Investigation of huge negative circular dichroism spectra of some nucleoproteins

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ABSTRACT

Under certain conditions of preparation, DNA, whether free or complexed with polylysine or histone KAP (I, f1), produce huge negative circular dichroism (CD) spectra with maxima at about 270nm. In order to investigate the cause of these spectra, reconstituted polylysine-DNA complex was used as a model system. It was found that the CD change of DNA in the complex is not a linear function of the fraction of base pairs bound. Such a CD spectrum is not changed despite dilution up to 128 folds for as long as 12 hours. Difference CD spectra taken between free DNA and any of the complexes are qualitatively the same, and are similar to those of free DNA and nucleohistone KAP (Fasman et al., Biochemistry 9, 2814-2822, 1970), free DNA and direct mixed polylysine-DNA complexes, or free DNA in high salt (Chang et al., Biochemistry 12, 3028-3032, 1973). The suggestion is made that this CD spectrum might be caused by specific conformational changes in DNA, perhaps belonging to the family of B to C transitions followed by a further structural distortion of DNA due to aggregation of the nucleoprotein molecules.

INTRODUCTION

Circular dichroism (CD) has been used extensively for the determination of DNA conformation in solution. In particular, three types of CD spectra have been theoretically and experimentally reported for the three common conformations in DNA, namely B, C and A forms (1-6). In protein-DNA complexes, a B spectrum has been reported for reconstituted nucleohistone GRK (IV, f2a1) (7,8) and nucleohistone KAP (I, f1) at low salt (9,10). A CD spectrum between that of B and C form DNA has been observed in chromatin (11-15), reconstituted nucleohistone KAS (IIB2, f2b) made by NaCl gradient dialysis with urea (16), direct-mixed polylysine-DNA (17), protamine-DNA (18) and polyarginine-DNA (19).

A CD spectrum similar to that of A form DNA was also found in reconstituted nucleohistone GRK (IV, f2a1) at intermediate salt (7) and nucleohistone KAS (IIB2, f2b) using GuCl gradient dialysis (20). In addition, another type of CD with a huge negative peak at about 270nm was reported for polylysine-DNA complex made by direct-mixing at 1.0M NaCl (21,22) or made by NaCl gradient (23), reconstituted nucleohistone KAP (1, f1) at intermediate salt (10,24), lithium films of DNA and dAT=AT copolymer at 92% or lower relative humidity (2) or pure DNA in poly (ethylene oxide) plus NaCl (25). This type of CD was called ψ spectrum (2,24,25).

Since light scattering or aggregation always accompanies the appearance of ψ spectrum in a nucleoprotein, it has been suggested that this CD is due to aggregation (2,23,25). Specifically it was suggested by Jordan et al (25) and Brunner and Maestre (2), that this ψ spectrum is caused by interhelical interaction among DNA molecules in B conformation which form a compact and ordered structure. This communication provides experimental results from reconstituted polylysine-DNA complexes and suggests an alternative interpretation of ψ spectrum: perhaps it is caused by a specific change in the secondary structures of DNA, such as is found in the family of B $\rightarrow$ C transitions; the change is then followed by a further structural distortion as aggregation of the nucleoprotein complexes occurs.

MATERIALS AND METHODS

Calf thymus DNA was purchased from Sigma Chemical Co. and was purified by phenol extraction. Poly (L-lysine) hydrochlorides (mol wt 170,000 and 15,500) were purchased from Schwarz/Mann. Reconstituted polylysine-DNA complexes were prepared by (A) step-wise NaCl gradient dialysis (26) and (B) continuous NaCl gradient dialysis from 2.0M to approximately 0.1M in about

one day. In each case 0.01M Tris, pH8.0 was used as a buffer. After the complexes were made they were dialyzed to 2.5×10^{-4} M EDTA, pH8.0 and studied by circular dichroism (CD) spectroscopy using Jasco Spectropolarimeter Model J-20. The CD results are reported as $\Delta\epsilon = \epsilon_L - \epsilon_R$, where ϵ_L and ϵ_R are respectively molar extinction coefficients for the left- and right-handed circularly polarized light. The units of $\Delta\epsilon$ are $M^{-1}cm^{-1}$ in nucleotide. DNA concentration in the complex was determined either by measuring absorbance at 260nm (A_{260}) in EDTA buffer and correcting light scattering contribution following the method of Leach and Scheraga (27) or by measuring A_{260} of the complex in EDTA buffer plus 2.0M NaCl under which condition polylysine is dissociated from DNA.

