Preparation of 11. A solution of **6** (12 mg, 20 μmol) in 5 ml of pyridine–water (1:1 v/v) was passed through a Dowex-50 pyridinium 5 cm × 1.2 cm column to replace the ammonium ion. Pyridine–water (1:1 v/v) (20 ml) was used to wash the column. The eluent was combined and evaporated to dryness under reduced pressure. The residue was then repeatedly evaporated with dry pyridine to drive off the moisture. A solution of the residue in 1 ml of pyridine was treated with mesitylenesulfonyl chloride (10 mg, 41 μmol) and polymer **10** (30 mg, 4.1 μmol of uridine dinucleotide). The suspension was allowed to stand at room temperature for 8 hr. Then ice–water (20 ml) was added, and after 14 hr the solid was separated by filtration. Water (100 ml) and methanol (150 ml) were used to wash the solid which then was dried under vacuum to give 29 mg of **11**.

Removal of Uridylyl-(3′-5′)-uridylyl-(3′-5′)-uridine from the Polymer 11. A sample of 4.6 mg of **11** was suspended in 1 ml of 0.5 *M* hydrazine in pyridine–acetic acid (4:1 v/v), and the mixture was shaken for 5 hr at room temperature. Ammonium hydroxide (0.5 *N*, 1 ml) was added, and the mixture was extracted with three 2-ml portions of ethyl ether. Then the aqueous layer was chromatographed on Whatman 3 MM paper in the solvent system S_1 for 1 day. The band of the slowest mobility (R_f 0.03) was eluted with water to give 9.2 OD units at 262 mμ of the trinucleotide, 48% yield based on the dinucleotide on the starting polymer. The trinucleotide was cleaved completely to uridine and uridine 5′-phosphate (1:1.90) by venom phosphodiesterase and underwent degradation to the extent of 95% upon pancreatic ribonuclease digestion to give uridine and uridine 3′-phosphate in the ratio 1:1.95.

The Conformation of Poly-β-alanine in Aqueous Solution from Proton Magnetic Resonance and Deuterium Exchange Studies[1]

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Abstract: Studies of poly-β-alanine (PBA) in D_2O solution were carried out on a highly branched, high molecular weight (>58,000) sample and a linear low molecular weight (18,000) sample using proton magnetic resonance (pmr) to observe the spin–spin coupling between α and β protons and deuterium exchange kinetics to test for conformational concealment of NH groups. Pmr spectra of PBA, β-alanine, *N*-acetyl-β-alanine ethyl ester (ABAEE), and *N*-acetyl-β-alanine-*N*′-methylamide (ABAMA) imply rapid internal rotation about the C^α–C^β bond with equal populations of the three staggered conformations. The pD dependence of the deuterium exchange rate of PBA is very similar to that of ABAEE and ABAMA, suggesting an absence of appreciable intramolecular hydrogen bonding in PBA, consistent with the random structure indicated by the pmr spectrum. The absence of hypochromism in the π–π^* band at 190 nm in a linear PBA (mol wt 58,000) is likewise consistent with the absence of ordered secondary structure.

The factors governing the stabilities of various chain conformations in poly-α-amino acids are gradually becoming clearer through studies of a wide variety of these materials, including both synthetic polymers and natural proteins.[2,3] The search for a full understanding of these factors is aided by studies of homologous polymers as well, since these provide additional tests of any hypothesis that may be put forward to explain conformational stability. The poly-β-amino acids are of particular interest for this purpose because of their close homology to the poly-α-amino acids.

A previous study[4] of poly-β-L-aspartic acid in aqueous solution suggested that this material in its uncharged form does take on an ordered conformation, possibly helical. Though this evidence was not conclusive, it encouraged further study of poly-β-amino acids, and the present work on poly-β-alanine, $(\mathrm{NHCH_2CH_2CO})_n$, seemed particularly relevant in view of its importance as the simplest representative of the family. Moreover, poly-β-alanine (PBA) is easily synthesized[5] and can be studied in aqueous solution when the sample is either highly branched or of low molecular weight.[6] The primary conformational tools used in the present study are proton magnetic resonance (pmr) spectroscopy and deuterium exchange kinetics.

