In the first place we investigated some well-known polypeptides. Measurements were carried out on an automatic recording spectropolarimeter which was constructed on the basis of a Jasco ORD-UV-5 spectropolarimeter. The spectral range was 0.5–2.3 μm, the maximum accuracy, 2×10^{-4} deg. The results for polypeptides are shown in Table I. It should be noted that in all cases the random form (coil) gave a value of $C_0 = 0$. In comparison with the α helix, the β form had an essentially greater value.

At present there are no methods permitting to evaluate the β-form content in polypeptides and therefore we had no possibility of obtaining the $C_0(\beta)$ value for the pure structural form. We measured C_0 for several globular proteins with a known structure. The C_0 value proved to correlate with the β-form content. Recalculating $C_0(\beta)$ to $C_0(100\%\ \beta)$ we obtained the same value $C_0 = -8.0$ (deg cm^2) dmol^{-1} for all the three investigated proteins (Table II). Thus the approximated values of C_0 for the different forms will be

Form	Random	α Helix	β Form
C_0 ((deg cm^2) dmol^{-1})	0	−1.0	−8.0

The selectivity of C_0 for the β form allows to identify it in proteins with very high precision. Thus, in lysozyme only twelve amino acid residues are in the β form and, as seen in Table II, C_0 is determined with an error of 25%, *i.e.*, the β conformation of three to five residues in a molecule consisting of 100–200 amino acid residues can be detected.

The practical application of the revealed effect is evident, and the theoretical explanation should be expected in the near future.

Optical Properties of Poly(L-azetidinecarboxylic acid) in Solution†

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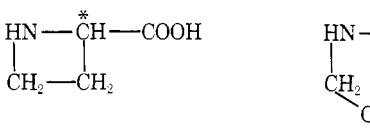
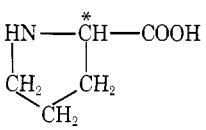
ABSTRACT: The effect of the rigidity of the four-membered ring of L-azetidinecarboxylic acid residue on the conformation of a new polymer structurally related to poly(L-proline) has been studied. As in the case of poly(L-proline), uv and CD results point to the existence of two different conformations in solution.

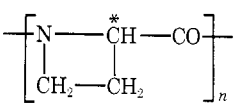
The replacement of L-proline with L-azetidine-2-carboxylic acid (L-Aze-COOH) in polypeptide chains of plant proteins and collagens results in drastic alterations of structures and biological functions, in spite of the close chemical similarity between the imino acids.[1-4] The "toxicity" of L-Aze-COOH has been attributed to the structural difference between its rigid four-membered ring and the more flexible pyrrolidine ring of proline, which would significantly affect the secondary and tertiary structure of polypeptide chains.[5] To elucidate these effects, the synthesis of a homopolymer of the following structure



azetidine-2-carboxylic acid



proline



has been recently accomplished.[6]

The usual *N*-carboxyanhydride procedure was not followed because the *N*-chloroformyl-L-azetidine-2-carboxylic acid intermediate could not be cyclized.[7,8] Poly(L-azetidinecarboxylic acid) (PLAze) was conveniently prepared by the polymeric self-condensation of an active ester (*i.e.*, the pentachlorophenyl ester of the imino acid), through the sequence shown in Scheme I.

The polymer was purified and fractionated by elution on Sephadex (G-50 fine). All spectroscopic measurements were performed using a fraction having a weight-average molecular weight, $\bar{M}_w$, of 7600 (~90 residues), determined by the Yphantis midpoint method.[6]

We report in this communication the uv adsorption and circular dichroism results of a preliminary study of PLAze conformation in solution.

