Salt and Base Sequence Specific Changes in the Chiroptical Properties of DNA

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Abstract. Chiroptical properties of natural DNA molecules differing in base composition were studied in solutions with high concentrations of monovalent sodium and caesium salts. It was found that the properties were dependent on the DNA base sequence and nature of both cations and anions. A comparison with the behaviour of the synthetic molecules of DNA demonstrated that the salt-induced changes in the natural molecules of DNA could not be accounted for by the appearance of the left-handed Z conformation. On the other hand, the tendency of the alternating A—T sequence to assume the novel X—DNA conformation seems to play a role even in the conformational properties of natural DNA.

Key words: Circular dichroism — High-salt solutions — DNA conformation— Z-DNA — X-DNA

Introduction

The left-handed Z-DNA conformation (Wang et al. 1979) has attracted much attention in the past four years. It was first detected in solutions of a synthetic DNA poly (dG—dC) .poly (dG—dC) in the presence of high concentrations of sodium salts (Pohl and Jovin 1972). Since that time, Z-DNA has been found even at physiological conditions in some plasmids (Singleton et al. 1982), in chromosomes of several organisms (Nordheim et al. 1981) and in rat tissues (Morgenegg et al. 1983). In these objects Z-DNA is stabilized by supercoiling and/or binding of some proteins (Nordheim et al. 1982). Linear, naked molecules of natural DNA depleted of proteins were studied less. Nevertheless, its specific retention in nitrocellulose filters has been observed at high-salt concentrations (Kuhlein et al. 1980). This effect was intepreted in terms of a salt-induction of left-handed Z regions in the natural DNA. This finding is rather surprising because it is not likely that natural DNAs contain blocks with a strictly alternating dG—dC sequence longer then the cooperative unit in the B—Z transition, i.e. 25 base pairs (Ivanov and Minyat 1981). It was thus of interest to study the conformation of other synthetic molecules of DNA at high concentrations of various salts (Vorlíčková et al. 1980, 1982a, 1983; Kypr et al. 1981). Results of these studies

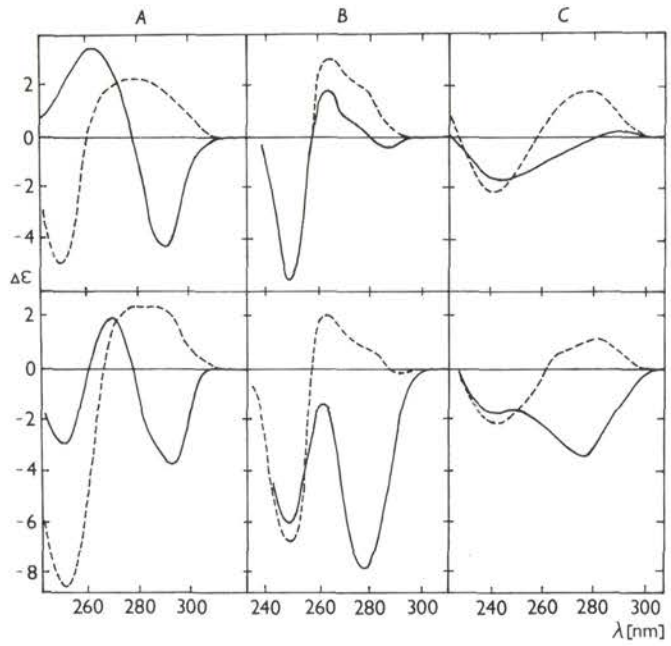

Fig. 1. CD spectral changes in the alternating purine-pyrimidine polynucleotides induced by high NaCl (*top*) and CsF (*bottom*) concentrations: – – – – low-salt. ———— high-salt. Solvent conditions: 0.05 mol/l sodium phosphate, pH 7, with the salt added:
A. poly(dG—dC).poly(dG—dC): 0.2 and 3.8 mol/l NaCl; 0.1 and 4.2 mol/l CsF.
B. poly(dA—dT).poly(dA—dT): 0.1 and 4.0 mol/l NaCl; 0.1 and 6.6 mol/l CsF.
C. poly(dA—dC).poly(dG—dT): 0.0 and 4.5 mol/l NaCl; 0.1 and 4.3 mol/l CsF.

significant for an interpretation of the data presented in this paper are summarized in Figure 1.

