Volume 2 number 6 June 1975 Nucleic Acids Research

Specifity of DNA-basic polypeptide interactions. Influence of neutral residues incorporated into polylysine and polyarginine.

Klaus Wehling, Hans-Adolf Arfmann, Karl-Heinz C.Standke+ and Karl G. Wagner

Gesellschaft für Molekularbiologische Forschung, D-3301 Stöckheim-Braunschweig, Abteilung Molekularbiologie, and +Forschungsanstalt für Landwirtschaft, D-3300 Braunschweig, GFR

Received 18 March 1975

ABSTRACT

An approach is described for evaluation of the specificity of basic polypeptides concerning the base pair composition of DNA. The polypeptides were covalently bound to CNBr activated agarose and two DNAs strongly different in base composition but of equal molecular weight were loaded and detached by a NaCl gradient. The difference in the NaCl concentrations between the elution maxima of the two DNAs was taken as a measure for the recognition specificity. - The results obtained confirmed the known AT- und GC-specificity of polylysine and polyarginine, respectively. Neutral residues incorporated into polylysine generally reduce the interaction affinity and also the AT-specificity of their host. This behavior is very pronounced with three homogeneous fractions of clupeine containing about one third of neutral aliphatic amino acids within clusters of arginine; the base pair specificity of these arginine copolymers was found to be practically nil.

INTRODUCTION

As early as 1955 Chargaff and his coworkers[1] began to use synthetic basic polyamino acids as models for studying the interaction of DNA with the basic proteins occurring "in vivo" in chromatin. In 1966 Leng and Felsenfeld[2] elaborated that simple polylysine interacts preferentially with AT-rich, whereas polyarginine favors GC-rich DNA. Since this time a large amount of work has been done to elucidate the features of this interaction (for review cf. [3,4]).

The investigations hitherto deal chiefly with the precipitation and the melting of polyamino acid-DNA complexes and circular dichroitic spectral observations. For the quantitative evaluation of the specificity an equilibrium method is required; this is difficult to perform with polymer-polymer interactions. As a possible approach the binding of DNA by basic polypeptides co-

valently fixed to agarose was investigated and the ionic strength required for the dissociation of the complex formed was taken as a measure of the coordinate binding strength. Using bacterial DNA of extremely different base composition, the degree of specificity can be expressed in terms of the ionic strength differences found for the maxima of the respective elution profiles, provided that DNAs of equal length are used. This method has its precursor in the basic polyamino acid-kieselgur columns applied for fractionation of different nucleic acids [5-8] In a similar manner as in the present work lysine-rich histone fractions were coupled to agarose for studying DNA-histone interaction [9,10]

MATERIALS AND METHODS

Poly(L-lysine) and the lysine copolymers containing different amounts of neutral aliphatic amino acids were synthesized as described earlier[11]. Poly(L-arginine) was purchased from Miles-Yeda Ltd. The apparent molecular weight of the polymers ranged between 40,000 and 90,000 daltons[11], while the manufactures of polyarginine reported 22,000 daltons.

Polypeptide Agarose was prepared using aliquots of a batch of CNBr activated sepharose from Pharmacia Fine Chemicals. After washing with 10^{-3} M HCl 2 g of Sepharose were added to 10 ml of a solution of 0.01 M Na_2HPO_4 (pH 8.5) and 0.5 M NaCl in which 20 mg of the basic polypeptide had been dissolved. The suspension was shaken at 4°C for 20 h, filtrated and washed with the above phosphate buffer. Analysis of the filtrates revealed that 85-100 % of the original polypeptides were coupled at these conditions. Thereafter the sepharose was treated with 1 M ethanolamine (pH 8) in order to block the unreacted active sites. The polypeptide-Sepharose was washed with 2.0 M NaCl, containing 0.1 M Na_2HPO_4 (pH 8) and 0.1 M sodium acetate (pH 4), respectively. A column bed of 1 x 8 cm was packed and equilibrated with the starting buffer.

DNA from Micrococcus lysodeicticus (ML) and Clostridium perfringens (CP) was obtained according to the procedure of Marmur[12]. Frozen ML-cells (strain M 1108/T4784) were purchased from Merck AG, Darmstadt and CP-cells (strain SR12) were harvested in the middle of the log-phase after

fermentation in a 50 l vessel.