The uv and CD spectra (Figures 1–3) of PLAze in water were recorded immediately after sample dissolution and registered repeatedly at regular time intervals. No time dependence was observed, excluding further conformational change. In the ethanol–water mixture (the polymer is insoluble in pure ethanol and, generally, in simple aliphatic alcohols), the spectra were recorded at the equilibrium conditions, *i.e.*, the changes of the circular dichroism at 219 nm were less than the uncertainties in the measurement. The similarity with the corresponding poly(L-proline) spectra, particularly in the curves recorded in the ethanol–water mixture, suggests that the conformation of PLAze in this medium resembles that of poly(L-proline I).[9-11]

† This paper is one of a group which was presented at the 10th Prague IUPAC Microsymposium on Macromolecules, August 28–31, 1972.

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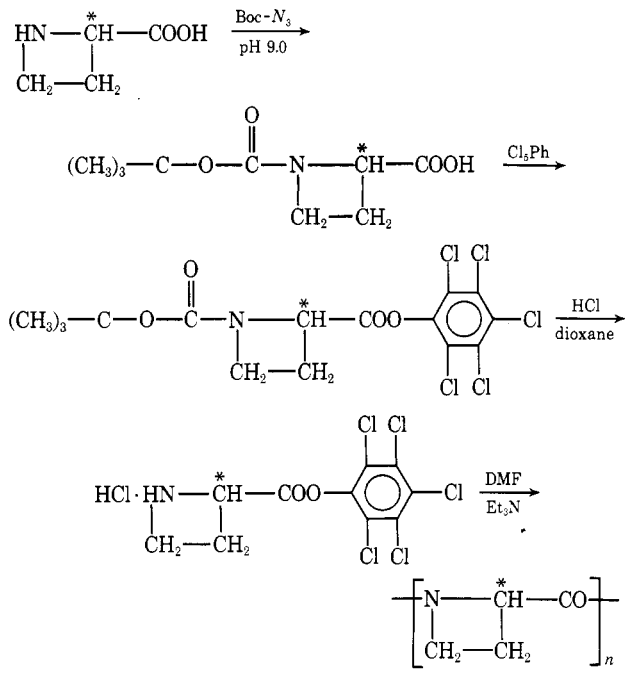
Scheme I[a]



[a] Cl_5Ph = pentachlorophenol.

Table I

Polymer	Band Position (nm)	$[\theta] \times 10^{-3}$ (deg cm^2) dmol^{-1}
Poly(L-proline) in trifluoroethanol[a,b]	232[a,b]	−5,900,[a] ~ −2,500[b]
	215[a,b]	86,000,[a] ~70,000[b]
	197[a,b]	−43,000,[a] ~25,000
Poly(L-proline) in 1-propanol[c]	236	−4,000
	214	58,000
	200	−29,500
Poly(L-proline) in water–1-propanol[b] (1:9)	232	~−5,500
	214	~50,000
	197	~−24,000
Poly(L-azetidine-carboxylic acid) in water–ethanol[d] (1:99)	219	77,000
	194	−112,000
Poly(L-proline I)[e] (calculated)	215	110,000
	199	−140,000
Poly(L-proline I)[f] (calculated)	219	135,000
	199	−130,000

[a] See ref 9. [b] See ref 11. [c] See ref 10. [d] This work. [e] See ref 12. [f] See ref 13.

In water, trifluoroethanol, and other fluorinated alcohols, the CD spectrum for the polymer does not even have a qualitative resemblance either to observed or calculated CD curve for poly(L-proline II).[9-13]

On the other hand, the results summarized in Figure 4, demonstrate that a reversible cooperative transformation between two stable conformations of the polymer can be induced by varying the relative percentage in the solvent mixture water–C_2H_5OH. Hence, we call PLAze II the conformation stable in water and fluorinated alcohols, supposing that the chain structure in these solvents is roughly similar to that of form II of poly(L-proline) (see discussion below).