Some of the properties of the PBA samples prepared by anionic polymerization of acrylamide have been reported elsewhere.[6] It was shown that chain branching often occurs due to the formation of the trifunctional β,β′-iminodipropionic acid residue in the polymer chain. Unbranched samples of high molecular weight are insoluble in water and other solvents favoring ordered conformations in poly-α-amino acids, while branching enhances water solubility enormously. The present study of aqueous solution properties is based largely on studies of a highly branched, high molecular weight sample and an unbranched, low molecular weight sample.

One of the early observations[6] suggesting an unusual polymer conformation (in a sample which later proved to be highly branched) was the strong flow birefringence of a dilute aqueous solution. This indicated a highly

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(1) Adapted from the Ph.D. Thesis of J. D. Glickson, Columbia University, New York, N. Y., 1968, on work performed at Columbia and Iowa State Universities.

(2) Many aspects of this subject are reviewed in G. D. Fasman, Ed., "Poly-α-amino Acids: Protein Models for Conformational Studies," Marcel Dekker, New York, N. Y., 1967.

(3) Conformational energy calculations are reviewed in G. N. Ramachandran and V. Sasisekharan, *Advan. Protein Chem.*, **23**, 283 (1968).

(4) J. Kovacs, R. Ballina, R. L. Rodin, D. Balasubramanian, and J. Applequist, *J. Amer. Chem. Soc.*, **87**, 119 (1965).

(5) D. S. Breslow, G. E. Hulse, and A. S. Matlack, *ibid.*, **79**, 3760 (1957).

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dissymmetric molecular shape, and raised the possibility of a rigid molecule with an ordered chain conformation. The subsequent search for further evidence of such a conformation, including the infrared dichroism studies mentioned earlier[6] and the additional work described below, proved entirely negative, however, and the major conclusion of this paper is that poly-β-alanine is an essentially disordered molecule in aqueous solution. It is possible to rationalize this result in terms of recently determined thermodynamic parameters for helix formation in poly-L-α-alanine, a chemically similar poly-α-amino acid. To this limited degree, the present results provide an interesting test of our understanding of factors governing helix stability.

Experimental Section

Poly-β-alanine. The preparation and chemical characterization of the poly-β-alanine samples used here have been previously described.[6] PBA-I is a water-insoluble sample of poly-β-alanine of at least 90% purity having a weight average molecular weight of about 58,000 based on its intrinsic viscosity. Solution of PBA-I in 90% formic acid followed by continuous dialysis against water for 24 hr yielded an aqueous solution of PBA-I which was concentrated enough for measurement of its amide π–π^* absorption in the uv. Upon standing at room temperature for a few hours, the PBA-I slowly precipitated. PBA-S6 is a water-soluble sample of poly-β-alanine prepared by partial hydrolysis of PBA-I to yield a weight average molecular weight of about 18,000. PBA-S1 is a highly branched water-soluble copolymer of β-alanine (45.8 mol %) and β,β-iminodipropionic acid (54.2 mol %) with numerous primary amide end groups and a few carboxyl end groups. Its molecular weight was shown by intrinsic viscosity measurements[6] to be greater than that of PBA-I.

***N*-Acetyl-β-alanine ethyl ester (ABAEE)** was prepared from β-alanine (Eastman Organic) by acetylation with acetic anhydride and esterification with ethanol.[7] The *N*-acetyl-β-alanine had a melting point of 72–78° after one recrystallization from ethyl acetate. The ester was fractionally distilled (bp 122–126° (1.3 mm)). *Anal.* Calcd for $C_7H_{13}NO_3$: C, 52.82; H, 8.23; N, 8.80. Found: C, 52.52; H, 8.47; N, 8.63.[8]

***N*-Acetyl-β-alanine-*N'*-methylamide** (ABAMA). ABAEE was treated with methylamine by the method of Mizushima, *et al.*,[9] to yield ABAMA. An aqueous solution of ABAMA was deionized through Amberlite MB-1 (Rohm and Haas), recrystallized twice from ethanol–ethyl acetate, and vacuum sublimed at 130° (0.5 mm); mp 148.3–148.8°. *Anal.* Calcd for $C_6H_{12}N_2O_2$: C, 49.98; H, 8.39; N, 19.43. Found: C, 49.96; H, 8.45; N, 19.39.

Ultraviolet spectra were recorded at room temperature on a Cary Model 15 spectrophotometer under nitrogen purge using 1-mm Suprasil cuvettes. Concentrations were determined by Kjeldahl nitrogen analysis.

