this has been done for a series of block copolymers with γ-benzyl L-glutamate.[30]

The predicted[11] helix sense of poly(β-benzyl L-aspartate) [as with that of other esters of poly(L-aspartic acid)] rests on a small free energy difference between two conformations having opposite helix sense and specified side-chain conformation. For the four low-energy conformations considered[11] (two RH, two LH), the conformational dependence of the side-chain energy terms (torsional, nonbonded, and electrostatic) is much in excess of the final energy difference between the total energy of the lowest energy RH and lowest energy LH form, *i.e.*, the predicted helix sense is critically dependent on the assumed side-chain conformations. The present results indicate considerable motion in the β-CH_2 group in chloroform solution, and this does not seem compatible with the degree of side-chain immobilization envisaged in the free energy calculations of ref 11. If the side chain possessed a dominant conformation, this would have been readily apparent from the nmr spectra. The helix sense of poly(aspartate esters) is clearly dependent on the statistical sum of a wide range of side-chain conformations.

(30) L. Paolillo, P. Temussi, E. Trivellone, E. M. Bradbury, and C. Crane-Robinson, *Biopolymers*, in press.

Acknowledgments. Our work is supported by the Science Research Council; one of us, H. G., is grateful to the Polytechnic governors for a research studentship. We thank Varian Associates for the opportunity to use the 300-MHz spectrometer.

Conformational Aspects of Polypeptide Structure. XXXIV. Amino Acid Substituted Poly-L-lysines

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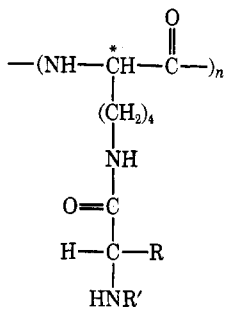
ABSTRACT: An investigation was carried out on several poly-L-lysines containing amino acid substituents on the N^{ϵ} position. The conformations of these polypeptides were examined using circular dichroism and 220-MHz nuclear magnetic resonance techniques in both aqueous and organic media. The results suggest that amino acid side chains can have important influences on the backbone conformation of a polypeptide. Specifically, we observed that whereas poly-L-lysine hydrobromide and poly(N^{ϵ}-glycyl-L-lysine hydrobromide) exist in a "random coil" conformation in distilled water, both poly(N^{ϵ}-L-phenylalanyl-L-lysine hydrobromide) and poly(N^{ϵ}-L-leucyl-L-lysine hydrobromide) are α helical.

The use of poly(α-amino acids) as models for proteins has yielded significant but often simplified information on the various factors affecting conformational stability. In an attempt to gain more detailed information concerning the stereochemical influence of peptide branches in proteins and cell membranes, we have undertaken a conformational investigation of a series of N^{ϵ}-substituted polylysines of the following form.



R = H, $CH_2C_6H_5$, $CH_2CH(CH_3)_2$

R′ = H(HBr), $COOCH_2C_6H_5$

Thus, derivatives of polylysines containing glycine, phenylalanine, and leucine residues on the N^{ϵ} position were examined. The class of compounds having R′ = Z will be referred to as "blocked" polymers, whereas when R′ = H(HBr) the compounds are usually called salts. In this paper we discuss the conformational analysis of these polypeptides using circular dichroism (CD) and 220-MHz nuclear magnetic resonance (nmr) techniques.

Experimental Section

(a) Preparation of Materials. Poly(L-lysine hydrobromide). Poly(L-lysine hydrobromide) was prepared according to the method of Katchalski.[2] The benzyloxycarbonyl protecting group was removed using hydrogen bromide in trifluoroacetic acid.

Poly(N^{ϵ}-*N*-benzyloxycarbonyl-glycyl-L-lysine) (I). Poly(L-lysine hydrobromide) (627 mg, 3 mmol) was dissolved in 4.5 ml of water and triethylamine (0.33 g, 3.3 mmol) was added. The resulting solution was stirred at 5–10° and a solution of *p*-nitrophenyl *N*-benzyloxycarbonylglycinate (1.38 g, 4.5 mmol) in 10 ml of dioxane was added dropwise. An additional 110 ml of dioxane was added in small portions, and the reaction was allowed to stir at room temperature for 40 hr. The resulting mixture was concentrated *in vacuo*. The residue so obtained was washed with ether, dried, and finally washed with water. After drying *in vacuo* over phosphorus pentoxide, a white amorphous powder (0.85 g, 89%) was obtained. The intrinsic viscosity determined in dichloroacetic acid at 25° was 0.19. This corresponds to a molecular weight of approximately 20,000.

