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Chain Branching in Poly- β -alanine

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a situation could arise simply from incomplete unfolding of the polypeptide chain or from the presence of salts which have been shown to affect the magnitude of the dichroic curves, presumably by a shielding effect.

The two-banded spectrum of PG and PL in the ionized form has a strong resemblance to the dichroic curves exhibited by polyproline II,^{3,6,15} which has been shown to exist in a threefold helix. This raises questions regarding the validity of assignment of the random form to the ionized polymer. In view of the facts that the electrostatic interactions of the side chains tend to favor extended chain configuration of the polymer, and that both extensive hydrodynamic¹⁻³ and recent nmr studies¹⁸ suggest that the charged polymer is indeed in extended configuration, and since the positions and the rotatory strengths of the optical bands (parameters specifically characterizing the conformation of a given system) of the charged polymers are significantly different from those exhibited by the threefold helical

model, polyproline II,^{3,6,15} and because of the absence of any other additional evidence suggesting to the contrary, we hold the opinion that the doubly inflected dichroic spectrum of the ionized polymers in a salt-free medium is that of the disordered form. It is still to be seen whether or not the disordered conformation of the ionized polymer indeed represents a true random form. A note of caution is further called for when speaking of "random" or "disordered" or "uncoiled" forms of polypeptides, since the conformation, and thus the optical parameters, can vary with size of polymer as well as conditions.

Acknowledgment. The author wishes to thank Dr. Frank A. Bovey of Bell Laboratories, Inc., Murray Hill, N. J., for helpful discussions and suggestions. For a preliminary report of this work, see ref 19. This work was supported by research grants from the American Cancer Society (P-482) and the National Science Foundation (GB-6964).

(18) R. E. Moll, *J. Amer. Chem. Soc.*, **90**, 4739 (1968).

(19) Y. P. Myer, *Polym. Preprints*, **10**, 307 (1969).

Chain Branching in Poly- β -alanine^{1,2}

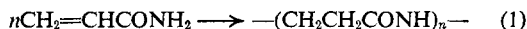
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Received July 31, 1969

ABSTRACT: Proton magnetic resonance and infrared absorption studies have been carried out on poly- β -alanines prepared by *t*-butoxide-initiated polymerization of acrylamide in pyridine, nitrobenzene, dimethyl sulfoxide, and dioxane solutions in the presence of phenyl- β -naphthylamine free-radical inhibitor. The water-soluble fractions were found to be branched copolymers of β -alanine and β,β' -iminodipropionic acid with primary amide end groups. Carboxyl end groups were detected as well, possibly resulting from the hydrolysis of primary amide groups. These carboxyl groups were the probable cause of a polyelectrolyte expansion effect in viscosity studies of a salt-free aqueous solution of a highly branched sample (PBA-S1) at pH 6. Flow birefringence studies showed that PBA-S1 consisted of readily orientable, nonspherical molecules in aqueous solution, suggesting that relatively short branches occur along a rather extended "backbone." The water solubility of the polymers increased with increasing branching and decreasing molecular weight. It is suggested that branch growth is initiated during polymerization by the formation of secondary amide anions on the polymer chain.

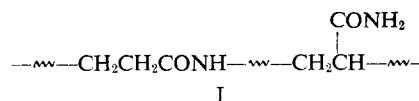
The *t*-butoxide-initiated polymerization of acrylamide in the presence of a free-radical inhibitor such as phenyl- β -naphthylamine or hydroquinone in an inert solvent has been reported to yield water-soluble and water-insoluble poly- β -alanine (reaction 1).³⁻⁵ In preparation for a study of the conformation



of poly- β -alanine in aqueous solution,⁶ we sought to determine directly the structure of the water-soluble samples.

Other investigators have noted from ir spectra that some of the products of reaction 1 contain anomalously

high proportions of primary amide groups.⁷⁻¹⁰ They suggested that these may arise from copolymers of polyacrylamide and poly- β -alanine, I.



We had been informed by Dr. M. Sloan, who supplied one of our samples, that β,β' -iminodipropionic acid was one of the hydrolysis products of polymers obtained by reaction 1. The pmr evidence reported here confirms this as well as the presence of anomalously high quantities of primary amide groups, and further shows that the primary amide groups are correlated with

(1) Adapted largely from the Ph.D. Thesis of J. D. Glickson, Columbia University, 1968.

(2) Most of the work for this article was carried out at Columbia and Iowa State Universities.

(3) D. S. Breslow, G. E. Hulse, and A. S. Matlack, *J. Amer. Chem. Soc.*, **79**, 3760 (1957).

(4) N. Ogata, *Bull. Chem. Soc. Jap.*, **33**, 906 (1960).

(5) N. Ogata, *Makromol. Chem.*, **40**, 55 (1960).

(6) J. D. Glickson and J. Applequist, to be published.

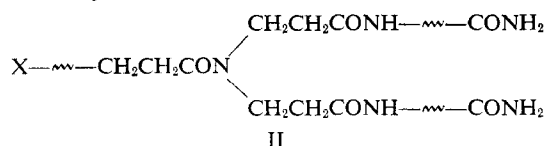
(7) N. Ogata, *J. Polym. Sci.*, **46**, 271 (1960).

