Side-Group Motions in Poly(α-amino acids)

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ABSTRACT: The dynamic mechanical relaxation behavior of some α-amino acid homopolymers and copolymers was studied over the temperature range 4.2–500°K. The polymers investigated included poly(L-leucine), poly(γ-methyl L-glutamate), poly(γ-benzyl L-glutamate), poly(N^ϵ-carbobenzoxy-L-lysine), and a series of copolymers of L-leucine and γ-benzyl L-glutamate. Each of the homopolymers exhibited a prominent relaxation process at 123°K (1 Hz) for poly(L-leucine), 262°K for poly(γ-methyl L-glutamate), 300°K for poly(γ-benzyl L-glutamate), and 303°K for poly(N^ϵ-carbobenzoxy-L-lysine). From the effects of both specimen orientation and the presence of a comonomer on the relaxation behavior, this relaxation process was attributed to side-group motion involving little or no participation of the helical backbone. Neighboring side-group interactions may be an important factor in determining side-group mobility, and this was seen to have a pronounced effect on the relaxation behavior of the copolymers.

The influence of intra- and intermolecular interactions on the flexibility of poly(α-amino acid) molecules in solution has been studied extensively. The mobility of chains or chain segments in the solid state and the effect of such motion on the solid state structure and properties have received less attention.

Nuclear magnetic resonance (nmr) measurements on solid poly(L-alanine), poly(L-leucine), poly(L-phenylalanine), and poly(γ-benzyl L-glutamate)[1] showed changes in the second moment with temperature which could be accounted for wholely in terms of side-group motion. Nmr measurements on the α- and ω-helical forms of poly(β-benzyl L-aspartate)[2] could also be explained by side-group motion, and it was suggested that rotation of the benzene ring might be an important factor in the stability of the ω structure. Anderson, *et al.*,[3] related the bulk tensile properties of copolymers of γ-benzyl L-glutamate and L-leucine to the molecular relaxation behavior. The mechanical relaxation behavior of the homopolymers poly(γ-methyl L-glutamate) and poly(γ-benzyl L-glutamate) has also been studied.[4,5] Fukada and coworkers[6,7] have recently shown that the relaxation processes in these two polymers coincide with dispersions in the temperature-dependent piezoelectric properties. In this paper, the mechanistic interpretation of the mechanical and nmr results in terms of side-group motions will be examined more closely in relation to the mechanical relaxation behavior of several more α-amino acid polymers and copolymers.

Experimental Section

The poly(α-amino acids) used in this study were prepared by polymerization of the respective α-amino acid N-carboxyanhydrides (NCA's). The preparation of the NCA's and the polymerization conditions, which are known to give high molecular weight polymers, have been described previously.[3] Films of poly(γ-benzyl L-glutamate) (PγBGlu) were prepared by two methods: (1) the polymer was precipitated from the polymerization solution with ethanol, dried under vacuum, and compression molded at 60° and (2) the polymer was cast from dioxane solution with a conventional film-casting knife. Films of poly(N^ϵ-carbobenzoxy-L-lysine) (PZLys) were molded at 80°, poly(γ-methyl L-glutamate) (PγMGlu) was molded at 95°, poly(L-leucine) (PLeu) was molded at 190°, and the copolymers of γ-benzyl L-glutamate and L-leucine were cast from dioxane solution. Films of the copolymers were oriented by drawing in steam. Infrared measurements showed the polymers to be predominately in the α-helical conformation, although some β structure in the drawn films was also indicated. All films were dried *in vacuo* at least 48 hr before using.

Poly(4-methylpent-1-ene) (P4MP1, TPX, Grade RTM/natural from Imperial Chemical Industries) was molded at 250° and quenched in ice–water.

Dynamic mechanical measurements in the temperature range 4.2–320°K were made with a free-oscillating torsional pendulum at 1 Hz.[8] The pendulum measurements were supplimented at higher temperatures, 250–450°K, with a DDV rheovibron.

Results

The relaxation curves of the four homopolymers are shown in Figures 1–4, where dynamic shear (Δ and G') and dynamic tension (tan δ and E') results are shown together for comparison. The relaxation maxima have been labeled α, β, γ in order of decreasing temperature to facilitate the discussion.

