Helix–Coil Stability Constants for the Naturally Occurring Amino Acids in Water. X. Tyrosine Parameters from Random Poly(hydroxypropylglutamine-*co*-L-tyrosine)[1]

R. K. Scheule,[2a] F. Cardinaux, G. T. Taylor,[2b] and H. A. Scheraga*

Department of Chemistry, Cornell University, Ithaca, New York 14853.
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ABSTRACT: The synthesis and characterization of water-soluble random copolymers containing L-tyrosine with N^5-(3-hydroxypropyl)-L-glutamine are described, and the thermally induced helix–coil transitions of these copolymers in water have been studied. The incorporation of L-tyrosine was found to increase the helix content of the polymers at all temperatures. The Zimm–Bragg parameters σ and s for the helix–coil transition in poly(L-tyrosine) in water were deduced from an analysis of the melting curves of the copolymers in the manner described in earlier papers. The large value of σ indicates that, in water, tyrosine has a tendency to promote helix–coil boundaries at all temperatures; the values of s indicate that this residue enhances helix growth at low temperature and reduces it at high temperature.

Much information about the conformational preferences of an L-tyrosine residue in polymers is presently available. Poly(L-tyrosine) has been investigated in organic solvents[3–7] and in water.[8–10] Copolymers of tyrosine and amino acids with ionized side chains have been studied in water,[11,12] but the presence of charged residues has complicated interpretation of the results. To eliminate this difficulty, and to gain insight into the expected conformational behavior of tyrosine residues in proteins, water-soluble random copolymers of tyrosine and a non-ionizable "host" [N^5-(3-hydroxypropyl)-L-glutamine (HPG)][13,14] have been synthesized, and the Zimm–Bragg helix–coil stability constants,[15] σ and s, of tyrosine determined. The results indicate that, in water at neutral pH, L-tyrosine has a pronounced ability to promote helix–coil boundaries at all temperatures, and that, depending on the temperature, it can either enhance or reduce helix growth.

The synthesis of water-soluble random copolymers of L-tyrosine with N^5-(3-hydroxypropyl)-L-glutamine is described in section I, and the experimental characterizations of these copolymers and their thermal helix–coil transitions in water are presented in section II. Finally, in section III, the data are analyzed by means of an appropriate form of the theory[16] to determine the helix–coil stability parameters of L-tyrosine in water; the theory is based on evidence that short-range interactions dominate in determining the local conformation of a polypeptide or protein.[17]

I. Experimental Section. Preparation and Characterization of the Copolymers

The copolymers were prepared by first copolymerizing the *N*-carboxyanhydrides of *O*-tetrahydropyranyl-L-tyrosine and γ-benzyl L-glutamate in dioxane with sodium methoxide as an initiator. The γ-benzyl ester groups were then substituted by 3-hydroxypropylamide groups and, lastly, the *O*-tetrahydropyranyl blocking groups were removed by treatment with mild aqueous acid.

A. Materials. All reagents and solvents used were identical in quality and preparation to those used in paper IX of this series.[18] The sodium methoxide solution was prepared and standardized as described by Blout and Karlson,[19] 2,3-dihydropyran was purified by the procedure of Hirschmann et al.,[20] and acetonitrile was shaken with Linde 4A Molecular Sieves, stirred with calcium hydride at room temperature, then refluxed with calcium hydride and fractionally distilled under nitrogen. L-Tyrosine was used as obtained from Schwartz-Mann.

Poly[N^5-(3-hydroxypropyl)-L-glutamine], poly[HPG], of degrees of polymerization $\overline{DP}_w$ = 700, 220, and 120 were fractions IIIB, IIB and IIC, respectively, of paper II[21] of this series.

B. Synthesis. *N*-Carboxyanhydrides. γ-Benzyl L-glutamate *N*-carboxyanhydride was prepared according to the general procedure described by Hirschmann et al.[20] with tetrahydrofuran as a solvent and recrystallization from ethyl acetate and hexanes. Synthesis of L-tyrosine *N*-carboxyanhydride followed the same procedure except for the use of acetonitrile instead of tetrahydrofuran as a reaction medium. L-tyrosine *N*-carboxyanhydride was found to be much more soluble in acetonitrile (more than 10 g/l. at 45°) than in tetrahydrofuran. L-Tyrosine is insoluble in either solvent. Thus, in acetonitrile, the end of phosgenation was indicated by the dissolution of all solids, whereas such an end point could not be observed in tetrahydrofuran.[20] The reaction mixture was concentrated under reduced pressure, and L-tyrosine *N*-carboxyanhydride precipitated by the addition of hexanes in 98% yield (decomposes without melting above 100°).

L-Tyrosine *N*-carboxyanhydride was converted to *O*-tetrahydropyranyl-L-tyrosine *N*-carboxyanhydride:[20] 16% yield after two recrystallizations; mp 88.5–89.5°; $[\alpha]^{23}589$ −75.3 (*c* 1.00, CH_2Cl_2). This product corresponds to one of the diastereoisomers reported:[20] $[\alpha]^{25}589$ −89.7 (*c* 1.00, CH_2Cl_2); ir (KBr) 3400 (s), 2900 (m), 1830 (s), 1760 (s) cm^{-1}; NMR ($CDCl_3$) δ 7.0 m (4 H), 6.3 m (1 H), 5.4 m (1 H), 4.5 m (1 H), 3.7 m (2 H), 3.1 m (2 H), 1.7 m (6 H). Anal: Calcd for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81. Found: C, 61.75; H, 5.92; N, 4.69.

The attempted synthesis of *O*-tetrahydropyranyl-L-tyrosine *N*-carboxyanhydride from *O*-tetrahydropyranyl-L-tyrosine is described in the Appendix. This procedure was unsuccessful since the *O*-tetrahydropyranyl protecting group was removed during phosgenation. Therefore, the protected tyrosine *N*-carboxyanhydride was prepared as described above.

Poly[γ-benzyl L-glutamate-*co*-*O*-tetrahydropyranyl-L-tyrosine], Poly[Glu(OBzl),Tyr(Thp)], Copolymers I–V. Random copolymers of γ-benzyl L-glutamate with up to 15% *O*-tetrahydropyranyl-L-tyrosine were synthesized by polymerization of the *N*-carboxyanhydrides in dioxane with sodium methoxide as initiator.[18] The polymerization reaction was monitored and found to be more than 90% complete in a few hours as observed previously.[19] The viscous reaction mixture was poured into 300 ml of vigorously stirred dry acetonitrile. The resulting white fibrous precipitate was centrifuged, washed with acetonitrile, collected on a filter funnel, and dried in vacuo over P_2O_5–NaOH. Yields were generally 80–85%. The chain lengths (determined, roughly, with the viscosity–molecular weight relationship of Fujita et al.[22]) of these copolymers are given in Table I.

Poly[N^5-(3-hydroxypropyl)-L-glutamine-*co*-L-tyrosine], Poly[HPG,Tyr], Copolymers 1–6. The protected copolymers I–V were treated with 3-amino-1-propanol to yield a series of water-soluble copolymers as described previously.[23] The course of the aminolysis was monitored, as described in section IC, and the reaction was terminated when more than 99.5% of the benzyl ester groups had been exchanged. The reaction mixture was poured into stirred, ice cold dilute aqueous hydrochloric acid and maintained at pH ≤3 (indicator paper) for 1 hr, for removal of the tetrahydropyranyl protecting group, and then dialyzed against water at 4° until amines could no longer be detected by a ninhydrin test[24] on 0.1 ml of dialyzate. The water soluble poly[HPG,Tyr] was recovered by lyophilization in 50–80% yield based on the number of

Table I
Characterization of Copolymers

Polymer No.	L-Tyr content of reaction mix, mol %	A/I[a]	$\overline{DP}$[b]	Temp of aminolysis reaction, °C	Fraction	L-Tyr content, mol %	$\overline{DP}_w$[c]	$\overline{M}_z/\overline{M}_w$	$\bar{v}$, cm³/g
I	0	20	890						
II	2.6	10	420						
III	5.0	15	640	60	1	5.8	220	1.2	0.786
					2	5.8	110	1.1	0.786
IV	10.0	22	1150	40/60[d]	3	11.2	470	1.3	0.782
					4	11.2	200	1.2	0.782
V	15.0	22	1310	60	5	14.1	206	1.2	0.780
					6	14.7	114	1.2	0.780

[a] Ratio of anhydride to initiator. [b] By viscometry, using the relation of Fujita et al.[22] for polymers in $HCCl_2COOH$. [c] By conventional sedimentation equilibrium (with an extrapolation to zero concentration). [d] The temperature was increased from 40 to 60° when the aminolysis reaction was found to be 90% complete.

moles of poly[Glu(OBzl),Tyr(Thp)]. The crude product was fractionated by the procedure described in paper II.[21] All polymers remained colorless throughout the entire synthesis.

C. Analytical Methods. Assay for *N*-Carboxyanhydrides. The extent of polymerization was determined by a modification of the procedure introduced by Miwa and Stahmann[25] for the quantitative analysis of *N*-carboxyanhydrides in aqueous solutions. An aliquot of the reaction mixture containing up to 4×10^{-5} mol of *N*-carboxyanhydride was added to 2 ml of 2 *M* hydroxylamine hydrochloride in a centrifuge tube (15 ml) with rapid mixing (Fisher Vortex-Genie). After exactly 10 min, 1 ml of 4 *M* hydrochloric acid followed by 1 ml of 0.37 *M* ferric chloride were added. Insoluble polymer was removed by centrifugation and filtration through a glass-wool plug in a disposable pipet. The optical density of the hydroxamic–ferric complex derived from the NCA (in the filtrate) was measured at 520 nm against a solvent and reagent blank. The relative decrease of monomer in the reaction mixture was taken as the optical density of a given aliquot relative to one removed prior to initiation of polymerization.

