QUANTITATIVE STUDY OF DYE BINDING TO DNA-POLYLYSINE AND DNA-POLYARGININE COMPLEXES

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SUMMARY: Soluble complexes of poly-L-lysine and poly-L-arginine with DNA were prepared by a salt gradient dialysis and were investigated on stoichiometric aspect using a dye binding technique. It was found that only partial cationic residues on polyarginine are effective for occupying DNA phosphate groups, while all residues in polylysine can occupy.

Basic chromosomal proteins, histone and protamine, have a high content of Lys and Arg residues and the residues are considered to be primarily responsible for the binding to DNA. On the other hand, the individual histone fractions containing varying content of Lys or Arg residue are suggested to have different physicochemical natures in interacting with DNA or roles on the maintenance of the chromatin structure (1-5). These might partly be explicable in terms of the difference in the binding properties of Lys and Arg residues to DNA. In fact polylysine and polyarginine change DNA structure differently upon complex formation (6), and circular dichroism spectra of DNA-polylysine complex resemble that of DNA-Lys-rich histone complex (4,5). We report in the present communication a stoichiometric study of the interaction of these polypeptide with DNA.

MATERIALS AND METHODS

Acridine orange (Eastman Organic Chemicals) was purified by the method described previously (7). Actinomycin D was obtained from Mann Research Laboratories, and 8-anilino-1-naphthalene sulfonic acid (ANS) from Tokyo Chemical Industry Co., Ltd. The concentrations of dyes were determined by the absorbancies using extinction coefficients 54,000 at 492nm for acridine orange, 25,000 at 440nm for actinomycin D and 4,950 at 350nm for ANS. Calf thymus DNA (Sigma Chemical Co., Type 1) was used without further purification and the extinction coefficient at 260nm per molar DNA phosphate

was determined to be 6,700. Poly-L-lysine·HBr (mol. wt. 75,000) and poly-L-arginine·$1/2H_2SO_4$ (mol. wt. 21,900) were purchased from Miles-Yeda Ltd., and the concentration was determined by the ninhydrin method after hydrolysis with 6N-HCl. DNA-polypeptide complexes were prepared by a salt gradient method as described in the previous work (6). In the present study the complexes were also formed in the presence of 5M urea and 2.5mM EDTA followed by exhaustive dialysis against 0.01M cacodylate buffer (pH 7.0) at final step. The prepared samples contained 72-77μM of DNA and had the same ratio of polypeptide cation to DNA phosphate (+/-) as the input ratios. Up to +/- ratio of 0.58, no precipitate was produced on further standing, and the experiments were therefore carried out with samples at +/- ratio of this value or lower. All samples of DNA-polypeptide-dye were prepared in 0.01M cacodylate buffer (pH 7.0) and test tubes were coated with silicone to minimize the amount of dye adsorbed. Absorption measurement and thermal melting experiments were performed in a Hitachi EPS-3T Spectrophotometer. Fluorescence spectra were measured with a Jasco FP2 spectrofluorometer.

RESULTS AND DISCUSSION

The thermal melting profiles of DNA-polypeptide complexes are shown in Fig. 1 without correction for light scattering. Typical biphasic transitions, assigned to the melting of free and complexed portions of DNA (8), are seen although the higher transitions are not clearly shown because of the high melting temperature unattainable in the present experimental conditions. The turbidity of the solution monitored at 340nm did not change up to 95°C for the sample prepared in the presence of urea in contrast with a gradual enhancement above 70°C for the one prepared in the absence of urea, and therefore the hyperchromicity over the lower transition of the former sample was corrected for tne contribution of the light scattering. The corrected hyperchromicities at 90°C showed 23, 48 and 61% decreases against that of free DNA for DNA-polylysine complexes with +/- ratios of 0.20, 0.42 and 0.58 respectively, while for DNA-polyarginine complexes of the same +/- ratios the corresponding decrease in hyperchromici-

