DIFFERENCE BETWEEN POLYLYSINE AND POLYARGININE IN CHANGING DNA STRUCTURE UPON COMPLEX FORMATION

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SUMMARY Soluble complexes of DNA with poly-L-lysine and poly-L-arginine were prepared by dialysis or mixing method, and their circular dichroism spectra and viscosities were measured. Both complexes formed by dialysis method had different circular dichroism spectra from that of free DNA respectively, i.e., the spectrum of the DNA-poly-L-lysine complex was similar to that of DNA at high salt concentration and the spectrum of the DNA-poly-L-arginine complex resembled that of DNA under melting at elevated temperature. The viscosity of the both complexes decreased pronouncedly with the increase in peptide to DNA ratio, although the viscosity of the DNA-poly-L-lysine complex was more rapidly decreased than that of the DNA-poly-L-arginine complex. The difference between the two polypeptides in changing DNA structure was observed also in the complexes formed by mixing method.

It would be important to know the conformation of DNA in nucleohistone, especially in relation to the regulation of gene function. Recently it has been suggested that the conformation of DNA in nucleohistone is affected markedly by slightly lysine-rich fraction, whereas the arginine-rich fraction has a little effect (1,2). In the present study, to clarify the difference between lysine and arginine residues in changing DNA conformation in nucleohistone, complexes between DNA and poly-L-lysine or poly-L-arginine were prepared by dialysis or mixing method, and their circular dichroism spectra and viscosities were measured.

MATERIALS AND METHODS Calf thymus DNA (Sigma Chemical Co., Type 1) was used without further purification. Concentration of DNA phosphate was determined by using the extinction coefficient at 260mμ per molar phosphate 6,700. Two specimens of poly-L-lysine HBr (Lot No. LY120; M.W. 75,000; average degree of polymerization (D.P.) 360 and Lot No. LY98; M.W. 5,500; D.P. 26) and poly-L-arginine · 1/2 H_2SO_4 (Lot No. AR35; M.W. 21,900; D.P. 96) were purchased from Miles-Yeada Ltd. Concentrations

of polypeptides in stock solutions and in dialysis tubes were determined by the ninhydrin method after hydrolysis with 6N-HCl. In dialysis method, a constant volume of peptide solution in 2M NaCl was added to an equal volume of DNA solution in 2M NaCl with constant stirring at room temperature to give the final concentration of DNA 10^{-4}M and various ratios of polypeptide cation to DNA phosphate (peptide/DNA), then the mixtures were dialysed at 4°C against 0.4M NaCl for 4h, 0.3M NaCl for 2h, 0.15M NaCl for 2h and finally overnight against 0.01M cacodylate buffer (pH 7.0). After dialysis, the concentration of DNA in the sample was measured by the absorbance at 260mμ of the redissolved samples in high salt concentration (2.35M NaCl) and no loss of DNA was observed. The loss of polypeptide in the dialysis tube was not also observed. Samples, in which any precipitate was produced on further standing ($>$peptide/DNA$\sim$0.58), were not used. In mixing method, a constant volume of DNA solution in 0.01M cacodylate buffer (pH 7.0) and an equal volume of polypeptide solution in the same buffer was mixed together by the procedure similar to that described above. Samples, in which no precipitate was produced, were used.

Circular dichroism was measured with a Jasco J-10 spectropolarimeter at 28°C. The concentration of DNA in samples was $0.67\sim1 \times 10^{-4}$M and the cell length was 2cm. Viscosity measurements were carried out in an Ostwald type viscometer of which the flow time for buffer was 210sec at 20°C, and the volume of sample was 4ml. The accuracy of the thermoregulation was ± 0.01°C and the thermometer was graduated to 0.05°C.

RESULTS AND DISCUSSION Thermal denaturation profile was measured to see the extent and the stabilization effect of complex formation, and it was confirmed that all complexes had enhanced thermal stability as reported by others (3-6).