Proton magnetic resonance spectra were recorded on a Varian A-60 spectrometer equipped with a variable temperature probe. The spectrum of β-alanine was obtained on a Varian 220-MHz spectrometer. The temperature was determined from the chemical shifts of ethylene glycol or methanol samples, which were inserted before and after the spectral measurements. Chemical shifts were referred to the methyl resonance of sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), the internal standard.

Deuterium Exchange Kinetics. Compounds to be studied were dried (P_2O_5, 77°) and rapidly mixed with ice-cold D_2O (Columbia Organic, 99.7%) under dry nitrogen in a test tube immersed in an ice bath. The pD (pH meter reading plus 0.40, using general purpose glass and calomel electrodes[10]) was adjusted with DCl and NaOD solutions. Approximate concentrations were 0.45 *M* for PBA-S1, PBA-S6, and ABAMA, and 0.87 *M* for ABAEE. The rate of exchange of amide hydrogen with deuterium was measured by following the appearance of HDO in the infrared absorption spectrum of the solution. The absorbance at 3400 cm^{-1}, due primarily to the OH stretch, was recorded as a function of time on a Beckman IR-4 spectrophotometer using an expanded absorbance scale and a 0.1-mm path length thermostated cell (12.0 ± 0.2°) with CaF_2 windows. For each of the samples, exchange rates were also obtained by monitoring the disappearance of the amide II band at 1550 cm^{-1}, and the results were consistent with those obtained at 3400 cm^{-1}. Because greater precision was obtained at the latter frequency it was preferred for general use. Exchange rate constants were determined from slopes of plots of log $(A_\infty - A)$ *vs.* time, where A and A_∞ are the absorbances at the given time and at equilibrium, respectively. At 3400 cm^{-1} there is a contribution to the absorbance from the disappearing NH stretch centered at about 3300 cm^{-1} as well as the OH stretch. Because the NH and OH concentrations are stoichiometrically related by the reaction under study, the rate constant for the increase in absorbance at 3400 cm^{-1} is the true rate constant for the exchange reaction NH $+ D_2O \rightarrow$ ND + HDO. Systematic errors due to drift in the baseline were found to be negligible over the periods of less than 1 hr required for the reaction. The precision of the measurement of $A_\infty - A$ was ± 0.002, as limited by instrumental noise. With observed changes in absorbance at 3400 cm^{-1} in the range 0.05–0.20 over the course of the reaction, this permitted determination of the rate constants to within 1–5% precision up to an extent of reaction of 80–90%, but beyond this extent the nature of the kinetics could not be determined. Likewise, the kinetics were not observed during the first 2–5 min after mixing with D_2O while the solutions were being manipulated.

Results

Pmr Spectra. Figure 1 presents the 60-MHz spectra of PBA-S6, ABAMA, and ABAEE, and the 220-MHz spectrum of β-alanine (all in D_2O, 39°). The 60-MHz spectrum of β-alanine has been reported elsewhere[6,11] and shows a more complex splitting pattern due to the smaller chemical-shift difference between α- and β-methylene protons in comparison to the other compounds studied. We show the higher frequency spectrum of β-alanine here in order to compare all compounds under conditions where the chemical-shift difference is large compared to the vicinal coupling constants.

The δ 3.75-ppm peak in the PBA-S6 spectrum originates from some residual methanol, the solvent from which this polymer was reprecipitated.

Assignment of the various resonances is accomplished by consideration of their chemical shifts, intensities, and spin coupling patterns. The ethyl group of ABAEE gives the usual triplet–quadruplet pattern and chemical shifts characteristic of other ethyl compounds.[12] The methylene resonances of PBA-S6 and β-alanine could be assigned unambiguously since they are the only nonexchanging protons in these compounds. In H_2O (pH 4.5, 39°) the low-field methylene resonances become an approximate quadruplet (the component peaks were spaced 6–7-Hz apart and had intensity ratios approximately 1:3:3:1), and a more complex multiplet for β-alanine, whereas the high-field methylene resonances are identical in D_2O and H_2O. Hence, the low-field methylene resonance must be associated with the β-CH_2, which couples to the vicinal NH and the α-CH_2, and the high-field resonance must be associated with the α-CH_2, which couples only to the β-CH_2. The α- and β-CH_2 resonances of ABAMA and ABAEE are assigned by the similarity of their chemical shifts to those of PBA-S6. These resonances in H_2O also resemble those of PBA-S6 in this solvent. The