RESULTS

Fig. 1 shows some typical CD spectra of reconstituted polylysine-DNA complexes with varied input ratio r of polylysine to DNA ($r = \text{lysine/nucleotide}$). It is seen that the CD is drastically changed when r is increased from 0 to 0.6. A huge negative peak at 283nm is observed for the complex with $r = 0.6$. These results are similar to those reported before (21-23). Fig. 2 shows the CD change at 270nm of DNA in the complex as a function of r . Contrary to what has been observed for a direct-mixed polylysine-DNA complex (17), with reconstitution, there is no linear relation between the difference CD and r , which, in this case, is equal to the fraction of DNA bound. In other words, conformational or CD change in a reconstituted polylysine-DNA complex is not a simple function of polylysine binding.

Since such a huge CD spectrum in general is accompanied by light scattering it has been suggested that intermolecular interaction among the complex molecules is the cause of this effect (2,23-25). In order to test whether this is the case

Fig. 1. CD spectra of reconstituted polylysine-DNA complexes prepared by continuous NaCl gradient dialysis. r values of the complexes are indicated.

Fig. 2. Dependence of difference CD at 270nm upon protein coverage on DNA. Reconstituted polylysine-DNA complexes with r in lysine/nucleotide (●). Reconstituted nucleohistone KAP (I, fl) with r in amino acid/nucleotide, complex concentration at $10^{-3}M$ (▲) and at $10^{-4}M$ (■) (reference 10, Fasman et al., 1970).

a complex ($r = 0.6$) was diluted by a factor of 2, 4, 16, 64 or 128. The CD spectra were recorded using cells with either 1.0cm or 10.0cm pathlength. Fig. 3 shows three CD spectra of the same complex in Fig. 1 ($r = 0.6$) but diluted 16, 64 or 128 folds. It is seen that, within experimental error, they are essentially identical to one another. It is further noted that some of these spectra were taken about 12 hours after those of the original solutions, so that a slow dissociation could have occurred. The identical CD spectra in Fig. 3 strongly suggest that these CD are not caused by reversible intermolecular interaction among polylysine-DNA complexes, because, if they were, dissociation by dilution should be expected to cause a CD change. Of course, if the intermolecular interaction were irreversible, this dissociation would not be apparent even though possible.

The method of reconstitution always yields cooperative binding of polylysine to DNA (26,28). The complex can be separated into two fractions by centrifugation, a pellet having most of DNA bound, and a supernatant having most of DNA free. Fig. 4 shows the CD spectra of the pellets of three complexes with $r = 0.2$, 0.4 and 0.6 respectively. These complexes were prepared by step-wise NaCl gradient dialysis, each step being longer than 3 hours. If a 3 hour period or less was used, complexes with the same r value showed smaller CD. Carroll (23) also reported a dependence of the final CD of a complex upon time used in gradient dialysis.

It is noted that, even though the CD of the original complex ($r = 0.2$) is positive at 280nm, the pellet has a negative CD whose shape is different from ψ spectrum. In addition, as expected, the CD spectra of the pellets of 0.4 and 0.6 complexes are more negative than those taken prior to centrifugation.

Fig. 3. CD spectra of a reconstituted polylysine-DNA complex ($r=0.6$ in Fig. 1) at various complex concentrations. If C_0 is the concentration in Fig. 1 ($C_0 = 1 \times 10^{-4}M$), $C = \frac{1}{16} C_0$ (—), $\frac{1}{64} C_0$ (---) and $\frac{1}{128} C_0$ (- · - ·).

