the form of its sodium salt (Na-PLGA); poly-L-lysine exists as the hydrobromide (PLL-HBr). Columns three and four of Table I indicate, respectively, the solvent media used and the temperatures of the experiments.

Intrinsic viscosities were measured using Ubbelohde viscometers thermostated to ±0.01°. The results are presented in column five of Table I. Concentrations of all PBLA solutions used in the viscosity measurements were determined from dry weight analyses and the densities of polymer[27] and solvent,[28] assuming no volume change on mixing. Stock solutions (2.5–3.0%) of both the Na-PLGA and the PLL-HBr were dialyzed at 37° against large excesses of their respective solvent mixtures for 48 hr., and solutions for viscosity determinations were obtained by gravimetric dilution of the dialyzed stock solutions with solvent from the respective dialysis experiments. The concentration of the stock solution of Na-PLGA was determined by Kjeldahl nitrogen analysis carried out in triplicate (mean deviation, 0.5%). The concentration of the PLL-HBr stock solution was determined similarly (mean deviation for four determinations, 0.6%.) The concentrations in g./dl. of the solutions used for the Na-PLGA and PLL-HBr intrinsic viscosity determinations were then calculated from the measured densities and weights of the solvent and stock solutions and the mean concentrations of the stock solutions assuming no volume change on mixing. Uncertainties in $[\eta]$ were estimated from reasonable alternatives to the best straight lines through the experimental points, taking into account the uncertainties in the points themselves.

Number-average molecular weights and osmotic second virial coefficients, shown in columns six and seven of Table I, respectively, were determined using a Mechrolab Model 502 high speed membrane osmometer. Number-average molecular weights reported for the ionic polymers correspond to the chemical formulas indicated in the first column of Table I. The experimental data were analyzed by plotting[29] $(\pi/c)^{1/2}$ *vs.* c where π is the measured osmotic pressure and c is the concentration. Concentrations of PBLA solutions in g./cm.^{3} were determined directly from the measured weights and the densities of the polymer[27] and solvent,[28] again assuming null volume change on mixing. Solutions of Na-PLGA and PLL-HBr were prepared from the same stock solutions used in the viscosity experiments, and concentrations were determined as described above. Uncertainties in M_n and A_2 were estimated using the methods previously described for assessing the uncertainties in $[\eta]$.

The weight-average molecular weight M_w of the PBLA sample, unfractionated, was *ca.* 250,000 as estimated from its intrinsic viscosities in dichloroacetic acid and in *m*-cresol, these being compared with plots of $[\eta]$ *vs.* M_w for unfractionated samples of PBLG in the former solvent[10] and for unfractionated PBLA samples in the latter.[27] For the sample of Na-PLGA, also unfractionated, $M_w \cong 136{,}000$ as estimated by the supplier using a graphical correlation of $[\eta]$ with M_w for unfractionated Na-PLGA. Using similar methods, the supplier estimated $M_w \cong 110{,}000$ for the sample of PLL-HBr. The values assumed for M_v for the three polymer samples, shown in column eight of Table I, were assigned on the basis of a comparison of the measured M_n and estimated M_w for each sample. The virtual coincidence of the measured M_n with the estimated M_w for the PLL-HBr justifies assigning $M_v = M_n$ for this sample. The similar assignment of M_v for the sample of PBLA may be a slight underestimate. The measured value of M_n for our sample of Na-PLGA was almost exactly one-half the estimated M_w. In view of the known propensity of PBLG to degrade when the side-chain ester groups are hydrolyzed to produce PLGA,[30] we have assumed the most probable molecular weight distribution for our sample of Na-PLGA. This distribution is characterized by $M_v/M_n = 1.67$ at the Θ-point,[31] and these are the conditions of relevance here.