(8) S. Okamura, T. Higashimura, and T. Senoo, *Kobunshi Kagaku*, **20** (218), 364 (1963); *Chem. Abstr.*, **61**, 13425a (1963).

(9) H. Nakayama, T. Higashimura, and S. Okamura, *Kobunshi Kagaku*, **23** (254), 433 (1966); *Chem. Abstr.*, **66**, 38266s (1966).

(10) H. Nakayama, *et al.*, *Kobunshi Kagaku*, **23** (254), 439 (1966); *Chem. Abstr.*, **66**, 38276t (1966).

the occurrence of β, β' -iminodipropionic acid. These facts suggest that the polymers are actually branched copolymers of β -alanine and β, β' -iminodipropionic acid; *e.g.*, a singly branched polymer would be represented by



where X is either an olefinic or *t*-butoxy ether group.¹¹ Thus primary, secondary, and tertiary amide groups would all be present. In the present study it is shown that the pmr spectra are quantitatively consistent with this structure, and with the absence of acrylic residues.

Samples studied in this laboratory ranged from an almost linear polymer PBA-I having at least 90% β -alanyl residues to a highly branched polymer PBA-S1 having 29% β -alanyl residues. The water-insoluble polymer was labeled PBA-I, and the water-soluble polymers were labeled in the order of decreasing water solubility (PBA-S1 > PBA-S2 . . . > PBA-S6). The structural studies focused on samples PBA-I and PBA-S1, which represented the extremes of solubility.

The mechanism of reaction 1 appears to be rather complex. Breslow, *et al.*, believed that polymerization was initiated by abstraction of a primary amide proton from acrylamide by a *t*-butoxide anion to yield $\text{CH}_2=\text{CHCONH}^-$. Ogata⁵ favored initiation by 1,4 addition of the *t*-butoxide anion to acrylamide followed by intramolecular proton transfer to yield the reactive species $(\text{CH}_3)_3\text{COCH}_2\text{CH}_2\text{CONH}^-$. In both mechanisms the primary amide anion end could participate in chain growth, but in that of Breslow, *et al.*,³ growth could also occur at the olefinic end, whereas Ogata's⁵ mechanism does not allow for growth at the *t*-butoxy end. Tani, *et al.*,¹¹ isolated from the reaction mixture different oligomers having olefinic and ether end groups, demonstrating that both types of initiation occur.

Breslow, *et al.*,³ favored a chain-transfer mechanism for chain growth, whereas Ogata⁵ favored continuation of the 1,4-addition mechanism. The controversy over the chain growth mechanism has still not been resolved.

The present study shows how slight modification of the proposed mechanisms for initiation could explain initiation of chain branching, and that chain growth could then proceed by either chain transfer or 1,4 addition.

The significance of the present study is its demonstration that processes accompanying reaction 1 yield branched products. A simple method for determining the degree of branching of the reaction products from their pmr spectra in aqueous solution is described. The solubility of the polymers in water is shown to increase with increasing degree of branching, and with decreasing molecular weight. It is suggested that hydrophilic primary amide and carboxyl groups may be important in enhancing water solubility. Polymers were prepared with high enough β -alanine content, and high enough water solubility, to permit con-

formational studies of poly- β -alanine in aqueous solution.⁶ Finally, this study showed that chain branching is consistent with previously proposed mechanisms for reaction 1.

Experimental Section

Poly- β -alanine Samples. A water-insoluble sample PBA-I (*Anal.* Calcd for $\text{C}_3\text{H}_5\text{NO}$: C, 50.69; H, 7.09; N, 19.71. Found: C, 48.77; H, 7.08; N, 18.07)¹² and a water-soluble sample PBA-S1 (*Anal.* Found: C, 48.79; H, 6.95; N, 18.31)¹² were gifts of Hercules Inc.¹³

A number of water-soluble fractions, synthesized by Ogata's method,⁴ were designated as PBA-S2 (100°, 10 min, pyridine), PBA-S3 (115°, 120 min, nitrobenzene), PBA-S4 (80°, 60 min, dioxane), and PBA-S5 (80°, 67 min, dimethyl sulfoxide), where the data in parentheses refer to the reaction temperature, reaction time, and solvent, respectively. All polymers were prepared using potassium *t*-butoxide initiator and phenyl- β -naphthylamine free-radical inhibitor. PBA-S6 was a water-soluble polymer prepared by hydrolysis of 2% PBA-I (w/v) for 74 min at 73.5° in a solvent prepared by bringing 50 ml of 37.4% HCl to 100 ml with 90% formic acid.

β, β' -Iminodipropionic acid was prepared by hydrolysis of β, β' -iminodipropionitrile (Eastman Organic) by Ford's method.¹⁴

β -Alanine (Eastman Organic) was recrystallized from a water-methanol-ether mixture and air dried.

Polyacrylamide was prepared by the method of Bovey and Tiers¹⁵ from vacuum-sublimed acrylamide (Eastman Organic).

