An A-Form of Poly(amino^{2}dA-dT)·Poly(amino^{2}dA-dT) Induced by Polyamines†

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ABSTRACT: The effect of the naturally occurring polyamines spermidine and spermine on poly(amino^2-dA-dT)·poly(amino^{2}dA-dT) conformation has been studied by UV, CD, and IR spectroscopies. It is shown that a conformational transition is induced in poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) by micromolar concentrations of the polyamines (30 μM) in low-salt aqueous solution. The analysis of our results, in view of previously published studies on conformational properties of the amino polynucleotide, indicates the resulting conformer to be an A-form. Interestingly, the polyamine concentration at the midpoint of the transition is the same in both cases. This provides further evidence that the coordination of positively charged counterions to DNA is determined largely from the DNA structure, probably with an important role for the sequence, and less from the nature of the ions.

The ubiquitous polyamines spermidine [$NH_2(CH_2)_3NH(CH_2)_4NH_2$] and spermine [$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] are structurally simple linear aliphatic compounds found in all living cells implicated in a variety of cellular reactions (Tabor & Tabor, 1984). These amines are found in millimolar concentrations in mammalian cells and may be implicated in the regulation of cellular growth and other processes, possibly by binding to and altering the conformation of DNA (Feuerstein et al., 1991). The interaction of polyamines with DNA may be crucial for polyamine regulation of normal growth; if the mechanisms of interaction were known in some detail, it might be possible to manipulate cell growth with polyamine analogs (Feuerstein et al., 1989). In view of the important biological role of polyamines, there is also a great concern to unravel these mechanisms to gain further insights in the effect of positively charged counterions in DNA conformation. Polyamines have also been shown to stabilize duplex DNA against thermal denaturation (Thomas & Bloomfield, 1984) and to convert synthetic polynucleotides from B-DNA to Z-DNA in a variety of cases (Behe & Felsenfeld, 1981; Chen et al., 1984; Rao et al., 1990). The interaction of polyamines with A-DNA has also been reported (Huse et al., 1978; Minyat et al., 1978; Jain et al., 1987).

Poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) has been the subject of recent studies undertaken to investigate the role of the amino exocyclic group in its conformational transitions. The conformation adopted by this polynucleotide in high-salt solutions has been the matter of some controversy. Previous studies proposed poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) in NaCl-containing solution (3.5 M) to be in a Z conformation (Howard et al., 1984). Later, NMR studies were interpreted as reflecting an A conformation (Borah et al., 1985, 1986). However, this interpretation was questioned by other authors who claimed that, in these conditions, the polynucleotide adopted the novel type of helix called X-DNA (Vorlickova et al., 1988a; Garriga et al., 1990), which could be stabilized with submillimolar concentrations of Mg^{2+}. Nevertheless, our latest FTIR[1] results, obtained with poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) in Mg^{2+}-containing solution and in films of low relative humidity (Garriga et al., 1992), are highly compatible with an A conformation for the polynucleotide, in agreement with the previously cited NMR studies (Borah et al., 1985, 1986).

We have used absorption and fourth-derivative UV (Garriga et al., 1990), FTIR, and CD spectroscopies to study the effect of spermine and spermidine on the conformational properties of poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT). At low ionic strength and neutral pH a conformational transition is induced with 30 μM spermine. Thermal denaturation of the spermine–polynucleotide complex is a cooperative process with a T_m of 94.5 °C. An analogous behavior is observed in the case of spermidine. The results reported show that spermine and spermidine induce the same conformational transition as previously obtained using Mg^{2+} and in dehydrated films (Garriga et al., 1992). The resulting conformer has the main characteristics of an A-form. The similar concentration of spermine and spermidine required to induce the conformational transition to the same concentration of polynucleotide would strengthen the current hypothesis that binding of positively charged counterions to DNA depends much more on the DNA structure and base sequence than on the nature of the ions. Our results provide further evidence that, in some cases, there is a more important contribution from a base-specific mechanism rather than from electrostatic effects.