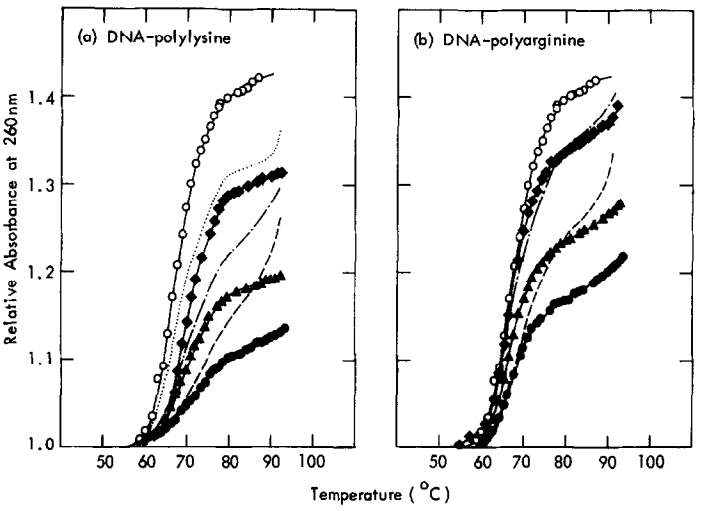

Fig. 1. Thermal melting profiles of DNA and DNA-polypeptide complexes. —o—o—, DNA; ....., +/-=0.20; —·—·—, +/-=0.42; -----, +/-=0.58 : samples prepared in the absence of urea, (DNA)=73μM. —■—■—, +/-=0.20; —▲—▲—, +/-=0.42; —●—●—, +/-=0.58 : samples prepared in the presence of urea, (DNA)=72μM.

ties were 8, 27 and 37%. This result shows that the smaller amount of base pairs are stabilized by complexing with polyarginine than with polylysine compared with samples at the same +/- ratio. Very similarly to the present data, Olins et al.(9) had observed a higher hyperchromicity of the lower transition of DNA-polyarginine complex.

Because of the favorable nature of a basic dye as having a strong or negligible affinity to DNA or to basic polypeptide respectively, we expected that the amount of free phosphate groups in DNA-polypeptide complex could be quantified by using acridine orange binding to the complex. The dye binds to DNA up to maximum amount as the ratio of DNA phosphate to bound dye reaches 1, where the absorbancy around 492nm attains minimum (10). In Fig. 2 the difference absorption spectra of acridine orange mixed with DNA at various ratios of DNA phosphate to dye (P/AO) against free dye are shown, and absorbancy difference at 492nm is plotted in Fig. 3. It is remarkable that all spectra at P/AO below 2 show similarly a negative peak at 492nm and the peak exhibits a minimum intensity at P/AO 1. When the dye was added to DNA-poly-

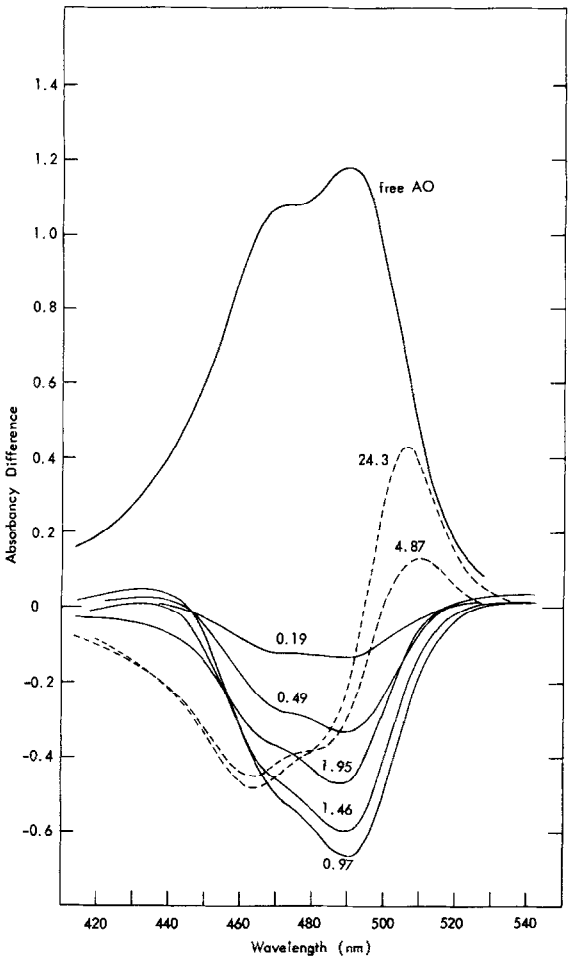

Fig. 2.