Analysis of PBA-S1 Hydrolysates. PBA-S1 was hydrolyzed under N_2 for at least 22.5 hr (the minimum time required for completion of hydrolysis, as determined by ninhydrin colorimetry studies) at 110° in 6 *N* HCl. β -Alanine was determined on a Beckman Model 120 B amino acid analyzer (β -alanine N/total N = $28.7 \pm 2.8\%$). Ammonia was determined by titration of ammonia that steam distilled from an alkaline solution of the intact PBA-S1 (ammonia N/total N = $37.4 \pm 0.7\%$). Total N was determined by the Kjeldahl method.

Viscosity measurements were made at 25.0°, using Cannon Ubbelohde dilution viscometers with solvent flow times of 30 sec or greater. A Hewlett-Packard Model 5901B automatic viscometer was used in some of the flow time measurements. No kinetic energy corrections were made.

PMR spectra were obtained on a Varian A-60 Analytical nmr spectrometer equipped with a V-6057 variable-temperature system. Temperatures were determined to $\pm 2^\circ$ from the separation of the peaks of an ethylene glycol sample placed in the probe before spectral measurements were performed. 2,2-Dimethyl-2-silapentane 5-sulfonate was used as the internal reference standard for chemical shifts, unless otherwise specified.

Infrared spectra were determined on a Beckman IR-12 spectrometer. Films were cast on Irtran II plates, and solution spectra were measured in a cell with CaF_2 windows (optical path 0.1 mm).

(12) Schwarzkopf Microanalytical Laboratory, Woodside 77, N. Y.

(13) We are indebted to Dr. M. Sloan of Hercules Inc., Wilmington, Del., for the sample of PBA-I (designated originally as PBA X-8208-49-16) and to Professor Paul Doty for the sample of PBA-S1 (designated originally as PBA X-8573-70A), which had also originated at Hercules.

(14) J. H. Ford, "Organic Syntheses," Coll. Vol. III, John Wiley & Sons, Inc., New York, N. Y., 1968, p 34; J. H. Ford, *J. Amer. Chem. Soc.*, **67**, 867 (1945).

(15) F. A. Bovey and G. V. D. Tiers, *J. Polym. Sci., Part A*, **1**, 849 (1963).

(11) H. Tani, N. Oguni, and T. Araki, *Makromol. Chem.*, **76**, 86 (1964).

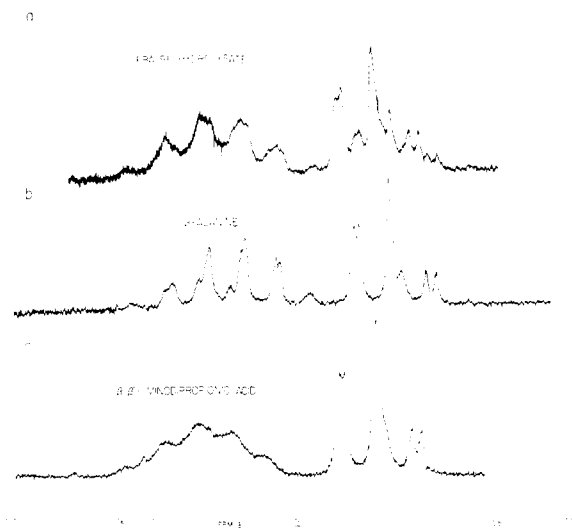


Figure 1. Pmr spectra of methylene resonances of (a) 7.81% PBA-S1 hydrolysate (w/v) in 5.15 M HCl; (b) 1.13 M β -alanine in 3.95 M HCl; (c) 0.620 M β,β' -iminodipropionic acid in 6 M HCl. Spectra were measured at 40°, using the methyl resonance of acetic acid as the internal standard with chemical shift 0.00.

Flow Birefringence. Extinction angle measurements were made in the Rao Instrument Co. flow birefringence apparatus in the laboratory of Professor P. Doty, Harvard University. A 0.6% solution of sample PBA-S1 in water was studied at 23.5° over the velocity gradient range 1700–7300 sec^{-1} .

Results

Hydrolysis of PBA-S1 and PBA-I. The low-field portion of the pmr spectrum of the total hydrolysate of PBA-S1 showed an ammonium resonance at δ 5.75 ppm (t, $J = 52.2$ Hz, NH_4Cl).¹⁶ Figure 1 illustrates that the high-field multiplet of the PBA-S1 hydrolysate (Figure 1a) was a superposition of the methylene resonances of β -alanine and β,β' -iminodipropionic acid (Figure 1b and c, respectively). The differing HCl concentrations had no effect on the spectra. The integral of the NH_4Cl triplet indicated that its concentration in the PBA-S1 hydrolysate was comparable to that of β,β' -iminodipropionic acid, which is seen from Figure 1 to be considerably greater than that of β -alanine. The spectra indicate that the only significant hydrolysis products are ammonia, β -alanine, and β,β' -iminodipropionic acid. The proportions of these judged from the spectra are in good agreement with those obtained from the chemical determinations of ammonia and β -alanine (Experimental Section), taking β,β' -iminodipropionic acid N/total N = $33.9 \pm 2.6\%$ by difference. Likewise, the ir spectrum (KBr pellet) of the total hydrolysate is in excellent agreement with that of a mixture of NH_4Cl , β -alanine \cdot HCl, and β,β' -iminodipropionic acid \cdot HCl in the mole ratio 37:29:34.