MATERIALS AND METHODS

Materials. Poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) was synthesized and characterized as described in detail previously (Vorlickova et al., 1988a). Spermine tetrahydrochloride and spermidine trihydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The polynucleotide and the polyamines were dissolved in 0.6 mM potassium phosphate buffer, pH 7.4, containing 0.045 mM EDTA (phosphate buffer).

UV Spectroscopy. UV spectra were recorded on a Perkin-Elmer 320 spectrophotometer attached to a microcomputer

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[1] Abbreviations: UV, ultraviolet; CD, circular dichroism; IR, infrared; FTIR, Fourier-transform infrared; T_m, melting temperature; EDTA, ethylenediaminetetraacetate.

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to allow posterior treatment of the absorption spectra. Absorption spectra were averages of four scans performed to improve the signal-to-noise ratio to prevent the appearance of artifacts in the derivative spectra. Absorption spectra were smoothed and derived according to the standard least-squares method by Savitzky and Golay (1964). Melting experiments were performed at a rate of 1 °C/min.

Circular Dichroism. Circular dichroism spectra, denoted by $\Delta\epsilon$, were obtained on a Jasco J-720 spectropolarimeter. Spectra of the samples, measured in thermostated 1-cm path length cells, were the result of two runs collected in the 220–350-nm interval with a microprocessor attachment.

Infrared Spectroscopy. Infrared spectra were obtained with a Matson Polaris FTIR spectrometer, Michelson's interferometer type, equipped with a liquid N_2-cooled, high-sensitivity, wide-range, low-gain mercurium–cadmium–telluride detector. The instrument was constantly purged with a dry-air pump (dew point better than –60 °C). Polynucleotide samples in the phosphate buffer aqueous solution were placed in a cell with a path length of 25 μm. Usually 250 scans at a resolution of 2 cm^{-1} and with a datapoint each of 0.5 cm^{-1} were coadded to ensure a good signal-to-noise ratio. A shuttle was used to permit a block averaging collection (five background scans vs five sample scans) to minimize spectral contributions due to the residual atmospheric water vapor content of the pumped dry air. All spectra were recorded using a personal computer interfaced as data station.

Sample Preparation. Samples for UV and CD spectroscopy were prepared at a concentration ranging from $A_{262} = 0.5$ to 1.4. The molar concentration of the polynucleotide solutions was calculated using an extinction coefficient of $\epsilon_{262} = 5280$ M^{-1} cm^{-1}. Concentrated solutions of the polyamines in phosphate buffer were prepared so that the volume of these solutions added to the polynucleotide solution could be kept at a minimum (less than 10% of the total volume). In any case, spectra were corrected for dilution when necessary.

Samples for IR spectroscopy were prepared at higher concentrations (20 mM) than those for UV and CD experiments. The required amount of polyamine was added to the concentrated DNA solution to induce the conformational transition. The polyamine over nucleotide molar ratio was the same as that in the UV and CD experiments (polyamine/nucleotide = 0.25). All spectra were recorded at 25 °C.

RESULTS

The binding of spermidine and spermine to poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) produces significant changes in the UV absorption and fourth-derivative spectra. The absorption and fourth-derivative spectra of the polynucleotide in the potassium buffer in the absence of polyamines (Figure 1) are the same as previously reported (Garriga et al., 1990). The addition of 30 μM spermine to the polynucleotide solution induces a conformational change which produces slight hyperchromism in the 290–310-nm region and hypochromism at the absorption maximum located at 262 nm in the absorption spectrum (Figure 1A). Fourth-derivative spectra undergo small but significant changes very similar to those reported in the previous study, where the conformational change was induced by Mg^{2+} (Garriga et al., 1990). The main changes in the derivative spectrum are the change in the ratio of the two long-wavelength derivative peaks at 287.0 and 299.0 nm, respectively, and the redshift of the 299.0-nm peak to 300.0 nm (Figure 1B). The same spectra are obtained with spermidine (not shown). In addition, we have performed thermal denaturation of the spermine– and spermidine–