Assay for Benzyl Esters. The progress of the aminolysis reaction may be expressed as the extent of substitution of hydroxypropylamide for benzyl ester groups in the γ-benzyl glutamate copolymers. A sample of the aminolysis reaction mixture was added with rapid mixing to 4 ml of acetonitrile in a stoppered centrifuge tube (10 ml) to precipitate the polymer (ca. 10 mg). After 10 min the mixture was centrifuged and the supernatant discarded. The residue was extracted with acetonitrile until free of benzyl alcohol as detected by its absorbance in the extract, and then the residue was dried to constant weight in vacuo over phosphorus pentoxide. Methanol (0.1 ml) was added to the dried polymer; after 10 min 1 ml of 2 *N* sodium hydroxide was added to saponify any benzyl esters, and the mixture was shaken vigorously for 1 hr. Solid sodium chloride was added to saturation and the mixture was extracted with 4.0 ml of cyclohexane. The concentration of benzyl alcohol derived from saponification of benzyl ester groups of the polymer was determined in the extract by absorbance at 258 nm (ϵ 188 M^{-1} cm^{-1}).

Ethyl acetate can be substituted for acetonitrile. The procedure described above will detect less than 0.5% of the benzyl ester groups originally present in a polymer. The sensitiyity can be increased by measuring the absorbance at 206 nm (ϵ 8400); however, absorption at this lower wavelength is less characteristic of benzyl alcohol.

Check for Deprotection of Tyrosine. When *O*-tetrahydropyranyl-L-tyrosine was dissolved in 0.01 *N* HCl, only L-tyrosine was found after 20 min at 23° by semiquantitative thin-layer chromatography on silica gel in chloroform, methanol, 17% NH_4OH (20:20:9 v/v) under conditions where 0.01% of the protected derivative would have been detected.

For copolymers with tyrosine, use was made of the different spectral characteristics of free and protected side chains. Tyrosine derivatives with unprotected phenolic hydroxyl groups in neutral aqueous solution have ϵ_{276}^{max} 1390, which shifts to ϵ_{294}^{max} 2240 when the pH is raised to 13. *O*-Tetrahydropyranyl-L-tyrosine was also found to have ϵ_{276}^{max} 1390, and showed no such shift with pH. Furthermore, the *O*-tetrahydropyranyl protecting group is completely stable against aqueous or nonaqueous base. Hence, loss of the tyrosyl protecting group is detected easily by the pH dependence of the uv spectrum. We found no loss (within an estimated error of 5–10%) of the tetrahydropyranyl groups throughout the synthesis until they were removed (quantitatively) with dilute aqueous acid.

Amino Acid Analysis. Each copolymer fraction was hydrolyzed according to the procedure of Moore and Stein[26] for 24 and 72 hr, respectively, and the results were corrected for the degradation of tyrosine relative to glutamic acid.

Assay for D-Amino Acids. The starting amino acids as well as the copolymers, poly[HPG,Tyr], were checked for the presence of D residues by the L-leucyl-dipeptide method of Manning and Moore.[27] The starting amino acids were found to contain less than 0.1% of their D isomers. We found it necessary to isolate tyrosine and glutamic acid from the acid hydrolyzates of the copolymers prior to derivatization to eliminate complications arising from other components and artifacts of hydrolysis. Complete separation of these two amino acids (in the hydrolyzate from 5–10 mg of polymer) was achieved with a scaled-down version of an ion-exchange separation by Hirs et al.[28] on a 60 mm × 5 mm column of Dowex AG1-X8 (200–400 mesh) in the acetate form contained in a disposable pipet, by elution with 0.5 *N* acetic acid at a flow rate of 15 ml/hr. Using this technique, the tyrosine and glutamic acid residues in the polymers were found to contain less than 2% of their D isomers after correcting for racemization during hydrolysis as determined on synthetic mixtures of L-tyrosine and L-glutamic acid.

Determination of Concentration. The concentrations of all copolymer solutions were determined by micro-Kjeldahl nitrogen analysis as described previously.[23] Combined with the composition obtained from amino acid analysis, an average value of the extinction coefficient of tyrosine was computed to be ϵ_{276} 1390 M^{-1} cm^{-1} in water at neutral pH and ϵ_{274} 1240 M^{-1} cm^{-1} in 2,2,2-trifluoroethanol. Subsequently, these experimental values of ϵ were used to check the results of individual amino acid and nitrogen analyses.

D. Molecular Weights. Weight- and z-average molecular weights of the water-soluble fractions were determined by the conventional sedimentation equilibrium method as reported earlier.[21] Determination of the initial concentration was made by calibrating the fringe shift against polymer concentration as in paper VII.[29] Some low-concentration equilibrium runs were analyzed by employing the absorption optics system of the Spinco Model E ultracentrifuge and a homemade electronic scanning system.[30]

The partial specific volumes $\bar{v}$ of the water-soluble copolymers were calculated as described previously.[29]

E. Viscosity, Optical Rotatory Dispersion, and Circular Dichroism Measurements. Viscosity, optical rotatory dispersion (ORD), and circular dichroism (CD) measurements were made as described earlier.[21]

II. Results

A. Synthesis and Characterization of Copolymers. The *O*-tetrahydropyranyl protecting group has not been used extensively heretofore in syntheses involving tyrosine. However, this protecting group was found to be ideal for the synthesis of the copolymers studied here. The demonstrated stability of the *O*-tetrahydropyranyl linkage in aqueous[31,32] and nonaqueous base (e.g., 3-amino-1-propanol) renders protected tyrosyl hydroxyl groups in the co-

polymer unreactive during the aminolysis reaction. Its extreme sensitivity toward mild aqueous acid has been reported previously,[31] and is illustrated by our unsuccessful attempts to prepare the *N*-carboxyanhydride from *O*-tetrahydropyranyl-L-tyrosine (see Appendix); i.e., even when precautions were taken to trap the evolved HCl,[20] the tetrahydropyranyl protecting group was lost. Tyrosyl hydroxyl groups could be deprotected quantitatively in the last step of the synthesis, using mild conditions that did not affect the rest of the copolymer.

Tyrosine copolymers synthesized with more widely used hydroxyl protecting groups were found to be unsatisfactory for various reasons. Use of the *O*-acetyl protecting group, an ester that is reactive under aminolysis conditions, resulted in colored polymers. The *O*-benzyl ether protecting group, although stable to aminolysis conditions, led to side reactions (ester formation and cross-linking) when recommended procedures[33,34] for its removal were applied to our poly[HPG,Tyr(Bzl)] copolymers.

Two important steps in our general procedures for synthesis of copolymers were improved (see section IC): previously, (1) we have not followed the course of the polymerization process analytically, and (2) the extent of aminolysis was measured only on the final, water-soluble copolymers. In the past, occasional difficulties were encountered in the latter process, leading to incomplete reaction, and the resulting polymers had to be discarded. By developing methods to monitor the *progress* of both of these reactions, we have been able to minimize reaction times, thereby reducing the possibility of side reactions, and to assure that the synthesis consistently led to high quality copolymers.

Table I summarizes the data for both the unfractionated poly[Glu(OBzl),Tyr(Thp)] copolymers and the fractionated, water-soluble poly[HPG,Tyr] copolymers that were investigated. Use was made of the nearly linear relation between the *A/I* ratio and the average degree of polymerization ($\overline{DP}$) of the resulting poly[Glu(OBzl),Tyr(Thp)] copolymers to synthesize copolymers of desired chain lengths. The extent of chain cleavage during aminolysis[21,35,36] can be seen by comparing $\overline{DP}$ values for the poly[Glu(OBzl),Tyr(Thp)] copolymers with the values of $\overline{DP}_w$ obtained for the fractionated poly[HPG,Tyr] copolymers. It was found that, by decreasing the reaction temperature during the initial stages of aminolysis, this breakage could be reduced, as has been suggested previously,[35,36] and is demonstrated by comparing the data in Table I for copolymers IV and V; a lowering of the initial reaction temperature by 20° doubled the chain length of the final polymer. For each poly[HPG,Tyr] copolymer, the two fractions reported bracket the range of molecular weights found.

Another observation apparent from the data in Table I is that the composition is independent of chain length for a given parent copolymer, i.e., the same average amount of tyrosine is found in the short chains as in the long chains. This, together with the fact that tyrosine was incorporated quantitatively into the polymers, indicates that there is little departure from randomness in these copolymers. Furthermore, it has been demonstrated in paper I[16] that the presence of relatively short blocks (of, say, tyrosine) does not influence the melting behavior of a random copolymer to any significant extent.

The presence in the copolymers of less than 2% of the tyrosine content as D-tyrosine (and similarly for glutamic acid) is not considered to be large enough to affect the computed values of σ and s for L-tyrosine significantly.

The concentration dependence of the apparent molecular weights of the fractions studied, illustrated in Figure 1, is quite small. This fact, plus the ability to obtain molecular weights at relatively low concentrations with absorption optics, allowed the extrapolation to infinite dilution to obtain $\overline{DP}_w$ to be performed with confidence. The magnitudes of $\bar{M}_z/\bar{M}_w$ shown in Table I indicate that the polymers used in this study were fairly homogeneous. The estimated precision of the values of $\overline{DP}_w$ was ±5%.