The pmr spectrum of the total hydrolysate of PBA-I in 50% H_2SO_4 (v/v) was in good agreement with the spectrum of an authentic sample of β -alanine in that solvent. There was only a trace of the ammonium

(16) The methyl resonance of acetic acid, the internal standard in this case, was assigned a chemical shift of 0.00 ppm.

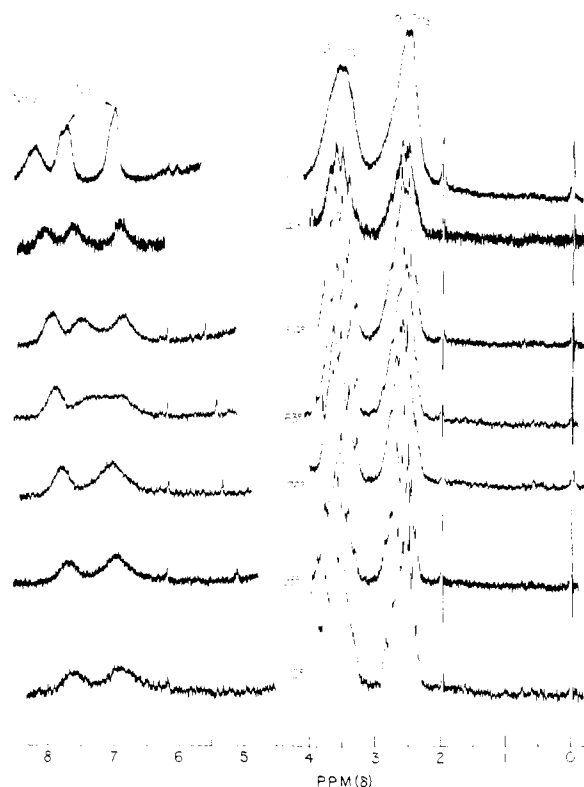


Figure 2. Pmr spectra of PBA-S1 in water, 31.9% (w/v), pH 5.33, at various temperatures.

triplet at low field. Comparison of the β -alanyl integral in the PBA-I hydrolysate with the integral of the methyl resonance of acetic acid, the internal standard, whose concentration was known, indicated that PBA-I was at least 90% poly- β -alanine.

PMR Spectra of PBA Samples. The pmr spectra of PBA-S1 at various temperatures in H_2O are shown in Figure 2 (the water resonance has been omitted). The lowest field resonance was designated as $\text{NH}(2^\circ)$ because it originated from secondary amide (β -alanyl) protons. This assignment was proved by the agreement of chemical shift of the lowest field resonance of PBA-S1 in 10 M LiCl, and the chemical shift of the single NH resonance of PBA-I (almost pure poly- β -alanine) in this solvent.

The next two low-field resonances were designated as $\text{NH}(1^\circ)$ because they originated from primary amide protons. The assignment of these resonances was made by comparison with the pmr spectrum of polyacrylamide (10% w/v, H_2O , pH 4.5, 40°) δ 7.63 (s, 1, $-\text{CONH}_2$), 6.91 (s, 1, $-\text{CONH}_2$), 2.20 (s, 1, $-\text{CH}-\text{CH}_2-$), and 1.68 ppm (s, 2, $-\text{CHCH}_2-$). At 40° the $\text{NH}(1^\circ)$ resonances of PBA-S1 in Figure 2b occurred at δ 7.65 and 6.93 ppm in good agreement with the chemical shifts of the primary amide resonances of polyacrylamide. The coalescence of the two $\text{NH}(1^\circ)$ resonances at about 63° is evidently due to the increasing rate of internal rotation about the C–N bond, as discussed by Bovey and Tiers¹⁵ for the case of polyacrylamide, where a similar coalescence occurred at about 70°.

The absence of acrylic resonances near δ 1.68 and 2.20 ppm in the spectra of PBA-S1 indicated that this

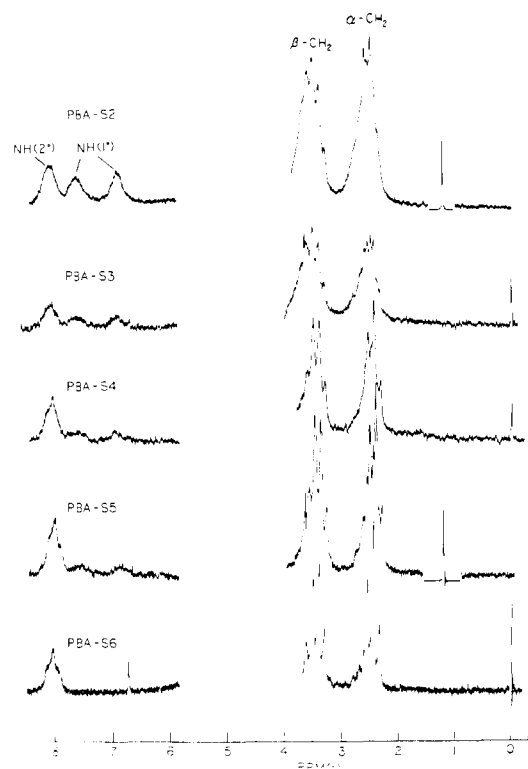


Figure 3. Pmr spectra of various water-soluble PBA samples, 10% (w/v) in water, pH about 5, 40°. The reference peak at $\delta = 0$ is 2,2-dimethyl-2-silapentane 5-sulfonate, and that at $\delta = 1.2$ is *t*-butyl alcohol.

polymer did not contain a measurable amount of acrylic residues.