Poly(γ-benzyl L-glutamate). Maxima in the damping curve of the compression-molded PγBGlu sample were observed at 396°K (110 Hz), 300°K (1 Hz), and 95°K (1 Hz), in good agrrement with previous studies. Peaks reported at 143°K[4] and 170°K[5] were not observed. The apparent activation energy of the β peak, calculated from the frequency shift, is 41.6 kcal/mol. The relaxation behavior of the PγBGlu film cast from dioxane was identical with that of the molded sample except that the cast film consistently broke at about 380°K, below the α relaxation. Differences in the relaxation spectra of molded and cast PγBGlu films have been reported previously[5] and were attributed to incomplete removal of the solvent. The film-casting technique used in the present study appears to have eliminated this problem.

Poly(γ-methyl L-glutamate). The damping curve of PγMGlu is similar to that of PγBGlu with maxima at 415°K (110 Hz), 262°K (1 Hz), and 95°K (1 Hz). The apparent activation energy of the β relaxation is 20.3 kcal/mol. PγMGlu does not exhibit a shoulder in the cryogenic region such as observed in the relaxation spectrum of PγBGlu. This difference suggests that local motion of the phenyl group may

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[1] J. A. E. Kail, J. A. Sauer, and A. E. Woodward, *J. Phys. Chem.*, **66**, 1292 (1962).

[2] F. Happey, D. W. Jones, and B. M. Watson, *Biopolymers*, **10**, 2039 (1971).

[3] J. M. Anderson, A. Hiltner, K. Schodt, and R. Woods, *J. Biomed. Mat. Res.*, in press.

[4] R. G. Saba, J. A. Sauer, and A. E. Woodward, *J. Polym. Sci., Part A*, **1**, 1483 (1963).

[5] J. V. Koleske and R. D. Lundberg, *Macromolecules*, **2**, 438 (1969).

[6] M. Date, S. Takashita, and E. Fukada, *J. Polym. Sci., Part A-2*, **8**, 61 (1970).

[7] T. Konaga and E. Fukada, *ibid.*, **9**, 2023 (1971).

[8] C. D. Armeniades and E. Baer, *ibid.*, **9**, 1345 (1971).

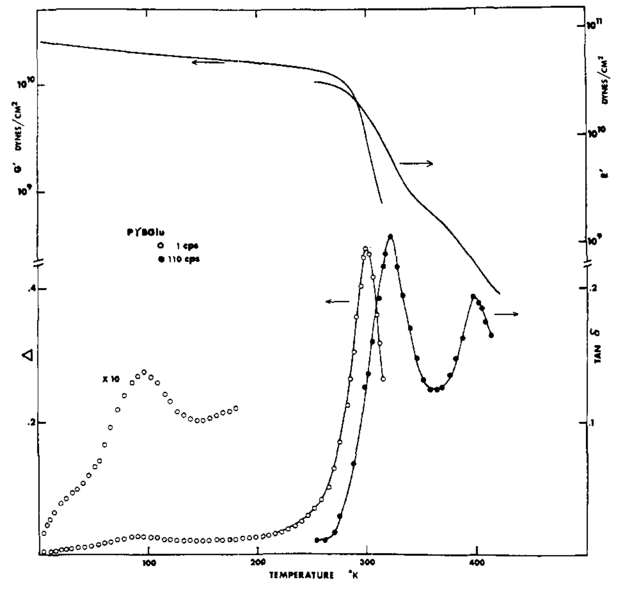

Figure 1. Temperature dependence of G' and Δ (1 Hz) and E' and tan δ (110 Hz) for poly(γ-benzyl L-glutamate).

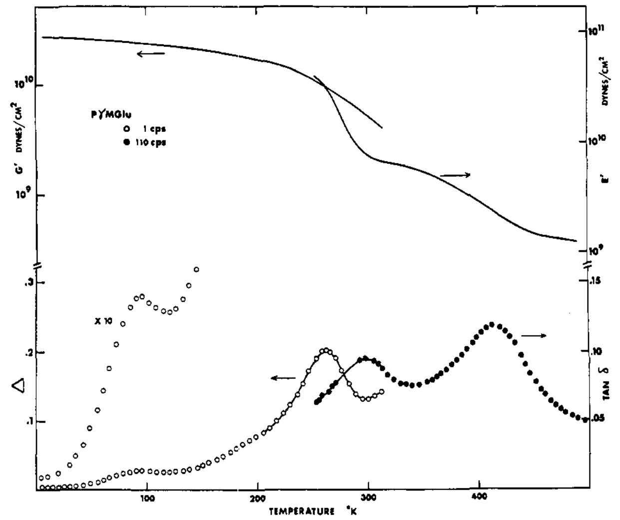

Figure 2. Temperature dependence of G' and Δ (1 Hz) and E' and tan δ (110 Hz) for poly(γ-methyl L-glutamate).

be responsible for the shoulder. A relaxation process attributed to phenyl group motion has also been observed in this temperature region in other polymers with pendant phenyl groups, for example, polystyrene.