Calf thymus DNA has a positive circular dichroism band with a maximum at 275mμ (Fig. 1(a)), and the circular dichroism of polypeptides is negligibly small above 240mμ. Since DNA-polypeptide complexes are turbid, the possible effects due to light scattering

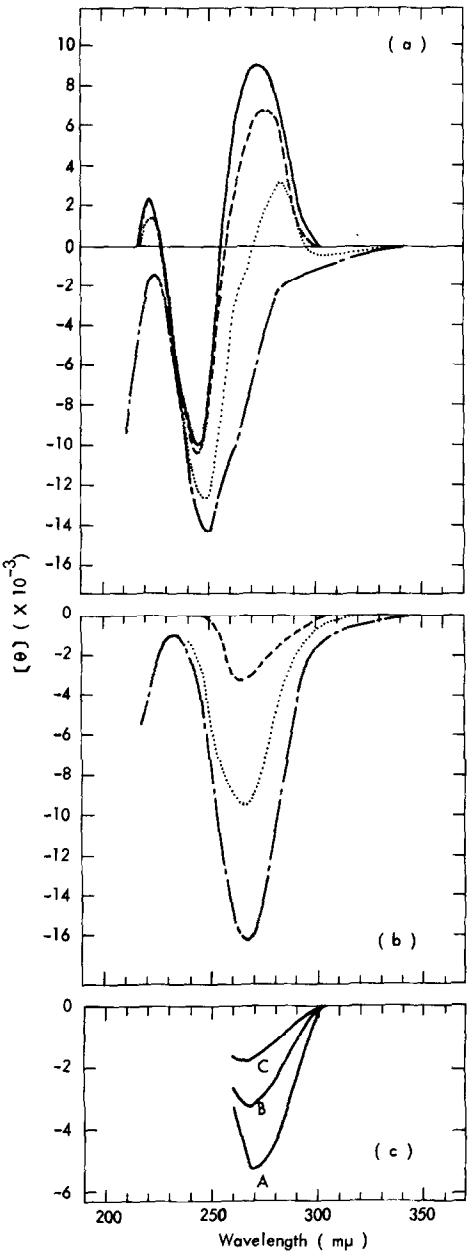

Fig.1 (a) Circular dichroism spectra of dialysed DNA-poly-L-lysine complex. (b) Difference circular dichroism spectra of dialysed DNA-poly-L-lysine complex against free DNA. Peptide/DNA ratios: ———, DNA alone; ------, 0.31; ....., 0.50; ——·——, 0.58. (c) Difference circular dichroism spectra of DNA at high salt concentrations against DNA at 0.01M cacodylate buffer (pH 7.0). Salt concentrations: curve A, 3.77; B, 1.89; C, 0.95M NaCl.

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was examined by using a colloidal sulfur suspension as a scattering material. However,

no influence on the measurements of circular dichroism was observed. In DNA-Lys (poly-L-lysine: D. P. 360 and 26) complex formed by dialysis method, the peak at 275mμ is drastically decreased and that at 245mμ increases slightly with the increase in peptide/DNA ratio as shown in Fig. 1(a). The difference circular dichroism spectrum, which was obtained by subtracting the spectrum of free DNA from that of the complex, has a characteristic negative band at 268mμ (Fig. 1(b)). The similar band is also shown in the difference circular dichroism spectrum for free DNA at high salt (NaCl) concentrations against DNA at low salt concentration (Fig. 1(c)), therefore the conformational change of DNA induced upon complex formation with poly-L-lysine by dialysis method might be similar to the change induced when salt (NaCl) concentration of DNA is brought to be higher. The resemblance to the circular dichroism spectrum at high salt concentration was also observed with the spectrum of DNA in nucleohistone (7,8). In the case of DNA-Arg (poly-L-arginine: D.P. 96) complex formed by dialysis method (Fig. 2), the magnitudes of the positive band