The transition of poly(dG—dC).poly(dG—dC) from the right-handed B to the left-handed Z form renders a complete inversion of the circular dichroic (CD) spectrum. The high concentrations of NaCl which induce the transition do not, however, substantially affect chiroptical properties of other alternating purine-pyrimidine polynucleotides poly(dA—dT).poly(dA—dT) and poly(dA—dC).poly(dG—dT). Unlike NaCl and many other salts, high concentrations of CaF not only transform poly(dG—dC).poly(dG—dC) to the Z form but also induce large changes in the chiroptical properties of poly(dA—dT).poly(dA—dT). The effect may originate in caesium cation specific alterations of DNA hydration (Vorlíčková et al. 1982b). The changes in the chiroptical properties reflect a transition of the polynucleotide to a novel conformation which was calléd X-DNA (Vorlíčková et al. 1983). It seems to differ from Z-DNA though NMR data indicate some

similarities in the overall architecture of both double helices (Kypr et al. 1981; Patel et al. 1981).

Remarkably, poly(dA—dC).poly(dG—dT) in which AT and GC pairs regularly alternate and bases are arranged in an alternating purine-pyrimidine sequence in both strands displays a CsF specific appearance of the negative long wavelength CD band like poly(dA—dT).poly(dA—dT). This implies that AT pairs in a certain sense control the behaviour of the GC pairs in the polynucleotide. Besides the CsF specificity poly(dA—dT).poly(dA—dT) and poly(dA—dC).poly(dG—dT) differ from poly(dG—dC).poly(dG—dC) in the kinetics of the salt-induced changes. While they are fast (their time course cannot be followed by CD) in the case of the two former polynucleotides it takes tens of minutes in NaCl (Pohl and Jovin 1972) and even hours in CsF (Vorlíčková, unpublished) for poly(dG—dC).poly(dG—dC) to attain equilibrium during the B—Z transition.

Unlike the alternating copolymers, homopolynucleotide duplexes poly (dA).poly(dT) and poly(dG).poly(dC) little change chiroptical properties with increasing salt concentrations and the changes do not significantly depend on the type of the salt employed (Pohl and Jovin 1972; Vorlíčková et al. 1980).

Here we report chiroptical properties of natural molecules of DNA from various sources at high concentrations of sodium and caesium salts. Our interest is mainly focussed on whether the Z or X conformation can be induced by the high-salt concentrations in linear molecules of natural DNA.

Materials and Methods

DNA from *Bacillus cereus* (33% GC) was isolated according to Marmur (1961). DNA from calf thymus (42% GC) and *Sarcina lutea* (72% GC) were gifts of Drs. E. Paleček and J. Boháček, respectively. The DNA preparations were characterized by differential UV absorbance melting curves thanks to the help of Dr. V. Kleinwächter.

CD messurements were carried out on Roussel Jouan, Model 185, and Jobin Yvon Mark III dichrographs. The CD spectra were taken in 1 cm path length cells at room temperature. Stock solutions of DNA (concentration 50—100 μg/ml) were prepared in 0.01 mol/l sodium acetate or 0.05 mol/l sodium phosphate, pH 7. Salt concentrations in the DNA solutions were increased by the addition of solid NaCl, $NaClO_4$ and CsCl or a 16 mol/l solution of CsF.