Poly(N^ϵ-carbobenzoxy-L-lysine). This polymer shows a very strong β relaxation at 303 °K (1 Hz) which is accompanied by a drop in modulus of almost two decades. The apparent activation energy is 46.3 kcal/mol. This relaxation has been observed in nmr measurements as a sharp decrease in the second moment between 300 and 330 °K.[9] In addition to the process at 303 °K, a secondary relaxation is observed at 135 °K (1 Hz) in the mechanical measurements.

Poly(L-leucine). PLeu shows a large γ relaxation at 123 °K (1 Hz) and a second less intense one at 215 °K (1 Hz). In the cryogenic region, the damping went through a minimum at 10 °K (Δ = 1.3 × 10^{-3}) and increased again to 4.2 °K (Δ = 1.5 × 10^{-3}). The mechanical relaxation spectrum of PLeu has not been reported previously; however, nmr measurements showed a marked decrease in the second moment between 77 and 120 °K and a more gradual decrease above 120 °K.[1] The relaxation spectrum of poly(4-methylpent-1-ene) (P4MP1), which also has an isobutyl side group, is included in Figure 4 and exhibits an intense γ relaxation at about the same temperature as PLeu, 108 °K (1 Hz).

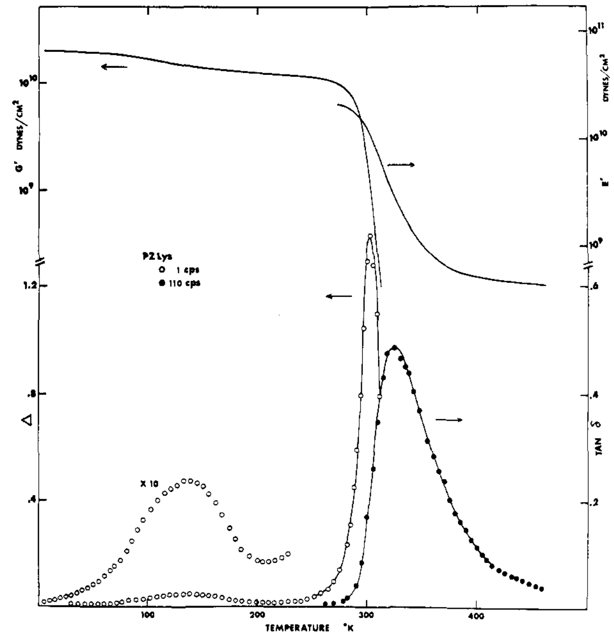

Figure 3. Temperature dependence of G' and Δ (1 Hz) and E' and tan δ (110 Hz) for poly(N^ϵ-carbobenzoxy-L-lysine).

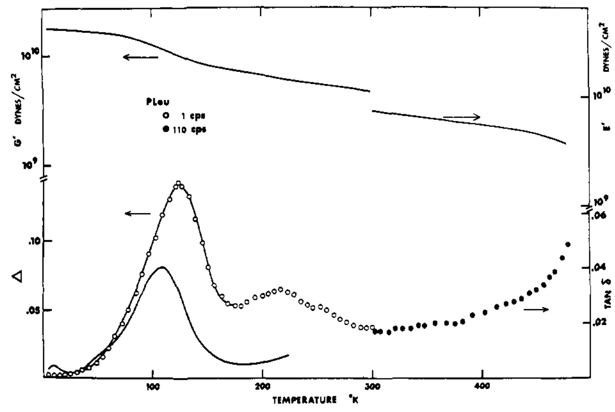

Figure 4. Temperature dependence of G' and Δ (1 Hz) and E' and tan δ (110 Hz) for poly(L-leucine). The solid curve is for poly(4-methylpent-1-ene) (Δ at 1 Hz).