Poly(N^{ϵ}-glycyl-L-lysine hydrobromide) (II). The blocked polymer (300 mg) was dissolved in 5 ml of a 45% solution of hydrogen bromide in acetic acid and allowed to stand at 20° for 30 min. Addition of a large excess of ether resulted in the precipitation of the

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(1) (a) Central Drug Research Institute; (b) Polytechnic Institute.

(2) E. Katchalski, *Methods Enzymol.*, 3, 540 (1957).

TABLE I
ANALYTICAL DATA ON SUBSTITUTED POLYLYSINES

Compound	Calculated, % C	H	N	Found, % C	H	N
Poly(Z-Gly-lysine)	60.18	6.63	13.16	59.87	7.00	12.84
Poly(Z-Phe-lysine)	67.45	6.65	10.26	66.97	7.11	9.93
Poly(Z-Leu-lysine)	63.97	7.78	11.19	63.54	7.71	11.47

Amino Acid Analysis[a]

Sample	Amino acids	Concn, μmol/cm³	Rel ratio
Poly(Gly-lysine HBr)	Lysine	1.041	1.00:1.15
	Glycine	1.373	
Poly(Leu-lysine HBr)	Lysine	0.2513	1.00:1.00
	Leucine	0.2504	
Poly(Phe-lysine HBr)	Lysine	0.1456	1.00:1.07
	Phenylalanine	0.1564	

[a] We wish to thank Dr. Arthur M. Felix of Hoffman-LaRoche Inc., Nutley, N. J., for performing these analyses. Each sample was dissolved in 0.01 *M* HCl to a total volume of 25.0 cm³. The sample was applied to the short column (for basic amino acids) and the long column (neutral and acidic amino acids) (1.00 cm³ for each analysis required). The standard citrate buffers were used (*i.e.*, short column, pH 5.28; long column, pH 3.25, with buffer change to pH 4.24 after 134 min). A standard containing 0.1 μmol/cm³ of each amino acid was used just prior to the samples. The quantitative calculations were made relative to that standard. We believe that the amino acid analyses attest to the complete substitution of the polylysine. We attribute the high value of the glycine in the poly(Gly-lysine HBr) sample to incomplete hydrolysis of the polymer before the amino acid analysis.

hydrobromide. The precipitated powder was thoroughly washed with ether and dried over phosphorus pentoxide and potassium hydroxide pellets *in vacuo*. The product was dialyzed against distilled water for 24 hr and lyophilized. The polymer was precipitated by addition of ethanol. The resulting hydrobromide (200 mg, 80%) was a yellow-white powder.

Poly(N^{ϵ}-*N*-benzyloxycarbonyl-L-leucyl-L-lysine) (III). Poly(N^{ϵ}-*N*-benzyloxycarbonyl-L-leucyl-L-lysine) (III) was prepared from poly-L-lysine and *p*-nitrophenyl *N*-benzyloxycarbonyl-L-leucinate using essentially the same procedure described for poly(N^{ϵ}-*N*-benzyloxycarbonyl-glycyl-L-lysine) (I). In view of the high solubility of poly(N^{ϵ}-*N*-benzyloxycarbonyl-L-leucyl-L-lysine) in ether, the crude product was washed with benzene to remove *p*-nitrophenol, followed by a minimal amount of cold ether. The final product was recovered in 88% yield.

Poly(N^{ϵ}-L-leucyl-L-lysine hydrobromide) (IV). Compound IV was prepared by treating poly(N^{ϵ}-*N*-benzyloxycarbonyl-L-leucyl-L-lysine) (III) with hydrogen bromide in acetic acid. The product was worked up using the procedure described in the preparation of poly(N^{ϵ}-glycyl-L-lysine hydrobromide) (II), yield 54%.