Results and Discussion

CD spectra of calf thymus DNA in solutions with high concentrations of monovalent sodium and caesium salts are presented in Figure 2. In accordance with the previous studies (Studdert et al. 1972; Ivanov et al. 1973; Zimmer and Luck 1974) high concentrations of all the salts brought about a depression of the long wavelength CD band while the short wevelength region was less affected. Note, however, the large quantitative differences in the long wavelength band depression at comparably high concentrations of the various salts. A comparison of the couples

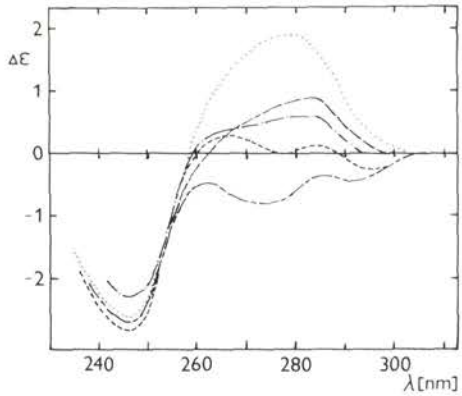

Fig. 2. CD spectra of calf thymus DNA in low- and high-salt solutions: .... 0.01 mol/l sodium acetate, pH 7, with the salt added: —.— 5.0 mol/l NaCl, — — — 7.0 mol/l $NaClO_4$, – – – – 6.0 mol/l CsCl, and — – — 5.6 mol/l CsF.

NaCl—$NaClO_4$, NaCl—CsCl, and CsCl—CsF shows that the nature of both the cations and anions plays a significant role.

Of the salts examined here and also those used in previous studies the largest changes were caused by CsF. This is illustrated in Figure 2 at 5.6 mol/l CsF when ellipticity of calf thymus DNA became negative in the whole wavelength range examined. Surprisingly, no more changes have been observed in the spectrum at higher concentrations of CsF as if conformational possibilities of the DNA were exhausted. It is interesting that the form V DNA (an arteficial molecule arising from annealing the single-stranded DNA rings of complementary sequence (Stettler et al. 1979) which has been found to bind Z—DNA specific antibodies (Pohl et al. 1982; Lang et al. 1982)) has the extreme negative ellipticity in the long wavelength part of the CD spectrum (Stettler et al. 1979; Pohl et al. 1982) comparable to that of calf thymus DNA in 5.6 mol/l CsF. A significant difference between the two CD spectra is in the exact position of the negative bands. The form V has the negative band located at 295 nm, i.e. like the Z form. Calf thymus DNA also has a local minimum of ellipticity at this wavelength but a global minimum is at 275 nm, i.e. at the negative band of the X form of poly(dA—dT).poly(dA—dT) (Figure 1).

The changes presented in Figure 2 cannot reflect a salt-induction of the left-handed Z conformation in, say, DNA regions with favourable sequences of bases for the following reasons: (i) After addition of any salt examined to the solution of calf thymus DNA no changes with time were observed in the CD spectra, implying that the induced conformational alterations are fast. This could not be the case if Z—DNA were formed. (ii) The ratio of the salt-induced changes

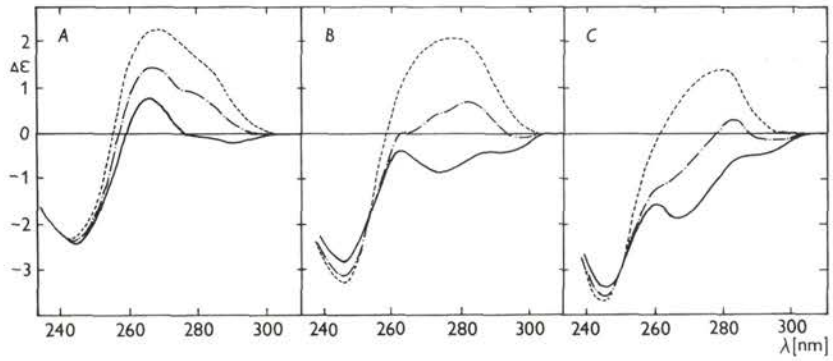

Fig. 3. CD spectral changes in natural DNA molecules with a different base composition induced by an increasing CsF concentration. Solvent conditions: 0.05 mol/l sodium phosphate, pH 7, 0.01 mmol/l EDTA, with the salt added: *A*. *Sarcina lutea* DNA (72 % GC): – – – 0.4, –.– 2.3, ——— 6.4 mol/l CsF; *B*. Calf thymus DNA (42 % GC): – – – 0.1, –.– 2.6, ——— 6.7 mol/l CsF; *C*. *Bacillus cereus* DNA (33 % GC): – – – 0.4, –.– 3.2, ———6.4 mol/l CsF.