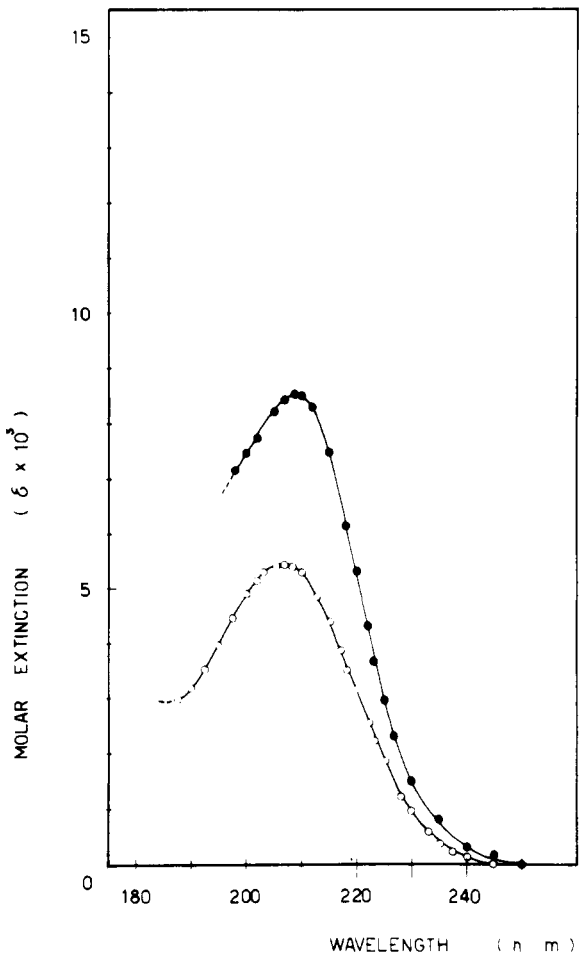
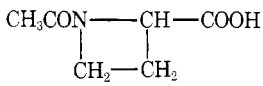


Figure 1. Ultraviolet absorption spectra of poly(L-azetidinecarboxylic acid I) in ethanol-water (●), 99:1 (v/v), and poly(L-azetidinecarboxylic acid II) in water (○).

The uv-spectra show well-defined bands centered at 206 nm in water (molar residue absorption 5500) and 209 nm in ethanol-water 99:1 (v/v) (molar residue absorption 8500), which we identify as the peptide $\pi \rightarrow \pi^*$ electronic transition. Both band maxima are displaced to the red with respect to the band maximum of the model compound



which is centered at 198 nm in aqueous solution.

The change of the solvent from ethanol-water to pure water is accompanied by a blue shift (~700 cm^{-1}) in the maximum and quite a large decrease in peak extinction coefficient. This corresponds to, but does not coincide with, the band maxima and intensity observed in the conversion of low molecular weight poly(L-proline I) to the form II in aqueous solution and in trifluoroethanol.[9,14]

As in the case of poly(L-proline), uv spectra show no clear evidence of the peptide $\pi \rightarrow \pi^*$ exciton splitting. The exciton band position for poly(L-proline II) has been calculated at 208 and 196 nm, and at 215 and 199 nm for poly(L-proline I), the parallel component being located at the longer wavelength in both cases.[12] This low-energy $\pi \rightarrow \pi^*$ exciton branch is expected to have a positive CD in both poly(proline) helices.[12]