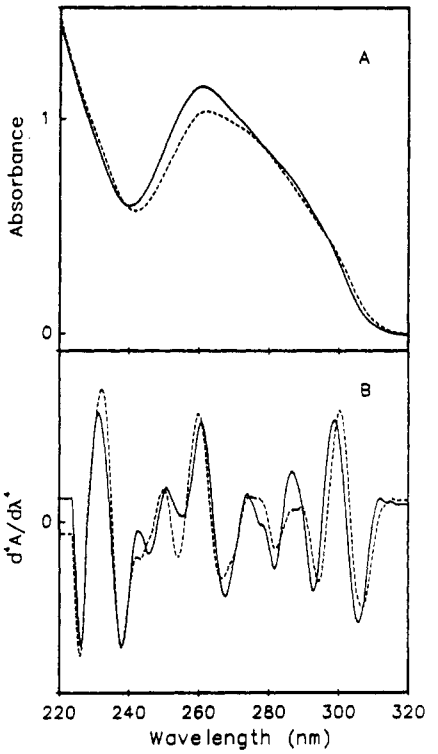

FIGURE 1: Absorption (A) and fourth-derivative (B) spectra of poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) (190 μM nucleotide) in 0.6 mM potassium phosphate, pH 7.4, containing 0.045 mM EDTA (—) and under the same conditions but with 30 μM spermine added (- - -).

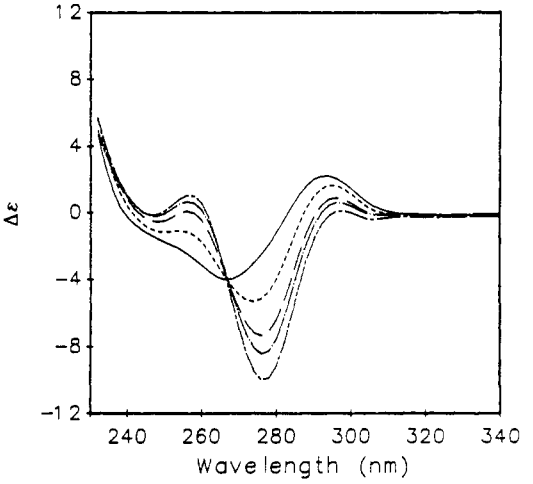

FIGURE 2: CD spectra of poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) (100 μM nucleotide) during its spermine-induced conformational transition. The polynucleotide in 0.6 mM potassium phosphate, pH 7.4, 0.045 EDTA was measured in the presence of the following concentrations of spermine: 0, —; 4 μM, - - -; 9 μM, – –; 17 μM, – · –; 30 μM, – – –.

polynucleotide complexes. The melting temperatures are 94.5 °C with spermine and 89.5 °C in the case of spermidine (polyamine over nucleotide molar ratio = 0.25). Absorption and fourth-derivative spectra of both denatured forms are nearly identical (not shown). The denaturation processes are reversible as judged by the identical absorption and fourth-derivative spectra obtained when the polynucleotide–polyamine complex was cooled back to 25 °C.

CD spectra reflect also the conformational transition clearly (Figure 2). The spermine-induced transition produces spectra identical to those obtained with NaCl (Howard et al., 1984; Vorlickova et al., 1988a) and with Mg^{2+} (Vorlickova et al., 1988b). Analogous results are obtained with spermidine. The

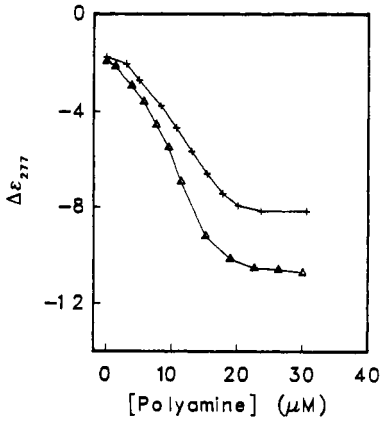

FIGURE 3: Changes in $\Delta\epsilon_{277}$ recorded during the polyamine-induced conformational transition. Δ, Spermine; +, spermidine. Solvent conditions were as in Figure 2. From the experimental results the maximum error in the $\Delta\epsilon_{277}$ values was determined to be within ±5%.

main change observed during the transition is the increase of the negative ellipticity at 277 nm. Note the isodichroic point at 266.5 nm, indicating that two distinct conformations of the polymer coexist in solution during the transition. A plot of the ellipticity at 277 nm vs polyamine concentration is shown in Figure 3. Interestingly, similar profiles are obtained for both spermine and spermidine. Changes are smaller in the case of spermidine, in an analogous way as reported previously for DNA (Minyat et al., 1978). The midpoint of the transition is at about 11 μM polyamine concentration in both cases. CD and UV spectra show no light scattering, indicating that there is no molecular aggregation as judged by the flat line—no tailing—observed above 320 nm in the CD spectra (Figure 2) and by the UV absorbance values above 320 nm (A = 0; Figure 1A).