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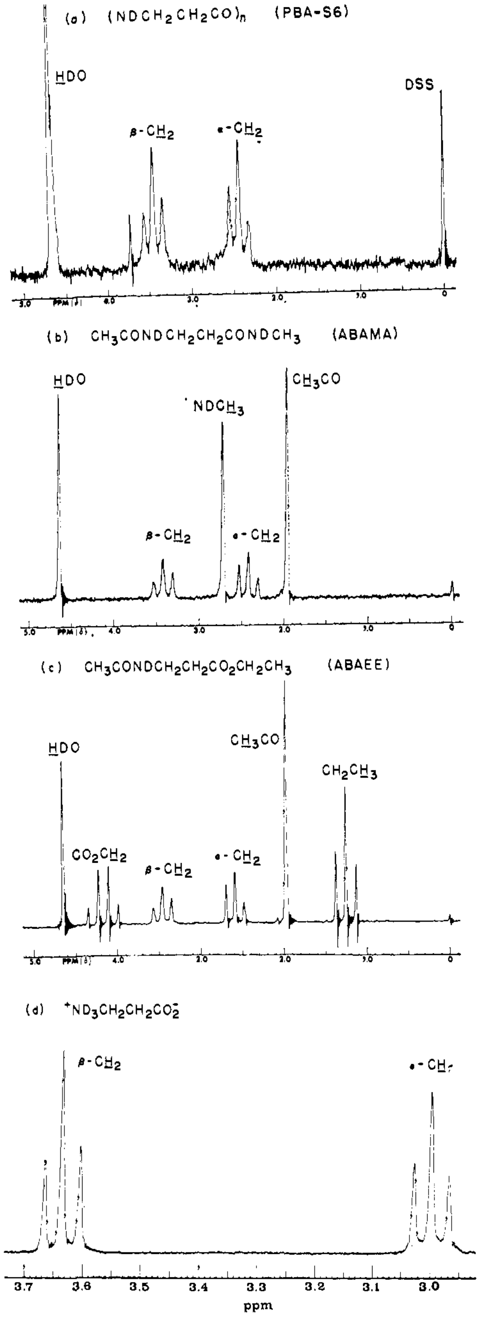

Figure 1. Pmr spectra in D_2O at 39°, 10% (w/v) concentration. The frequency is 60 MHz for a, b, and c, and 220 MHz for d.

acetyl methyl group of ABAMA is assigned from its intensity and also from its lack of splitting in H_2O. By contrast the NCH_3 resonance becomes a doublet (J = 5 Hz) in H_2O.

In Table I are listed the chemical shifts for the α- and β-methylene groups, determined from each spectrum as the position of the central peak in the triplet. The coupling constant J_{av} in Table I is the spacing between peaks in either triplet. The significance of this quantity is discussed below.

In H_2O the NH resonance of PBA-S6, δ 8.0 (J = 5-7 Hz), is a broadened triplet, whereas the NH resonances of ABAMA, δ 7.9, and ABAEE, δ 8.0, are too broad to reveal any spin coupling.

The pmr spectrum of sample PBA-S1 has been shown elsewhere.[6] Since it is more complex than that of PBA-S6 due to the effects of branching in the former, it will not be considered in the present paper.

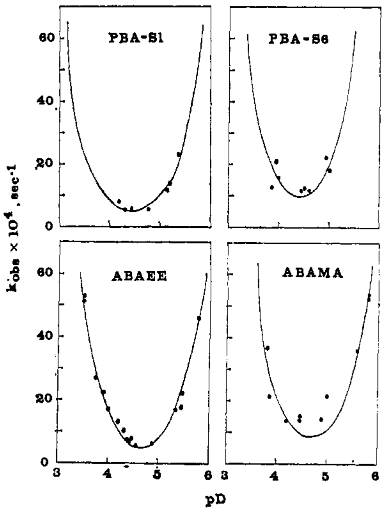

Figure 2. Pseudo-first-order rate constants for deuterium exchange in D_2O at 12°.