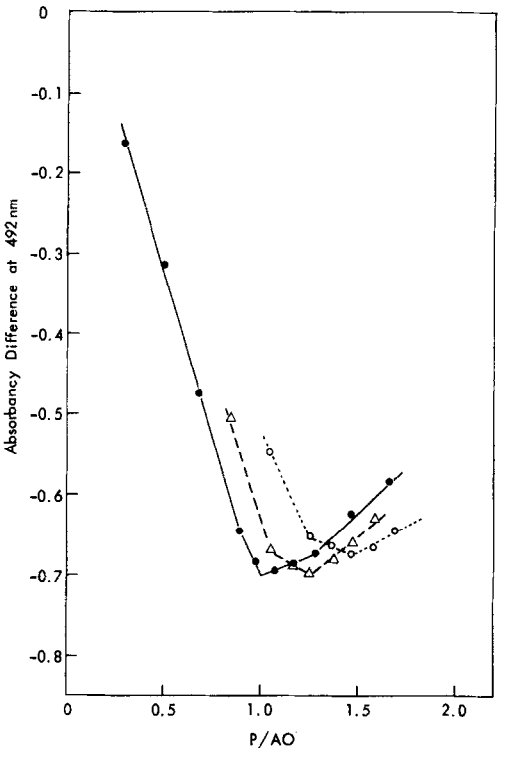

Fig. 3.

Fig. 2. Difference absorption spectra of acridine orange mixed with DNA at various P/AO ratios (0.19 – 24.3) against the spectrum of free acridine orange (free AO). Concentration of the dye : 25μM.

Fig. 3. The plot of absorbancy difference at 492nm between bound and free dyes against P/AO. —●—●—, DNA; ··o··o··, DNA-polylysine +/-=0.32; --△--△--, DNA-polyarginine +/-=0.32. Concentration of the dye : 25μM.

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peptide complexes, similar difference spectra to those in Fig. 2 were obtained suggesting that the same mode of dye binding as free DNA-dye system occur in DNA-polypeptide-dye system. In this case, absorption spectra of turbid samples above +/- 0.3 were meas-

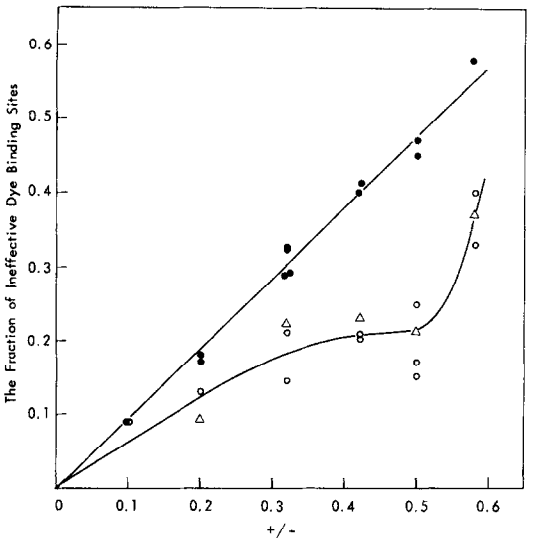

Fig. 4.

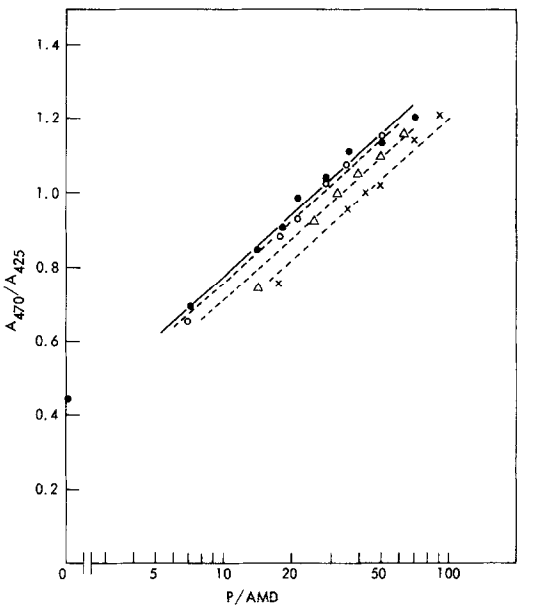

Fig. 5.

Fig. 4. The fractions of ineffective binding site of acridine orange on DNA-polypeptide complexes with various +/- ratios. (●), DNA-polylysine prepared in the absence of urea; (o), DNA-polyarginine prepared in the absence of urea; (△), DNA-polyarginine prepared in the presence of urea.