The assignment of the NH(1°) and NH(2°) resonances was confirmed by partial hydrolysis of PBA-S2 for 5 min in 4 *N* HCl at 100°. The spectrum of PBA-S2 at pH 5.6 and 40° indicates that, like PBA-S1, it has intense NH(1°) and NH(2°) resonances (Figure 3). After partial hydrolysis a spectrum of PBA-S2 in 4 *N* HCl showed a strong ammonium triplet as the only low-field resonance, indicating that primary amide groups had hydrolyzed, but the high-field methylene resonances were unchanged, indicating that little if any hydrolysis of secondary and tertiary amides had occurred. Under the same conditions acetamide, *N*-methylacetamide, and *N,N*-dimethylacetamide were hydrolyzed to the extent of 100.0, 8.1, and 7.4%, respectively, confirming that these conditions should produce significant hydrolysis only of primary amide groups. Removal of the HCl and ammonia and adjustment of the pH to 5.6 resulted in a spectrum identical with that of the original sample (Figure 3a) except for the absence of the NH(1°) resonances, indicating that these resonances must have originated from the primary amide groups. This experiment also illustrates the ease with which primary amide groups on PBA samples could be replaced by carboxyls.

The two high-field resonances in Figure 2 were designated as β -CH₂ and α -CH₂, respectively, because they originated from the β - and α -methylene groups of PBA-S1. The methylene resonances of the β -alanyl spectra could not be resolved from the corresponding resonances of β,β' -iminodipropionyl residues. The

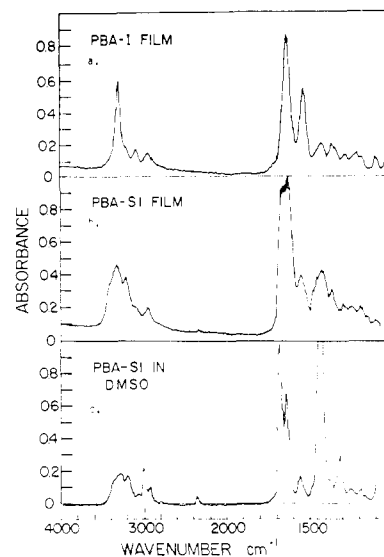


Figure 4. Ir spectra of PBA-I and PBA-S1 films (a and b, respectively), and of PBA-S1 in DMSO solution (c).

TABLE I
RELATIVE INTEGRALS OF AMIDE NH RESONANCES OF
WATER-SOLUBLE PBA SAMPLES IN H₂O, 40°, AND pH ~5

Sample	NH(2°)	NH(1°) _{obsd}	NH(1°) _{calcd}
PBA-S1	0.28	0.36	0.36
PBA-S2	0.35	0.27	0.33
PBA-S3	0.42	0.25	0.29
PBA-S4	0.65	0.18	0.18
PBA-S5	0.68	0.16	0.16
PBA-S6	0.98	0.00	0.01

assignment of the methylene resonances was possible because their chemical shifts agreed exactly with the chemical shifts of the corresponding methylene resonances of *N*-acetyl- β -alanine-*N'*-methylamide (CH₃-CONHCH₂CH₂CONHCH₃), where the assignment was made by comparing splitting patterns of the *N*-deuterated and nondeuterated forms.⁶

The pmr spectra of the remaining water soluble samples are shown in Figure 3. In Table I are listed the relative integrals of the NH resonances, calculated with respect to a value of 4.00 for the total integral of the α - and β -methylene resonances. In column four are listed the values of the NH(1°) integral calculated for a polymer with branching of the type indicated in structure II and having an NH(2°) integral given in the second column of Table I.

IR Spectra. Figure 4 shows the ir spectra of a film of PBA-I cast from formic acid solution, of a film of PBA-S1 cast from aqueous solution, and of a solution of PBA-S1 in DMSO. The peaks at 1420 and below 1100 cm⁻¹ are artifacts arising from solvent absorptions. An attempt was made to prepare an oriented film of PBA-S1 by stroking a film being cast from aqueous solution. Polarized infrared spectra showed no evidence of orientation.