The temperature dependence of the spectra was examined for PBA-S6 and β-alanine in water between 40 and 98° and for ABAEE in deuteriochloroform and acetone-d_6 between −31 and 39° and as the pure liquid between 39 and 172°. It is significant that very little temperature dependence of the spectra was observed. In particular, the value of J_{av} (Table I) remained constant above 40°, and for ABAEE diminished gradually below this temperature to 5.9 Hz at −31°

Table I. Chemical Shifts and Coupling Constants

Compd	δ, ppm α-CH_2	δ, ppm β-CH_2	J_{av}, Hz
PBA-S6	2.43	3.43	6.5
ABAMA	2.42	3.43	6.7
ABAEE	2.59	3.46	6.5
β-Alanine	2.997	3.633	6.7

Deuterium Exchange Kinetics. The exchange of the NH protons of PBA-S6, PBA-S1, ABAMA, and ABAEE in D_2O at 12° obeyed simple first-order kinetics for at least 75% of the exchange reaction. Figure 2 shows the pD dependence of the pseudo-first-order rate constants k_{obsd}. The data are typical of those showing both acid and base catalysis, and following Leichtling and Klotz[13] we interpret the observations in terms of the equation

$$k_{obsd} = k_0 + k_a[D^+] + k_b[OD^-] \qquad (1)$$

The solid curves in Figure 2 were calculated assuming k_0 = 0 and using the values of k_a and k_b in Table II, obtained by a least-squares fitting method. The concentrations $[D^+]$ and $[OD^-]$ were obtained from the relationships pD = $-\log[D^+]$ and $[OD^-] = K_w/[D^+]$, respectively, where $K_w = [D^+][OD^-] = 2 \times 10^{-15}$.[14] Table II also lists k_{min}, the minimum value

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(14) R. W. Kingerly and V. K. La Mer, *J. Amer. Chem. Soc.*, **63**, 3256 (1941).

Table II. Deuterium Exchange Rate Constants in D_2O, 12°

Compd	k_a, l. mol^{-1} sec^{-1}	$k_b \times 10^{-6}$, l. mol^{-1} sec^{-1}	$k_{min} \times 10^4$, sec^{-1}	pD_{min}
PBA-S1	7.4 ± 2.4	4.5 ± 0.2	5.1	4.46
PBA-S6	11.2 ± 2.4	9.3 ± 1.3	9.2	4.39
ABAMA	20.3 ± 2.9	4.3 ± 0.5	8.3	4.69
ABAEE	16.1 ± 0.3	3.7 ± 0.1	6.9	4.67

of k_{obsd}, and pD_{min}, the pD corresponding to k_{min}, as calculated from the relationships

$$k_{min} = 2(K_w k_a k_b)^{1/2} + k_0$$

$$pD_{min} = {}^1/_2 \log (k_a/k_b K_w)$$

which are derived by letting $dk_{obsd}/d[D^+] = 0$ in eq 1.

It was found possible to obtain a somewhat improved fit to the data by allowing k_0 to take nonzero values, but the uncertainties in all of the rate constants become large by this method, and the values of k_0 in particular are too uncertain to be meaningful.

In the case of PBA-S1, 72% of the NH protons originated from primary amide end groups, and the remainder from β-alanyl NH groups.[6] Thus, it was necessary to determine which amide groups were involved in the observed exchange. In H_2O at pH close to 5, and temperatures below about 60°, three distinct resonances occur in the low-field portion of the pmr spectrum of PBA-S1.[6] The β-alanyl NH is associated with the lowest field resonance, and the cis and trans primary amide NH's are associated with the other two resonances. (It is not known which is cis and which is trans.) Four minutes after adding D_2O to a sample of PBA-S1, the pmr spectrum (10°, pD 5.32) showed a distinct β-alanyl NH resonance at δ 8.3 ppm, and only a faint trace of the primary amide NH resonances at δ 7.9 and 7.3 ppm. The β-alanyl NH slowly disappeared, but could still be observed 13 min after initiating the exchange. This experiment indicates that, at least at pD 5.32, the exchange of primary amide NH was too rapid to follow, whereas the exchange of β-alanyl NH was observable. At other pD's the deuterium exchange of PBA-S1 followed simple first-order kinetics for at least 75% of the observed reaction, which is consistent with the belief that only one chemically distinct form of NH contributes to the observed kinetics. Moreover, estimates of the number of protons contributing to the measured rate of exchange of PBA-S1 agreed with the concentration of β-alanyl NH but not with the concentration of primary amide NH. We thus feel safe in ascribing the observed exchange rates to β-alanyl NH.

For ABAMA, first-order kinetics were observed for at least 75% of the reaction, as with the other compounds. This indicates that both NH protons in ABAMA exchange at nearly the same rate, in spite of minor differences in environment.