Fig. 5. The ratios (A_{470}/A_{425}) of absorbancy at 470nm to that at 425nm of actinomycin D added to DNA and DNA-polyarginine complexes with various +/- ratios prepared in the absence of urea. +/- ratios: (●), 0 (DNA); (o), 0.20; (△), 0.42; (x), 0.58. Concentration of the dye was 0.8μM.

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ured using an integral sphere, an attachment to eliminate the light scattering. Besides the similarlity of the spectrum, the minimum absorbancy at 492nm was attained at larger P/AO ratios as shown in Fig. 3. The fact would be simply accounted for by the diminution of dye binding sites. The fraction of ineffective dye binding sites (S_{ineff}) in each DNA-polypeptide complex was calculated from the P/AO ratio giving minimum absorbancy at 492nm ($(P/AO)_{min}$) as $S_{ineff}=1-1/(P/AO)_{min}$. The value of S_{ineff} thus calculated is plotted against +/- ratio in Fig. 4. It is apparent that the fractions of ineffective sites are equal to +/- ratios for DNA-polypeptide complex, but smaller than +/- ratios for

DNA-polyarginine complex. The results indicate that all Lys residues bind completely to DNA phosphates with 1:1 stoichiometry while Arg residues bind incompletely. The same results are obtained for both series of samples prepared in the absence or presence of urea. The data coinside with the result of the thermal melting experiments described above.

Similar process was also repeated using another dye, actinomycin D. The dye binds preferentially to G-C pair in DNA double helix and upon binding the visible absorption band shifts to red resulting in the decrease and increase in the absorbancies at 425 and 470nm (A_{425} and A_{470}) respectively (11). A constant amount of the dye was added to DNA or DNA-polypeptide complex solutions to give various ratios of total DNA phosphate to dye (P/AMD) and the absorption spectra were measured. A relatively low concentration of dye (0.8μM) was used, since a slight interaction between the dye and polypeptide was observed at higher concentrations, e.g. 10μM of the dye and 0.1mM of polypeptide. The resultant spectral change of the dye was represented by the change of the A_{470}/A_{425} ratio with P/AMD (e.g. Fig. 5 for DNA and DNA-polypeptide complexes). Here the concentration of light scattering was corrected simply by subtracting the scattering of DNA-polypeptide complex from the observed absorbancy. Obviously, the plots for DNA-polypeptide complexes shift to the larger side of P/AMD, and from the extents of the shift the fractions of ineffective sites were calculated using essentially the same procedure as that in the case of acridine orange. The values obtained are summarized in Table 1 together with those obtained by acridine orange binding for comparison. Note that in all samples the fractions of ineffective sites are nearly equal for the two dyes, and therefore the incomplete binding of polyarginine would not be attributable to base specificity of the binding.

The presence of the remaining free Arg residues, suggested from the smaller fraction of ineffective dye binding site in DNA-polyarginine complex, was pursued using a fluorescent probe ANS, an acidic dye of which fluorescence around 515nm is markedly strengthened when adsorbed to polypeptides (12). Contrary to our expectation, the result

TABLE 1

The decrease of dye binding sites on DNA by binding with polypeptides

Sample	+/-	ineffective sites / total sites	
		acridine orange*	actinomycin D**
DNA-polylysine	0.10	0.10	
	0.20	0.19	0.04
	0.32	0.30	
	0.42	0.40	0.37
	0.50	0.48	
	0.58	0.55	0.50
DNA-polyarginine	0.10	0.06	
	0.20	0.12	0.06
	0.32	0.18	
	0.42	0.21	0.22
	0.50	0.21	
	0.58	0.37	0.39

*, The values were obtained from the plots in Fig. 4.
**, The samples for the experiment were prepared in the absence of urea.

showed that free Arg residues do not exist, at least, in a state as to interact with ANS molecules. The unbound Arg residues might be caught very close to DNA. Since the higher tendency of polyarginine to aggregate than polylysine was suggested from its larger turbidity at high ionic strength, one possible explanation for the lower effeciency in covering DNA phosphates of polyarginine may be that it results from the interaction between DNA and aggregated polypeptide chains at higher ionic strengths in the course of gradient dialysis. In fact it was suggested from our another melting experiment that Arg residues bind to phosphate groups with 1:1 stoichiometry when the complex is prepared by direct mixing at an ionic strength of 0.01, at which the polypeptide chain would be in stretched non-aggregated structure. This supposition, however, leave unexplained the observation that the presence of urea in preparation did not cause any change in the results.

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