Poly(N^{ϵ}-*N*-benzyloxycarbonyl-L-phenylalanyl-L-lysine) (V). Poly(N^{ϵ}-*N*-benzyloxycarbonyl-L-phenylalanyl-L-lysine) (V) was prepared in 86% yield from poly-L-lysine and *p*-nitrophenol *N*-benzyloxy-L-phenylalaninate as described for poly(N^{ϵ}-*N*-benzyloxycarbonyl-glycyl-L-lysine).

Poly(N^{ϵ}-L-phenylalanyl-L-lysine hydrobromide) (VI). Compound VI was prepared from poly(N^{ϵ}-*N*-benzyloxycarbonyl-L-phenylalanyl-L-lysine) (V) in 66% yield using the procedure described to prepare poly(N^{ϵ}-glycyl-L-lysine hydrobromide) (II).

The solvents used in the spectroscopic investigations, hexafluoro-2-propanol (Columbia Organic), dimethyl-d_6 sulfoxide (Diaprep), and deuterium oxide (Diaprep) were of the highest purity available. These solvents were all used without further purification.

(b) Apparatus and Measurements. Circular Dichroism Studies. Circular dichroism studies were carried out using a Cary 60 spectropolarimeter modified with a Model 6001 circular dichroism attachment. The experimental solutions were prepared by weighing the desired sample into a volumetric flask and adding the solvent. The spectra were obtained using 0.1- and 0.5-mm path length cells. Dry prepurified nitrogen was employed to purge the instrument before and during the experiments. All spectra were recorded at ambient temperature.

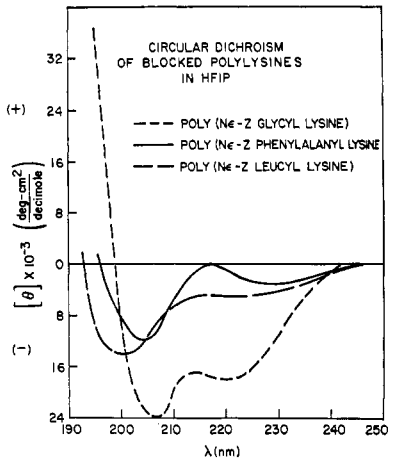

Figure 1. CD of blocked polylysines in hexafluoro-2-propanol.

Nuclear Magnetic Resonance Studies. Spectra were recorded on a Varian Associates HR-220 instrument. The samples were dissolved in either DMSO-d_6 or D_2O to a concentration of approximately 2% (w/v). Tetramethylsilane was used as the internal standard in DMSO, whereas sodium 2,2-dimethyl-2-silopentane-5-sulfonate (DSS) was employed in water. All solutions were prepared immediately prior to recording the spectra. All spectra were recorded at ambient temperature.

Results and Discussion

Poly-L-lysine[3-7] has been found to exist in β, disordered, and helical conformations depending on solvent, temperature, and pH. In addition, comparative studies[8,9] on poly(L-α,γ-diaminobutyric acid), poly-L-ornithine, and poly-L-lysine have shown that the side chain has an important influence on the backbone conformation. At present, however, no information is available on the effect of amino acid side chains on the polylysine backbone. Secondary associations of amino acid branches and side chains may have an important influence on the stereochemistry of proteins and membranes. In order to gain information about such effects, we undertook a conformational analysis of several N^{ϵ} amino acid substituted polylysines.

We first examined the CD of the various blocked polymers in hexafluoro-2-propanol. The results of this study are presented in Figure 1 and Table II.[10] As can be seen, although there are definite differences, these polymers all exhibit spectral parameters similar to those observed for helical polypeptides. Poly(N^{ϵ}-*N*-Z-glycyl-L-lysine) is the most helical in this medium. Comparison of its spectral patterns with those

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(7) B. Davidson and G. D. Fasman, *Biochemistry*, **6**, 1616 (1967).
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TABLE II
COMPARISON OF CIRCULAR DICHROISM PARAMETERS OF VARIOUS POLY-L-LYSINES IN ORGANIC SOLVENTS

Compound	$[\theta]$ of $\pi \rightarrow \pi^*$	$[\theta]$ of $n \rightarrow \pi^*$	Position of crossover, nm	$\frac{[\theta]_{n\rightarrow\pi^*}}{[\theta]_{\pi\rightarrow\pi^*}}$
Poly(Z-Gly-lysine)	−24,000 (207 nm)	−18,300 (220 nm)	199	0.75
Poly(Z-Phe-lysine)	−12,000 (205 nm)	−3,000 (227 nm)	196	0.25
Poly(Z-Leu-lysine)	−15,000 (200 nm)	−5,200 (222 nm)	193	0.33
Poly(Z-lysine)[a]	−43,000 (208 nm)	−34,800 (220 nm)	~201	0.81
Poly(Z-lysine)[b]	−39,000 (209 nm)	−20,300 (223 nm)	200–201	0.73

[a] In TFE taken from ref 11. [b] In HFIP taken from ref 10.