Infrared spectra in H_2O, obtained as described under Materials and Methods, are presented in Figure 4. Interest was centered in the 1300–1100-cm^{-1} region corresponding mostly to the phosphate and deoxyribose vibrations. In this region, marker bands for C2′-endo/anti conformation of thymine, C3′-endo/anti conformation of adenine and A-form deoxyribose can be detected (Taillandier et al., 1987). In our case, the spectrum of poly(amino²dA-dT)·poly(amino²dA-dT) in aqueous phosphate buffer without polyamines (Figure 4A) shows the characteristic spectrum of B-form with bands at 1294 and 1281 cm^{-1}, the latter being associated to thymine in C2′-endo/anti conformation (Taillandier et al., 1987). The antisymmetric phosphate vibration ν_s PO_2^- is located at 1225 cm^{-1} with a shoulder at about 1211 cm^{-1}. In addition, there is a shoulder of relatively weak intensity at 1187 cm^{-1}. In view of this shoulder, we have previously suggested the presence of mixed sugar puckers with some of the sugar moieties in the C3′-endo/anti conformation (Garriga et al., 1992). This was proposed when our results were compared with those reported for poly(dA)·poly(dT) films at low relative humidity which were interpreted in terms of a heteronomous duplex structure (Taillandier et al., 1987). Our proposal is in accordance with the tendency shown by AT-containing copolymers to adopt mixed sugar puckers (Rao & Kollman, 1985). Upon addition of spermine to the polynucleotide solution, the spectrum undergoes significant changes (Figure 4B). The band at 1294 cm^{-1} has disappeared, while the phosphate band has been shifted to 1235 cm^{-1}. Furthermore, the band at 1281 cm^{-1} has been shifted to 1277 cm^{-1} and the shoulder at 1187 cm^{-1} is now a clear, definite band. The same results were obtained in a previous FTIR study of conformational changes induced in poly(amino²dA-dT)·poly(amino²dA-dT) by Mg^{2+} and by

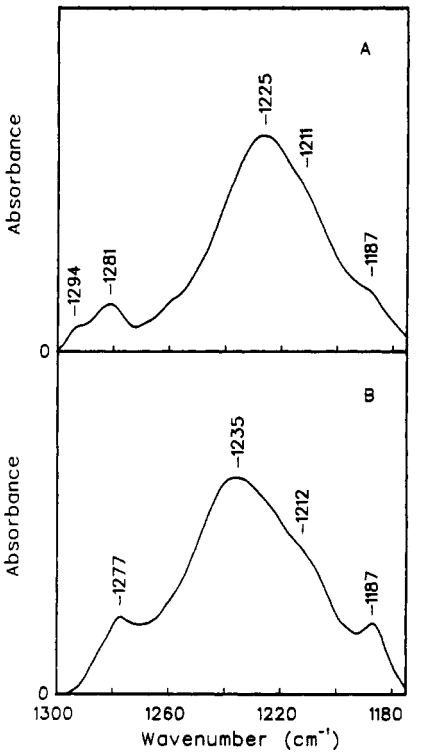

FIGURE 4: Infrared absorption spectra in the phosphate and deoxyribose vibration modes region of poly(amino²dA-dT)·poly(amino²dA-dT). (A) Polynucleotide in 0.6 mM potassium phosphate, pH 7.4, containing 0.045 mM EDTA; (B) same as (A) but in the presence of spermine (spermine over nucleotide molar ratio, S/P = 0.25). Nucleotide concentration was 20 mM.

dehydration (Garriga et al., 1992). The addition of spermidine to the polynucleotide solution produces the same spectrum of Figure 4B, indicating that the conformations obtained with both polyamines are the same.

