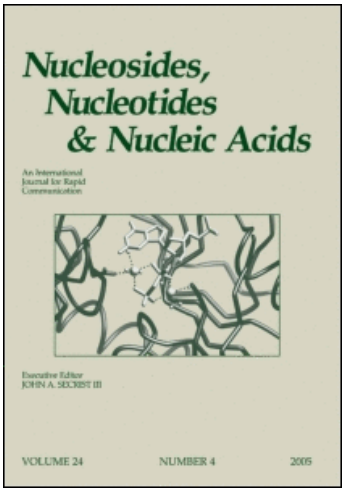

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Interaction of RKRK Tetrapeptide with Polynucleotides

Ajay Kumar[a]
[a] National Institute Of Immunology, New Delhi, INDIA

To cite this Article Kumar, Ajay(1995) 'Interaction of RKRK Tetrapeptide with Polynucleotides', Nucleosides, Nucleotides and Nucleic Acids, 14: 9, 1823 — 1829
To link to this Article: DOI: 10.1080/15257779508010706
URL: http://dx.doi.org/10.1080/15257779508010706

NUCLEOSIDES & NUCLEOTIDES, 14(9&10), 1823-1829 (1995)

INTERACTION OF RKRK TETRAPEPTIDE WITH POLYNUCLEOTIDES

AJAY KUMAR
National Institute Of Immunology,
Aruna Asaf Ali Marg,
New Delhi 110067, INDIA.

ABSTRACT : The interactions of RKRK tetrapeptide with polynucleotides using circular dichroism spectroscopy are described.

INTRODUCTION

The conformation of DNA depends on its sequence as well as on the counterions and other small molecules which interact with it. It is now becoming apparent that the predominant conformation of DNA in living cells is the B conformation. However, various other conformations of DNA (such as A , Z and C) have been observed under the influence of high salt (1) alcohol (2) and spermine (3) etc. It has been observed that the CD spectra of poly d(A-T). poly d(A-T) in the presence of $Co(NH_3)_6^{+}$, high concentration of CsF, alcohol and spermidine have a negative 275 nm band against a positive band around 262 nm in their absence (4-7). It has been pointed out by Vorlickova et.al. (8) that X-DNA is more readily induced in poly d(A-(Ethyl ^{5})-U).poly d(A-(Ethyl ^{5})-U) and especially poly d(A-(Butyl ^{5})-U).poly d (A-(Butly ^{5})-U) than in poly d(A-T).poly d(A-T) or poly d(A-U).poly d(A-U) . It has been shown that

poly d(A ^{2} Amino-T).poly d(A ^{2} Amino-T) also undergoes a transition from the B form to the unusual X form (9). A-X isomerization of poly (dA-dT) and B-X isomerization of poly (dA ^{2} Amino-dT) have been studied by fourth derivative spectrophotometry (10). The circular dichroism studies of a family of poly (dA-dU(Y^{5}))polynucleotides (Y= H, methyl, ethyl, propyl, butyl or pentyl) conducted in water- alcohol solution containing sodium or cesium counterions showed that the polynucleotides denatured or adopted A or X - DNA conformations depending on the concentration and type of the alcohol, type of counterions and the length of the aliphatic substituent in place of the thymine methyl group (11). Recently it has been demonstrated that poly (dI-dC) adopts X-DNA in concentrated CsF, but the transition is evidently incomplete (12). Vorlickova et.al. have recently shown that a halogen atom in place of the thymine methyl in poly (dA-dU (bromo)^{5}) and poly (dA-U(iodo)^{5}) hinders the isomerization into X-DNA (13). An understanding of the parameters affecting the conformation of nucleic acid is necessary to understand the function of nucleic acid in vivo. One such parameter is the side chain of the basic amino acid in the protein. One of the strongest and most obvious interactions between proteins and the DNA backbone is the electrostatic attraction between positively charged residues (e.g.lysine and arginine) and the negatively charged backbone phosphates (14, 15). Model studies with polycationic proteins such as polylysine and oligo/polyarginine usually feature a markedly higher duplex stability (16-19). It has also been shown that oligolysine molecules induce a B-X conformational transition in poly d(A-T).d(A-T) (20). The B-X transition in poly (dA-dT) has been observed by the binding of tetralysine (21). Protamines and protamine like protein (PLP) contain large number of lysine and arginine residues (22). Therefore in order to understand the effect of lysine and arginine repeats ,the interaction of RKRK tetrapeptide with the two polynucleotides using circular dichroic spectroscopy have been investigated. Under the influence of RKRK tetrapeptide, poly d(A-T).poly d(A-T) exhibits a transition from the B form to the unusual X form, whereas poly d(G-C).poly d(G-C) undergoes a B-C transition.