Uv Spectra. The extinction coefficients ϵ_{max} of PBA, ABAMA, and *N*-methylacetamide at λ_{max}, the wavelength of maximum absorption of the amide π–π^* transition, are summarized in Table III. For comparison, some comparable data of Rosenheck and Doty[15] for various conformations of poly-L-lysine and poly-L-glutamic acid are also included.

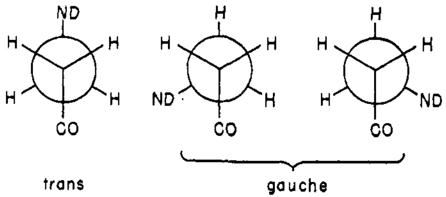

Figure 3. Staggered conformations of the C^α–C^β bond in β-alanine derivatives.

Discussion

Interpretation of Pmr Spectra. The spectrum of the α- and β-methylene groups in all compounds (Figure 1) is a pair of uniformly spaced triplet peaks which are mirror images of each other. This is the simplest form of the spectrum of two pairs of equivalent, or nearly equivalent, nuclei with spin–spin interactions between the pairs (A_2X_2 or ABXY in the notation of Pople, Schneider, and Bernstein[16]). Such a spectrum offers a clue to the extent of internal rotation about the C–C bond, and we consider here the possible conclusions that may be drawn.

Table III. Data on π–π^* Absorption in Polyamino Acids and Related Compounds in Water

Compd	λ_{max}, nm	ϵ_{max}, l. mol^{-1} cm^{-1}
PBA-I	190	7530 ± 400
PBA-S1	187.5	7610 ± 400
ABAMA	187	8210 ± 430
N-Methylacetamide	187	8730 ± 460
Poly-L-lysine[15]		
Coil	192	7100
α helix	192	4400
β structure	194	7800
Poly-L-glutamic acid[15]		
Coil	190	7100
α helix	190	4200

For simplicity, we regard the possible conformations about the C^α–C^β bond as being limited to the three staggered forms shown in Figure 3. If all three conformations are equally populated and are rapidly interconverting, then the α- and β-methylene protons comprise an A_2X_2 system with equal vicinal coupling constants J and J' (the two coupling constants betwen A and X nuclei), which are weighted averages over the existing conformations.[17] The spectrum predicted[16] for this case is a symmetrical pair of triplet peaks as observed, with the positions of the central peaks in each triplet locating the chemical shifts of nuclei A and X, and the spacing between peaks in either triplet being equal to the conformation-average vicinal coupling constant J_{av} ($= J = J'$), in accordance with the way the data in Table I were obtained. Thus the simplest interpretation of the spectra is that rapid internal rotation with nearly equal populations of the three staggered conformations is occurring about the C^α–C^β bond in all of the compounds. For poly-β-alanine this would imply that the chains are essentially disordered.

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(16) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High-Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959, Chapter 6.

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However, there are known cases of A_2X_2 spectra which have the appearance of triplet peaks even though J and J' differ significantly, due to the near coincidence of certain lines and low intensities of others.[18] Thus the above interpretation is not the only possible one, and we mention here the alternatives, any of which would allow a considerable degree of order in poly-β-alanine. The cases corresponding to an A_2X_2 system with unequal J and J' are (a) a nonuniform mixture of rapidly interconverting trans and gauche conformations, such as might arise if regions of helical order were in rapid equilibrium with random regions; (b) the exclusively trans conformation; and (c) the exclusively gauche conformation with rapid interconversion between the two forms. A poly-β-alanine helix in case c would necessarily exist in right- and left-handed forms in rapid equilibrium. In addition, it is possible that the ABXY system represented by gauche conformations which interconvert slowly could account for the observed triplet splitting if the criteria of Abraham and Bernstein[18] for the ABXY case are met. In any of the cases mentioned the observed coupling constant J_{av} is the arithmetic mean of the four possible vicinal coupling constants.[18]

A case not included above is that of a slowly interconverting mixture of trans and gauche. The observed spectrum would then be a superposition of the spectra of the two forms, and these should be distinct. Since only one spectrum is observed, this possibility seems remote.