DISCUSSION

The results presented in this paper show that spermine and spermidine induce a conformational transition in poly(amino²dA-dT)·poly(amino²dA-dT) in low ionic strength buffer (0.6 mM potassium phosphate, 0.045 mM EDTA, pH 7.4). This transition is characterized by changes in the absorption, fourth-derivative, CD, and FTIR spectra. Changes in absorption and fourth-derivative spectra are the same as those previously described (Borah et al., 1986; Garriga et al., 1990). CD spectra clearly show that the polynucleotide undergoes a two-state conformational transition upon binding of spermine and spermidine. CD spectra obtained, characterized by the formation of a negative band at 277 nm with an isodichroic point at 266.5 nm, are the same as previously reported (Howard et al., 1984; Vorlickova et al., 1988a,b). The transition is reversible, and the B-DNA spectra can be recovered upon addition of 0.1 M NaCl.

Let us now address which conformation of poly(amino²dA-dT)·poly(amino²dA-dT) is induced by polyamines. The effect of these polyamines in polynucleotide conformation has been previously described in a variety of cases; they induce the B–Z transition of different synthetic alternating polynucleotides such as poly(dG-m⁵dC)·poly(dG-m⁵dC) (Behe & Felsenfeld, 1981), poly(dG-dC)·poly(dG-dC) (Rao et al., 1990), and poly(dA-dC)·poly(dG-dT) (Thomas & Messner, 1986). In our case the UV absorption and CD spectra induced in poly(amino²dA-dT)·poly(amino²dA-dT) by polyamines are coincident with the spectra previously reported using Na^+

(Howard et al., 1984; Borah et al., 1986; Vorlickova et al., 1988a) and Mg^{2+} (Vorlickova et al., 1988b). It is noteworthy that some controversy has arisen about the involved conformation. Initially it was proposed to be a Z-form (Howard et al., 1984), but later NMR evidence brought the authors to propose that it was an A-form (Borah et al., 1985, 1986). Later, however, it was proposed that this conformation was the novel type of helix called X-DNA (Vorlickova et al., 1988a). This conclusion was reached by the authors upon comparison of circular dichroic spectra of the polynucleotide with those of its parent compound poly(dA-dT)·poly(dA-dT) (Vorlickova et al., 1988a). Recently, we have performed an absorption and fourth-derivative spectrophotometric study of the conformational transitions undergone by poly(dA-dT)·poly(dA-dT) and poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) (Garriga et al., 1990). The results reported in this fourth-derivative study brought us to postulate an X-form for poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) in Mg^{2+}-containing solution in view of the previously cited CD studies (Vorlickova et al., 1988a,b). Notwithstanding, our latest infrared results are highly consistent with an A-form for the polynucleotide in Mg^{2+}-containing solution and in dehydrated films (Garriga et al., 1992), in agreement with NMR studies (Borah et al., 1985, 1986). The infrared spectra of the polynucleotide in the absence and in the presence of spermine and spermidine obtained in the present study are the same as those recently obtained with Mg^{2+} and in dehydrated films (Garriga et al., 1992). Infrared spectra of the polynucleotide in the presence of polyamines are characterized by a band at 1277 cm^{-1} which can be assigned to the thymidine C3′-endo/anti conformation (Ghomi et al., 1990) and a band at 1187 cm^{-1} highly characteristic of A conformation (Adam et al., 1986; Ghomi et al., 1990) and assigned to a deoxyribose motion (Pohle & Fritzsche, 1980). This new A-DNA marker band located at around 1185 cm^{-1} is now well established and is used to characterize the conformation of DNA (Taillandier et al., 1985). In view of the reported data, we conclude that poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) undergoes a B to A transition in the presence of spermine and spermidine.