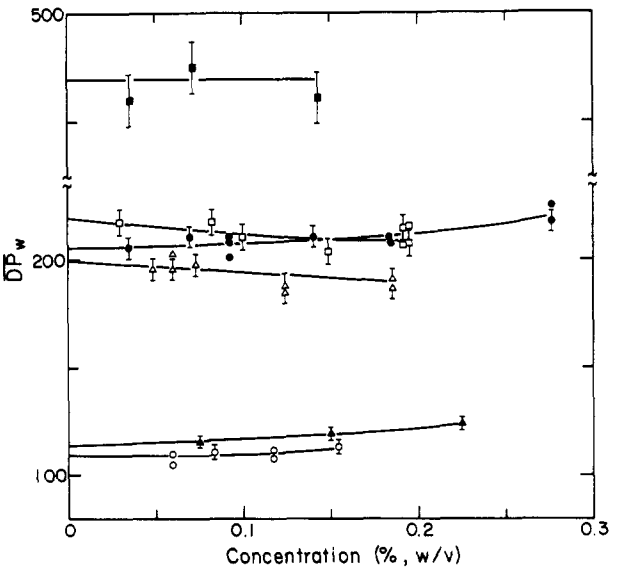

Figure 1. Concentration dependence of molecular weights for fractions used for analysis to obtain σ and s: (■) 11.2% Tyr, $\overline{DP}_w$ = 470 (fraction 3); (□) 5.8% Tyr, $\overline{DP}_w$ = 220 (fraction 1); (●) 14.1% Tyr, $\overline{DP}_w$ = 206 (fraction 5); (△) 11.2% Tyr, $\overline{DP}_w$ = 200 (fraction 4); (▲) 14.7% Tyr, $\overline{DP}_w$ = 114 (fraction 6); (○) 5.8% Tyr, $\overline{DP}_w$ = 110 (fraction 2). The error symbols represent the experimental error in each measurement.

B. ORD and CD Data for the Copolymers. The ORD and CD data for representative fractions of poly[HPG,Tyr] in water are shown in Figure 2. Both the ORD and the CD data are interpretable[37–39] in terms of combinations of right-handed α-helix and random coil, the relative contribution of each conformation being a function of both temperature and composition. Contributions to these spectra arising from tyrosine side-chain transitions are not evident, and amount to at most a very small perturbation on the large amide background. This result is consistent with the available experimental evidence, e.g., that of Ramachandran et al.,[40] which seems to indicate that large tyrosyl side-chain rotational strengths are associated with systems in which the aromatic side chains are specifically oriented. Because of the low mole fractions of tyrosine in our copolymers, and because of the nature of our host amino acid, any specific orienting of tyrosine side chains should be minimal and result in small side-chain rotational strengths. An upper limit of possible tyrosine side-chain contributions to b_0 and their effect on the computed conformational parameters of tyrosine are discussed in section IIC. In order to minimize contributions to b_0 measurements (Figure 3) from the long-wavelength (L_b) tyrosyl transition, ORD spectra were extended only down to 290 nm. The procedures used to obtain the curves of Figure 3 were otherwise the same as shown in paper VII.[29] No concentration dependence was observed, and all curves were reproducible. The error symbols in Figure 3 reflect two standard deviations in b_0 (or in helix content θ_h) calculated[41] from standard deviations in the concentration ($\pm 0.025 b_0$) and in the slope of the Moffitt–Yang plot (±3, in b_0 units). The transitions were demonstrated to be reversible in all cases.

The thermally induced melting curves demonstrate several things. First, at low temperature, incorporation of L-tyrosine greatly increases the helix content of a copolymer

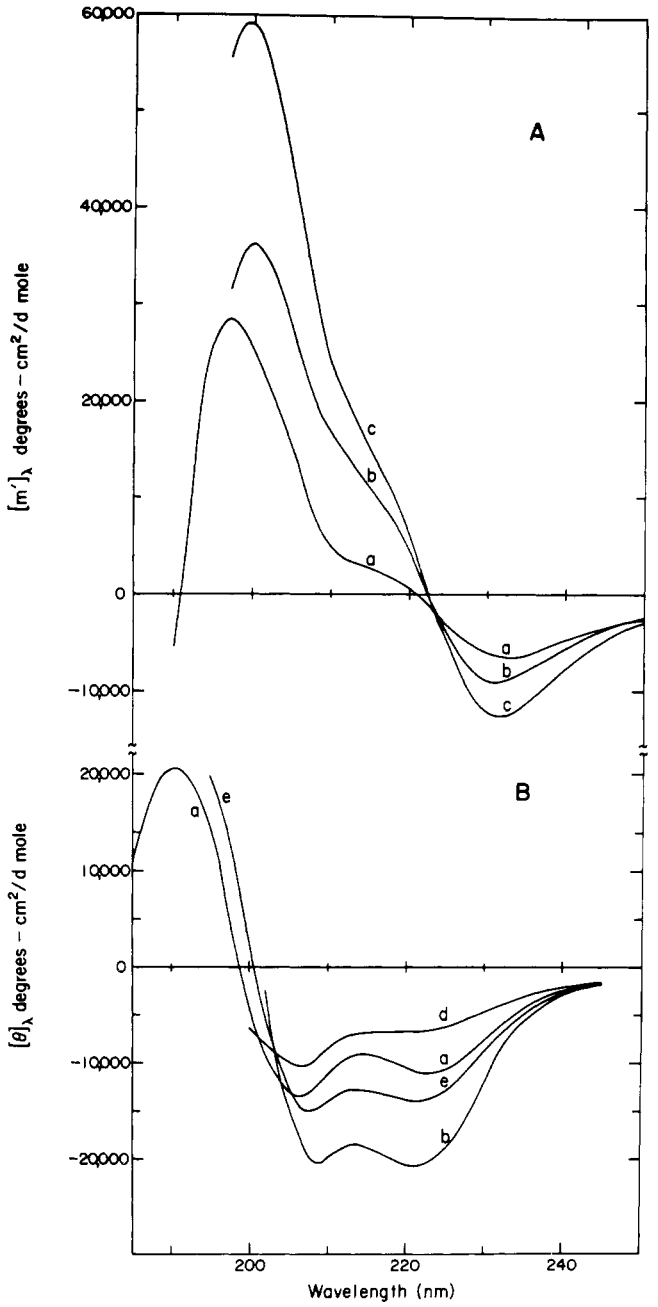

Figure 2. (A) ORD and (B) CD data in water for representative samples of tyrosine copolymers: (a) 5.8% Tyr, $\overline{DP}_w$ = 110 (fraction 2) at 25°; (b) 14.7% Tyr, $\overline{DP}_w$ = 114 (fraction 6) at 0.8°; (c) 11.2% Tyr, $\overline{DP}_w$ = 470 (fraction 3) at 0.8°; (d) 14.7% Tyr, $\overline{DP}_w$ = 114 (fraction 6) at 50°; (e) 14.7% Tyr, $\overline{DP}_w$ = 114 (fraction 6) at 25°.

relative to that of the corresponding homopolymer (i.e., the one of similar chainlength). Only at high temperatures do the helix contents of copolymer and homopolymer approach each other. Second, for similar chainlength copolymers at a given temperature, the ability of tyrosine to increase helix content is not linear in the mole fraction of tyrosine. For example, in fractions of DP ~ 200, increases in the mole fraction of tyrosine have an increasingly smaller effect on the overall helix content. These aspects of the copolymer transition behavior will be delineated with the aid of the helix–coil transition parameters obtained for tyrosine in section III.

C. b_0 for Complete Helix and Complete Coil. For the homopolymers poly[HPG] and poly[HBG] studied in paper II,[21] the value of b_0 for the complete helix was taken to be −750. For the complete coil a value of zero was used. Because these values may vary with the nature of the side chain,[42] and especially because there may possibly be contributions to b_0 from the tyrosyl side-chain chromophore, several fractions were examined in trifluoroethanol (TFE), with b_0 corrected for the dispersion of the refractive index of the solvent.[43] The results are shown in Table II, together with values of b_0 for homopolymers of poly[HPG] of corresponding chainlengths.[44]

Values of $-b_0$ less than 750 for the *homopolymers* indicate that the infinite-chain behavior (100% helix content) is attained only when the chainlength approaches 1000 residues. Small chainlengths have a correspondingly smaller fraction of helical residues.

The values of $-b_0$ obtained for the *copolymers* in TFE indicate several things. First, there is the expected decrease in the values of $-b_0$ with decreasing chainlength, for a given composition. Second, the values of $-b_0$ decrease with increasing tyrosine content, for a given chainlength; we cannot determine whether these decreases in $-b_0$ reflect a loss of helix content (from the action of tyrosine as a possible helix breaker) or a positive contribution to the optical rotatory strength from the tyrosyl side-chain chromophore, or both. Third, whatever the cause of the low values of $-b_0$, several of these values are the same (within an experimental error of ±15–20 b_0 units) as those of the host homopolymer of the corresponding chainlength; e.g., the values of $-b_0$ = 710 and 704 for fractions 3 and 1, respectively, are similar to values 730 and 690 for homopolymers of the same chainlength; thus, the effect of tyrosine incorporation on $-b_0$ is indeed small, even though we cannot specify the reason for this effect.

