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Unperturbed Random Coil Dimensions of Two Tricopolypeptides Containing Glycine, Glutamic Acid, and Lysine

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ABSTRACT

Two tricopolypeptides with varying amounts of glycyl, ϵ-carbobenzoxy-L-lysyl, and γ-benzyl-L-glutamyl residues were synthesized and characterized by determining their intrinsic viscosity, refractive index increment, molecular weight, and second virial coefficient in the random coil solvent dichloroacetic acid. The characteristic ratio (C_∞) of the two polypeptides was determined using the Brant-Flory equation. C_∞ decreased from 8.1 to 4.8 on changing the content of glycine from 27.3 to 46.0 mole %. These values of C_∞ are markedly higher than those expected from a consideration of the effect of glycine on random coil dimensions of copolypeptides.

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Statistical mechanical treatment by Flory, Mandelkern, and their co-workers [1-3] of polypeptidic systems in the unperturbed random conformation has led to the evaluation of their unperturbed random coil dimension, and also the characteristic ratio (C_∞) given by $\langle r_0^2 \rangle / n_p l_p^2$, where $\langle r_0^2 \rangle$ is the unperturbed mean square end to end distance, n_p is the degree of polymerization, and l_p is the length of the "virtual" bond in the transpeptide unit of the polypeptide backbone. The characteristic ratio would be a measure of the hindrance to free rotation around the virtual bond, or of the rigidity of the chain. Numerical values for C_∞ were calculated for some homopolyamino acids, like polyglycine, polyalanine, and polyproline, and also for a few random and sequential copolypeptides [1-3]. These studies indicate that the characteristic ratio of a copolypeptide containing glycine should largely be governed by the content of glycine, which having no β-carbon atom offers the least hindrance for free rotation around the α-carbon atom. Experimental data on the characteristic ratios of heteropolypeptides is rather limited [1, 2, 4, 5]. The present report deals with the synthesis and evaluation of C_∞ of two glycyl tricopolypeptides.

EXPERIMENTAL

Synthesis of Polypeptides

Two tricopolypeptides (PP3-1, PP3-2), with different relative ratios of glycycl, lysyl, and glutamyl residues, were synthesized as follows.

The N-carboxy-α-amino acid anhydrides (NCA) were obtained by phosgenation of solutions of γ-benzyl-L-glutamate, ϵ-carbobenzoxy-L-lysine, and glycine in dioxane. All the solvents were purified by standard methods [6] and dried. They were freshly distilled over a suitable dehydrating agent before use.

Polymerization of the NCAs was done as follows:
To a 4% solution of a mixture of the recrystallized NCAs in dioxane-benzene medium (1:1, v/v), the initiator, n-butylamine was added (A/I = 200) and the polymerization was allowed to proceed for 4 days at 25 ± 0.05°C, with pure nitrogen gas bubbling through the reaction mixture. The precipitation of polymerized material was completed with the addition of petroleum ether (60 to 80°C), and the polymer was washed free from any unreacted monomers with hot ethyl acetate.

Amino acid analysis of the two tricopolypeptides was done using a Beckman amino acid analyzer. The mole percent composition of the two polypeptides is given in Table 1.

TABLE 1

Sample	Composition in mole %			dn/dc (ml/g)
	Glu	Lys	Gly	
PP3-1	37.4	35.2	27.3	0.1138
PP3-2	32.6	21.4	46.0	0.1121
Poly-γ-benzyl-L-glutamate [15]				0.1000
Polyglycine				0.1016
Poly-ϵ-carbobenzoxy-L-lysine				0.1037

Viscosity

An Ubbelhode-type dilution viscometer was used. Temperature of the thermostatic bath was maintained at 25 ± 0.05°C. The flow time of solvent was 180 to 200 sec, the long efflux time making kinetic energy correction unnecessary. Time of flow was measured with a stopwatch, reading directly to 0.1 sec. Density correction at various dilutions was not made. Dichloroacetic acid (DCA) was used as the random coil solvent immediately after distillation.