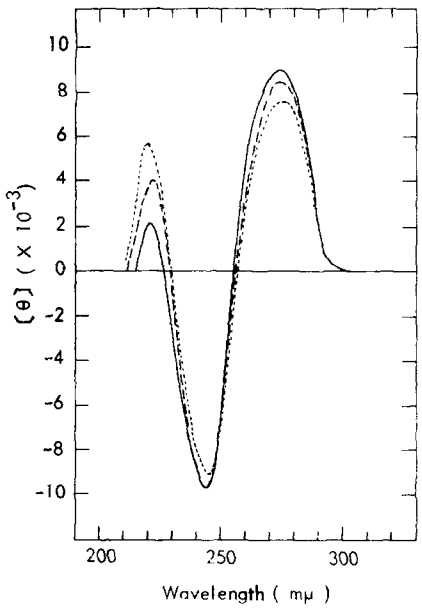

Fig. 2 Circular dichroism spectra of dialysed DNA-poly-L-arginine complex. Peptide/DNA ratios: ———, DNA alone; ------, 0.42; ——·——, 0.58. Solvent: 0.01M cacodylate buffer (pH 7.0).

at 275mμ and of the negative one at 245mμ decrease slightly accompanying a small red shift of the latter band with increasing peptide/DNA ratio and the difference circular dichroism spectrum has no clear band. The same tendency of the changes in circular dichroism spectrum has also been observed in DNA under melting at high temperature (9), therefore it is considered that the secondary structure of DNA is somewhat disordered by binding with poly-L-arginine. The hydrogen bonds between the complementary strands, however, would not be broken because the stabilization effect of this polypeptide against thermal denaturation was observed in the complex.

In the case of mixed complexes, the difference between the two polypeptides in changing the circular dichroism spectrum of DNA was also observed(Fig. 3). The magnitude of

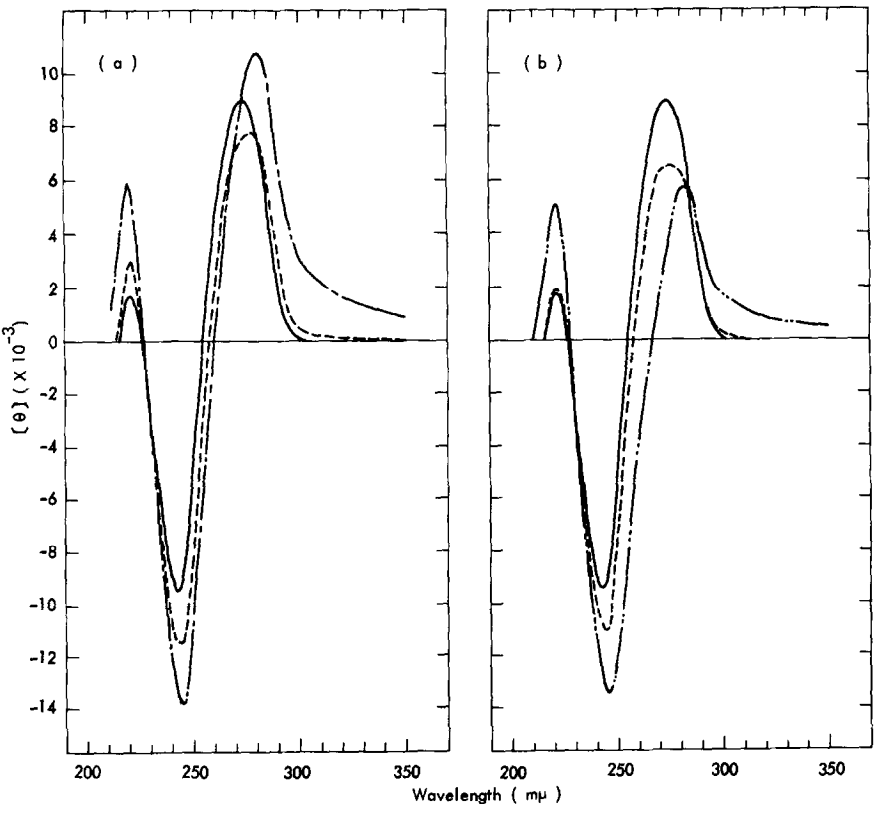

Fig. 3 Circular dichroism spectra of mixed DNA-polypeptide complex. (a) DNA-poly-L-lysine complex. (b) DNA-poly-L-arginine complex. Peptide/DNA ratios: ———, DNA alone; ------, 0.42; —·—, 0.58; —··—, 1.00. Solvent: 0.01M cacodylate buffer (pH 7.0).

the positive peak at 275mμ changes differently with peptide/DNA ratio for each complex although both show a slight red shift of the peak and new circular dichroism beyond 310mμ.