TABLE III
COMPARISON OF CIRCULAR DICHROISM PARAMETERS FOR SEVERAL WATER-SOLUBLE POLY-L-LYSINES

Compound	Position, nm			Ellipticity		
	$\pi \rightarrow \pi^*$	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	$\pi \rightarrow \pi^*$	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$
Helical polylysine[a]	190.5	207	221	+71,500	−38,900	−39,800
Helical polylysine[a]		207	222	+50,000	−33,000	−32,000
Poly(L-lysine HBr)		190	220	−33,000		+4,000
Poly(Gly-lysine HBr)		192	217	−26,000		+2,000
Poly(Phe-lysine HBr)	195	207	225	+55,000	−7,000	−18,000
Poly(Leu-lysine HBr)	190	208	222	+60,000	−22,000	−26,000

[a] Taken from ref 4.

or poly(N^{ϵ}-Z-L-lysine)[11] in TFE shows that the positions and relative intensity of the Cotton effects associated with both the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions are very similar. The differences in absolute values for the molar ellipticities could be due to specific interactions between main-chain and side-chain amide chromophores which result in a lower rotational strength. In any event our results suggest that the three polylysine derivatives investigated are all partially helical. Absorption by the aromatic side chain of the phenylalanyl analog in the amide chromophore region makes it difficult to comment about this polymer. The position and intensity of the CD parameters of poly(N^{ϵ}-*N*-Z-leucyl-L-lysine), however, suggest that it is less helical than the glycyl analog. We believe that the reduced helicity of this compound and probably of the phenylalanyl derivative may be explained in terms of the steric interactions among the bulky side chains of these polymers. Such interactions would be much more prominent in the phenylalanyl and leucyl polylysines than in the corresponding glycyl compound and might be expected to result in less stable helical structures. The blocked polymers were also examined using nmr. Results of this investigation will be discussed later in this paper.

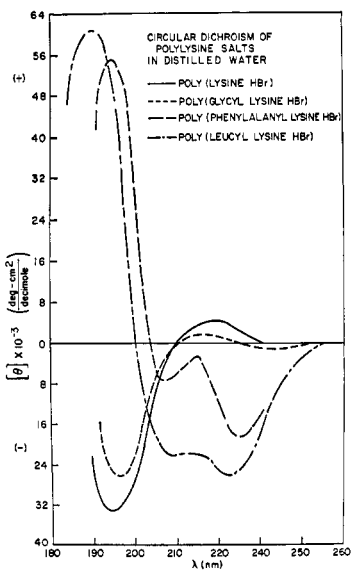

Figure 2. CD of polylysine salts in distilled water.

After completing our CD examination of the blocked polypeptides, we carried out a similar conformational analysis on the corresponding hydrobromide salts. Since these latter salts are water soluble, we hoped that we would gain information which would be more applicable to biologically active systems. The results of CD studies on these salts in distilled water are presented in Figure 2. The spectral patterns indicate that there are conformational differences among the four polymers investigated. Poly(L-lysine hydrobromide) and poly(N^{ϵ}-glycyl-L-lysine hydrobromide) exhibit spectra which are typical of polypeptides in a disordered conformation. In sharp contrast, poly(N^{ϵ}-L-phenylalanyl-L-lysine hydrobromide) and especially poly(*N*-L-leucyl-L-lysine hydrobromide) display spectral patterns which resemble those of known helical polypeptides. Studies using model systems demonstrated that the differences between the former and latter species cannot be attributed to the optical activity of the leucine and phenylalanine side chains. In fact, one of the reasons for the odd shape of the spectral patterns of the phenylalanyl-substituted polylysine is that the aromatic amino acid residue gives a positive contribution to the CD in the 210–230-nm region. A comparison of the spectral parameters of our polymers and those reported for helical poly-L-lysine is presented in Table II. It should be noted that the apparent pH of all of these polymers was measured prior to and subsequent to the CD studies. In all cases this apparent pH was between 5 and 6. In fact, of the four polymers examined the unsubstituted poly-L-lysine hydrobromide was the least acidic. Nevertheless, it is in a "random coil" conformation in solution. We conclude that the differences in conformation cannot be attributed to differences in side-chain ionization.