In order to show that this effect does not influence the computed values of σ and s, we will test the assumption that the decrease in $-b_0$ in TFE results only from a positive contribution of the tyrosyl side-chain chromophore to the rotational strength; i.e., we will tentatively assume that the decrease in $-b_0$ from fraction 1 to 4 (both having the same chainlength) arises from a more positive contribution to b_0 at the higher tyrosine content of fraction 4 and that, if a correction were made for this positive contribution, then $-b_0$ for fraction 4 would have the larger value observed for fraction 1 (a similar situation arises when comparing fractions 2 and 6, with the lower-tyrosine-content fraction 2 having a value of $-b_0$ close to that of the homopolymer of similar chainlength). Thus, we will assume that b_0 = −750 for the complete helix in the copolymer, and correct the observed values $(-b_0)_{obsd}$ by adding a correction, $b_0'(T)$, to obtain the corrected value $(-b_0)_{corr}$ which, when divided by 750, gives θ_h [which will be higher than the value of θ_h computed by omitting the correction term $b_0'(T)$].

A crude measure of $b_0'(T)$ was obtained from the *magnitude* of the tyrosyl L_b circular dichroism band.[45] The molar ellipticity of the tyrosyl L_b band of fraction 3 (11.2% Tyr; DP = 470) in TFE at ~0° was found to be −610 (deg cm²)/dmol, while its b_0 value was −710. In order to maximize the value of $b_0'(T)$, and hence its effect on the computed values of σ and s, we will assume that $-b_0$ for this fraction would have been 40 units higher, i.e., 750 instead of 710, if the contribution of the tyrosyl side-chain chromophore (manifested by the observed ellipticity) were absent. Thus, we take $b_0'(T)$ as

$$b_0'(T) = \frac{X_i \cdot [\theta]_{276,i}(T)}{(0.112)(-610)} \quad (40)$$

where X_i is the mole fraction of tyrosine in copolymer fraction i, $[\theta]_{276,i}(T)$ is its molar ellipticity in *water* at 276 nm at temperature T, and 0.112 and −610 are the mole fraction and molar ellipticity (in TFE), respectively, of fraction 3.

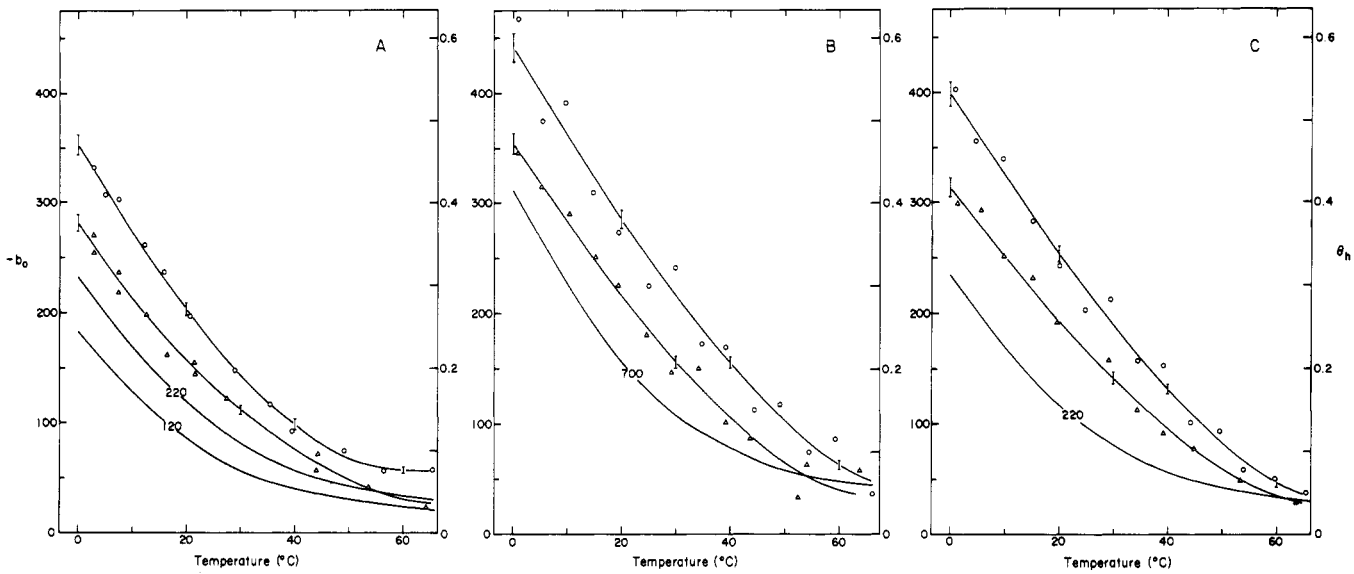

Figure 3. Temperature dependence of b_0 for poly[HPG,Tyr] copolymers in water. Poly[HPG] (lines without experimental points) of $\overline{DP}_w$ = 120 (fraction IIC of paper II[21]), $\overline{DP}_w$ = 220 (fraction IIB of paper II), and $\overline{DP}_w$ = 700 (fraction IIIB of paper II) are included for comparison: (A) (○) 5.8% Tyr, $\overline{DP}_w$ = 220 (fraction 1), (△) 5.8% Tyr, $\overline{DP}_w$ = 110 (fraction 2); (B) (○) 11.2% Tyr, $\overline{DP}_w$ = 470 (fraction 3), (△) 14.1% Tyr, $\overline{DP}_w$ = 206 (fraction 5); (C) (○) 11.2% Tyr, $\overline{DP}_w$ = 200 (fraction 4), (△) 14.7% Tyr, $\overline{DP}_w$ = 114 (fraction 6). The points are the experimental ones, and the lines represent the smoothed experimental curves. The size of the error symbols reflects the experimental errors in θ_h arising from errors in the determination of concentration and in the slope of the Moffitt–Yang plot.

Table II
Values of b_0 for Copolymers in Trifluoroethanol and $CHCl_2COOH$

Fraction	L-Tyr content, mol %	Solvent	Temp, °C	b_0 ($\overline{DP}_w$)	b_0[a] ($\overline{DP}_w$)
3	11.2	Trifluoroethanol	0.8	−710 (470)	−740 (860)
1	5.8	Trifluoroethanol	1.2	−704 (220)	−720 (300)
2	5.8	Trifluoroethanol	2.0	−678 (110)	−690 (210)
4	11.2	Trifluoroethanol	0.8	−641 (200)	−660 (150)
6	14.7	Trifluoroethanol	0.8	−616 (114)	−650 (100)
1	5.8	$CHCl_2COOH$	22.6	−10 (220)	
6	14.7	$CHCl_2COOH$	25.0	−10 (114)	

[a] Maximum values of b_0 at ~5° for samples of poly[HPG] in methanol, with the indicated $\overline{DP}_w$'s, from Figure 3 of Okita et al.[44]

Thus, all values of $(-b_0)_{obsd}$ were corrected to $(-b_0)_{corr}$ by addition of $b_0'(T)$, and converted to θ_h by dividing by 750. The values of σ and s were computed from these "corrected" melting data by the same procedure (to be presented in section III) used to analyze the "uncorrected" melting data [i.e., by dividing $(-b_0)_{obsd}$ by 750]. Despite its intentionally exaggerated value (designed to overestimate any tyrosyl side-chain contribution to b_0), this correction had only a moderate effect on the calculated thermodynamic quantities (leading to an increase in s and a decrease in σ), their variation being within the originally determined limits of experimental error. This correction of the experimental helix contents also did not lead to any better agreement with the recomputed theoretical melting curves, either collectively (as measured by the parameter τ, see below) or in groups (e.g., for groups of polymers of specific tyrosine content). For these reasons, the Discussion section which follows will focus on the results obtained using values of b_0 = −750 and 0 for the complete helix and coil, respectively, together with the experimentally measured b_0 values, $(b_0)_{obsd}$.

III. Discussion

A. Helix–Coil Parameters for L-Tyrosine. The melting curves of the copolymers described in section II were analyzed according to the LAPS (Lifson–Allegra–Poland–Scheraga) hierarchy of approximations to obtain σ and s for poly(L-tyrosine) in water. This procedure has been discussed extensively in earlier papers of this series.[16,21,47] To conserve computer time, the first approximation, corresponding to the theory of Lifson,[48] was used initially; then better values of σ and s were obtained with the second approximation, corresponding to the theory of Allegra.[49] The data from the Allegra approximation were checked with the exact theory of Lehman and McTague[50] in three representative cases.[51] The results of these calculations are shown in Table III along with the original experimental data for comparison. Both the second-order (Allegra) and the first-order (Lifson, not shown in Table III) approximations give results which agree well with those obtained from the Lehman–McTague method. The higher order Allegra approximation will be used in all subsequent discussion of the tyrosine parameters.