Fig. 4. CD spectra of pellets of reconstituted polylysine-DNA complexes prepared by step-wise NaCl gradient dialysis. The pellets were obtained by centrifuging the complexes with $r = 0.2, 0.4$ and 0.6 at 10,000rpm in a Szwall SS-34 rotor for 10 min.

If the difference CD spectra were taken between those of the pellets in Fig. 4 and that of pure DNA (Fig. 1), a striking similarity is found among them with a maximum at about 270 to 275nm (Fig. 5) and a minimum at about 230 to 240nm. We were then interested to see whether similar difference CD spectra could be obtained from those CD spectra reported earlier in other systems. Fasman et al (10) reported that reconstituted nucleohistone KAP (I, fl) in 0.14M NaF showed a progressive red-shift of the positive CD band near 275nm which finally disappeared and became negative at $r = 1.0$ amino acid residue per nucleotide. At $r = 3.0$, a huge negative CD with its peak at 270nm was also observed. The apparent CD spectra are too complicated for interpretation. However, difference CD spectra prove to be much more informative as has been successfully demonstrated before in other systems (17-19) and also in Fig. 5 in this report. With this in mind, difference CD spectra between DNA and nucleohistone (Fig. 2 in reference 20, Fasman et al., 1970) were measured and are shown in Fig. 6. Though the original CD spectra look complicated (20), the difference CD spectra are qualitatively the same among themselves with a maximum at 260 to 270nm and a minimum at 240nm. The negative peak is more enhanced and slightly red-shifted as more histone is complexed to DNA. These results are strikingly similar to those shown in Fig. 5 for the pellets of reconstituted polylysine-DNA complexes.

Difference CD spectra between B spectrum of DNA and those of DNA in various concentrations of poly (ethylene oxide) (PEO) and NaCl (Fig. 2 in reference 25, Jordan et al., 1972) were also taken. They are essentially the same as those shown in Fig. 5 for reconstituted polylysine-DNA complexes and in Fig. 6 for reconstituted nucleohistone KAP in 0.14M NaF. There is one negative peak which is progressively enhanced and red-shifted from 265 to 273nm with a minimum at 235 to 240nm.

Fig. 5. Difference CD spectra between free DNA and the pellets of the complexes in Fig. 4.

Fig. 6. Difference CD spectra between free DNA and reconstituted nucleohistone KAP (I, fl). r values of the complexes are indicated. These spectra were calculated from those in Fig. 2 of reference 10, Fasman et al., 1970.

DISCUSSION

The appearance of ψ spectrum with a huge negative CD at 270 to 280nm is mysterious. Aggregation has been implied as a possible cause for this CD (2, 22-25). More specifically, Jordan et al (25) has proposed that this CD is not due to changes in the secondary structures of DNA but rather to a compact and ordered arrangement of DNA molecules in B conformation which have a strong interhelical interaction. These authors further suggest that this interhelical interaction is the origin of the ψ spectrum both in their system (25) and in nucleoproteins (21-24). If a specific interhelical interaction were indeed the cause of this ψ spectrum, it would be difficult to imagine that such interaction is independent of the absence or the presence of different types of proteins on DNA. For instance, in protein-bound regions of a nucleoprotein, there is one amino acid residue per nucleotide in reconstituted polylysine-DNA complex but there are three amino acid residues per nucleotide in reconstituted nucleohistone KAP (29). How could these bound proteins not interfere with a very close and specific interaction among DNA molecules?

It was reported by Haynes et al. (22) that X-ray diffraction of polylysine-DNA complex in 1.0M NaCl showed B conformation. This report was used as the major evidence against the suggestion of conformational change in DNA as a possible cause for ψ spectrum (2,25). Our re-examination of this report indicates that this conclusion was not definitive. These authors reported that the X-ray diffraction diagrams of the complexes were poorly defined, though sometimes slight orientation of the polylysine-DNA gels could be achieved. The amplitude of ψ spectrum of polylysine-DNA complex depends greatly upon many factors, such as molecular weight of polylysine and input ratio of polylysine to DNA (23). The variation in

