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.

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# 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 $\alpha$-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 $\zeta$-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 $\alpha$ 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 $\alpha$-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 $\alpha$ 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]

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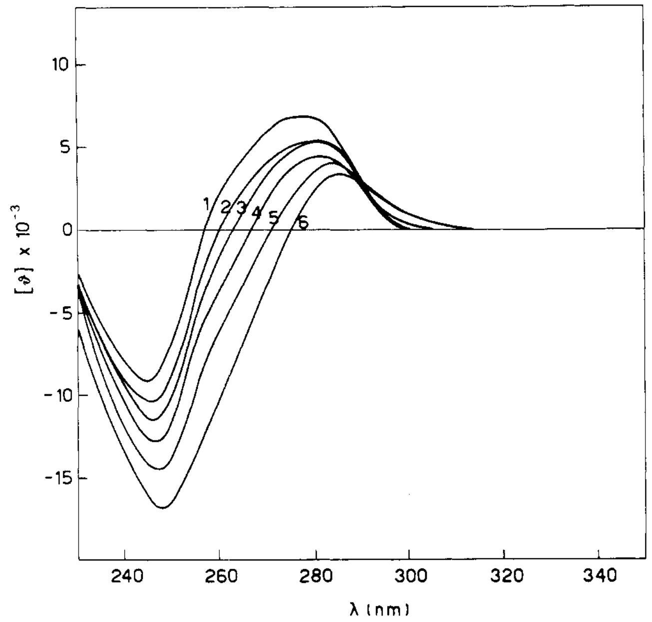

**Figure 1.** Circular dichroism spectra of complexes between DNA and poly-L-lysine at increasing neutralization ratio $N^+/P^-$. Calf thymus DNA from Worthington and poly-L-lysine from Miles were used. DNA concentration was $1.5 \times 10^{-4}$ *M*. Solutions were made by mixing in $5 \times 10^{-4}$ *M* NaCl and $5 \times 10^{-5}$ *M* sodium citrate (pH 7.5): (1) DNA, (2) DNA + PL (+/− = 0.1), (3) DNA-PL (+/− = 0.2), (4) DNA + PL (+/− = 0.3), (5) DNA-PL (+/− = 0.4), and (6) DNA-PL (+/− = 0.6).

and spectroscopic techniques[6-8] leading to different structural hypotheses. In fact the X-ray results seem to leave little doubt that DNA exists in the high humidity B-type form, whereas the large changes in optical activity suggest (if asymmetric scattering phenomena may be excluded) a number of hypotheses which involve modifications in the DNA conformation.

We have therefore investigated the stereochemical conditions of a regular interaction between DNA and polylysine on the basis of the theoretical conformational analysis. This technique, which was developed in the last few years, has contributed either to clarify the main forces stabilizing the conformations of synthetic and biological molecules and polymers or to predict the structure in favorable cases. We have been interested in this area and have recently achieved good success in the prediction of the molecular structure of complex polypeptide systems like gramicidin S and actinomycin and very promising results in the study of the complex between DNA and actinomycin.[9] On this basis the structure of the complex between DNA and polylysine was explored.

We have selected the most stable conformations of the polypeptide chain, including the $\beta$ carbon atoms, which are able to interact coherently with the DNA double helix, namely, those which have the same helical symmetry as DNA. This condition is suggested by the stoichiometry of interaction, corresponding to nucleotide/peptide = 1, in the case of the DNA-poly-L-lysine complex. We assumed two peptide units as the repeating unit because of the double-strand structure of DNA and selected the most stable conformations characterized by the same repeat (3.4 Å) and number of monomer units per turn[10] as DNA and an average radial cylindrical coordinate between 6 and 10 Å.