The copolymer melting data were analyzed by finding the best value of σ by application of the "goodness of fit" criterion, expressed in terms of the parameter τ defined in paper II.[21] With σ taken as independent of temperature, the best fit of the copolymer data was obtained by minimizing τ. Figure 4 shows that the second-order (Allegra) theory gives a minimum in τ at $\sigma = 66 \times 10^{-4}$. The error in this parameter was found by calculating σ for each fraction at each temperature individually using the best[52] value of s found for that particular temperature. To represent accu-

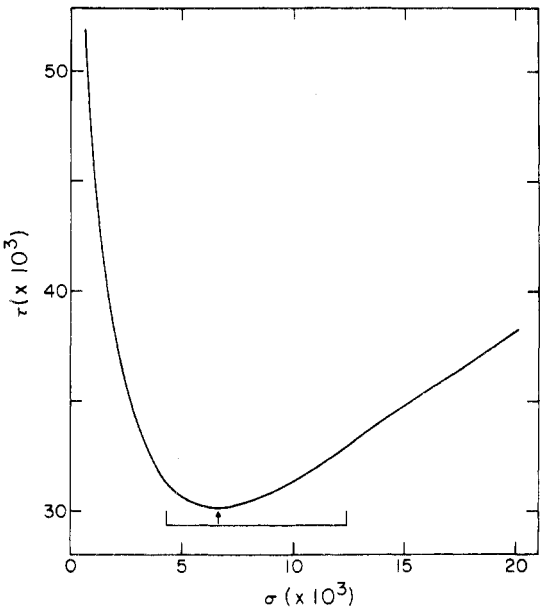

Figure 4. Determination of the best temperature-independent value of σ as the one which corresponds to the lowest value of τ for the tyrosine copolymers, using the Allegra theory. The arrow represents this best value, while the brackets represent the limits of the error in σ (discussed in section IIIA).

Table III
Comparison of the Value of θ_h, Calculated with the Approximate and Exact Theories[a] for Finite Chains

L-Tyr content, mol fraction	$\overline{DP}_w$	Temp, °C	$(\theta_h)_{exp}$	$(\theta_h)_{theor}$ Allegra[b]	$(\theta_h)_{theor}$ Lehman–McTague[b]
0.058	220	0	0.471	0.452	0.450
		30	0.191	0.187	0.187
		60	0.077	0.062	0.062
0.112	470	0	0.588	0.550	0.549
		30	0.288	0.247	0.247
		60	0.084	0.066	0.066
0.147	114	0	0.417	0.458	0.452
		30	0.189	0.217	0.214
		60	0.048	0.058	0.058

[a] The parameters used for poly[HPG] were those of Table II in paper II.[21] [b] The parameters used for L-tyrosine were obtained by fitting the data by the Allegra theory with $\sigma = 66 \times 10^{-4}$.

Table IV
Values of the Zimm–Bragg Parameter s for Poly(L-tyrosine) in Water from 0 to 60°[a]

Temp, °C	s	Temp, °C	s
0	1.12	40	0.88
10	1.07	50	0.77
20	1.02	60	0.65
30	0.96		

[a] Computed with the Allegra theory[49] with $\sigma = 66 \times 10^{-4}$.

rately the skewness of the resulting distribution of σ values, an arithmetic average of all values of σ greater than the best value[52] was computed. Similarly, an arithmetic average was computed for all values of σ less than the best value. These arithmetic averages are shown as error limits in Figure 4. The temperature dependence of σ was computed by finding the "best" value of σ at each temperature for *all* fractions. The resulting variation of σ with temperature

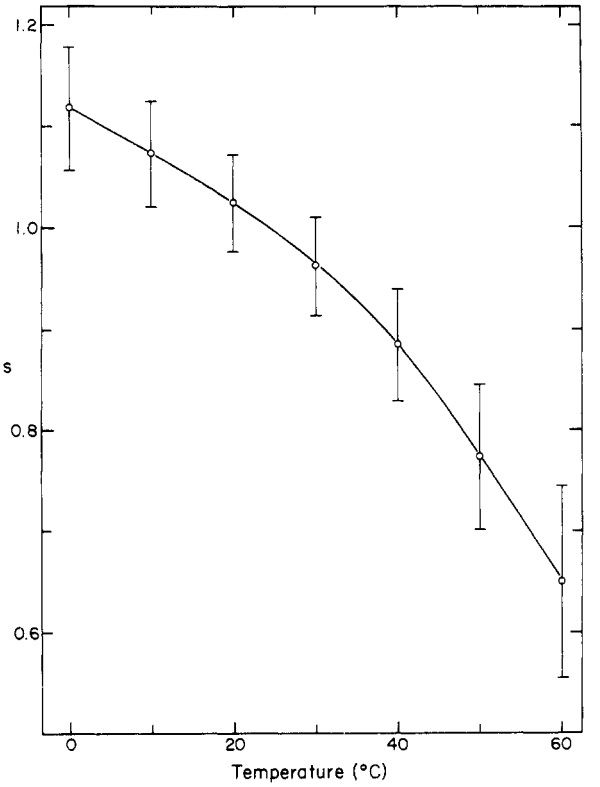

Figure 5. A plot of s vs. T for poly(L-tyrosine) in water. The error symbols are described in section IIIA. The solid line is drawn to pass through all the points.

was found to be well within the limits of error of a temperature-*in*dependent σ. As a consequence, σ was taken to be independent of temperature in the determination of $s(T)$ for tyrosine.

The best value of s at each temperature was found using the Allegra theory with $\sigma = 66 \times 10^{-4}$. These values are shown in Table IV and in Figure 5. The error symbols on the computed values of s are standard deviations in s at a given temperature as calculated from the values of s found (with σ held fixed) when each fraction was fit individually at that particular temperature. The values of s calculated in this way were not found to vary in any regular way with composition, implying that there are no discernable tyrosine–tyrosine interactions in these copolymers. Such interactions should indeed be minimal for truly "random" copolymers of the compositions employed in these studies.

Figure 6 shows the computed melting curves (using the best-fit Allegra values) along with the experimental points. The error symbols on the computed curves arise from errors in composition (±2.5%) and molecular weight (±5%). The agreement between the calculated and experimental values of θ_h is reasonably good in most cases.

The thermodynamic quantities $\Delta G°$ (the free energy), $\Delta H°$ (the enthalpy), and $\Delta S°$ (the entropy) for the conversion of a coil residue of L-tyrosine to a helical one at the end of a long helical sequence can be obtained from the values of s and its temperature dependence. Figure 7 shows a plot of $\Delta G° (= -RT \ln s)$ vs. temperature with error symbols calculated from the standard deviations in s. The thermodymamic parameters $\Delta H°$ and $\Delta S°$ determined from this plot at 20° are listed in Table V. The deviations listed for these thermodynamic quantities are those derived from the procedure used to find the slope of the $\Delta G°$ vs. temperature curve at 20°.

B. Comparison with Other Results. Although no other experimentally determined numerical values of the helix–

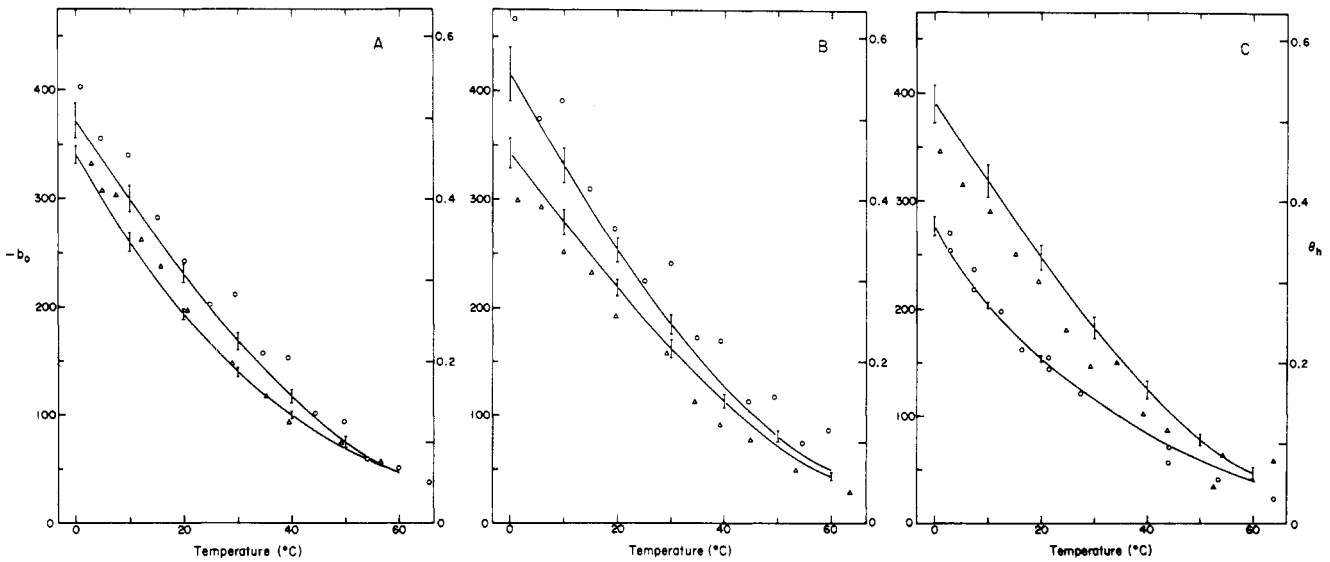

Figure 6. Comparison of the calculated melting curves, obtained from the parameters of the Allegra theory (with $\sigma = 6.6 \times 10^{-3}$) for L-tyrosine, with the experimental points: (A) (○) 11.2% Tyr, $\overline{DP}_w = 200$ (fraction 4), (△) 5.8% Tyr, $\overline{DP}_w = 220$ (fraction 1); (B) (○) 11.2% Tyr, $\overline{DP}_w = 470$ (fraction 3), (△) 14.7% Tyr, $\overline{DP}_w = 114$ (fraction 6); (C) (△) 14.1% Tyr, $\overline{DP}_w = 206$ (fraction 5), (○) 5.8% Tyr, $\overline{DP}_w = 110$ (fraction 2). The error symbols indicate errors in the calculated values of θ_h arising from errors in composition and chain length.