in the short and long wavelength band was substantially smaller than expected if the long wavelength band were depressed as a consequence of the transition of certain regions in the DNA to the Z form. (iii) Among the salts examined here $NaClO_4$ transforms poly(dG—dC).poly(ddG—dC) to Z—DNA at the lowest concentration (the transition midpoint is at 1.8 mol/l). Then comes NaCl (2.5 mol-/l) and finally caesium salts (CsF 3.5 mol/l and CsCl 5.0 mol/l) (Pohl and Jovin 1972; Vorlíčková, unpublished). With calf thymus DNA the most pronounced changes were, on the contrary, observed with the caesium salts, especially CsF, then NaCl and finally $NaClO_4$ (Figure 2). Interestingly, the salts affect chiroptical properties of poly(dA—dT).poly(dA—dT) in the same order (Vorlíčková et al. 1980).

It was indicated several times above that AT pairs were responsible for the extreme effect of CsF on the chiroptical properties of natural DNA. To clarify this point we carried out an examination of the chiroptical properties of other two natural molecules of DNA, one with very high and the other with very low GC content, in the CsF solutions. The results are given in Figure 3 and show that the depth of the negative long wavelength CD band induced by high concentrations of CsF is becoming larger with the portion of AT pairs in the DNA. The result suggests that AT pairs and their inclination to assume the X form are really responsible for the strong dependence of the chiroptical proporties of natural DNA on the concentration of CsF. There are some more lines of indirect evidence supporting this suggestion: (i) Note the difference between the effect of high concentrations of CsCl and CsF on the chiroptical properties of calf thymus DNA

(Figure 2). A similar difference has also been observed with poly(dA—dT).poly-(dA—dT) in which CsF but not CsCl induces X—DNA (Vorlíčková et al. 1983). (ii) The transition of poly(dA—dT).poly(dA—dT) to X—DNA takes place within a relatively broad range of CsF concentrations. This indicates a low cooperativity of the transition, which implies that even a few base pairs with a favourable sequence can be transformed to the X form even if the rest of the molecule remained in B—DNA (Vorlíčková et al. 1983). Occassional GC pairs interrupting the alternating AT stretches would not restrict their unique conformational properties because poly(dA—dC).poly(dG—dT) displays a CsF specific conformational transition of a low cooperativity like poly(dA—dT).poly(dA—dT) (Vorlíčková et al. 1982).

The integral nature of the CD spectrum does not permit to draw an unambiguous conclusion concerning the manner by which the AT pairs affect the conformation of natural DNA in the concentrated solutions of CsF. More can say ^{31}P NMR in the spectrum of which X—DNA gives a diagnostic resonance (Vorlíčková et al. 1983). We performed ^{31}P NMR measurements of the AT rich Bacillus cereus DNA. The measurements were not, however, successful because DNA in high concentrations inevitable in NMR studies precipitated at high concentrations of CsF. Hence it is desirable to seek other probes that would help in clarifying the problem. Furthermore it is necessary to find ways to stabilize X—DNA in conditions that are closer to the physiological environment than the high concentrations of CsF. This would not only contribute to a better understanding of its physico-chemical properties but also make its biochemical and biological investigations possible.