The shape of the CD spectrum of PLAze in ethanol–

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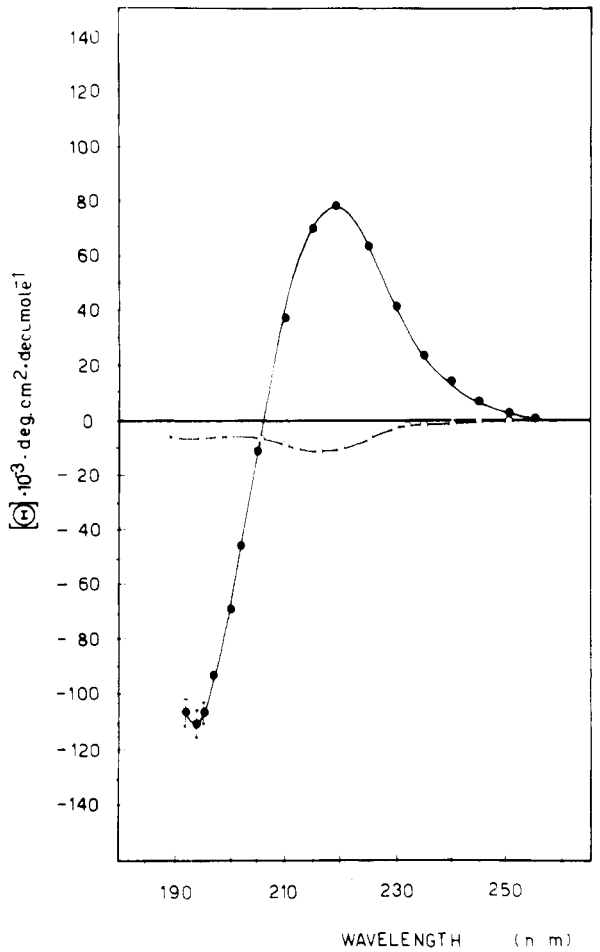

Figure 2. Circular dichroism of poly(L-azetidine-2-carboxylic acid I) in ethanol–water (●), 99:1 (v/v), and poly(L-azetidinecarboxylic acid II) in water (○).

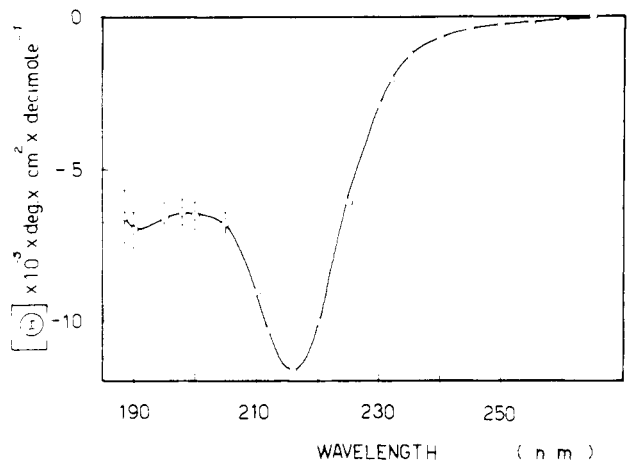

Figure 3. Circular dichroism of poly(L-azetidine-2-carboxylic acid) in water (expanded scale).

water, 99:1 (v/v), apart from the small negative band near 230 nm observable in poly(L-proline) spectra, is similar to that of poly(L-proline I) in trifluoroethanol and 1-propanol.[9,10] A strong positive absorption at 219 nm is followed by a more intense negative CD maximum centered at 194 nm. We assign the observed strong CD bands to the lobes of the exciton splitted $\pi \rightarrow \pi^*$ component polarized parallel to the helix axis, in analogy with the assignment of bands in poly(L-proline I).[12] The intensity of the observed circular dichroism is higher than that observed for poly(proline I) and is closer to that calculated for this helix.