Viscosity Measurements. The concentration dependence of the reduced specific viscosity η_{sp}/c of PBA-S1 in water in salt-free solution at pH 3.57 and pH 6, and in 0.1 *M* KCl at pH 6 is shown in Figure 5, where *c* is the concentration and η_{sp} is the specific

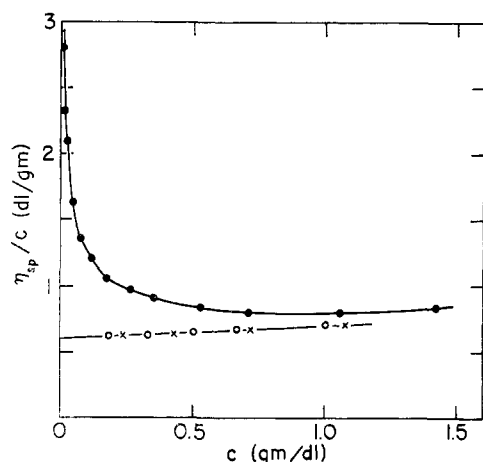


Figure 5. Concentration dependence of the reduced specific viscosity of PBA-S1 (●) at pH 6, no salt added; (×) at pH 6, 0.1 M KCl; (○) at pH 3.56, no salt added.

viscosity. At pH 6 in salt-free solution there was a marked deviation from linearity, whereas the other two cases showed good agreement with the Huggins equation (eq 2),¹⁷ where $[\eta]$ is the intrinsic viscosity

$$\eta_{sp}/c = [\eta] + k'[\eta]^2c \quad (2)$$

and k' is the Huggins constant. The linear plots yielded $[\eta] = 0.602$ dl/g and $k' = 0.27$.

In 90% formic acid both polymers gave linear Huggins plots. PBA-S1 had $[\eta] = 1.176$ dl/g and $k' = 0.303$, and PBA-I had $[\eta] = 0.420$ dl/g and $k' = 0.495$.

Titration of PBA-S1. After exhaustive dialysis, titration of PBA-S1 with standard base indicated that $1.98 \pm 0.06\%$ of the residues bore covalently bound acidic groups with intrinsic $pK_a = 4.42$ (probably carboxyl groups) and $0.24 \pm 0.06\%$ of the residues bore covalently bound groups with $pK_a \cong 9$ (probably amino groups). These titratable groups presumably arose through partial hydrolysis of the original polymer. The apparent pK_a , pK_{app} is given by

$$pK_{app} = pH - \log \frac{\alpha}{1 - \alpha} \quad (3)$$

where α is the degree of ionization. The intrinsic pK_a of the carboxyl groups was found by extrapolating pK_{app} to $\alpha = 0$. This was done fairly accurately, since the dependence on α was rather small, the value of pK_{app} at $\alpha = 1$ being 4.65.

Flow Birefringence. PBA-S1 showed a remarkably strong flow birefringence in aqueous solution. The extinction angle measurements were converted to values of the rotational diffusion constant Θ by means of the tables of Scheraga, Edsall, and Gadd¹⁸ giving $\Theta = 304\text{--}650$ sec⁻¹ over the flow gradient range 1695–7257 sec⁻¹. A prolate ellipsoid of revolution of axial ratio p and length L obeys Perrin's equation

$$L^3 = \frac{3kT}{2\pi\eta\Theta} (2 \ln 2p - 1) \quad (4)$$

(17) M. L. Huggins, *J. Amer. Chem. Soc.*, **64**, 2716 (1942).

(18) H. A. Scheraga, J. T. Edsall, and S. Gadd, *J. Chem. Phys.*, **19**, 1101 (1951).

where k is Boltzmann's constant, T is the absolute temperature, and η is the viscosity of the solvent. Assuming $p = 30$, we find that the length L of the equivalent ellipsoid varies from 3690 to 2860 Å over the observed gradient range, the length decreasing with increasing gradient as is normal for a polydisperse system.

Discussion

The results of the hydrolysis of PBA-S1 are in both qualitative and quantitative agreement with structure II. Hydrolysis of primary amide end groups should yield ammonia. Hydrolysis of secondary amide groups of the polymer should yield β -alanine, and hydrolysis of the tertiary amide groups at the branch points should yield β, β' -iminodipropionic acid. For high molecular weight polymers the concentrations of other hydrolysis products originating from olefinic or *t*-butoxy ether end groups should be too low to observe. The pmr and ir spectra of the hydrolysates confirmed that, within spectroscopic sensitivity, ammonia, β -alanine, and β, β' -iminodipropionic acid were the only hydrolysis products.

The number of primary amide groups per molecule having structure II should exceed by one the number of β, β' -iminodipropionyl groups per molecule. A small degree of hydrolysis of the primary amide groups and a high degree of branching will tend to equalize the yields of ammonia and β, β' -iminodipropionic acid in the hydrolysate. The approximate equality of the yields of ammonia ($37.4 \pm 0.7\%$) and β, β' -iminodipropionic acid ($33.9 \pm 2.6\%$) in the PBA-S1 hydrolysate is therefore consistent with structure II.

The pmr spectra of the polymers in Figures 2 and 3 showed that the polymers contained primary and secondary amide groups and α - and β -methylene residues, but no acrylic residues, which would yield two peaks whose integral ratio was 1:2, and which should have appeared at considerably higher field than the α -CH₂ resonance. These results are likewise consistent with structure II and are inconsistent with structure I.