(11) M. Terbojevich, M. Acampora, A. Cosani, E. Peggion, and E. Scoffone, *Macromolecules*, 3, 618 (1970).

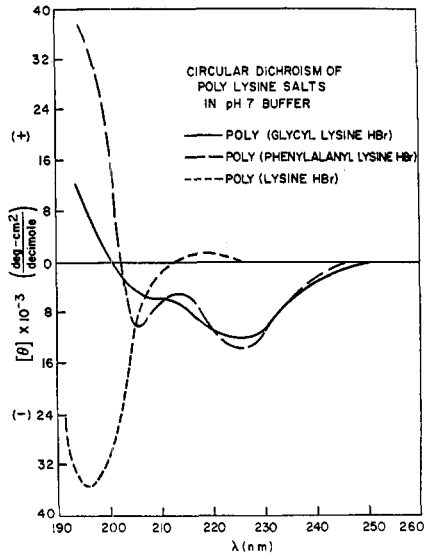

Figure 3. CD of polylysine salts in pH 7 buffer.

The CD of several of these polymers was also studied in an aqueous solution buffered at pH 7 (Table III). Under these conditions poly(L-lysine hydrobromide) is still completely soluble. Both derivatives examined, however, were not completely soluble and resulted in turbid solutions. The results of these CD experiments appear in Figure 3. They suggest that whereas the unsubstituted polymer remains random, the glycyl derivative has undergone a conformational change to a different structure in solution. The spectral pattern of poly(N^{ϵ}-glycyl-L-lysine hydrobromide) is not typical of an α helix. It is known, however, that poly-L-lysine undergoes a temperature-induced helix $\rightarrow$ β transition at pH 11. It is possible that such a transition occurs at pH 7 for the glycyl-substituted polylysine. Thus, the spectral patterns of poly(N^{ϵ}-glycyl-L-lysine hydrobromide) could be due to a mixture of β and helical species. The presence of associated molecules would also explain the low solubility exhibited by this polypeptide.

We found that the addition of potassium chloride caused the turbid suspension of poly(N^{ϵ}-glycyl-L-lysine hydrobromide) in pH 7 buffer to clear up immediately. Figure 4 presents CD spectral patterns of this polypeptide at different pH's and salt concentrations. We observed that the addition of 0.2 *M* KCl to a solution of this glycyl derivative caused a blue shift of the $\pi \rightarrow \pi^*$ minimum and the crossover position. It should be realized that in its ionized form poly(N^{ϵ}-glycyl-L-lysine hydrobromide) behaves as a polyelectrolyte, and

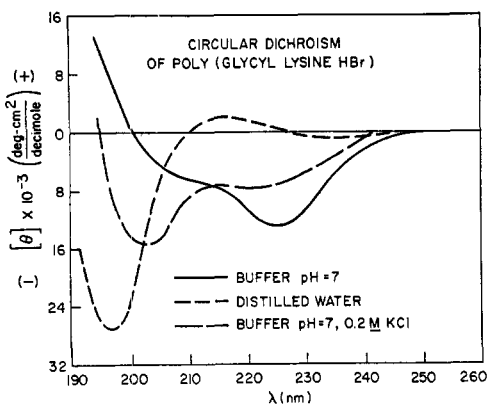

Figure 4. CD of poly(N^{ϵ}-glycyl-L-lysine hydrobromide).

