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DIFFERENT BINDING MODES OF SPERMINE TO A-T AND G-C BASE PAIRS MODULATE THE BENDING AND STIFFENING OF THE DNA DOUBLE HELIX.

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The study of the interactions between multivalent cations and DNA was undertaken several years ago in order to clarify the energetical contributions and mechanisms of DNA compaction into bacteriophage heads. A detailed knowledge of the interaction modes of DNA with the naturally occurring polyamines is of special importance. The large electrostatic repulsion between the DNA phosphates can be overcome by tri- and tetravalent cations leading to torus-shaped collapsed particules [1]. It has been shown that short DNA restriction fragments become spontaneously curved upon spermine binding, and we observed a loss of flexibility of the DNA chain upon spermine interaction [2-3]. In order to gain more insight into the mechanisms responsible for the bending and stiffening of the DNA chain, we have examined the interactions of spermine with synthetic polynucleotides containing different bases contents and sequences.

MATERIAL AND METHODS.

The synthetic polynucleotides poly(dG-dC), poly(dA-dT) and poly(dA).poly(dT) were obtained from Boehringer, dialysed twice against a buffer containing 1 mM EDTA, 1 mM sodium cacodylate, pH 6.5 and then twice against 1 mM sodium cacodylate, pH 6.5.

The second derivative spectra were recorded on a Perkin-Elmer lambda 5 spectrophotometer using a derivation interval of 4 nm.

All spectroscopic measurements were made at an absorbance of 1 except for bipolar pulses experiments (absorbance = 0.5). The results are expressed as a function of Sp/P, the spermine over mononucleotide molar ratio.

RESULTS.

1. U.V. Spectra and 2^d derivative U.V. spectra.

At low Sp/P ratios (<0.20), only very small absorption and turbidity changes occurred with the A-T containing polymers, while a red-shift of the absorbance maximum from 252.8 to 256.1 nm was already observed at low Sp/P ratios with poly (dG-dC).

More detailed information was obtained from second derivative spectra with very little turbidity perturbation. For each polymer, the U.V. band at 245-275 nm could be resolved into two (poly(dG-dC)) or three (the other polynucleotides) main contributions while several small transitions were detected at longer wavelengths. Among the three polynucleotides, only poly(dG-dC) displayed significant changes in the U.V. derivative spectra at Sp/P<0.18. Its U.V. band was resolved into two main contributions at 251 and 260 nm. Upon addition of spermine, a decrease of the 250 nm contribution, compared to the 260 nm one was observed, explaining the red-shift observed in the direct spectrum. At $0.22 < Sp/P < 0.25$ the reverse trend was observed and a higher spermine concentration aggregation was detected. The absorption modifications thus indicate that spermine interacts with the aromatic rings of the bases in poly(dG-dC), but not in the other polymers.

2. Electro-optical results.

Natural and synthetic DNAs are mainly oriented in electric fields by fast induced dipoles. Therefore they only display small transients when submitted to pulses of alternated polarity [1,3]. Upon addition of spermine, an important induction of a permanent dipole is generally observed at low Sp/P [1]. The A•T containing polynucleotides show a behaviour similar to that of calf thymus DNA [1], while with poly(dG-dC) almost no change in the orientation mechanism was observed. Thus, the binding of spermine to poly(dA-dT) and to poly(dA).poly(dT) is partially assymetric, while the binding of spermine to poly(dG-dC) did not give rise to a permanent dipole contribution. At higher Sp/P ratios the permanent dipole contribution disappeared as a consequence of condensation into toroidal particules for poly(dA)-poly(dT) and poly(dA-dT) [1,3].

The field-free decay of the dichroism allowed us to obtain information about the hydrodynamic behaviour of the polynucleotides. The relaxation times ($\bar{\tau}$) of both A-T containing polymers were reduced by the addition of the polyamine due to either inherent bending or to an increase of flexibility (thermal bending) even at low Sp/P ratios. On the contrary, poly(dG-dC) displayed a two-fold increase of the mean relaxation time at very low Sp/P (< 0.05), followed by a further increase to a plateau for $0.05 < Sp/P < 0.25$. The increase of $\bar{\tau}$ reflects a stiffening (loss of flexibility) of this polynucleotide when interacting with spermine. At Sp/P = 0.30, poly(dA-dT) collapsed into toroids producing a decrease of $\bar{\tau}$ while multimolecular aggregates were obtained for poly(dG-dC).

CONCLUSIONS.

Spermine binds to the bases of poly(dG-dC) and produces a stiffening of the molecule without induction of a permanent dipole. The stiffening of the DNA chain hinders the condensation into toroidal particles. No B-Z transition has been observed neither by C.D. nor by U.V. spectroscopy. Spermine does not affect the absorption characteristics of the A-T containing polynucleotides and binds assymetrically probably to the phosphates, inducing a permanent dipole. This interaction induces flexibility and/or bending of the double-helix leading to torus-shaped condensed particles.

Different binding modes of spermine to A.T and G.C base pairs thus produced different effects on the bendability and flexibility of the polynucleotides. This finding probably explains the dual effect of spermine on restriction fragments of natural DNA : bending [2,3] and stiffening [3] occuring simultaneously.

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