Various pieces of evidence suggest that none of the alternatives to a random conformation applies. (i) The similarity of the coupling constants in Table I strongly suggests that very nearly the same conformational equilibrium exists in the polymer and the model compounds. Thus it is unlikely that long-range interactions or cooperative effects, such as are usually present in helical polymers, play any role in the conformation of the polymer. (ii) The value of J_{av} for equal population of the three staggered conformations in 103 substituted ethanes was found by Abraham and Pachler[17] to be given approximately by

$$J_{av} = 17.97 - 0.796\sum_{i=1}^{6} E_i$$

where E_i is the electronegativity of the ith atom on the C–C fragment. Taking the electronegatives[19–21] for C, H, and N as 2.55, 2.15, and 3.00, respectively, we find J_{av} = 6.68 Hz for the NCH_2CH_2C group, in good agreement with the observed values in Table I. This suggests that the three conformations are in fact nearly equally populated. (iii) The dielectric studies of β-alanine and related dipolar ions by Wyman[22,23] were interpreted to mean that there is essentially free rotation about the C^α–C^β bond in aqueous solution. (iv) We observed identical pmr spectra for β-alanine in D_2O in the presence and absence of 1 M NaCl, indicating the absence of any preferred conformation due to electrostatic interactions in this dipolar ion. (v) The pmr line widths observed for PBA-S6 are comparable to those in the low molecular weight model compounds, indicating the absence of dipolar broadening effects such as are observed for backbone protons of poly-α-amino acids in rigid ordered conformations.[24]

The fact that the pmr spectra were not very sensitive to temperature (over the range −31 to 172° for ABAEE) is also consistent with equal populations of the C^α–C^β conformations over most of the temperature range, though it is equally consistent with the alternative of a large excess of trans or gauche through the same range due to a large energy difference between the two forms. The evidence cited above argues against the latter possibility.

The pmr spectra are thus best interpreted to mean that there is rapid internal rotation about the C^α–C^β bond with nearly equal populations of staggered conformations in all of the compounds and that the low molecular weight polymer PBA-S6 is randomly coiled in aqueous solution in the temperature range studied (40–98°).

Deuterium Exchange. The relevance of deuterium exchange kinetics in this conformational study comes from the finding that many of the NH groups in native proteins and helical poly-α-amino acids exchange more slowly by orders of magnitude than NH groups in randomly coiled polymers or simple model peptides.[25] It is presumed that reduced rates result from hydrogen bonding or other steric concealment of the NH groups. The interpretation of a measured rate is actually complicated by the various catalytic effects studied by various workers (reviewed in ref 13). In this study we adopt the point of view of Leichtling and Klotz[13] that the study of the pD dependence of the exchange rate provides the most suitable comparison between a polymer and simpler model compounds. By this criterion, the data in Figure 2 and Table II show that the model compounds and polymers are all remarkably similar in their exchange behavior. We conclude that the accessibility of NH groups to solvent is essentially the same in the polymers as in the model compounds.

Two pieces of evidence indicate that the model compounds chosen are not involved in interamide hydrogen bonds. First, the pmr spectra discussed above appear to rule out any cyclic structure resulting from intramolecular hydrogen bonds. Second, the occurrence of intermolecular hydrogen bonds should be governed by equilibrium constants comparable to that for N-methylacetamide, in which hydrogen bonding was found by Klotz and Franzen[26] to be negligible in H_2O at 25° at concentrations under 1 M (as used here at 12°).

The similarity in rate constants in Table II therefore indicates that the poly-β-alanine samples are also largely non-hydrogen-bonded, and most likely nonhelical. The deuterium exchange and pmr evidence is thus mutually consistent. Since the conclusions from pmr apply to PBA-S6 but not necessarily to the highly branched PBA-S1, we leave open the possibility that the latter may contain a portion of highly motile, nonexchanging

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(22) J. Wyman, Jr., *Chem. Rev.*, **19**, 213 (1936).

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secondary structure, whose effect would be merely to reduce the measured rate constants in proportion to the amount of such structure present.[27] The data in Table II do not show any clear-cut reduction for PBA-S1, but they also are not precise enough to rule out the presence of such a motile structure or other small steric effects such as might arise from branching. It is likewise possible that a small portion of permanently slowly exchanging NH groups (not exceeding 14%) escaped detection due to the fact that the kinetics could not be followed with sufficient accuracy to more than 86% completion in the PBA experiments.