Specific viscosities of the complex and free DNA showed no measurable dependency on the concentration. The fact might be attributed partly to the low concentration of the sample and/or to the relatively high shear rate of the viscometer. It is apparent, however, that the viscosity of the complex pronouncedly decreases with increasing peptide/DNA ratio. In Fig.4 the ratio of the specific viscosity of the complex to that of free DNA at 0.75×10^{-4}M DNA concentration is plotted against peptide/DNA, and it is shown that the viscosity of DNA-Lys complex decreases more rapidly than that of DNA-Arg complex irrespective of the method of complex formation. DNA-Lys complex would have a more marked tendency to aggregate or to fold than DNA-Arg complex. In this plot, complexes formed by dialysis method have a characteristic shoulder at low peptide/DNA ratio (~0.1) whereas complexes formed by mixing method have not.

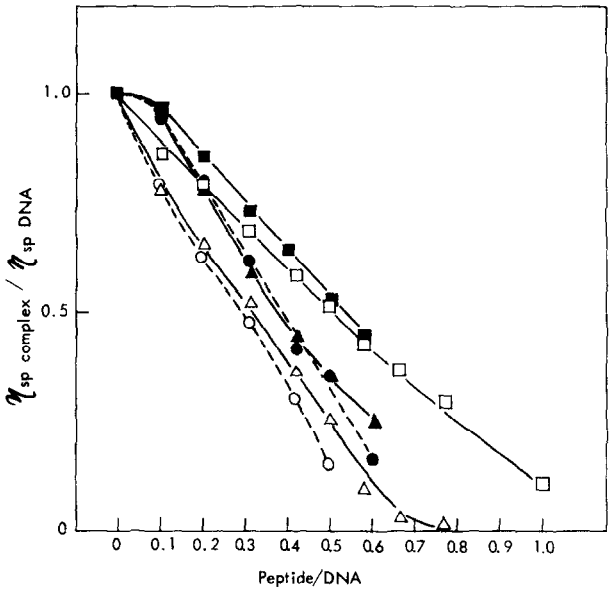

Fig.4 Dependence of $\eta_{sp\ complex} / \eta_{sp\ DNA}$ on the ratio of peptide/DNA in the DNA-polypeptide complex.
--O--O--, mixed DNA-Lys26; --●--●--, dialysed DNA-Lys26; —△—△—, mixed DNA-Lys360; —▲—▲—, dialysed DNA-Lys360; —□—□—, mixed DNA-Arg96; —■—■—, dialysed DNA-Arg96. DNA concentrations: 1×10^{-4}M for mixed complex, and 0.75×10^{-4}M for dialysed complex. Solvent: 0.01M cacodylate buffer (pH 7.0).

These results suggest that polylysine changes the conformation of DNA more considerably than polyarginine. This is interesting since other workers (1,2) have reported that the conformation of DNA in nucleohistone is affected markedly by slightly lysine-rich fraction, whereas the arginine-rich fraction has a little effect. It has been pointed out that bound water is necessary to keep the DNA structure (10), and that the B-form DNA structure begins to break down when the hydration of bases is altered (11). On the other hand, the origins of the effects of high salt, diminishing the Cotton effects of optical rotatory dispersion (12) and reducing the circular dichroism band at 275mμ (13) were suggested to be the change of the hydration or humidity of DNA molecules. Therefore, the different effects on the conformational changes of B-form of DNA by the two polypeptides might be attributed to the different effect on the hydration around bases in either or both of size and type between the two polypeptides through the interaction of the basic group of each polypeptide and the phosphate group of DNA.

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