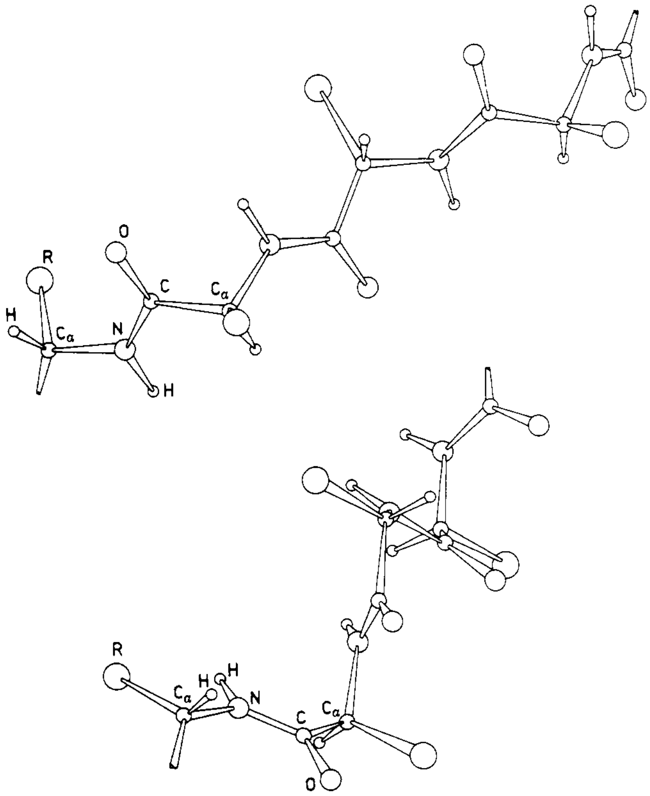

**Figure 2.** Three-dimensional projections containing the helical axis of the polypeptide chain: (a, top) $\beta$ conformation; (b, bottom), alternate sequence of right- and left-handed $\alpha$-helix conformation.

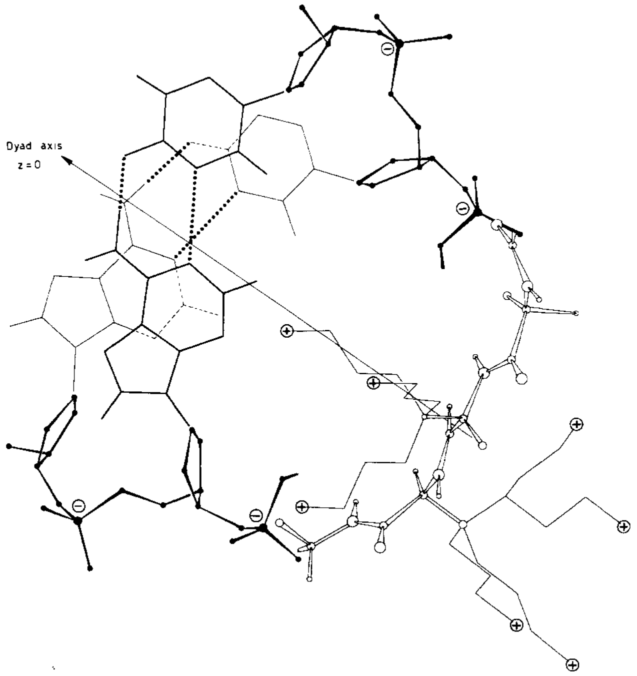

**Figure 3.** Three-dimensional projection of a cylindrical section perpendicular to the helical axis of DNA-poly-L-lysine complex. The polypeptide chain is in a $\beta$ conformation and the possible positions of the charged amino groups are indicated.

Two types of conformation are shown in Figure 2a,b.

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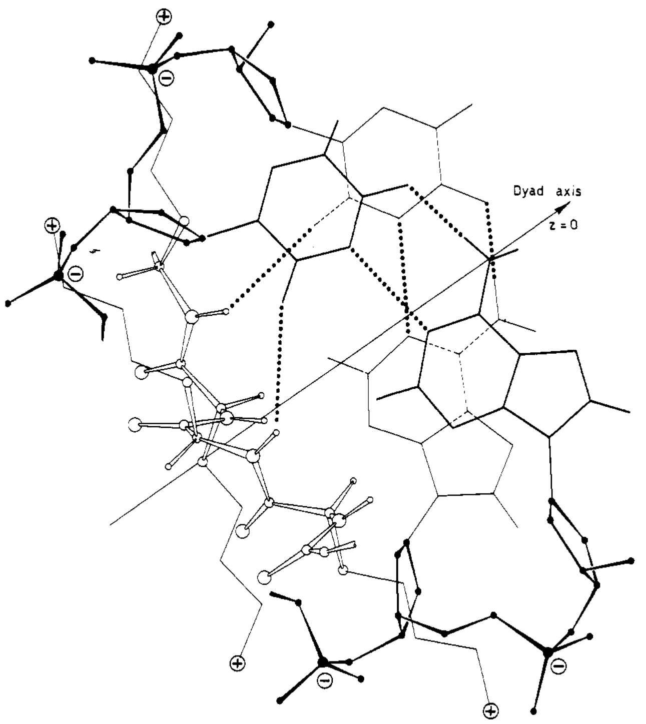

**Figure 4.** Three-dimensional projection of a cylindrical section perpendicular to the helical axis of DNA-poly-L-lysine complex. The polypeptide chain is in a right-handed-left-handed α-helix alternate conformation; the favorable positions of the charged ζ-amino groups are indicated.