Ultraviolet Spectra. The extinction coefficient for the amide $\pi-\pi^*$ transition in PBA-I (Table III) does not show the marked hypochromism apparent in helical poly-α-amino acids. This rules out the possibility of any geometrical arrangement of amide groups approximating that in the α helix, while it permits either a random structure or an amide arrangement comparable to that in β structures. This is the only conformational evidence we have obtained on a high molecular weight, unbranched sample in aqueous solution. The spectrum of the branched sample PBA-S1 is slightly different due to the presence of primary and tertiary amides, and is correspondingly less informative regarding the status of the secondary amides.

Thermodynamic Considerations. The apparent instability of an ordered PBA structure in water may be rationalized by comparison with poly-α-L-alanine, $(NHCHCH_3CO)_n$. Recent studies[28,29] on the latter polymer in water have given the following thermodynamic parameters for helix formation: $\Delta H^\circ \simeq -190$ cal/residue-mol, $\Delta S^\circ \simeq -0.55$ cal deg^{-1} residue-mol^{-1}, and $\Delta G^\circ \simeq -30$ cal/residue-mol at 25°. We feel justified in drawing a rough analogy between PBA and poly-α-L-alanine because our molecular model studies show possible PBA helices in which the geometries (and probably the interactions) are rather similar to those in the α helix. A major additional factor in the PBA case is the internal rotation about the C^α–C^β bond. If we assume that this bond has three equally probable conformations in the random chain (as the pmr evidence indicates) and only one in a helix, then there is a contribution to ΔS° for helix formation in the amount $-R \ln 3 = -2.2$ cal deg^{-1} residue-mol^{-1}. Assuming that the remaining contributions to ΔS° and ΔH° are the same as for poly-α-L-alanine, we find $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -190 + 298(0.55 + 2.2) = +630$ cal/residue-mol for PBA at 25°. A PBA helix would then be quite unstable. This estimate is very rough, but it does show the importance of the entropic effect of one additional single bond in the residue backbone. One might also consider that an energetically unfavorable conformation about the C^α–C^β bond in a helix would act to destabilize it. The 3.4 residue/turn poly-β-amino acid helix mentioned elsewhere[4] places the C^α–C^β bond in a precisely staggered gauche conformation, and in this case the energy contribution from this source would not be significant. The above calculation, while rather oversimplified, provides a convenient rationale for our experimental conclusions.

Acknowledgments. We are indebted to Dr. W. D. Phillips at E. I. du Pont de Nemours & Co., Inc., for helpful consultation on the pmr data and for making available a 220-MHz nmr spectrometer, to Dr. V. Damle for writing a computer program used in the analysis of deuterium exchange data, to Dr. R. W. King for inspiration and advice during the pmr studies, and to Dr. Joan Field for her early exploratory work on deuterium exchange in PBA. This investigation was supported by a research grant (GM-13684) and a predoctoral fellowship (GM-21103) from the National Institute of General Medical Sciences, U. S. Public Health Service.

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Communications to the Editor

Lanthanide-Induced Shifts in Proton Nuclear Magnetic Resonance Spectra. I. Europium-Induced Shifts to Higher Fields

Sir:

Much of the work reported in the literature[1] using lanthanide shift reagents (LSR's) to induce large pseudocontact shifts in proton nmr spectra has employed tris(dipivalomethanato)europium(III) [Eu(DPM)$_3$]. Other lanthanides, notably praseodymium, and other organic ligands have also been investigated.[1c] The proton nmr shifts induced by these LSR's have been qualitatively discussed, and sometimes at least semiquantitatively rationalized, in terms of a two-parameter form of the pseudocontact shift equation,[2] conveniently expressed as

$$\Delta\delta_i = \delta_{i,[\mathrm{Eu}\neq 0]} - \delta_{i,[\mathrm{Eu}=0]} = k(3\cos^2\theta_i - 1)(1/R_i^3) \qquad (1)$$

where $\Delta\delta_i$ is the (incremental) shift in parts per million of proton i, δ_i is the chemical shift of proton i (in parts per million from internal TMS), k represents a collection of constants, θ_i is the angle describing the position of proton i relative to the assumed effective symmetry axis of the europium complex, and R_i is taken as the proton–europium distance.

Hitherto reported *europium lanthanide induced shifts* (*LIS's*) have all been *to lower applied fields* (higher

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(2) (a) H. M. McConnell and R. E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1958); (b) G. N. La Mar, *ibid.*, **43**, 1085 (1965).