(γ-Benzyl L-glutamate)-*co*-L-leucine Copolymers. The relaxation behavior of the γBGlu–Leu copolymers in the temperature region of the PγBGlu β peak is shown in Figure 5. The most striking feature is the manner in which the magnitude but not the temperature of the β process is influenced by the copolymer composition. This behavior was confirmed by the torsional pendulum results. An α peak was observed in only one copolymer, the one of highest γBGlu content. The temperature of the maximum was shifted 70° higher than for the homopolymer, and presemably the other copolymers would also show an α peak if the integrity of the samples could have been maintained to higher temperatures.

The relaxation behavior of the copolymers in the temperature region of the PLeu γ process is shown in Figure 6. Al-

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(9) A. Tsutsumi, K. Hikichi, and M. Kaneko, *Rep. Progr. Polym. Phys. Jap.*, 13, 331 (1970).

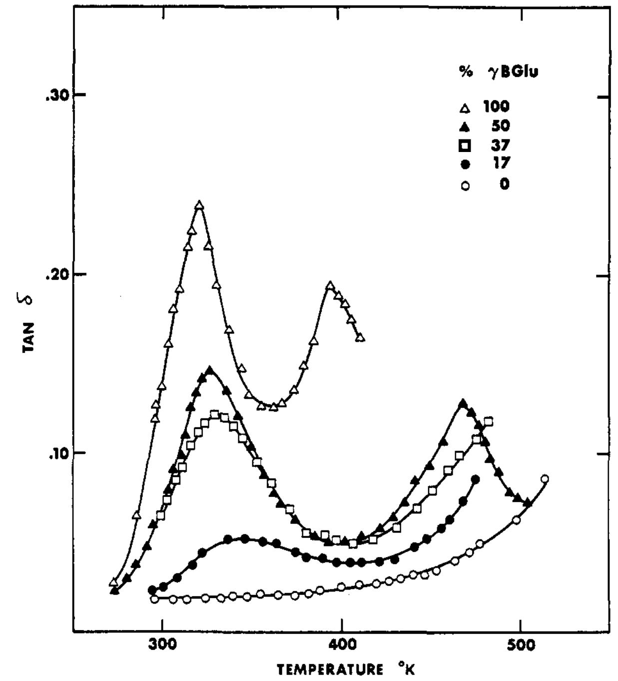

Figure 5. Temperature dependence of tan δ (110 Hz) for copolymers of L-leucine and γ-benzyl L-glutamate in the region 300–500°K.

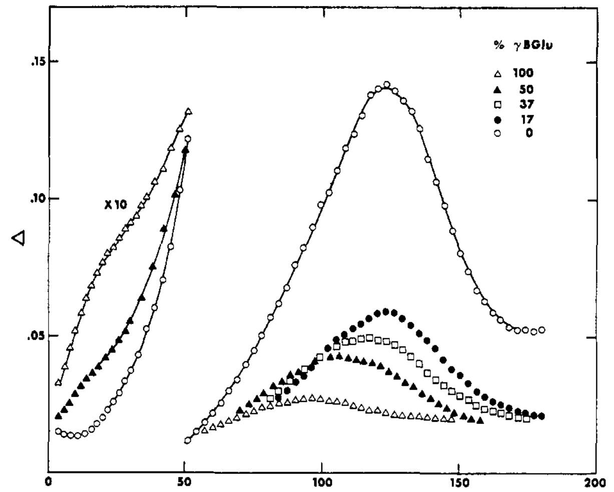

Figure 6. Temperature dependence of the logarithmic decrement Δ (1 Hz) for copolymers of L-leucine and γ-benzyl L-glutamate in the region 4.2–200°K.

though interpretation of the spectrum is complicated by the weak overlapping relaxation in PγBGlu at 95°K, it can be seen that even the smallest amount of γBGlu (17%) drastically decreases the intensity of the γ peak.

Orientation. Films of the γBGlu–Leu 50–50 copolymer were used in the orientation study because these films could be drawn to larger extensions than were attainable with wide films of the PγBGlu homopolymer. The results in Figure 7 show that the relaxation curve for the oriented sample tested at 90° to the orientation direction is the same as that for the undeformed film, while the β peak is much less intense for the sample tested parallel to the direction of orientation. There appears to be less directional dependence to the α process, although highly oriented specimens tested at 0° consistently broke before a maximum as observed.