Our conclusion is feasible in view of previous studies on A-forms of polynucleotides induced by polyamines. An early CD study reported the induction of an A conformation in calf thymus and *Micrococcus luteus* DNA by spermine and spermidine in solution with analogous results as those reported in our present study (Minyat et al., 1978). Later, it was proposed that the preparation of polymers containing 2-aminoadenine instead of adenine would make it possible to convert them to the A-form readily (Drew & Dickerson, 1981). The role of the extra amino group disrupting the minor-groove hydration spine would be in the basis of the transition observed in our work. In fact, poly(dA-dT)·poly(dA-dT), i.e., the same polymer but lacking the 2-aminoadenine group, does not undergo any conformational transition to a distinct type of double helix upon addition of spermine (Marquet & Houssier, 1988).

More recent studies are also compatible with our assignment of the polyamine-induced conformation to A-DNA. Jain et al. have recently obtained an A-DNA octamer in the presence of spermine (Jain et al., 1987, 1989). In addition, cyanophage S-2L DNA (which contains 2-aminoadenine instead of adenine) has been shown to adopt an A conformation in the presence of millimolar concentrations of CsCl (Vorlickova et al., 1991). Furthermore, a recent X-ray diffraction study has shown that a sodium poly(amino^{2}dA-dT)·poly(amino^{2}dA-dT) fiber adopted an A conformation in the relative humidity interval between 44 and 98% (Konyukhov et al., 1992).

In our study, we have shown that both spermine and spermidine induce the conformational transition to the A-form with approximately the same concentration at neutral pH (see Figure 3). Moreover, the profiles obtained, although not identical, are quite similar in shape. These data point to the same mechanism of interaction for both spermine and spermidine. The differences observed in both curves (see Figure 3) and in particular the different value of $\Delta\epsilon$ at the plateau could be related to the formation of two different structures within the A-DNA family with somewhat different helical parameters (Jain et al., 1991).

With respect to the mechanism of interaction of polyamines with DNA, the first attempt of explanation was made, in the study of the condensation of DNA by these cationic ligands (Wilson & Bloomfield, 1979), using the counterion condensation theory as developed by Manning (1978). This theory treats counterions as point charges whose interactions depend only on the charge. According to this theory, it was demonstrated that spermine (with a +4 charge) collapses DNA at a lower concentration than spermidine due to its expected larger binding constant (Wilson & Bloomfield, 1979). The condensation counterion theory neglects specific ion and structural effects (Manning, 1978). However, structural effects have been reported (Stewart, 1988; Plum & Bloomfield, 1990; Plum et al., 1990). In addition, increasing evidence has been accumulating in the past years which has led to the certainty that it is incorrect to consider polyamines as just several cationic charges covalently linked together (Egli et al., 1991).

In the present work, if the mechanism of interaction between the polyamines and the amino polynucleotide was expected to implicate charge–charge interactions with the polynucleotide phosphates, as proposed, for example, in the pure-spermine form of Z-DNA recently reported (Egli et al., 1991)—and in view of the larger binding constant of spermine (Wilson & Bloomfield, 1979)—different concentrations of spermine and spermidine would be required to induce the transition. Interestingly, a surprising feature of the structural model proposed by Jain et al. (1989) is the lack of any direct interaction of the polyamine with the phosphate groups of DNA. Thus, a base-only binding mode of spermine to A-DNA, in the major groove, has been proposed via hydrogen bonding to the bases and water molecules. Furthermore, the authors state in this study that spermidine can bind in the same way as spermine (Jain et al., 1989). The same was proposed in an early CD study of B to A transition of DNA in solution (Minyat et al., 1978). Our results, which show a very similar ability of both spermine and spermidine in inducing the transition, would be compatible with the base-only binding mode in the case of A-DNA. Another interesting feature of the model by Jain et al., which could be present in our case, is the presence of extra Watson–Crick cross-strand hydrogen bonds between purine residues in adjacent base pairs. This kind of unusual cross-strand hydrogen bond has also been observed in a B-DNA decamer with a G·A mismatch (Privé et al., 1987). In our case, the possibility exists that a similar purine–purine cross-strand hydrogen bond could be formed. This could occur, in the minor groove, between the N2 hydrogen of a 2-aminoadenine base and N3 of the 2-aminoadenine in the adjoining base pair. Nevertheless, the flexibility of the interacting molecules allows the possibility of other base-specific modes of binding (Jain et al., 1989). A similar three-centered hydrogen bond, involving N2—from an amino group—and N3 of adjoining guanines, has been

recently found in the crystal structure of four-stranded *Oxitricha* telomeric DNA (Kang et al., 1992).