MATERIALS AND METHODS

Poly d(A-T).poly d(A-T), and poly d(G-C).poly d(G-C) were purchased from Pharmacia. Sodium chloride and EDTA-Na were from Merck (India). For Circular dichroism spectroscopy, sample at a polynucleotide-phosphate concentration of 37.5 μmole were used. The RKRK tetrapeptide used was synthesised by a solution phase method. The concentration of RKRK tetrapeptide was determined by amino acid analysis using norluceine as an internal standard. Circular dichroic specta were recorded at 22° C on a Jasco J-20 spectropolarimeter. Cells of 1.0 cm path length were used. All the CD spectra were recorded in 1 mM EDTA, 10 mM Tris, 100 mM NaCl solution (pH 7.5). Increasing amounts of RKRK tetrapeptide were added from its stock solution to the polynucleotide solution to give the desired peptide residue : nucleotide (P/N) ratios. The concentration of polynucleotide was kept constant. The solution was thoroughly stirred before recording the spectra. No precipitation was observed on addition of tetrapeptide to the polynucleotide solutions.

RESULTS AND DISCUSSION

CD spectra of poly d(A-T).d(A-T) in 1 mM EDTA, 10 mM Tris, 100 mM NaCl solution as a function of RKRK tetrapeptide concentrations are shown in Fig. 1. The CD spectrum of poly d(A-T).poly d(A-T) shows a positive peak at 269 nm and a negative peak at 242 nm, which is characteristic of the B conformation of DNA (16). On addition of RKRK tetrapeptide to poly d(A-T).poly d(A-T) (P/N= 0.02) the ellipticity of the positive band decreases to about one third and no other change take place. On further addition of tetrapeptide to poly d(A-T).poly d(A-T) (P/N=0.04) a small negative band at 280 nm along with a positive peak at 243 nm is observed. At (P/N =0.10) a negative peak at 268 nm and a more intense negative peak at 243 nm is observed. On further adition of tetrapeptide (P/N=0.20) the intensity of both the negative peaks is increased. At (P/N= 0.40) negative peaks at 245 nm and 268 nm were observed. This spectrum resembles the spectrum of X form of poly d(A-T).poly d(A-T) which has been observed in 3mM

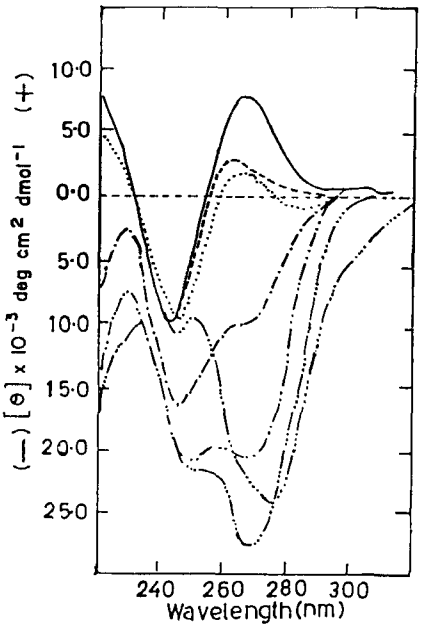

Fig.1 Circular dichroic spectra of poly d(A-T).poly d(A-T) (-) 1 mM EDTA, 10 mM , 10mM Tris, 100mM NaCl solution (pH 7.5) ;poly (A-T).poly d(A-T)-RKRK tetrapeptide complex at different peptide residue:nucleotide ratio (P/N), 0.02,(---);0.04,(....);0.10,(-·-);0.20,(-··-) ;0.40 (-···-); 0.60,(-····-).

CsCl-62% methanol (6). At P/N=0.60 a intense band at 278 nm, and a hump at 245 nm along with a tailing at 290 nm was observed. The tailing beyond 290 nm appears to be due to the aggregation in poly d(A-T).poly d(A-T). The interesting feature of RKRK- poly d(A-T).poly d(A-T) interaction is that the change in CD spectra brought about with increasing quantities of peptide showed no isodichroic point indicating that there is a continuous change in conformation and not a shift from an initial conformation to a final conformation.

CD spectra of poly d(G-C).poly d (G-C)-RKRK tetrapeptide complex in 1 mM EDTA, 10 mM Tris, 100 mM NaCl are shown in Fig.2. The CD spectrum of poly d(G-C).poly d(G-C) shows a positive peak at 272 nm and a negative peak at 250 nm, which is

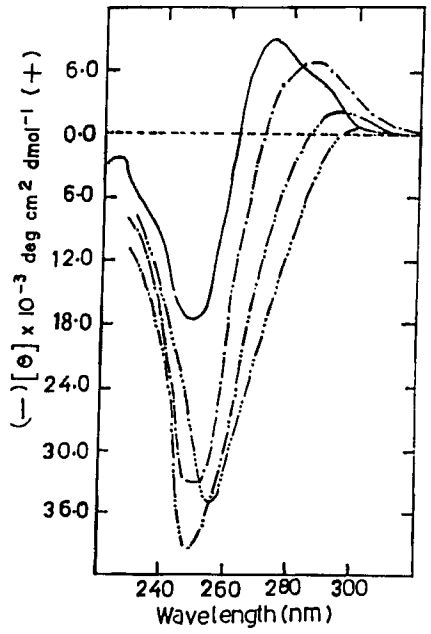

Fig.2 Circular dichoric spectra of poly d(G-C). poly d(G-C),(-), 1 mM EDTA, 10 mM Tris, 100 NaCl solution (pH 7.5),poly (G-C).poly d(G-C)-RKRK tetrapeptide complex at different peptide residue :nucleotide ratio (P/N) 0.2,(-•-);0.4,(-··-);0.66,(-•••-).