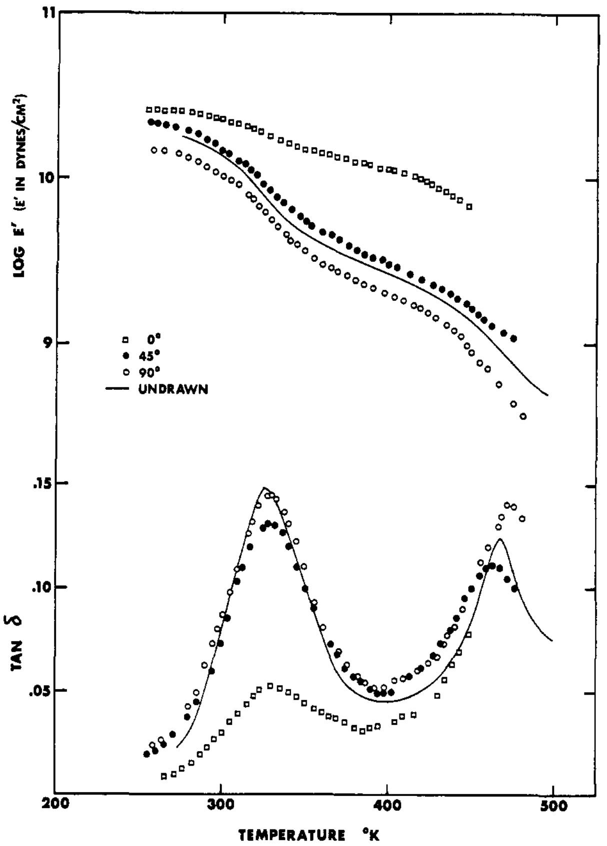

Figure 7. Temperature dependence of *E'* and tan δ (110 Hz) for oriented (γ-benzyl L-glutamate)-*co*-L-leucine 50–50 copolymer.

Discussion

Poly(γ-benzyl L-glutamate). The relaxation behavior of the polymers studied is summarized in Table I. Previous investigators have suggested that the relaxations at 300 and 262°K in PγBGlu and PγMGlu, respectively, should be attributed to the onset of side-group motions, while the higher temperature α process may involve small segments of the backbone which are in a random conformation.[6,7] The results presented here support these assignments. The intensity of the β peak increases as the length of the side group increases, that is, as the side groups account for a larger volume fraction of the polymer (tan $\delta_{\max}$ = 0.095, 0.22, and 0.48 for PγMGlu, PγBGlu, and PZLys, respectively). Likewise, the activation energy of the process increases with increasing side group size.

Evidence that the side groups relax independently of the backbone is provided by the results for the copolymers. The random inclusion of a comonomer did not change the temperature of the β relaxation, while the intensity was proportional to the per cent γBGlu. If a segment of the backbone were involved, the known effect of the Leu residues to stabilize the α helix[10] would be reflected as an increase in the temperature of the β process, as is observed for the α process, and would probably also result in a more complex relationship between the composition and the β peak intensity.

With the assumption that the side groups relax independently of the backbone, it is of interest to consider the effect of orientation on the β process. The oriented polymer can be pictured as a "composite" of a rigid backbone compo-

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[10] G. D. Fasman, C. Lindblow, and E. Bodenheimer, *Biochemistry*, 3, 155 (1964).

Table I
Relaxation Temperatures for Poly(α-amino acids), –(NHCHRCO)–

Polymer	R	T_α (110 Hz), °K	T_β (1 Hz), °K	T_γ (1 Hz), °K
Poly(γ-benzyl L-glutamate) (PγBGlu)	$CH_2CH_2COOCH_2C_6H_5$	396	300	95
				20 (sh)[a]
Poly(γ-methyl L-glutamate) (PγMGlu)	$CH_2CH_2COOCH_3$	415	262	95
Poly(N^ϵ-carbobenzoxy-L-lysine) (PZLys)	$(CH_2)_4NHCOOCH_2C_6H_5$		303	135
Poly(L-leucine) (PLeu)	$CH_2CH(CH_3)_2$			123
				215
(γ-Benzyl L-glutamate)-*co*-L-leucine 50–50		466	300	105
(γ-Benzyl L-glutamate)-*co*-L-leucine 37–63		>480	300	116
(γ-Benzyl L-glutamate)-*co*-L-leucine 17–83		>480	300	123