In order to obtain fractions with about the same average molecular weight, the DNA was sonicated and fractionated by gel filtration on Sepharose 4 B. The original DNAs (not sonicated) revealed rather unsymmetric elution profiles with an appreciable fraction in the exclusion volume. The different DNAs were sonicated for different periods and the elution profiles on the agarose were recorded. Rather symmetric and comparable elution profiles were obtained, when ML-DNA was sonicated for 2x2.5 min and CP-DNA for 2x2 min at 40 W with the Branson sonifyer J17A in an ice-bath. Usually solutions containing 2 mg DNA in 10 ml of 1 M NaCl, 0.01 M sodium acetate and 1 mM EDTA pH 6.8 were sonicated, added to a 2.5x40 cm Sepharose 4 B column equilibrated with the same buffer and the DNA eluted with this buffer at 4° C, at a rate of 40 ml per hour. Fractions symmetrically located around the peak maximum were collected. The sedimentation coefficient and the hypochromicity in the above buffer were determined and the following results were obtained: ML-DNA 5.8_5 S and 23.5 %, CP-DNA 6.0_7S and 30.4 %.

The base composition of the DNA fractions was obtained from measuring the melting point in 0.15 M NaCl and 0.015 M sodium citrate of pH 7.0 according to the procedure of Marmur and Doty[24]. The T_m values obtained of 82° and 100° C correspond to 69 mole % AT for CP-DNA and 25 mole % for ML-DNA, respectively, when using the equation of Ref. 24. These data agree with those from other authors (cf. Ref. 23).

To analyse the DNA-basic polypeptide interaction, 1 ml of DNA (0.05 to 0.15 µ mol nucleotides), dialysed against the starting buffer, was added onto the preequilibrated polypeptide-Sepharose column. The elution was performed with linear NaCl gradients in 0.01 M sodium acetate pH 6.7 at 4° C, at a rate of 8.4 ml per hour; NaCl gradients 0.5-2.0 M were taken with the protamines and the copolymers, 1.2-2.5 M with polylysine and 1.5-3.5 M with polyarginine. Fractions (1.8 ml) were analysed for absorbance at 260 nm and for conductance, to determine the NaCl concentration; LKB Conductolyzer 5300 B was used.

RESULTS AND DISCUSSION

To ascertain that the agarose does not markably contribute to the basic polypeptide-DNA interaction, ethanolamine-Sepharose was produced under the same conditions as reported above. The DNA loaded with 0.5 M NaCl buffer was completely eluted without retention. Further we checked that the NaCl concentration at which the complex on the column was performed, has no influence upon the elution behavior of the DNA. Polylysine-Sepharose was mixed with ML-DNA at 2.5 M NaCl and slowly dialyzed to 1.2 M NaCl; then a column was prepared and the DNA eluted. There was no difference in the elution profile and its position relative to the experiment where DNA was directly added with the starting buffer.

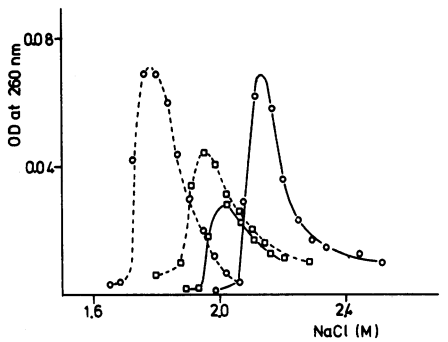

Figure 1: DNA base pair specificity of basic polyamino acids. The elution profiles of ML-DNA (o) and CP-DNA (□) obtained with Sepharose coated with polylysine (----) and polyarginine (——), respectively, are shown.

Fig. 1 shows the elution profiles of ML- and CP-DNA from polylysine- and polyarginine-Sepharose, respectively. With the polylysine column CP-DNA (69 mole % AT) was eluted at higher NaCl concentrations relative to ML-DNA (25 mole % AT), exhibiting the known AT-specificity of polylysine, whereas polyarginine reveals GC-specificity[2,8,13]. Fig. 1 also shows that with polyarginine-Sepharose the DNAs are eluted at higher NaCl concentration relative to polylysine-Sepharose; obviously the interaction affinity is stronger with polyarginine.