Table V
Thermodynamic Parameters for L-Tyrosine

ΔG°_{20}, cal/mol	−14 ± 27
ΔH°_{20}, cal/mol	−930 ± 100
ΔS°_{20}, eu	−3.1 ± 0.3
σ	66×10^{-4}

coil stability constants for L-tyrosine in water at neutral pH have been reported, a few related qualitative results are available. Tyrosine has been incorporated into unfractionated random copolymers using charged lysine[11] or glutamic acid[12] as the water-solubilizing "host". The solvent-induced transition behavior of poly[Lys,Tyr] copolymers in methanol–water was interpreted as demonstrating that tyrosine residues "stabilize" the polylysine helix, with the implication[11] that the value of s for tyrosine should be equal to or slightly less than that of phenylalanine at 30°. The results of this series of papers indicate that, in water, phenylalanine[29] and tyrosine have values of s of 1.07 and 0.96, respectively, at 30°. The pH-induced helix–coil transition of an unfractionated poly[Glu,Tyr] copolymer in water–dioxane (2:1 v/v) containing 0.2 M sodium chloride suggested[12] that tyrosine might have a lower value of σ than that of glutamic acid. Indeed, the values of σ found in this series for *uncharged* glutamic acid[18] and tyrosine in *water* are 0.010 and 0.0066, respectively. This apparent agreement may well be fortuitous, however, since the treatment of the poly[Glu,Tyr] data assumed that the helix–coil transition parameters of glutamic acid are independent of the state of ionization, an assumption which has since been shown to be unjustified.[18]

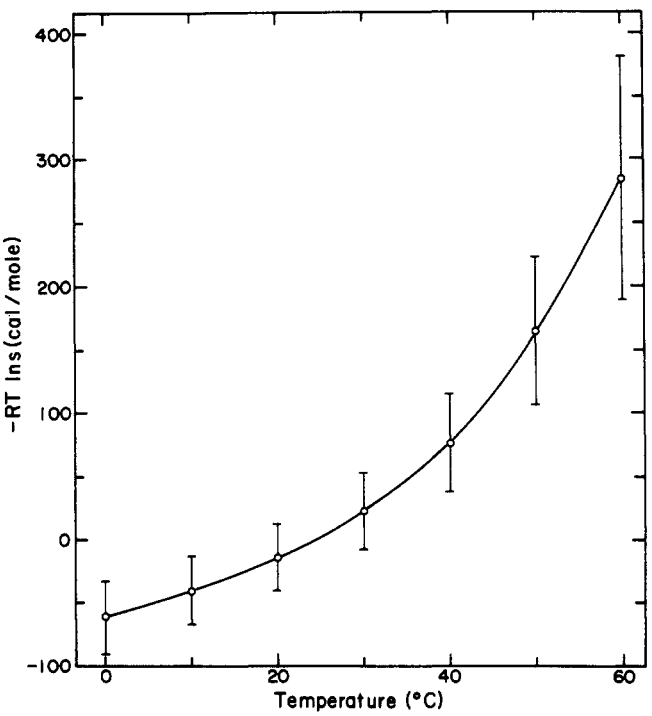

Figure 7. A plot of $-RT \ln s$ (i.e., ΔG°) vs. T for poly(L-tyrosine) in water. The solid line has been drawn through the points obtained from the Allegra analysis (with $\sigma = 66 \times 10^{-4}$). The error symbols were calculated as described in section IIIA.

C. Implications. Experimentally, copolymers of poly[HPG,Tyr] have been shown to be more helical than homopolymers of poly[HPG] of corresponding chainlength. The same theory employed to deduce the σ and s parameters for tyrosine from these experimental data can be used for further calculations designed to improve our understanding of the behavior of a tyrosine residue in these synthetic copolymers or in a protein. For example, in Table VI are compiled computed[53,54] values of the average length of a helical sequence ($\bar{L}_h$) and the average number of these sequences ($\bar{n}_h$) to be found for equal chainlengths of poly[HPG] and poly[HPG^{90}Tyr^{10}]. For poly[HPG], the melting occurs, essentially, by decrease in the length of a *single* helical segment. However, poly[HPG^{90}Tyr^{10}] has a higher helix content, distributed among four helices at 273°K, and melts by decrease in the length and number of these helical segments; i.e., the incorporation of tyrosine increases the helix content by promoting several smaller helices, not by increasing the length of the one original helix. Even at low temperatures, where $s_{Tyr} > 1$, the large value of σ_{Tyr} acts to make $\bar{L}_h$ smaller (and $\bar{n}_h$ larger) in the copolymer compared to the homopolymer.

It is of interest to examine how both σ and s affect the helix content of a homopolymer or copolymer. It is well

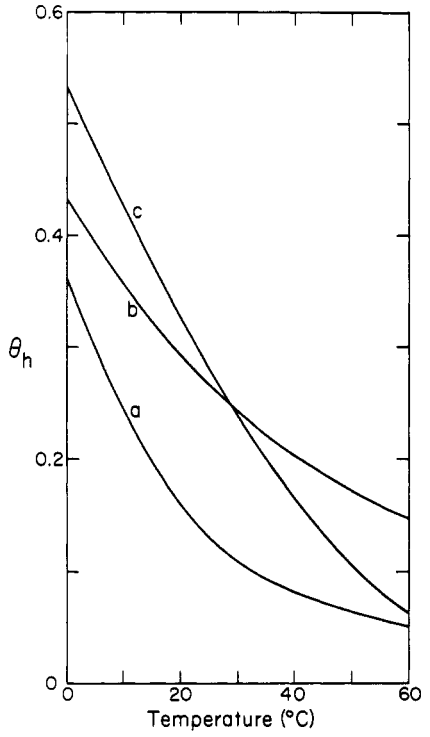

Figure 8. Computed[53] melting curves for polymers of DP = 256. Curve a, homopolymer poly[HPG]. Curve b, copolymer of HPG (with σ and s from Table II of paper II[21]) and 10% of a guest residue having values of s for HPG but a value of σ for Tyr (66×10^{-4}). Curve c, same as curve b except that the values of $s(T)$ for the guest residues are those of Table IV of this paper.

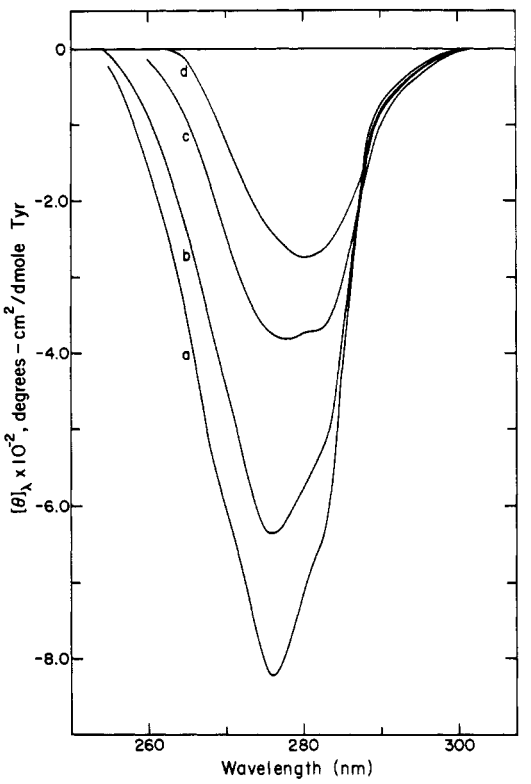

Figure 9. Temperature dependence of the ellipticity of the tyrosyl L_b band in water, 11.2% Tyr, $\overline{DP}_w$ = 470 (fraction 3) at: (a) 0.8°; (b) 19.5°; (c) 39.6°; (d) 59.0°.

Table VI
Computed[53] Influence of Tyr on Transition Behavior of Poly[HPG][a]

	0% Tyr			10% Tyr		
T, °K	$\overline{L}_h$[b]	$\overline{n}_h$[c]	θ_h[d]	$\overline{L}_h$[b]	$\overline{n}_h$[c]	θ_h[d]
273	52	1.5	0.36	34	4.0	0.53
283	38	1.5	0.24	28	3.9	0.43
293	30	1.4	0.16	23	3.6	0.33
303	24	1.1	0.11	19	3.2	0.24
313	21	0.9	0.08	16	2.7	0.16
323	18	0.8	0.06	13	2.1	0.10
333	16	0.6	0.05	10	1.6	0.06

[a] For a copolymer of DP = 256. [b] The average length of a helical section, defined as the ratio of the fraction of helical residues to (½) the fraction of helix–coil boundary residues. [c] The average number of helical sections, defined as the product of the DP and (½) the fraction of helix–coil boundary residues. [d] $\theta_h = \overline{L}_h \cdot \overline{n}_h / DP$.