Intrinsic viscosity, $[\eta]$, of the tricopolypeptides was determined from plots of η_{sp}/c vs c where η_{sp} is the specific viscosity of the solution and c is the concentration.

The plots of η_{sp}/c vs c showed, at concentrations lower than 0.2 to 0.3 g/dl, an abnormal behavior, in that the value of η_{sp}/c increases sharply with a decrease of concentration. Intrinsic viscosity was determined by extrapolating the straight line part of the curve and neglecting data in the low concentration region—a region in which normally data is not obtained and, consequently, the abnormal behavior could be missed. Details of this abnormal behavior, which was also observed in helix-promoting solvents, like dimethyl formamide, would be reported elsewhere.

Refractive Index Increments

The refractive index increments were measured with a Brice-Phoenix Differential Refractometer which was calibrated with standard solutions of KCl.

Turbidity

The random coil solvent DCA and polypeptide solutions in this solvent were clarified by filtration through sintered glass (G-4). Turbidity measurements were made at 436 nm with a Brice-Phoenix Universal Light Scattering Photometer. The Photometer was calibrated with a standard sample of polystyrene, and also with solutions of Ludox.

The molecular weight and second virial coefficient of the two systems were determined using Eq. (1). Zimm plots were not made as turbidities were fairly independent of the scattering angle.

$$\frac{Hc}{\tau} = \frac{1}{M_w} + A_2 c \qquad (1)$$

where $H = 32\pi^3 (dn/dc)^2 / 3\lambda^4 N$, c is the concentration, τ is the turbidity, A_2 is the second virial coefficient, (dn/dc) is the specific refractive index increment, λ is the wavelength of light in vacuum, and N is the Avogadro number.

Measurements of individual refractive index increments were reproducible within ±2% while those of intensity of light measurements were reproducible to ±1%. As turbidity involves a square term in (dn/dc), M_w and A_2 values would be correct within ±5%.

RESULTS AND DISCUSSION

The values of intrinsic viscosity, second virial coefficient, and weight-average molecular weight are given in Table 1. The molecular weight of the two polymers is comparable to that of many enzymes.

The chain expansion factor, α which is needed to convert the intrinsic viscosity data obtained in the good solvent DCA, to the theta state, could be evaluated from either [7, 8]

$$\frac{A_2 M_v}{[\eta]} = 200\left(1 - \frac{1}{\alpha^3}\right) \quad \text{(Krigbaum [7])} \qquad (2a)$$

or

$$\frac{A_2 M_v}{[\eta]} = 414 \log[1 + 0.886(\alpha^2 - 1)] \quad \text{(Orofino and Flory [8])} \qquad (2b)$$

Several authors [3, 4, 5, 9, 10] used Eq. (2a) or Eq. (2b) to determine α. The values of α calculated from Eq. (2a) are about 5 to 6% higher than those from Eq. (2b). In the present work, we were interested in obtaining relative values of C_∞ for two polypeptides containing different amounts of glycine, and to this end we used Eq. (2a).

Viscosity-average molecular weight (M_v) appears in the above equation. The ratios of M_w to M_n (by end group analysis [11]) for the two tricopolypeptides were about 1.2 and 1.4. Further, as $M^{1/3}$ occurs in the equation for α, M_w was used in place of M_v.

The characteristic ratios were evaluated [12] from

$$C_\infty = \frac{\langle r_0^2 \rangle}{\eta_p l_p^2} = \left[\frac{[\eta]}{\phi M_v^{1/2} \alpha^3} \right]^{2/3} \left[\frac{M_0}{l_p^2} \right] \tag{3}$$

where M_0 is the mean residue weight and ϕ is a constant. There is a certain amount of uncertainty in the value of the constant ϕ to be used; the two extreme values [2] assigned to it are 0.0021 and 0.0025. In the present report the lower value of ϕ was used; the higher value of ϕ would decrease C_∞ by a few percent only.