also a characteristic of the B-conformation of DNA. On addition of RKRK tetrapeptide (P/N=0.2) a long wavelength peak red shift from 272 nm to 286 nm is observed and the intensity of 250 nm negative peak is increased. At (P/N =0.4) a small hump at 293 nm and a negative peak at 250 nm with an increase in intensity is observed. This spectrum resemble the spectrum of poly d(G-C).poly d(G-C) obtained at 7.1 M CsCl or at 3.8 M LiCl (2) and may be attributed to the C form of DNA (2). Recently this type of change has also been observed in conformation of DNA on addition of $(Arg\text{-}Val\text{-}Gly)_n$ (23). On further addition of RKRK tetrapeptide the positive peak vanished and the negative peak red shifted from 250 nm to 255 nm. This change in the spectrum on further addition of the RKRK tetrapeptide may be due to the aggregation of poly d(G-C).poly d(G-C). This spectrum resembles the spectrum of T-7 DNA lysine rich histone (F-1) complex (24).

CONCLUSIONS

A comparision of the CD spectrum of the poly d(G-C).poly d(G-C) -RKRK complex and of the poly d(A-T).poly d(A-T)-RKRK complex at P/N =0.4 suggests that the mode of interaction of RKRK tetrapeptide are different with these polynucleotides.

REFERENCES

1. Pohl,F.M. and Jovin,T.M. (1972) J.Mol.Biol. **67** ,375.
2. Pohl,F.M.(1976) Nature **260**, 365.
3. Ivanov,I.V and Minyat, E.E. (1981) Nucleic Acids Res! **9**, 4783 .
4. Vorlickova, M.; Sklenar,V. and Kypr,J.(1983) J.Mol. Biol. **166**,85.
5. Zimmer,C.; Mark,C. and Guschlbauery, W. (1983) FEBS Lett. **154**, 156.
6. Vorlickova,M.;Minyat,E.E. and Kypr, J. (1984) Biopolymers **23**,1.
7. Thomas,T.J. and Bloomfield,V.A. (1985) Biopolymers **24**, 2185.
8. Vorlickova,M.; Kypr,J.;Sagi,J.;Laszlo,O. and Vladimir,S. (1987) Int. J.Biol. Macromol. **9**, 131.
9. Vorlickova,M.; Sagi,J.;Szaboics,A. and Szemzo,A. (1988) J.Biom. Stru. Dyn. **6**, 503.
10. Garriga, P.;Sagi,J.;Garcia-Quintana,D.; Sabes, M. and Manyosa,J. (1990) J.Biomol Stru. Dyn.**7**, 1061.
11. Vorlickova,M.;Sagi,J.;Hejtmankova,I. and Kypr,J. (1991) J.Biomol Structure Dyn. **9**, 571.
12. Vorlickova,M. and Sagi,J. (1991) Nucleic Acids Res. **19**, 2343.
13. Vorlickova,M.;Chladkova,J. and Kypr, J. (1992) Nucleic Acids Res. **20**, 1109.
14. Saenger,W.(1984) Principle of Nucleic Acids Structure, Springer Verlag, New York, Chap,18.
15. Ollis, D.L. and White, W.S. (1987) Chem Rev. **87**, 981.
16. Tsuboi,M. (1967) In Conformation of Biopolymers, Vol.2, Academic Press, New York, p682.
17. Von Hippel,P.H. and McGhee, J.D. (1972) Ann.Rev.Biochem.**41**, 231.
18. Helen,C.and Maurizot,J.C. (1981) VRC Crit Rev. Biochem. **10**,213.

19.Willmitzer,L. and Wagner,K.G. (1980) Biophy. Struct. Mech. 6, 95.

20.Kumar, G.S.;Atreyi, M.Rao,M.V.R. (1987) Current Science, **56**,163.

21.Atreyi,M.;Rao,M.V.R.; Kumar,G.S. and Kumar,S., 14th (1988) International Congress of Biochemistry, Prague, Abstract,Vol V, p 83 .

22.Libertini, L.J.; Ausio,J.; Holde,K.E.V. and Small,E.W. (1988) Biopolymers **27**, 1459.

23.Panou-Pomonis,E.; Sakarellos,C. and Sakarellos- Daitsiotis,M (1986) Eur.J. Biol. **57**,437 .

24.Donalt,E.O. and Ada,L.O.(1971) J.Mol.Biol. **57** ,43 .

Received March 15, 1994
Accepted July 21, 1995