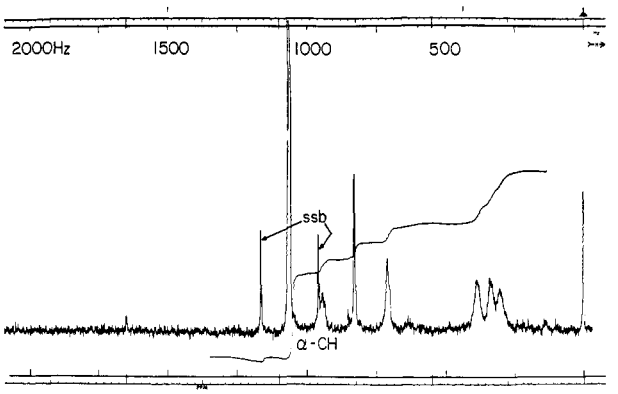

Figure 5. Nmr (220 MHz) of poly(N^{ϵ}-glycyl-L-lysine hydrobromide) in D_2O

electrostatic repulsion between charged ammonium groups is probably responsible for disruption of helical conformations. In light of this, the addition of simple salts is expected to shield the charges and thus stabilize the more compact helix. Comparison of the spectral patterns obtained in distilled water with those found in pH 7 and 0.2 *M* KCl solution indicates that the glycyl-substituted polymer is more helical in the latter medium. In fact the addition of 1.0 *M* KCl to a solution of poly(N^{ϵ}-glycyl-L-lysine hydrobromide) in distilled water also seemed to result in a conformational transition. (It should be noted that at this high salt concentration it was not possible to observe the spectral region of the $\pi \rightarrow \pi^*$ chromophore. Enhancement of the n $\rightarrow$ π^* transition, however, was observed, thus giving evidence for a structural change.) It is difficult, however, to explain the position and intensity of the Cotton effects for the glycyl derivative at pH 7 both in the presence and absence of added salt. At present, we can only conclude that added salt causes a significant change in the CD patterns and that poly(N^{ϵ}-glycyl-L-lysine hydrobromide) undergoes a conformational transition somewhere between pH $\simeq$5 and 7.

We attempted to confirm our CD results using 220-MHz nmr spectroscopy. Since all of the hydrobromide salts were very soluble in D_2O, we were able to utilize aqueous solutions for measurements in these studies. Thus, except for concentration differences, the nmr and CD investigations were completely comparable. As expected, the N–H protons of all polymers exchanged almost immediately. Therefore, the N–H region of the nmr could not be used in our analysis. The α-CH proton region, on the other hand, was observable in all cases and yielded conformationally relevant information. Figures 5 and 6 present the resonances of poly(N^{ϵ}-glycyl-L-

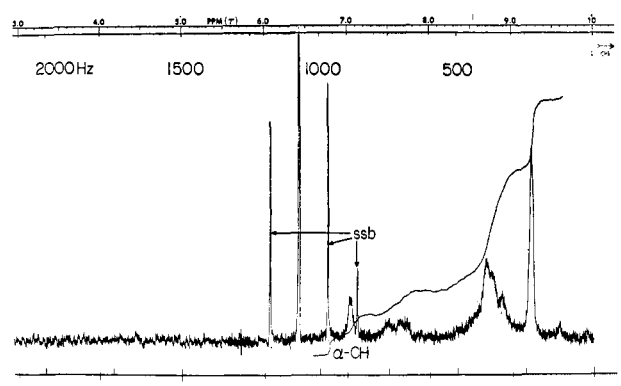

Figure 6. Nmr (220 MHz) of poly(N^{ϵ}-L-leucyl-L-lysine hydrobromide) in D_2O.

TABLE IV
NUCLEAR MAGNETIC RESONANCE PARAMETERS OF N^{ϵ}-SUBSTITUTED POLYLYSINES IN D_2O

Compound	Position from DSS, ppm						
	α-CH	Downfield $-(CH_2)-$	Other $-(CH_2)-$	C_6H_6	$C_6H_5CH_2$	$(CH_3)_2C-$	H_2O
Poly(L-lysine HBr)	4.40	3.05	1.98 1.63				4.89
Poly(glycyl-L-lysine HBr)	4.33	3.21	1.75 1.52 1.37				4.84
Poly(Phe-L-lysine HBr)	4.08	3.22	1.91 1.31	7.25	3.04		4.86
Poly(Leu-L-lysine HBr)[a]	4.04	3.18	1.75 1.54			1.05	4.89

[a] It should be noted that no DSS was used in this experiment. We believe the constancy of the position of the water resonance for the different polylysines allows this to be used as a standard.

lysine hydrobromide) and poly(N^{ϵ}-L-leucyl-L-lysine hydrobromide). Stereochemically, the most important feature of these spectra is the upfield shift of the α-CH resonance in the leucyl-substituted polymer as compared with the corresponding glycyl derivative.