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References

Ivanov V. I., Minchenkova L. E., Schyolkina A. K., Poletayev A. I. (1973): Different conformations of double-stranded nucleic acids as revealed by circular dichroism. Biopolymers **12,** 89—110

Ivanov V. I., Minyat E. E. (1981): The transitions between left- and right-handed forms of poly(dG—dC). Nucleic Acids Res. **9,** 4783—4798

Kuhlein U., Tsang S. S., Edwards J. (1980): Cooperative structural transition of PM2 DNA at high ionic strength and its dependence on DNA damages. Nature **287,** 363—364

Kypr J., Vorlíčková M., Buděšínský M., Sklenář V. (1981): Strange double helix of poly(dA—dT) in high-salt solution. Biochem. Biophys. Res. Commun. **99,** 1257—1264

Lang M. C., Malfoy B., Freund A. M., Daune M., Leng M. (1982): Visualization of Z sequences in form V of pBR 322 by immuno-electron microscopy. EMBO J. **1,** 1149—1153

Marmur J. (1961): A procedure for the isolation of deoxyribonucleic acid from Microorganisms. J. Mol. Biol. **3,** 208—218

Morgenegg G., Celio M. R., Malfoy B., Leng M., Kuenzle C. C. (1983): Z—DNA immunoreactivity in rat tissues. Nature **303,** 540—543

Nordheim A., Pardue M. L., Lafer E. M., Moller A., Stollar B. D., Rich A. (1981): Antibodies to left-handed Z—DNA bind to interband regions of *Drosophila* polytene chromosomes. Nature **294,** 417—422

Nordheim A., Tesser P., Azorin F., Kwon Y. H., Moller A., Rich A. (1982): Isolation of Drosophila proteins that bind selectively to Z—DNA. Proc. Nat. Acad. Sci. U.S.A. **79,** 7729—7733

Patel D. J., Kozlowski S. A., Suggs J. W., Cox S. D. (1981): Right-handed alternating DNA conformation: Poly(dA—dT) adopts the same dinucleotide repeat with caesium, tetraalkylammonium and 3alfa, 5beta, 17beta dipyrrolidinium steroid dimethiodide cations in aqueous solutions. Proc. Nat. Acad. Sci. U.S.A. **78,** 4063—4067

Pohl F. M., Jovin T. M. (1972): Salt-induced cooperative conformational change of a synthetic DNA: Equilibrium and kinetic studies with poly(dG—dC). J. Mol. Biol. **67,** 375—396

Pohl F. M., Thomae R., Di Capua E. (1982): Antibodies to Z—DNA interact with form V DNA. Nature **300,** 545—546

Singleton C. K., Klysik J., Stirdivant S. M., Wells R. D. (1982): Left-handed Z—DNA is induced by supercoiling in physiological ionic conditions. Nature **299,** 312—316

Stettler V. H., Weber H., Koller T., Weismann C. (1979): Preparation and characterization of form V DNA, the duplex DNA resulting from association of complementary, circular single-stranded DNA. J. Mol. Biol. **131,** 21—40

Studdert D. S., Patroni M., Davis R. C. (1972): Circular dichroism of DNA: Temperature and salt dependence. Biopolymers **11,** 761—779

Vorlíčková M., Kypr J., Kleinwächter V., Paleček E. (1980): Salt-induced conformational changes of poly(dA—dT). Nucleic Acids Res. **8,** 3965—3973

Vorlíčková M., Kypr J., Štokrová Š., Šponar J. (1982a): A Z-like form of poly(dA—dC).poly(dG—dT) in solution? Nucleic Acids Res. **10,** 1071—1080

Vorlíčková M., Sedláček P., Kypr J., Šponar J. (1982b): Conformational transitions of poly(dA—dT). poly(dA—dT) in ethanolic solutions. Nucleic Acids Res. **10,** 6969—6979

Vorlíčková M., Sklenář V., Kypr J. (1983): Salt-induced conformational transition of poly(dA—dT). poly(dA—dT). J. Mol. Biol. **166,** 85—92

Wang A. H. J., Quigley G. J., Kolpak F. J., Crawford J. L., van Boom J. H., van der Marel G., Rich A. (1979): Molecular structure of a left-handed double helical DNA fragment at atomic resolution. Nature **282,** 680—686

Zimmer C., Luck G. (1974): Conformation and reactivity of DNA. IV. Circular dichroism studies of salt-induced conformational changes of DNAs of different base composition. Biochim. Biophys. Acta **361,** 11—32

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