Table I also contains the data of Doty, Bradbury, and Holtzer[10] for the intrinsic viscosity, molecular weight, and second virial coefficient of PBLG in dichloroacetic acid at 25°. In the absence of information regarding the molecular weight distribution of this sample, we have assumed the distribution to be sufficiently narrow so that $M_n \cong M_v \cong M_w$. Expansion

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(30) W. G. Miller, private communication.
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factors α for all four polymers have been calculated according to eq. 3, and they are given in column nine of Table I. The reported uncertainties were estimated from the uncertainties in A_2, M_n, and $[\eta]$.

Discussion

Values of $\langle r^2\rangle_0/n_p l_p^2$ calculated from eq. 2 are presented in the next to the last column of Table I. These results are subject to errors of about $\pm 10\%$; the estimates of M_v constitute the largest source of uncertainty. The similarity of results for the four diverse systems is striking. It strongly suggests that specific interactions involving side chains or solvents exert little influence on the *unperturbed* dimensions of the polypeptide random coil in the systems examined.

The four polypeptides listed in Table I are characterized by large side chains. They have been investigated in aqueous, phenolic, and acidic solvents. The absence of any measurable dependence of the polypeptide unperturbed dimensions on the amino acid side chains casts doubt on the importance of interactions between *first neighbor* side chains in proteins.

Also worthy of note is the fact that even in dichloroacetic acid the unperturbed dimensions of PBLG are in close agreement with those for the other polymers. If protonation of the amide group occurs in this solvent, as seems likely,[26] the planar, *trans* conformation of the peptide group evidently is not destroyed by the proton transfer.

The measured values of the mean square unperturbed end-to-end distance are compared in the final column of Table I with the quantity $\langle r^2\rangle_{0,f}$ calculated for a polypeptide chain having all amide bonds *trans*, with free rotation prevailing about the other chain bonds.[12,15] The ratio $\langle r^2\rangle_0/\langle r^2\rangle_{0,f}$ for polypeptides is typical of values found for other polymers.[13]

Theoretical interpretation of these results in terms of polypeptide structure is presented in the following paper.[17]

Acknowledgments. Support of this work by the Directorate of Chemical Sciences, Air Force Office of Scientific Research Contract No. AF49(638)-1341, and by the National Institutes of Health through a postdoctoral fellowship for D. A. Brant is gratefully acknowledged.

The Configuration of Random Polypeptide Chains. II. Theory

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The experimentally measured dimensions of polypeptide chains in the unperturbed, random coil form have been successfully correlated with the chain structure using the rotational isomeric state model and statistical mechanical methods applicable to linear systems of interacting subunits. The polypeptide chain with all its amide groups in the trans conformation may be treated as a sequence of virtual bonds of fixed length connecting successive α-carbons; the mutual orientation of a pair of adjoining virtual bonds is determined by the angles of rotation about the single bonds at the intervening α-carbon atom. Contributions to the configuration energy from bond rotation (torsional) potentials and interactions between nonbonded atoms and groups have been assessed and subsequently evaluated using approximate analytical expressions. From the chain geometry and the character of these interactions, it is shown that the mutual orientation of a given pair of adjoining virtual bonds is sensibly independent of the orientations of all other virtual bonds in the chain. Electrostatic interactions between amide groups, heretofore ignored, markedly affect the configuration. Satisfactory agreement between theoretical calculations and experimental results is achieved only by taking account of these dipolar interactions, in addition to the relevant torsional and van der Waals energies The dependence of the polypeptide unperturbed dimensions on chain length has been investigated also.

Introduction

The unperturbed mean dimensions of a number of linear polymers have now been successfully correlated with their chain structures and associated impedences to bond rotations. The basis for these correlations is provided by the rotational isomeric state model for chain molecules.[1] Mathematical methods applicable to this model are furnished by the well-known theory originally developed for a linear array of interacting magnetic dipoles.[2,3] They have been adapted to the treatment of the unperturbed mean square end-to-end distance and dipole moment of linear polymer molecules by Birshtein and Ptitsyn,[4] Lifson,[5] Nagai,[6] and Hoeve.[7] These methods, which take account of the interdependence of nearest-neighbor rotations, have been applied to polyethylene,[8] poly(dimethylsiloxane),[9]

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