The presence of this kind of cross-strand hydrogen bonds would provide a possible mechanism by which spermine elevates the T_m of the DNA duplex. The T_m of poly(amino²-dA-dT)·poly(amino²dA-dT) in phosphate buffer is 47 °C (Garriga et al., 1990). In the presence of spermine and spermidine we have obtained T_m values significantly higher than that obtained when the transition was induced with Mg^{2+} (Garriga et al., 1990). The increased stability against denaturation could be explained by the specific binding mode of the polyamines and by the presence of the putative extra cross-strand hydrogen bonds proposed before.

The structure proposed might involve a geometrical arrangement with the 2-aminoadenine groups close to each other and evidences again the important role of the exocyclic amino groups in the minor groove in the conformational transitions of DNA. This observation would be in accordance with the proposed increase in the stacking interactions, and coupling of electronic transitions related to 2-amino adenines, described in our previous fourth-derivative spectrophotometric study (Garriga et al., 1990). It is known that there is increased overlap area between stacked base-pairs in the A-form when compared to the B-form, and a decrease in flexibility for A-DNA has been reported recently (Charney et al., 1991).

The possible biological significance of stabilization of the A-form by polyamines can be considered. It could play a role in the formation of DNA–RNA hybrids (Minyat et al., 1978; Jain et al., 1989). Furthermore, in those places where polyamines are present in vivo (in the phage heads, chromosomes, RNA polymerase–DNA complexes, etc.) the water activity is probably diminished (Minyat et al., 1978). This fact could make the A-form relevant in certain conditions in the cell. In our case, the dehydrating effect of the amino group in position 2 of adenine in the polynucleotide minor groove would be the basis of the conformational transitions observed in the present study and would be connected with the possible biological significance of the A conformation in vivo.

In summary, our results indicate that binding of spermine and spermidine to poly(amino²dA-dT)·poly(amino²dA-dT) induces and stabilizes the A-form of the polynucleotide. Furthermore, the results reported are in accordance with the proposal that the coordination of positively charged counterions to DNA is determined largely from its structure and sequence and less from the nature of the ions (Gessner et al., 1989).

REFERENCES

Adam, S., Bourtayre, P., Liquier, J., & Taillandier, E. (1986) *Nucleic Acids Res. 14*, 3501–3513.

Behe, M. J., & Felsenfeld, G. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 1619–1623.

Borah, B., Cohen, J. S., Howard, F. B., & Miles, H. T. (1985) *Biochemistry 24*, 7456–7462.

Borah, B., Howard, F. B., Miles, H. T., & Cohen, J. S. (1986) *Biochemistry 25*, 7464–7470.

Charney, E., Chen, H.-H., & Rau, D. C. (1991) *J. Biomol. Struct. Dyn. 9*, 353–362.

Chen, H. H., Behe, M. J., & Rau, D. C. (1984) *Nucleic Acids Res. 12*, 2381–2389.

Drew, H. R., & Dickerson, R. E. (1981) *J. Mol. Biol. 151*, 535–556.

Egli, M., Williams, L. D., Gao, Q., & Rich, A. (1991) *Biochemistry 30*, 11388–11402.

Feuerstein, B. G., Pattabiraman, N., & Marton, L. J. (1989) *Nucleic Acids Res. 17*, 6883–6892.

Feuerstein, B. G., Williams, L. D., Basu, H. S., & Marton, L. J. (1991) *J. Cell Biochem. 46*, 36–47.

Garriga, P., Sági, J., Garcia-Quintana, D., Sabés, M., & Manyosa, J. (1990) *J. Biomol. Struct. Dyn. 7*, 1061–1071.

Garriga, P., Sági, J., Garcia-Quintana D., & Manyosa, J. (1992) *J. Biomol. Struct. Dyn. 5*, 899–910.

Gessner, R. V., Frederick, C. A., Quigley, G. J., Rich, A., & Wang, A. H.-J. (1989) *J. Biol. Chem. 264*, 7921–7935.