The values of α and C_∞ are given in Table 2. The characteristic ratios of PP3-1 and PP3-2 are found to be 8.1 and 4.8, respectively. The glycine content of PP3-1 was 27.3 mole % whereas the other polypeptide has a higher content of glycine (46 mole %). The values of C_∞ appear to be very much on the high side as compared to those one would expect from the following considerations. Both glutamyl and lysyl residues have side chains with atoms beyond the β-carbon. Earlier theoretical studies on unperturbed dimensions of polyamino acids (e.g., polyalanine, poly γ-benzyl glutamate) indicate that atoms beyond the β-carbon atom in the side chain do not have a significant

TABLE 2

Sample	$[\eta]$ (dl/g)	$M_w \times 10^{-3}$	$A_2 \times 10^4$ (cc mole/g^2)	α	C_∞
PP3-1	0.146	14.7	2.9	1.05	8.1
PP3-2	0.139	16.9	6.9	1.19	4.8

influence on the conformation of the polypeptide backbone [9, 13]. In other words, such amino acid residues could be considered as equivalent for purposes of discussion of conformation of the backbone of polypeptide chains. Glycine, with no β-carbon atom, is a special case. Then the two tricopolypeptide systems could be looked upon as consisting in part of glycine, and the rest, of amino acid residues with a β-carbon atom. For such systems, Flory [9] has shown that the characteristic ratio would largely be dependent on the content of glycine; C_∞ of polyalanine is expected to decrease sharply (and nonlinearly) with a slight (random) incorporation of glycine in it. The numerical values of C_∞ expected from the above considerations are about 4.5 for PP3-1 and 3.0 for PP3-2. There is a marked difference between the expected and observed values, although PP3-2, with a higher concentration of glycine, does have a lower value of C_∞ (4.8) than that of PP3-1 (8.1).

According to Benoit [14], the evaluation of M_w for copolymers by light scattering is beset with difficulties, mainly due to chemical heterogeneity of a copolymer. The numerical value (and sign) of the refractive index increments of the homopolymers of the different constituent monomers of a copolymer may be 1) nearly the same or 2) widely different depending upon the solvent used; in the latter case, highly erroneous values of M_w would be obtained. We, therefore, synthesized polyglycine and poly-ϵ-carbobenzoxy lysine and determined their refractive index increments in DCA and compared them with the literature value [15] of poly-γ-benzyl glutamate. All the three increments (Table 1) are close to each other, indicating that chemical heterogeneity would not have any significant effect on the molecular weight determination of the polypeptides in DCA solutions.

Kinetic data on the copolymerization of various amino acid-NCAs is rather meagre [16-18]. However, homopolymerization studies in dioxane medium, with n-hexylamine as initiator, by Wiengarten [19] have shown that the glycine-NCA polymerizes about nine times faster than γ-benzyl glutamyl NCA. But, on changing the solvent from dioxane to benzene, the rate constant for γ-benzyl glutamyl-NCA increases tenfold; no data for glycine-NCA (or for ϵ-carbobenzoxy lysine NCA) in benzene is available. The kinetic behavior of amino acid NCAs in the dioxane-benzene medium used by us was also not studied. However, it was noted that during tercopolymerization the solutions were homogeneous for more than 48 hr and only thereafter was some phase separation observed. In contrast to this, an early separation occurs during the homopolymerization of glycine-NCA in dioxane-benzene. The above suggests the absence of large blocks of glycyl residues in the tercopolymer chains. It seems that, in the systems under report, the sequence of residues would not be too far off from that of an idealized random sequence. An explanation for the

significant difference between the experimental and expected values for C_∞ for PP3-1 and PP3-2 then, perhaps, has to be sought in terms of either side chain-side chain interactions, or in terms of an existence of a conformation which is not entirely random in dichloroacetic acid [20].

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