Using model compounds and data obtained on the previously discussed blocked polymers, we were able to assign most of the important resonances of these polypeptides. A summary of the assignments appears in Table IV. In general, the assignments were easily made. It should be noted, however, that in the leucyl and phenylalanyl polylysines the side-chain CH_2 resonances were broadened and overlapped with each other. In such cases, peak positions were assigned using the center of the observed envelope. We also found that the backbone and side-chain α-CH resonances overlapped for poly(N^{ϵ}-L-phenylalanyl-L-lysine hydrobromide). This overlap is apparently a consequence of both the upfield shift of the backbone proton resonance on helix formation and the downfield shift of the CH proton of the phenylalanyl side chain in the presence of the aromatic ring.

The results in Table IV show that the backbone α-CH resonance is shifted approximately 0.3 ppm to higher field for both leucyl and phenylalanyl derivatives with respect to poly(L-lysine hydrobromide). The α-CH resonance for the glycyl-substituted polylysine, on the other hand, is shifted less than 0.1 ppm. These results are consistent with the CD studies and confirm that the leucyl and phenylalanyl polymers are helical, while the corresponding glycyl derivative is disordered. Previous studies on the helix–coil transition of polypeptides in mixed organic solvents[12-15] have reported similar upfield shifts on helix formation. There have not been many studies of the helix–coil transition using nmr in aqueous solution. Markley, Meadows, and Jardetzky[12] reported that the α-CH of poly(L-glutamic acid) shifts approximately 0.15 ppm to higher field on helix formation. Bradbury[16] and coworkers observed similar upfield shifts for poly(L-glutamic acid) and poly-L-lysine while reporting that a helix-forming copolypeptide showed virtually no shift in α-CH position. It should be noted that both of these investigations were carried out on 60-MHz instruments and that the peaks were fairly broadened. In addition, the transitions from coil to helix were caused by changing the pH of the medium. More recently, Joubert, Lotan, and Scheraga[17] studied the high-resolution nmr (100 MHz) of several poly(N^5-(ω-hydroxyalkyl)-L-glutamines) in aqueous solution. They reported that the α-CH proton resonance of the helix-forming peptides shifted upfield by 0.16 ppm during the coil–helix transition. In our study, the α-CH resonances were well resolved and fairly easy to assign. All polymers studied were also at approximately the same pH. In light of the many different factors between ours and the previously reported studies, not the least of which is the presence of various amino acid side chains, we believe that the larger upfield shift that we found is quite reasonable. We conclude that the nmr studies support our previous CD results on the hydrobromide salts.

We also attempted to confirm our CD results on the blocked polylysines using 220-MHz nmr spectroscopy. Since HFIP has a strong absorbance in the α-CH region of the spectrum we conducted our investigation in dimethyl-d_6 sulfoxide. The results found for poly(N^{ϵ}-N-Z-glycyl-L-lysine) are presented in Figure 7. Using model compounds we were able to assign all peaks except those for the backbone α-CH and N-H protons. Later, integration of these spectra indicated that the α-CH resonance is very broad in this solvent and appears between 4.1 and 3.7 ppm. Because of this broadness an exact assignment of position is not possible. Since the broadening of the backbone N-H resonance is generally greater than that of the α-CH resonance, we do not believe

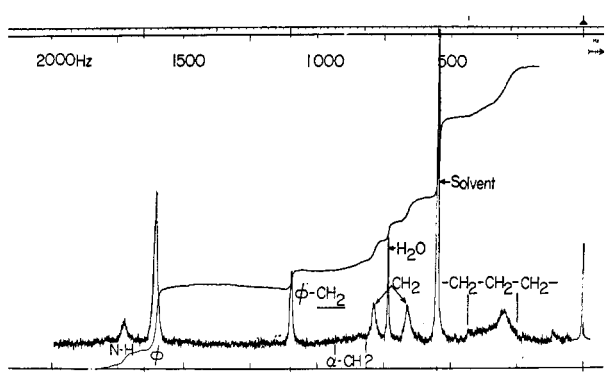

Figure 7. Nmr (220 MHz) of poly(N^{ϵ}-N-Z-glycyl-L-lysine) in DMSO-d_6.