Each branch in structure II introduces a $-\text{CH}_2\text{CH}_2\text{CONH}_2$ and a $-\text{CH}_2\text{CH}_2\text{CON}<$ residue in addition to the expected β -alanyl residues. The former two residues contain the same atoms as two β -alanyl residues. Hence, the branched structure is isomeric with poly- β -alanine. As a consequence the sum of the relative integrals of the NH resonances should be unity for all polymers with structure II, *i.e.*

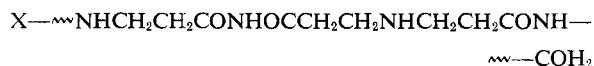
$$2\text{NH}(1^\circ) + \text{NH}(2^\circ) = 1 \quad (5)$$

where $\text{NH}(1^\circ)$ denotes the integral of one of the primary amide resonances, and $\text{NH}(2^\circ)$ denotes the integral of the secondary amide resonance. Equation 5 was used to calculate $\text{NH}(1^\circ)_{\text{calcd}}$ from the observed value of $\text{NH}(2^\circ)$ in Table I. The good agreement between the observed and calculated values of $\text{NH}(1^\circ)$ gave further support for the validity of structure II.

The difference between $\text{NH}(1^\circ)_{\text{obsd}}$ and $\text{NH}(1^\circ)_{\text{calcd}}$ should equal the fraction of carboxyl end groups, if these arise by hydrolysis of primary amides. Table I indicates that PBA-S2 and PBA-S3 may have about 6 and 4% carboxyl end groups, respectively.

The 1.98% carboxyl end groups determined by titration of PBA-S1 is consistent with the data in Table I within the accuracy of the integrals.

As an alternative to structure II one might have proposed a linear structure such as the following to account for the presence of β,β' -iminodipropionic acid and ammonia in the hydrolysate. Ammonia would result from



hydrolysis of the imide group as well as the primary amide end group. Equimolar amounts of ammonia and β,β' -iminodipropionic acid would be expected if the end group contribution is minor, consistent with the observations. However, the following evidence indicates that all the ammonia arose through hydrolysis of primary amide groups: (i) the yields of ammonia in the hydrolysates of PBA-S1 and PBA-S2 (37.4 ± 0.7 and $27.2 \pm 0.5\%$, respectively) agreed with the observed pmr integrals of the primary amide peaks (0.36 and 0.27, respectively); (ii) the disappearance of primary amide peaks in the pmr spectrum of PBA-S2 on partial hydrolysis corresponded with the appearance of ammonia peaks. This evidence is thus essential support for the branched structure. Incidentally, the same evidence shows that the extent of branching can be determined from the pmr spectrum of the polymer without resorting to hydrolysis and the subsequent tedious analysis. Thus the $\text{NH}(2^\circ)$ integral in PBA-S1 (0.28) agrees with the yield of β -alanine on hydrolysis (28.7%), in addition to the agreement between ammonia and primary amide groups noted above.

The ir spectra of PBA-I and PBA-S1 shown in Figure 4 give further qualitative information about their structures. Thus, the spectrum of PBA-I is similar to that observed for other nylons (poly- β -alanine is nylon 3),¹⁹ and is therefore consistent with the conclusion that this polymer is essentially pure poly- β -alanine. In addition to the expected *trans*-NH stretching absorption at 3310 cm^{-1} , PBA-S1 has a shoulder at 3200 cm^{-1} arising from *cis*-NH stretching.²⁰ Since secondary amides are expected to be *trans*,²¹ the latter absorption must originate from primary amide groups. In contrast to the sharp amide I absorption of PBA-I (Figure 4a) at 1639 cm^{-1} originating from hydrogen bonded secondary amides, the PBA-S1 film (Figure 4b) showed a broad peak in this region resulting probably from the overlap of amide I absorptions of primary, secondary, and tertiary amides, and the amide II of primary amides. In DMSO solution two peaks are resolvable in this region in the PBA-S1 spectrum (Figure 4c), the lower frequency peak most likely being the amide II band of the primary amides. The secondary amide II absorption of PBA-S1 at 1550 cm^{-1} is relatively weak in this polymer as compared to PBA-I because of the much lower β -alanine content of PBA-S1.

The water solubility (judged qualitatively by the tendency to go into or remain in solution) of the

polymers listed in Table I decreases from top to bottom. This trend is paralleled by an increase in β -alanine content and a decrease in the number of end groups. Hence, water solubility was enhanced by branching and/or hydrophilic end groups.

Poly- β -alanine may form water-insoluble aggregates stabilized by intermolecular hydrogen bonds. The ir spectrum of the PBA-I film indicates that all the amides are hydrogen bonded,²⁰ and various types of intermolecularly hydrogen bonded structures have been observed in other nylons.^{19,22} Because of their irregular shapes, branched polymers would be expected not to form as stable intermolecularly hydrogen bonded structures as would linear polymers. The improved packing of linear polymers would also produce increased hydrophobic interactions stabilizing insoluble aggregates. It is reasonable, therefore, that water solubility should be enhanced by branching.