[a] Sh = shoulder.

nent, with the chains oriented parallel to the draw direction, and a relaxing γBGlu side-group component. Following the method Takayanagi,[11] testing the oriented polymer at 0 and 90° to the draw direction can be represented by parallel and series coupling, respectively, of the two components. The appropriate modulus expressions have been derived,[11] and if reasonable values for the moduli of the individual components are assumed, the model *qualitatively* predicts both the higher modulus and smaller modulus drop for the 0° specimen. Any *quantitative* application of the model is prohibited by the use of copolymers in the experimental study and by the relatively poor orientation that can be achieved by drawing wide films.

Poly(L-leucine). The γ peak in P4MP1 at 108°K has been identified as a side-group process[12] and the relaxation in PLeu at 123°K would appear to arise from a similar mechanism. Although side-group motions may be responsible for the most prominent relaxation process in each of the poly(α-amino acid) homopolymers, that is, the γ process in PLeu and the β process in PγMGlu, PγBGlu, and PZLys, the motions involved may be quite different in the two cases, as evidenced by the effect of a comonomer on the β and γ processes, respectively. While the intensity of the γBGlu β peak depended linearly on the γBGlu content, the effect of γBGlu on the Leu γ peak was far out of proportion to the amount present. One interpretation of this latter effect is that the mobile unit responsible for the γ process includes the side group plus a segment of backbone. For this effect to be important, especially in the lowest γBGlu copolymer, several monomer units must participate. If a segment of this length were involved, however, the flexibility of the backbone should significantly affect the process, and the γ peak in PLeu would be expected at a much higher temperature than in P4MP1 because of the greater rigidity of the H-bonded α helix compared to the polyolefin helix. For example, P4MP1 has a glass transition at about 300°K, while no glass transition has been observed in PLeu.

An alternative explanation for the effect of copolymerization is that the mobility of the leucine side group is profoundly affected by neighboring side-group interactions. Interactions of side groups, *e.g.*, through van der Waals forces and dipole–dipole interactions, have been suggested as an important contribution to the stability of the PLeu α helix.[13] The possible importance of such interactions suggests that the relative orientations of the side groups would also be interrelated, and therefore alteration in the position of one side group, *e.g.*, by rotation, may induce a corresponding change in the position of neighboring side groups. The γ relaxation is therefore proposed to be a "cooperative" process in the sense that the motion of one side group is coupled to similar motion of nearby groups. A similar situation might be expected in P4MP1, since this polymer also adopts a helical conformation with the side groups, producing a helical ridge along the side of the molecule. This arrangement also affords considerable opportunity for neighboring-group interactions. Neighboring-group interactions are thought to be much less important in the case of polymers with a blocked functional group in the side chain, *e.g.*, PγBGlu.[14] However, it is difficult to draw any conclusions regarding the "cooperative" nature of the PγBGlu β process from the copolymer results because the large γBGlu side groups are not isolated or buried by the comonomer side groups and may still be able to interact with other γBGlu side groups.

Conclusions

The most prominent relaxation process in each of the four poly(α-amino acid) homopolymers studied was attributed to side-group motion. These polymers do not exhibit primary glass transitions which can be associated with large-scale motion of the backbone, and even the side-group processes do not appear to involve significant backbone participation. Therefore, it is concluded that the relaxation spectrum of a poly(α-amino acid) in a rigid conformation will be determined primarily by the nature of the side groups. Neighboring side-group interactions may be an important factor in determining the side-group mobility, and this is seen to have a pronounced effect on the relaxation behavior of the copolymers.

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(11) M. Takayanagi, K. Imada, and T. Kajiyama, *J. Polym. Sci., Part C*, No. **15**, 263 (1966).

(12) M. Takayanagi and N. Kawasaki, *J. Macromol. Sci. Phys.*, **1**, 741 (1967).

(13) S. E. Ostroy, N. Lotan, R. T. Ingwall, and H. A. Sheraga, *Biopolymers*, **9**, 749 (1970).

(14) G. D. Fasman in "Poly-α-amino Acids," G. D. Fasman, Ed., Marcel Dekker, New York, N. Y., 1967, Chapter 11.