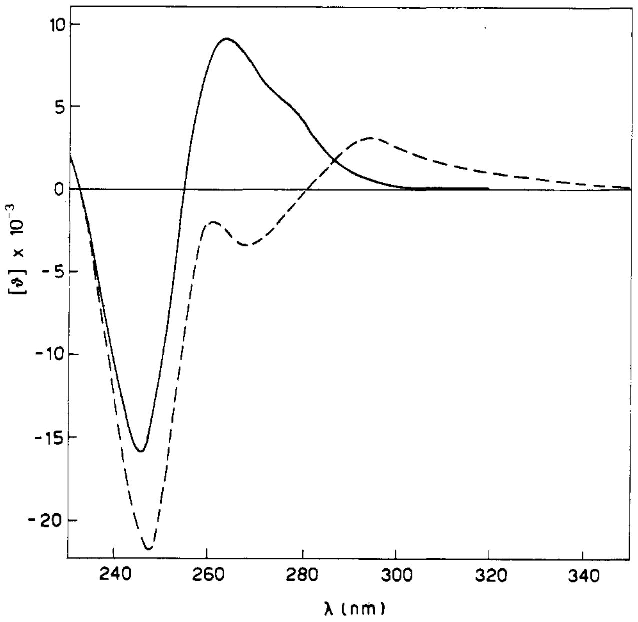

**Figure 5.** Circular dichroism spectra of poly[d(A-T)] (—) and poly[d(A-T)]-poly-L-lysine complex (---). Poly[d(A-T)] was from General Biochemicals; neutralization ratio (+/− = 0.5). Other experimental conditions were as described in Figure 1.

The first (Figure 2a) is a distorted β extended chain; the second (Figure 2b) is characterized by a sequence of right-handed and left-handed α-helical conformations. We have studied their interactions with the DNA B form by fitting the polypeptide chain into the two grooves and localizing the deepest minima of the van der Waals interaction energy.

In the first step of the analysis, no account was taken of long-range electrostatic forces. In fact, as the results of this analysis show, the steric interactions leave a limited mobility to the mutual arrangement of polynucleotide and polypeptide chains in the narrow groove of DNA, where X-ray data indicate the polypeptide chain is localized.[4,5,10] On the other hand, the cationic lysine side chains possess a large degree of freedom which might allow attainment of the geometrical requirements for interaction with the phosphate groups without strong perturbations on the backbone. In the case of the β chain, the best model obtained in the first step of the analysis is schematically shown in Figure 3. As may be seen, one type of the charged side chains of polylysine can interact either with the bases or with the phosphates, whereas the other one is in such a position that a strong interaction with the phosphate groups seems difficult to achieve, assuming trans and gauche sequences of conformations along the side chain.

In addition, it should be noted that this conformation is not specific for the large or the narrow groove of the double helix, in contrast with some chemical evidence and X-ray results.[4,5,10] This is due to the fact that β-type structures are characterized by large (~10 Å) radial cylindrical coordinates, which results directly from the geometrical condition that the polypeptide chain have the same symmetry as DNA. On the contrary the second conformation, shown in Figure 4, interacts with the inner regions of the narrow groove of DNA, by electrostatic and hydrogen bond interactions with the phosphate according to evidence,[5] and is able to form hydrogen bonds with the pyrimidines. Moreover, the overall structure is stabilized by van der Waals and hydrophobic forces, which accounts for the stability of the complex even in salt solutions.

It should be noted that the electrostatic interactions with phosphate groups reflect the presence of a quasidyad axis (which relates the two strands), whereas hydrogen bonds are formed between the peptide NH groups and the CO groups of the bases of one strand of DNA. Finally, the intimate fitting of the polylysine chain into the narrow groove of DNA accounts for the quasi-invariance of the X-ray equatorial spacings of DNA-polylysine fibers[5] with respect to DNA.

Although a strict comparison between the stability of the two structures discussed above cannot be made at present without taking into account the solvent and the salt free-energy contributions, nevertheless the general experimental evidence is consistent with the second proposed model. This model is at present undergoing a refining process which minimizes the total conformational energy by perturbing the conformations of both the DNA and polypeptide chains. The preliminary results indicate slight changes of the tilting and of the distance from the helical axis of the base pairs. These features can form the basis of an explanation of the changes observed in the CD spectra, together with the possible perturbation due to the hydrogen bonds of the n-π* optically active transitions. The X-ray diagrams would not be sensitive to these structural modifications. Furthermore, only thymine seems to fit all the steric requirements for a hydrogen-bond interaction with the polypeptide chain which can explain the selectivity of polylysine for (A + T)-rich DNA under a variety of conditions.[8] In this connection, it is very interesting that the CD spectrum of poly[d(A-T)] is strongly modified by interaction with polylysine, as can be seen in Figure 5. Finally, it should be noted that a regular sequence of right-handed and left-handed α-helical conformations can be adopted by poly-L-lysine as well as by poly-D-lysine, in agreement with the similar affinity for DNA of these two polymers.[8]