amplitude is also significant among different preparations using the same method. Such a variation in the spectrum has also been reported by Brunner and Maestre (2) in different preparations of DNA film. The reason is not clear. Because of these facts, it is difficult to determine whether the X-ray diffraction results reported (22) came from the sample with a huge $\downarrow$ spectrum or not. In addition, since the complexes studied by Haynes et al. (22) had $r = 0.5$ lysine per nucleotide, in these complexes, 50% of DNA were not bound and admittedly would have had a B conformation. The presence of a mixture of DNA in varied conformations can perhaps be an obstacle to X-ray diffraction studies. In addition, heterogeneity among polylysine-bound DNA, which has been demonstrated by thermal denaturation (28,30,31), can cause further difficulty in X-ray work.

The above reasoning seems to indicate that the suggestion of a specific and ordered arrangement of B form DNA as a cause for $\downarrow$ spectrum is not well supported. Consequently an alternative view is presented here suggesting that the $\downarrow$ spectrum could be caused by a conformational change in DNA, perhaps a B to C transition followed by a further structural distortion due to aggregation of the nucleoprotein complexes.

In a heterogeneous system such as nucleoprotein, a measured CD spectrum is the sum of the CD spectra of various species, such as those from protein free or bound regions. Even within protein-bound regions it is possible to have different CD spectra depending upon how the protein molecules bind on a DNA molecule, for example, either cooperative or random binding. Therefore, the measured CD spectrum may not be clearly informative. However, if difference CD spectra are taken, the same type of interaction will give only one type of difference CD spectrum, with its amplitude proportional to the extent of this interaction. We have taken advantage of this fact and calculated the CD spectra

of protein-bound regions in direct-mixed polylysine-DNA (17), protamine-DNA (18) and polyarginine-DNA (19). If different types of interaction are involved, the shape of the difference CD spectrum will be changed and the amplitude will not be proportional to the extent of interaction.

As shown in Fig. 2, either in reconstituted polylysine-DNA or nucleohistone KAP (10), the difference CD is not a linear function of extent of binding. The results in Fig. 5 for the pellets are more significant. Because of cooperative binding, the fractions of DNA base pairs bound by polylysine in the three samples shown in Figs. 4 and 5 are respectively 60%, 75% and 87% for the pellets obtained from complexes with $r = 0.2, 0.4$ and 0.6 which were determined by the method of thermal denaturation (29). On the other hand, the difference CD at 270nm (Fig. 5) for the same r values are $-4.5, -14$ and -53 respectively. It seems that the spectrum is greatly affected by how closely the DNA molecule approximates its full coverage by polylysine. However, this is not the only factor responsible for this CD, because, in EDTA buffer, a direct-mixed polylysine-DNA complex with 95 to 100% coverage still shows a positive CD near 280nm (unpublished results). Therefore, in salt gradient dialysis, both cooperativity and specificity in binding seems to be required for this ψ spectrum to occur. Perhaps cooperative and specific binding occurs slowly and only at certain NaCl concentration during reconstitution, such that when the change of NaCl gradient is not slow enough, the resulting CD spectrum of the complex is much smaller (23, 30).

Although they differ in amplitude, the difference CD spectra of reconstituted polylysine-DNA (Fig. 5) and nucleohistone KAP (Fig. 6) are very similar to the difference CD spectra of DNA in NaCl or DNA in direct-mixed polylysine-DNA (17), protamine-DNA (18), and polyarginine-DNA complexes (19). Since the binding of proteins to DNA is random in these systems, CD changes were interpreted to

be a result of structural alteration in those base pairs bound by the proteins, rather than ordered arrangements of DNA molecules through specific interhelical interaction. The similarity in difference CD spectra suggests that the spectral changes found in reconstituted polylysine-DNA (Fig. 5) and nucleohistone KAP (Fig. 6) could also be caused by a similar structural alteration of DNA base pairs, perhaps in B to C transitions, such as is observed in direct-mixed polylysine-DNA, protamine DNA and polyarginine-DNA complexes (17-19). The enhancement of the amplitude in ψ spectra could possibly be caused by an additional structural change in the DNA molecule due to aggregation of these nucleoprotein molecules.

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