The advantage of the present method is that the interaction specificity of the basic polypeptides directed towards the DNA base pairs can be expressed in a semiquantitative manner. The differences in the maxima of the

Agarose coated with	elution maximum ML-DNA 25	CP-DNA 69 mole % AT	Δ_{AT}
	(M)		(M)
Polylysine	1.78	1.95	.17
Polyarginine	2.13	2.00_5	$-.12_5$
Poly(Ala^{10}, Lys^{90})	1.16	1.23	.07
Poly(Gly^{10}, Lys^{90})	1.14_5	1.24	$.09_5$
Poly(Nle^{10}, Lys^{90})	1.11_5	1.15	$.03_5$
Poly(Pro^{5}, Lys^{95})	1.13	1.19	.06
Clupeine Z	1.06_5	1.05_5	-.01
Clupeine YI	1.00_5	1.00	$-.00_5$
Clupeine YII	1.13	1.10_5	$-.02_5$
	1.22_5	1.23	$-.00_5$

Table 1: DNA base pair specificity of different basic polypeptides. The NaCl concentration of the elution maxima of two DNAs with strongly different base composition are listed; Δ_{AT} is obtained by subtracting the NaCl concentration of the ML-DNA maximum from that of the CP-DNA maximum.

elution profiles of the different DNAs defined as differences in the NaCl concentration scale are listed in Table 1; when negative this value means GC-specificity. A series of lysine copolymers containing neutral, aliphatic amino acid residues, like Gly, Ala,Nle and Pro, respectively, were also investigated and the results included in Table 1. In comparison to polylysine the neutral residues decrease the interaction affinity as a consequence of diluting the positive charges along the polymer. However, the interaction specificity is also markedly decreased; it decreases in the series Gly>Ala> Nle. This dependency is rather unexpectet; with polylysine the specificity has been correlated with hydrophobic interactions of the lysine side chain with the AT base pair surface [13,14], hence one should expect that hydrophobic norleucine side chain would favor AT specificity. However, the replacement of a lysine residue by norleucine obviously decreases the AT-specificity more strongly than a glycine residue.

Fig. 2 illustrates the effect of increasing amounts of alanine in a lysine copolymer upon the interaction affinity and specificity towards DNA. Whereas the interaction affinity decreases continuously after a steep decline at the beginning, the interaction specificity remains practically constant revealing a reduced AT-specificity. By precipitation experiments at different NaCl concentrations it was reported that the sequential polypeptides poly-L-(Lys-Ala), poly-L-(Ala-Lys-Pro) and poly-L-(Lys-Pro-Lys) reveal AT-specificity[15,16].

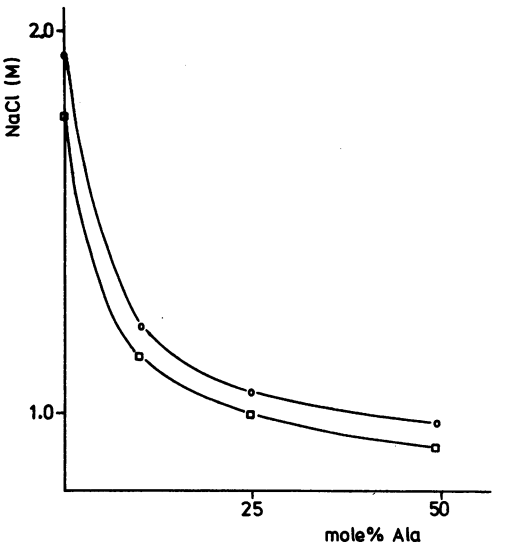

Figure 2: The influence of alanine residues incorporated into polylysine upon the interaction affinity with DNA. The NaCl concentration of the DNA elution maxima are plotted against the alanine content of Sepharose coated with lysine-alanine copolymers; ML-DNA (□) and CP-DNA (o).

The sperm-specific protamines obtained from several fishes represent copolymers of arginine containing about 30 % neutral, aliphatic amino acid residues, with the arginines mainly as clusters[17]. With the present method the three species found in clupeine from herring were investigated with respect to their interaction specificity towards DNA. Table 2 shows the results which indicate that the neutral amino acids, and probably also the size of the protamine, significantly decrease the interaction affinity towards DNA relative to polyarginine (Table 1). Furthermore the GC-specificity of polyarginine is almost completely suppressed with the protamines. This

illustrates their function to block the non-vegetative DNA in the sperm chromosomes in an unspecific manner. In contrast the lysine-rich histone KAP (F1) is reported to show AT-specificity[10,18,19], whereas the two arginine-rich histones were found to reside in situ at GC-rich regions of the DNA[20].

CONCLUSION

In the present work a procedure is developed for the estimation of the interaction affinity and specificity of DNA towards basic polypeptides. For comparing the interaction affinities of different polypeptides, the conditions of coupling of the polypeptides to Sepharose must be standardized. The polypeptide density on the Sepharose grains and the relative number of lysine residues reacted with CNBr activated centers should influence the affinity towards DNA. We therefore used aliquots of the same batch of CNBr-activated and stabilized Sepharose, and also applied the same Sepharose polypeptide concentration ratio. There may be also differences in the reaction rates for lysine and arginine residues, respectively. However, these factors should mainly influence the absolute interaction affinity but not the interaction specificity obtained from the relative affinities using the same column and different DNAs. On the other hand, interaction specificity is affected when DNAs of different average length are used; thus the DNA samples must be standardized with respect to this.