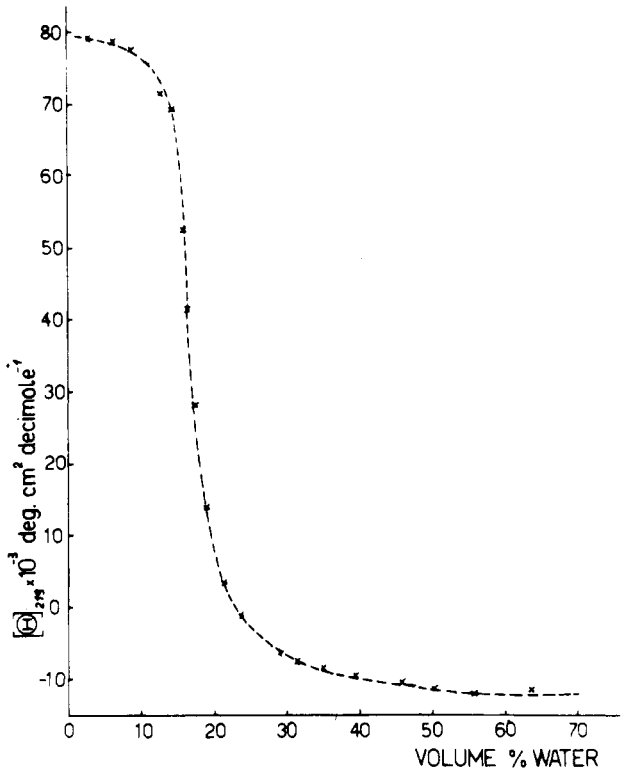

Figure 4. Solvent-induced transition for poly(L-azetidine-2-carboxylic acid). Ellipticity at 219 nm has been measured after equilibrium between the two forms had been established in different mixtures of water and ethanol.

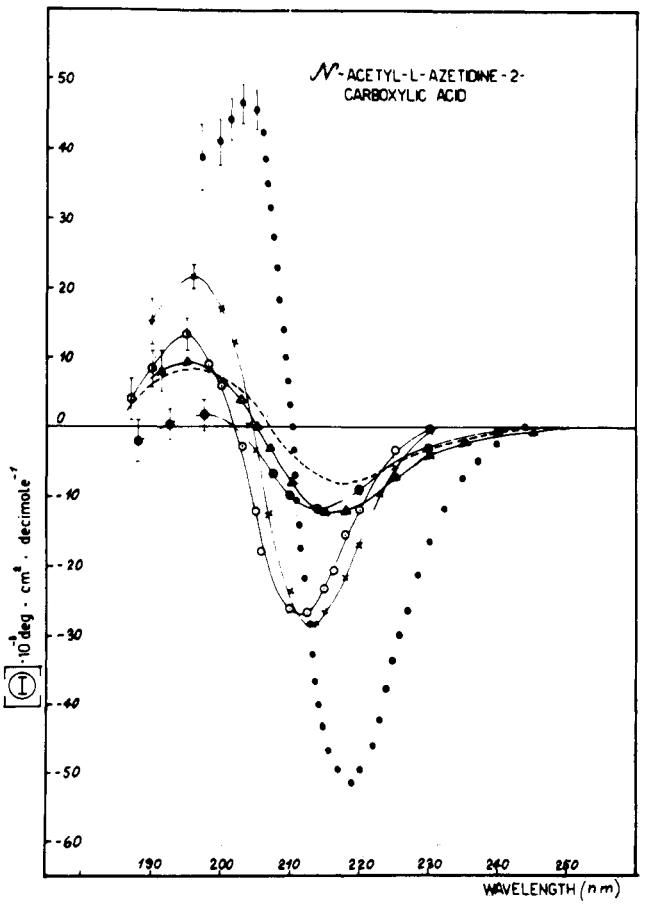

Figure 5. Circular dichroism of *N*-acetyl-L-azetidine-2-carboxylic acid in: (-⊙-) water, (-×-) methanol, (-△-) ethanol, (- - -) trimethyl phosphate, (·····) chloroform, (-◐-) 0.1 *N* H_2SO_4.

Table I summarizes the observed and calculated CD parameters for PLAze and poly(proline) in solvents supporting form I. On the basis of the results of Table I, we believe that in ethanol–water, 99:1 (v/v) poly(L-azetidinecarboxylic acid) takes up a tightly wound helical conformation similar to that of the poly(L-proline I) helix. The difference in intensity of the CD extremes observed near 215 and 195 nm in the two polymers could be explained as the result of a greater flexibility of the poly(proline I) helix, which, in turn, originates from the greater conformational freedom of the pyrrolidine ring compared to the rigidity of the azetidine ring. The high circular dichroism of PLAze I could be alternatively explained as being due to less favorable interactions of the solvent with the solute molecule, so that an intramolecular intrinsically stable conformation is favored.