known[15] that, for a homopolymer at a temperature where $s > 1$, an increase in σ decreases the helix content whereas, at a temperature where $s < 1$, an increase in σ increases the helix content; this behavior arises because, in both cases, a residue with a high value of σ induces more boundaries between helix and coil regions. In the case of the copolymers studied here, the helix content is low, i.e., $\theta_h < 0.5$ over most of the transition. Thus, a tyrosyl residue will increase the helix content significantly over that of the poly[HPG] homopolymer of comparable chainlength because the large value of σ_{tyr} increases the number of helix–coil boundaries. This helix-promoting effect of a large value of σ_{tyr} is modified by the influence of s_{tyr}. At temperatures where $s > 1$, the effect of s_{tyr} is to reinforce that of σ, i.e., to increase the helix content, but [because the tyrosine content is low, making it relatively improbable that the high concentration of tyrosyl residues in the random copolymer, required for helix growth(s), is present] the effect of s_{tyr} is not as great as that of the large value of σ_{tyr}; a large value of σ_{tyr} can manifest itself in helix nucleation even when the local concentration of tyrosyl residues is low. If the tyrosine concentration were higher, the influence of s_{tyr} would become greater. At temperatures where $s < 1$, the effect of s_{tyr} is to favor coil regions, but the large value of σ_{tyr} predominates, i.e., the helix content is increased. The net effect of both parameters, as can be seen in Figure 3, is that the helix content is increased over the *whole* temperature range by introducing tyrosyl residues. The separate effects of σ and s are illustrated in Figure 8 for polymers of DP = 256. Curve a is that for the homopolymer poly[HPG]. Curve b was computed for a copolymer with an HPG host and a guest having the same values of s as the host, but with a value of σ larger (and equal to that found here for tyrosine) than that of the host. It can be seen that, since $\theta_h < 0.5$, the larger value of σ for the guest residue (and hence the larger number of helical sequences) increases θ_h (of course, in the region of $\theta_h > 0.5$, curve b would lie below curve a). The *additional* effect of s of the guest residue is evident in curve c (computed by assigning to both σ and s of the guest the values found here for tyrosine). At low temperature, where $s > 1$ for the guest residue, a further increase in helix content is observed (i.e., curve c lies above curve b). At high temperature, where $s < 1$ for the guest residue, the effect of s of the guest on the helix content partially offsets that of its large value of σ, and curve c lies below curve b. Thus tyrosine induces an increase in the number of helix–coil borders because of its high value of σ and, in the larger number of (smaller) helical segments, the presence of tyrosine residues increases or decreases the length of these segments depending on whether the temperature is low or high, i.e., depending on whether s_{tyr} is >1 or <1, respectively. Thus, the term "helix making" is an ambiguous one, since the in-

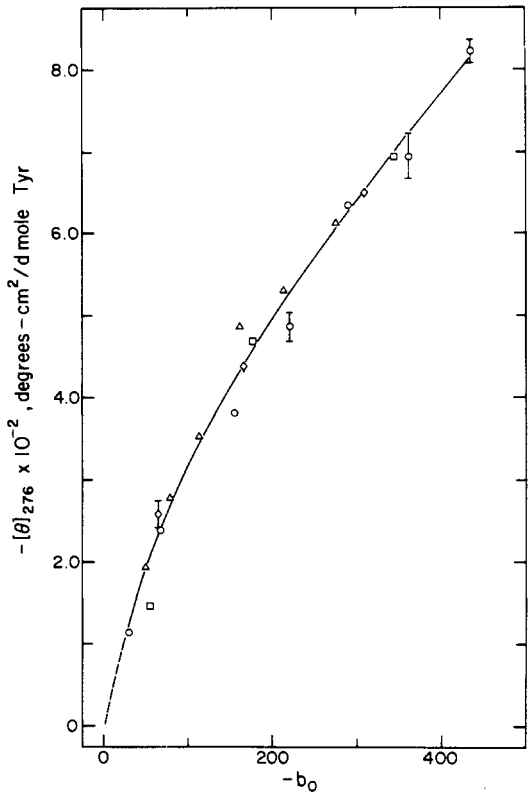

Figure 10. A plot of ellipticity at 276 nm vs. b_0 for several copolymers in water: (○) 11.2% Tyr, $\overline{DP}_w$ = 470 (fraction 3); (△) 5.8% Tyr, $\overline{DP}_w$ = 110 (fraction 2); (□) 5.8% Tyr, $\overline{DP}_w$ = 220 (fraction 1); (◇) 14.7% Tyr, $\overline{DP}_w$ = 114 (fraction 6). Error symbols indicate experimental uncertainty arising from signal/noise ratio and instrumental instability.

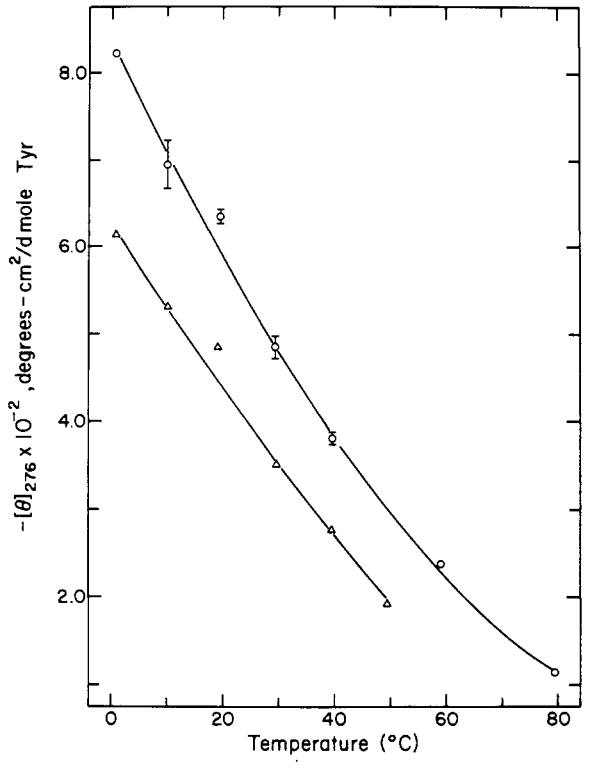

Figure 11. Temperature dependence of the ellipticity at 276 nm in water for two copolymer fractions with different helix contents: (○) 11.2% Tyr, $\overline{DP}_w$ = 470 (fraction 3); (△) 5.8% Tyr, $\overline{DP}_w$ = 110 (fraction 2). Error symbols indicate experimental uncertainty arising from signal/noise ratio and instrumental instability.

crease in helix content, for a given type of guest amino acid residue, depends on the behavior of *both* σ and s, and both parameters must be used to specify the conformational preferences of a residue.

Thermodynamically, the process of converting a tyrosine residue in the coil state to a helical state at the end of a long helical sequence is favored enthalpically but unfavored entropically (see Table V). At 25°, the large unfavorable entropy is exactly compensated for by a large favorable enthalpy. At higher temperatures, the coil state of a tyrosine residue becomes increasingly more entropically stabilized. Spectroscopic evidence cited below suggests that this entropic stabilization of the coil state, in part, might be the result of an enlargement of the conformational space (ϕ, ψ, χ^1, χ^2) available to a tyrosine residue when it assumes a coil conformation. Theoretically, the rotational strength of a tyrosyl side-chain transition depends on the relative orientations of the side chain and backbone.[55] Experimentally, the magnitude of the circular dichroism of the tyrosyl L_b band of these copolymers in water is seen to decrease with both increases in temperature (Figure 9) and decreases in overall helix content (Figure 10). A partial resolution of these two effects (Figure 11, where the upper curve corresponds to the copolymer with the higher helix content) demonstrates that, at a *fixed* temperature, a tyrosine residue in the coil state exhibits a smaller ellipticity than one in the helical state. If it can be assumed that the rotational strength of tyrosyl side-chain bands decreases with increases in relative side chain backbone rotational freedom,[56] then these observations would indicate *more relative* side chain backbone rotational freedom for a tyrosine residue in a random coil than one in a helical environment.

The theory used here[16] is based on the dominance of short-range interactions, and indicates that a tyrosine residue at 25° (where s = 1.00) has equal probabilities of being in the helix and coil states. However, in proteins of known structure, several studies of frequencies of occurrence of amino acids in various conformational regions have shown that tyrosine has a low probability of being found in the α-helical region. In three such studies, α-helical frequencies for tyrosine were found to be: 0.31 (based on 8 proteins[57]), 0.22 (based on 15 proteins[58]), and 0.18 (based on 21 proteins[59]). Similar conclusions were reached on the basis of a more complete statistical analysis.[60] Apparently, in proteins, conformational states of tyrosines are *not* determined *exclusively* by short-range, nearest-neighbor interactions but also by specific medium- and possibly long-range interactions which are not present in the random copolymers studied here. The disparity between experimental tendencies in random copolymers and conformational frequencies in proteins may reflect the presence of longer-range interactions involving tyrosine in proteins. A similar conclusion was reached in the case of ionized glutamic acid.[18] In that case, it was shown that interactions between glutamic acid in the ith position in a protein and a positively charged residue in positions $i \pm 4$ increased the tendency of glutamic acid residues to be helical in proteins compared to their behavior in random copolymers.[61]

Although the initiation parameter σ found here for tyrosine also conceivably may be modified in proteins by medium- and long-range interactions, its role in promoting boundaries may persist because of its large magnitude.

IV. Conclusions

Water-soluble random copolymers containing L-tyrosine and N^5-(3-hydroxypropyl)-L-glutamine were synthesized and characterized. From an analysis of the thermally induced helix–coil transitions of these copolymers, the

Zimm–Bragg parameters σ and s were determined. Based on its value of s, L-tyrosine was shown to have a high probability of being helical at low temperature but a very low probability of existing in a helical region at high temperature. The large value of σ found for tyrosine indicates a marked ability to promote helix–coil boundaries.

Acknowledgment. We are indebted to Mr. H. Chan and Mr. G. Davenport for their technical assistance.