The high water solubility of linear polyacrylamide and polyacrylic acid illustrates the hydrophilic nature of primary amide and carboxyl groups. These groups may contribute to the water solubility of PBA samples. The importance of carboxyl groups was indicated by a marked decrease in the water solubility of PBA-S1 after esterification of its carboxyls with diazomethane. The effect of carboxyls on the conformation of PBA-S1 is illustrated by the anomalous viscosity of this polymer in salt free solution at pH 6 (Figure 5). As the concentration of counterions is lowered by dilution of the solution, the repulsion between ionized carboxyls is strong enough to cause a marked expansion of the polymer which in turn causes an increase in the viscosity with dilution. The small variation in pK_{app} during titration of PBA-S1 with base (about 0.2 pH unit) indicates that after the initial expansion of the polymer the carboxyl groups are too far apart to strongly perturb each other's dissociation constants. Hence, the carboxyl groups are probably widely separated in the polymer network. These observations illustrate how carboxyl groups, despite their low concentration (about 2%), can profoundly affect the solubility and other solution properties of the polymers.

A highly dissymmetric molecular shape of PBA-S1 has been indicated by the flow birefringence study. The observations were made at 0.6% concentration in a salt-free solution, and according to the viscosity data, Figure 5, polyelectrolyte expansion effects are minimal under these conditions. It, therefore, seems unlikely that polyelectrolyte effects could be solely responsible for the highly extended shape. Furthermore, the molecular length is sufficient to expect relatively easy orientation in a stroked film, yet the absence of infrared dichroism in such a film shows that the absorbing groups are randomly oriented. A possible explanation of these results is that the pattern of branching is a series of short branches extending from a much longer "backbone," so that orientation of the molecules does not necessarily result in orientation of the absorbing groups.

The intrinsic viscosity of PBA-S1 (1.176 dl/g) is sufficiently greater than that of PBA-I (0.420 dl/g) to

(19) E. M. Bradbury and A. Elliot, *Polymer*, **4**, 47 (1963).

(20) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," Methuen, London, 1959, Chapter 12.

(21) L. Pauling, "The Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, p 498.

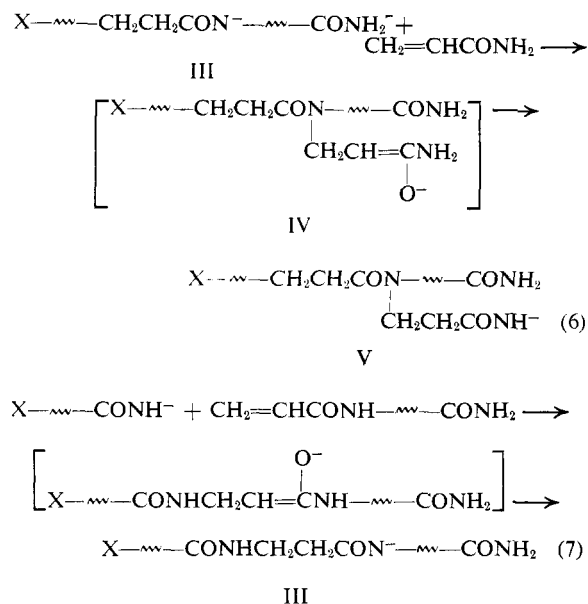
(22) C. H. Bamford, A. Elliot, and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York, N. Y., 1956.

prove that PBA-S1 has at least as large a weight-average molecular weight as PBA-I. From the light scattering and viscosity measurements of Breslow, *et al.*,³ it is found that PBA-I has a weight-average molecular weight of about 58,000, and that of PBA-S1 is probably considerably greater than this. Hence, the difference between the water solubility of PBA-S1 and PBA-I cannot be attributed to a lower molecular weight of the former, but is rather a result of their differing structure.

That a decrease in molecular weight enhances water solubility was illustrated by the partial hydrolysis of the water insoluble PBA-I (with weight average molecular weight 58,000) to yield the partially water-soluble PBA-S6 (with weight average molecular weight about 18,000, based on viscosity measurements). Primary structure appears to exert a more profound effect than molecular weight on the solubility of polymers with at least moderate degrees of polymerization.

As noted above the mechanism of reaction 1 is not well understood, and the observation of chain branching serves only to show that the mechanism may be even more complex than had been previously supposed. It has been proposed that the reactive species initiating linear polymerization of acrylamide, according to eq 1, is a *primary amide anion*. The reactive species which initiates branching could be a *secondary amide anion* III, which undergoes 1,4 addition with acrylamide forming the intermediate IV, which then undergoes intramolecular proton transfer to yield the primary amide anion V by the reaction scheme given in (6). Chain growth could then continue either by chain transfer, as proposed by Breslow, *et al.*,³ or by 1,4 addition as proposed by Ogata.⁵ The secondary amide anion III could be formed either by direct proton abstraction of a secondary amide proton by the *t*-butoxide ion, in a manner analogous to Breslow, *et al.*'s, mechanism for initiation,³ or III could be formed by the reaction scheme given in (7).

A referee of this paper has called our attention to



the fact that acrylamide is known²³ to disappear in the early stages of polymerization, and that reaction 6 would accordingly account for branching only in the early stages. He suggests that a reaction of III with unsaturated polymer would be a reasonable mechanism for branching in the later stages. We thank the referee for these suggestions.

Acknowledgment. The authors wish to thank Dr R. W. King for his advice on the pmr studies. This investigation was supported by Public Health Service Grants GM-21103 and GM-13684 from the division of General Medical Sciences, Public Health Service.

(23) L. W. Bush and D. S. Breslow, *Macromolecules*, **1**, 189 (1968).