Ghomi, M., Letellier, R., Liquier, J., & Taillandier, E. (1990) *Int. J. Biochem. 22*, 691–699.

Howard, F. B., Chen, C. W., Cohen, J. S., & Miles, H. T. (1984) *Biochem. Biophys. Res. Commun. 118*, 848–853.

Huse, Y., Mitsui, Y., Iitaka, Y., & Miyaki, K. (1978) *J. Mol. Biol. 122*, 43–53.

Jain, S., Zon, G., & Sundaralingam, M. (1987) *J. Mol. Biol. 197*, 141–145.

Jain, S., Zon, G., & Sundaralingam, M. (1989) *Biochemistry 28*, 2360–2364.

Jain, S., Zon, G., & Sundaralingam, M. (1991) *Biochemistry 30*, 3567–3576.

Kang, C., Zhang, X., Ratliff, R., Moyzis, R., & Rich, A. (1992) *Nature 356*, 126–131.

Konyukhov, D. A., Volkova, L. I., Alexeev, D. G., Akimenko, N. M., Sagi, J., Vorlickova, M., Kypr, J., & Skuratovskii, I. Y. (1992) in *DNA Structure and Protein Recognition* (Klug, A., & Subirana, J. A., Eds.) p 92, Centre for International Meetings on Biology, Madrid.

Manning, G. S. (1978) *Q. Rev. Biophys. 11*, 179–246.

Marquet, R., & Houssier, C. (1988) *J. Biomol. Struct. Dyn. 6*, 235–246.

Minyat, E. E., Ivanov, V. I., Kritzyn, A. M., Minchenkova, L. E., & Schyolkina, A. K. (1978) *J. Mol. Biol. 128*, 397–409.

Plum, G. E., & Bloomfield, V. A. (1990) *Biopolymers 29*, 13–27.

Plum, G. E., Arscott, P. G., & Bloomfield, V. A. (1990) *Biopolymers 30*, 631–643.

Pohle, W., & Fritzsche, H. (1980) *Nucleic Acids Res. 8*, 2527–2535.

Privé, G. G., Heinemann, U., Chandrasegaran, S., Kan, L. S., Kopka, M. L., & Dickerson, R. E. (1987) *Science 238*, 498–504.

Rao, M. V. R., Atreyi, M., & Saxena, S. (1990) *Biopolymers 29*, 1495–1497.

Rao, S. N., & Kollman, P. (1985) *J. Am. Chem. Soc. 107*, 1611–1617.

Savitzky, A., & Golay, M. J. E. (1964) *Anal. Chem. 36*, 1627–1639.

Stewart, K. D. (1988) *Biochem. Biophys. Res. Commun. 152*, 1441–1446.

Tabor, C. W., & Tabor, H. (1984) *Annu. Rev. Biochem. 53*, 749–790.

Taillandier, E., Liquier, J., & Taboury, J. (1985) *Adv. Infrared Raman Spectrosc. 12*, 65–114.

Taillandier, E., Ridoux, J. P., Liquier, J., Leupin, W., Denny, W. A., Wang, Y., Thomas, G. A., & Peticolas, W. L. (1987) *Biochemistry 26*, 3361–3368.

Thomas, T. J., & Bloomfield, V. A. (1984) *Biopolymers 23*, 1295–1306.

Thomas, T. J., & Messner, R. P. (1986) *Nucleic Acids Res. 14*, 6721–6733.

Vorlickova, M., Sagi, J., Szabolcs, A., Szemzo, A., Otvos, L., & Kypr, J. (1988a) *Nucleic Acids Res. 16*, 279–289.

Vorlickova, M., Sagi, J., Szabolcs, A., Szemzo, A., Otvos, L., & Kyrp, J. (1988b) *J. Biomol. Struct. Dyn. 6*, 503–510.

Vorlickova, M., Khudyakov, I. Y., Hejtmankova, I., & Kypr, J. (1991) *J. Biomol. Struct. Dyn. 9*, 81–85.

Wilson, R. W., & Bloomfield, V. A. (1979) *Biochemistry 18*, 2192–2196.