The CD curve of PLAze in water and fluorinated alcohols does not resemble either the observed curve for poly(L-proline) in these solvents or the calculated CD spectra for poly(L-proline II)[12,13] (Figures 2 and 3). The outstanding feature of this spectrum is the low intensity of its dichroism.

A negative band centered at 216 nm replaces the small positive band observed at around 220 nm in the CD spectrum of poly(proline) in water. In the wavelength range from 208 to 188 nm an almost constant negative dichroism of low intensity is observed. The shape of the CD curve is not altered in 4 M $CaCl_2$, 0.1 N H_2SO_4, and 0.01 N $HClO_4$. On the other hand, the CD spectrum of the model compound N-acetyl-L-azetidine-2-carboxylic acid is distinctly different from that of the polymer in a large number of solvents (Figure 5).

At the present it is problematic to assign the 216-nm CD band of the polymer spectrum to an electronic transition, as it is difficult to speculate about the conformation of PLAze in solution in the above solvents. However, the low intensity of the circular dichroism suggests that, in these highly solvating media, the resulting optical activity originates from a distribution of geometries in solution.

Acknowledgments. We are grateful to Drs. F. Galluzzi and E. Gratton for several enlightening discussions and to Mr. E. De Gregoriis and Mr. A. Farina for technical assistance.

Conformational Studies on Deoxyribonucleic Acid–Polylysine Complexes†

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ABSTRACT: The structure of the complex between DNA and polylysine is analyzed on the basis of conformational calculations and experimental findings and possible connections with the local structure of nucleohistones are discussed. A molecular model characterized by alternant peptide sequences of right-handed and left-handed α-helix-type conformations, winding along the narrow groove of DNA in the B-type form, is proposed. This model is stabilized by hydrophobic, electrostatic, and hydrogen-bond interactions between the ζ-amino groups and the phosphate residues of both the strands of DNA and by hydrogen bonds between the NH peptide groups and the bases of one strand of the double helix.

The complexes between polylysine and DNA have been widely studied in order to obtain informations on the molecular mechanism of the interaction between basic proteins and nucleic acids. Although polylysine (which in the physicochemical conditions of interaction with DNA has a random-coil conformation) cannot represent the behavior of nuclear proteins which contain secondary structure, it is rather reasonable to assume that this basic polypeptide interacts with DNA predominantly through similar mechanism as the segments rich in basic amino acids of histones. This hypothesis is strongly supported by the recent findings on nucleohistones by Boublik *et al.*[1] From their results a general pattern of behavior emerges in that the basic regions of the polypeptide chains are the primary sites of interaction with DNA, while the regions which contain a high proportion of apolar and acidic residues are folded in specific globular structures.

We have recently investigated[2,3] a simple system which can represent a rough model of histones, namely, polylysine with increasing per cent of acetylated side chains, which contains increasing fractions of α helix under the physicochemical conditions of interaction with DNA. We have measured the circular dichroism (CD) of complexes between DNA and polylysine at different neutralization ratios as well as of DNA and acetylated polylysine with different α-helix contents.

As it is shown in Figure 1 the CD spectrum of DNA is progressively modified by increasing the per cent of the polypeptide. CD spectra of complexes between DNA and acetylated polylysine present similar trends by varying the per cent of α helix at a fixed neutralization ratio, indicating a similar mechanism of perturbation. Therefore, a scheme of structure of these complexes may be drawn in which DNA interacts with the fractions rich in basic residues, whereas the more hydrophobic regions are possibly folded in globular arrangements.

The object of this paper is to examine the structural basis of the primary sites of interaction between basic proteins and DNA which can be simulated by the complex between polylysine and DNA. These complexes have been investigated by different authors by means of X-ray[4,5]

† This paper is one of the group presented at the 10th Prague IUPAC Microsymposium on Macromolecules, August 28–31, 1972.

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