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the absorption at 7.83 ppm can be assigned to this proton. Rather, we feel that it is more likely attributable to either the N^{ϵ} NH or the carbobenzoxy N–H protons. Since it was not possible to assign the peak positions of the backbone resonances with any confidence, the nmr studies could not be used to differentiate between the relative helicity of the various blocked polymers. They were useful in assigning peak positions which were applied in the analysis of the corresponding hydrobromide salts.

Broadening of backbone protons can arise from any structural feature which will inhibit rotational motion of the molecule. Certainly helicity causes such an effect. We therefore use our nmr results as supporting evidence for the helicity in the glycyl and other blocked polylysines. However, the primary support for our contention is based on our CD results.

The conformational analysis on the free poly-L-lysine compounds suggests that amino acid branches can have an important influence on the backbone conformation of proteins. In our simple model systems, we observed that significant stereochemical differences existed among the various polylysines.

The effect of moving the charge on a polyelectrolyte away from the backbone is to lower the excess electrostatic repulsive energy, thus stabilizing the less expanded conformation. Since it is such electrostatic repulsion which is mainly responsible for destabilizing the helical form of poly-L-lysine, this effect is primarily responsible for the differences between the substituted and unsubstituted polylysines. It was also observed, however, that significant conformational differences exist between poly(N^{ϵ}-glycyl-L-lysine) on one hand and the phenylalanyl and leucyl analogs on the other. These differences indicate that the effect of these substituents is not solely to remove the charge from the peptide backbone. The stereochemical variations among the substituted polylysines could be due to changes in solvation because of the nature of the side chain or interactions between side chains which stabilize less expanded forms. These are especially prevalent when the solvent is polar and the side chains are hydrocarbon-like in nature. The hydrocarbon nature of the phenylalanyl and leucyl derivatives is expected to have a much different effect on the solvation of the peptide than that of the glycyl or unsubstituted polylysines. It is also known that the presence of aliphatic and aromatic groupings can affect the local dielectric constant in an aquous solution. The effect of such groups is to lower the dielectric constant in their immediate environment. According to Coulomb's law, such a decrease in dielectric constant would be expected to increase the interaction between neighboring charges, thereby leading to a more expanded conformation. We observe, however, helical structures for the leucyl- and phenylalanyl-substituted polylysines. The existence of these compact species suggests that the hydrophobic side chains may be attracting each other in these aqueous solutions. Such interactions are apparently sufficient to overcome any increase in the electrostatic repulsion and thus result in the stabilization of helical species.

Finally, it should be noted that interactions between bulky hydrocarbon side chains can result in opposite effects in organic and aqueous media. In the latter, "hydrophobic bonding" of these groups prevents the expansion of the polymer and stabilizes helical species. In the former, however, steric interactions between these same groupings can result in destabilization of the helical conformation.

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Unperturbed Dimensions of Some 1–4-Linked Homopolysaccharides

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ABSTRACT: The characteristic ratios of a number of 1–4-linked homopolysaccharides are calculated using the methods developed by Flory and coworkers. It appears that the characteristic ratio is related to the freedom of rotation about the glycosidic bonds but also to the type of bonding (*e.g.*, axial–axial, equatorial–axial, etc.) between the monomers.

In recent years a number of calculations have been made of the characteristic ratios of polysaccharides such as amylose,[1] cellulose,[2] and alginic acid.[3] However, no serious attempt has been made to investigate the characteristic ratios of a series of such polysaccharides in order to draw general conclusions on the influence of types of bonding or positions of substituents on the characteristic ratios. In this paper characteristic ratios are calculated for a number of 1–4-linked polysaccharides with the sugar rings in the pyranose form. In practice, the details of the ring geometry and the bridge parameters will vary with the different polymers, and it has been shown[2] that the calculated value of the characteristic ratio is quite sensitive to the assumed bridge parameters. However, at least the general qualitative findings of this paper should not be influenced by small changes in these parameters or in the potential function and, at this stage, it appears to

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