Appendix. Synthesis of *O*-Tetrahydropyranyl-L-tyrosine

L-Tyrosine Benzyl Ester Hydrochloride. This compound was prepared by the procedure of Erlanger and Hall:[62] yield 47%; mp 203–204° (lit.[62] 205°); R_f 0.77; butanol, acetic acid, water (40:30:30 v/v) [BAW] on silica gel.

***N*-Benzyloxycarbonyl-L-tyrosine Benzyl Ester.** L-Tyrosine benzyl ester hydrochloride (5.0 g, 16.2 mmol) was dissolved in a mixture of 125 ml of chloroform and 150 ml of water containing 4.2 g (50 mmol) of $NaHCO_3$. The mixture was stirred rapidly at room temperature while carbobenzoxy chloride (t ml, 30 mmol) was added dropwise over a period of 1.5 hr. The reaction was allowed to proceed overnight, after which the chloroform layer was removed and the aqueous phase extracted several times with chloroform. The combined chloroform extracts were washed with dilute $NaHCO_3$, dilute HCl, and water (until neutral), and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give a light-green oil which was diluted with dry ethyl acetate and filtered; hexane was added to induce crystallization, and the product was recrystallized from ethyl acetate–hexane: yield 75%; mp 117–118°; R_f 0.62 (chloroform–acetic acid 95:t), R_f 0.69 (BAW); ir (KBr) 4320 (s), 3320 (s), 3030 (m) cm^{-1}.

***N*-Benzyloxycarbonyl-*O*-tetrahydropyranyl-L-tyrosine Benzyl Ester.** Finely ground, dry *N*-benzoyloxycarbonyl-L-tyrosine benzyl ester (3.9 g, 9.7 mmol) was placed in a 25-ml round-bottom flask equipped with a drying tube. Freshly distilled 2,3-dihydropyran (10 ml, 107 mmol) was added along with 30 μl (0.97 mmol) of 12 *N* HCl as a catalyst, and the reaction was stirred at room temperature to give a clear, light yellow-green solution after 45 min. Ether was added to the reaction mixture after an additional 3 hr of stirring and the solution at 4° was washed three times each with 2 *N* NaOH, water, and a saturated solution of NaCl, and dried over anhydrous Na_2SO_4. Solvents were removed under reduced pressure and the residual oil was crystallized from ethyl acetate–hexanes as colorless needles, which were dried over P_2O_5–NaOH in vacuo: yield 45%; mp (corr) 84.0–84.5°; R_f 0.61 [chloroform–acetone (80:20)]; ir (KBr) 3420 (s, absence of ν(OH) at 3320); 3030 (m), 2940 (s) [ν(CH)] cm^{-1}.

***O*-Tetrahydropyranyl-L-tyrosine.** *N*-Benzyloxycarbonyl-*O*-tetrahydropyranyl-L-tyrosine benzyl ester (3.0 g, 9.2 mmol) was dissolved in 160 ml of anhydrous methanol, 0.3 g of Pd (10% on charcoal) was added, and the mixture was hydrogenated at 3 atm in a Parr apparatus. No unreacted material could be detected by thin-layer chromatography of a sample after 1.5 hr. Water (70 ml) was added to redissolve the product which had precipitated, and this suspension was filtered through a bed of Celite to remove the catalyst. The solvents were azeotropically removed with ethanol under reduced pressure and *O*-tetrahydropyranyl-L-tyrosine was crystallized from a concentrated ethanolic solution: yield 95%; mp (decomposes without melting above 160°); ir (KBr) 2940 (s), 1520 (s) cm^{-1}; NMR (0.1 *N* NaOD) δ 7.0 m (4 H), 5.4 m (1 H), 4.6 m (3 H), 2.8 m (2 H), 1.7 m (6 H).

Attempts at Phosgenation of *O*-Tetrahydropyranyl-L-tyrosine. *O*-Tetrahydropyranyl-L-tyrosine in tetrahydrofuran was treated with phosgene in the presence of silver cyanide to remove the hydrochloric acid generated in the process.[20] Only L-tyrosine *N*-carboxyanhydride could be isolated from the reaction mixture.

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(1) (a) This work was supported by research grants from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service (AM-08465), and from the National Science Foundation (BMS71-00872A04). (b) Presented before the Division of Physical Chemistry at the 169th National Meeting of the American Chemical Society, Philadelphia, April, 1975, Abstracts, p Phys 019.
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Moments and Distribution Functions for Poly(dimethylsiloxane) Chains of Finite Length

Paul J. Flory* and Vincent W. C. Chang

Department of Chemistry, Stanford University, Stanford, California 94305.
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ABSTRACT: The persistence vector $\mathbf{a} \equiv \langle \mathbf{r} \rangle$ and the "center-of-gravity" vector $\langle \mathbf{g} \rangle$ have been calculated for PDMS chains, both vectors being expressed in a reference frame with X axis along the initial Si–O bond and Y axis in the plane defined by this bond and the following one. The respective vectors converge with increase in chain length to the limiting persistence $\mathbf{a}_\infty$ of magnitude 7.35 Å and direction virtually coincident with the X axis. Cartesian tensors up to the sixth rank formed from the displacement vector $\boldsymbol{\rho} = \mathbf{r} - \mathbf{a}$, where $\mathbf{r}$ is the end-to-end vector for the chain of n bonds, are evaluated as the average over all configurations for n = 2–100. For $n \leq 20$, the second moment tensor $\langle \boldsymbol{\rho}\boldsymbol{\rho}^{\mathrm{T}} \rangle$ is highly asymmetric; the asymmetry decreases with n but remains appreciable, even at n = 100. Comparison of the components of the tensors of higher rank formed from the reduced vector $\tilde{\boldsymbol{\rho}} = \langle \boldsymbol{\rho}\boldsymbol{\rho}^{\mathrm{T}} \rangle^{-1/2}\boldsymbol{\rho}$ (i.e., the vector measured in the axes of the second moment ellipsoid) with the corresponding components for the freely jointed model chain establishes the equivalence of 17.0 bonds of the real chain to one of the model for $n > 50$. Spatial densities of the distribution $W_{\mathrm{a}}(\boldsymbol{\rho})$ of $\boldsymbol{\rho}$ about $\mathbf{a}$ are calculated from the three-dimensional Hermite series truncated at the term in the polynomials involving the tensor of sixth rank. The distribution is aspherical for finite n, and, unlike the polymethylene chains investigated previously, does not display cylindrical symmetry about any axis. It is negatively skewed along the direction of $\mathbf{a}$.

In order to acquire a proper appreciation of the configurational characteristics of chain molecules of a given polymer, analysis of finite sequences of units is essential. It does not suffice to consider only those parameters that characterize the chain in the limit of infinite length, e.g., the limiting moments of the chain vector $\mathbf{r}$ as embodied in the characteristic ratio $C_\infty = \lim_{n\to\infty} (\langle r^2 \rangle_0 / nl^2)$ where n is the number of bonds and l is the bond length, or the radius of gyration ratio $\langle s^2 \rangle_0 / nl^2$ in the same limit, and so on. This is implicit in the fact that the distribution function $W(\mathbf{r})$ for vector $\mathbf{r}$ invariably becomes Gaussian for an unperturbed chain of sufficient length and of finite flexibility, regardless of its chemical structure in all other respects. Hence, the single parameter $\langle r^2 \rangle_0$ characterizes the distribution function and related properties in this limit; all higher moments of $\mathbf{r}$ are then calculable from $\langle r^2 \rangle_0$. The spatial configurations of chains of various chemical structures are too diverse to be described by a single parameter. Noteworthy in this connection is the incapability of any universal model to represent faithfully the configurations of the various polymeric chains encountered in practice.[1,2] Direct analysis of each real chain is essential, without resort to artificial models equipped with adjustable parameters.

Features of the configuration peculiar to chains of a given type are manifested in full in finite sequences, typically at lengths up to about 200 skeletal bonds. Thus, for example, the ratios of moments $\langle r^4 \rangle_0 / \langle r^2 \rangle_0^2$, etc., depart markedly in this range from their values in the limit $n \to \infty$.[2-4] They reflect the structural and conformational description of the chain in question and they delineate departures of $W(\mathbf{r})$ from Gaussian.

The configurations of finite chains or of finite sequences of units in long chains, and the distribution function $W(\mathbf{r})$ describing these configurations, are matters of direct importance to x-ray and neutron scattering by high polymers in bulk and in solution at intermediate values of the magnitude of the wave vector $\kappa = (4\pi/\lambda)$ in $(\vartheta/2)$, i.e., for κ = 0.05 to 0.3.[5] The subject is relevant also to the formation of cyclic conformations within long chains; to the formation of cyclic oligomers by elimination of sequences of units, directly or indirectly, from long chains;[6-9] to the analysis of the hydrodynamic behavior of polymeric chains of any length; and to any of the physical properties of relatively short chains ($n < \sim 200$).

The finite sequence within a long chain differs from the finite chain of the same length as the sequence, owing to configurational correlations with neighboring units in the former situation. Differences arising on this account usually turn out to be small.[10,11] Hence, it is legitimate to ignore the distinction and to view results for finite chains in the broader context that includes finite sequences within long chains.

A deeper understanding of the configurations of short chains, or sequences, can be acquired by expressing the chain vector $\mathbf{r}$ and its distribution in a reference frame fixed within the chain molecule.[12-14] An internal reference frame defined by the first two bonds of the chain is a con-