The origin of the base pair directed specificity of lysine and arginine residues, respectively, is unclear. One should keep in mind that this is a property of the native DNA surface, for with nucleotides, where the bases are accessible, both polyarginine and polylysine prefer guanine over adenine[21]. The higher affinity of DNA towards polyarginine relative to polylysine is in accord with other evidence but in contrast to the interpretation of the melting behavior of DNA-polyamino acid complexes (cf. Ref. 22 and citation herein).

Recognition specificities as observed between DNA and polylysine or polyarginine can certainly not explain the functional aspects of histone-DNA interactions. The great conservation of the amino acid sequences in histones are in favor of different functional implications. However model studies, as des-

cribed in the present work, are approaches to the great goal i. e. the understanding of how base sequences can be recognized by interaction of the DNA surface with specific proteins of which the lac-repressor is the classical example but which also exist in chromatin. The rather low level of specificity reached by the present protein models can certainly be elevated by introducing aromatic amino acids. Experiments in these lines are in progress.

ACKNOWLEDGEMENTS

We are very grateful to Dr. V. Wray for linguistic advice, to the government of the German Federal Republic (BCT 16) and the Deutsche Forschungsgemeinschaft (Wa 91/5) for grants, and our biotechnology department for fermentation of the Clostridium perfringens.

REFERENCES

1. Spitnik, P., Lipshitz, R. and Chargaff, E. (1955) J. Biol. Chem. 215, 765 - 775
2. Leng, M. and Felsenfeld, G. (1966) Proc. Nat. Acad. Sci. USA 56, 1325-1332
3. Yarus, M. (1969) Ann. Rev. Biochem. 38, 841-873
4. von Hippel, P.H. and McGhee, J.D. (1972) Ann. Rev. Biochem. 41, 231-300
5. Ayad, S.R. and Blamire, J. (1968) Biochem. Biophys. Res. Comm. 30, 207-212
6. Helleiner, C.W. (1969) Can. J. Biochem. 47, 1199-1202
7. Jawis, D., Loeser, R., Herrlich, P. and Röschenthaler, R. (1970) J. Chromatog. 52, 158-161
8. Helleiner, C.W. (1972) J. Chromatog. 72, 400-402
9. Ayad, S.R. and Parker, M.C. (1973) J. Chromatog. 82, 343-348
10. Panusz, H., Bartkowiak, J., Krzywiec, A., Joustra, M. and Plucienniczak, A. (1974) Nucleic Acids Res. 1, 1143-1151
11. Arfmann, H.-A., Labitzke, R., Lawaczeck, R. and Wagner, K. G. (1974) Biochimie 56, 53-60
12. Marmur, J. (1961) J. Mol. Biol. 3, 208-218
13. Shapiro, J.T., Leng, M. and Felsenfeld, G. (1969) Biochemistry 8, 3219-3232
14. Latt, S.A. and Sober, H.A. (1967) Biochemistry 6, 3307-3314
15. Privat, J.-P., Spach, G. and Leng, M. (1972) Eur. J. Biochem. 26, 90-95
16. Sponar, J.,Stokrova, S., Koruna, I. and Blaha, K. (1974) Coll. Czeck. Chem. Comm. 39, 1625-1645
17. Ando, T., Yamasaki, M. and Suzuki, K. (1973) Protamines, Springer-Verlag, Berlin, Heidelberg, New York
18. Sponar, J. and Sormova, Z. (1972) Eur. J. Biochem. 29, 99-103

19. Pluciennicak, A., Bartkowiak, J., Krzywiec, A., and Panusz, H. (1974) Nucleic Acids Res. 1, 1675-1690
20. Clark, R.J. and Felsenfeld, G. (1972) Nature New Biol. 240, 226-229
21. Wagner, K.G. and Arav, R. (1968) Biochemistry 7, 1771-1777
22. Wagner, K.G. and Arfmann, H.-A. (1974) Eur. J. Biochem. 46, 27-34
23. Handbook of Biochemistry (1970) Sober, H.A. ed., 2nd edn., The Chemical Rubber Co., Cleveland
24. Marmur, J. and Doty, P. (1962) J. Mol. Biol. 5, 109-118