Short communication

Two Distinct Conformers Coexist in a Synthetic DNA Poly(dA-dT). Poly(dA-dT) in Low-Salt Aqueous Solution

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Circular dichroism spectroscopy (CD) sensitively reflects even slight alterations of the base stacking geometry in DNA (Tinoco et al. 1980; Johnson et al. 1981). However, the alterations cannot be unambiguously interpreted in conformational terms so that, for example, the question concerning the origin of the relatively extensive temperature-induced changes in the CD spectrum of a synthetic DNA poly(dA-dT) . poly(dA-dT) still remains unanswered (Gennis and Cantor 1972; Studdert et al. 1972; Brahms et al. 1976). These changes are interesting from the biological point of view because some proteins participating in gene expression cause the same changes in the CD spectrum of poly(dA-dT) . poly(dA-dT) as do temperature alterations (Shimer et al. 1988; Schnarr and Daune 1984). We report two new results relevant to this problem, obtained from comparative CD analysis of a number of poly(dA-dT) . poly(dA-dT) analogs.

A careful analysis of the CD spectra of poly(dA-dT) . poly(dA-dT) recorded at different temperatures suggests that they intersect in isodichroic points located at about 241 and 278.5 nm (Fig. 1). This possibility is interesting because isodichroic points point to the presence of two distinct species in solution (Kypr and Vorlíčková 1986), in this case two polynucleotide conformers. The temperature-induced changes are fast, completely reversible, and the isodichroic points slightly shift if NaCl concentration in the polynucleotide solution is increased (not shown). We synthesized a number of poly(dA-dT) . poly(dA-dT) analogs differing from the parent polynucleotide by the base pair exocyclic substituents, and examined the temperature dependences of their CD spectra. Figure 1 shows CD spectra of poly(dA-ethyl^{5}dU) . poly(dA-ethyl^{5}dU) obtained at different temperatures. In this case, the existence of the insodichroic points (at 224 and 272 nm) is even more evident than with poly(dA-dT) . poly(dA-dT). We conclude from these experiments that two distinct B-DNA type conformers with slightly different base stacking geometries coexist in poly(dA-dT) . poly (dA-dT) and poly(dA-ethyl^{5}dU) . poly(dA-ethyl^{5}dU) and that the relative sta-

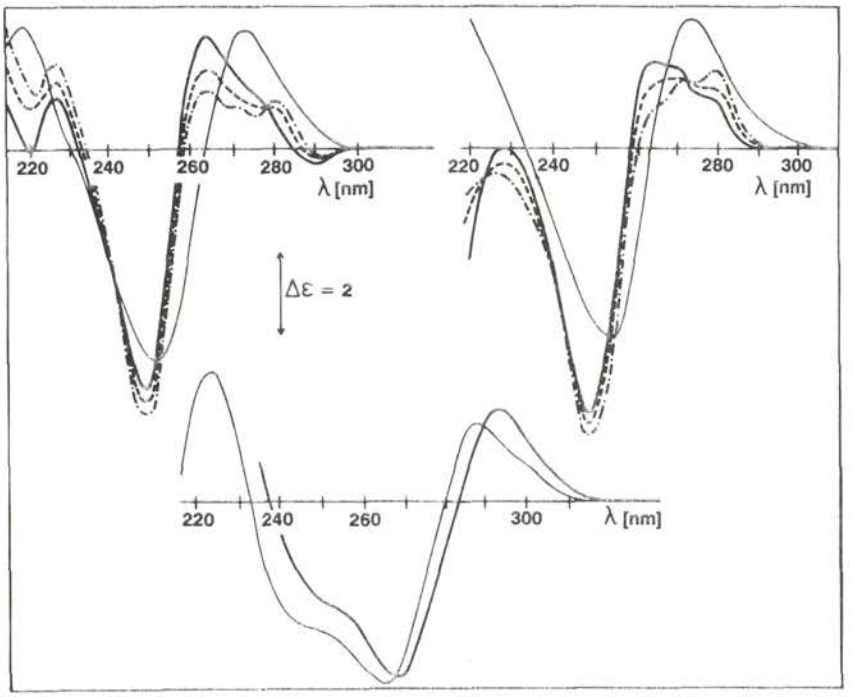

Fig. 1. CD spectra of (*top left*) poly(dA-dT), (*top right*) poly(dA-ethyl^{5}dU) and (*bottom*) poly(amino^{2}dA-dT). The spectra presented as thin lines correspond to single-stranded polynucleotides. The other spectra were recorded at various temperatures prior to denaturation. Poly(dA-dT) was measured in 0.6 mmol l potassium phosphate, pH 6.8 and 0.03 mol/l EDTA at – · – 1, – – – 8, ——— 22.5, and ——— 38 °C. Poly(dA-ethyl^{5}dU) was in 0.02 mmol/l sodium acetate, pH 6.8 in the presence of 0.01 mmol l EDTA at – . – 3.5, – – – 16.5, ——— 27 and ——— 41 °C. Poly(amino^{2}dA-dT) was dissolved in 0.6 mmol l potassium phosphate, pH 6.8, 0.03 mmol l EDTA, ——— from 4 to 32 °C, ——— 48 °C. The measurements were performed using a Jobin-Yvon dichrograph Mark IV in 1 cm pathlength cells placed in a thermostatted holder. The polynucleotides used in this study were synthesized, purified and characterized as described previously (Sági et al. 1977; Vorlíčková et al. 1988).

bilities of these two conformers are delicately controlled by temperature. Coexistence of two slightly different B-DNA conformations in poly(dA-dT). .poly(dA-dT) has also been indicated by 2D NMR studies (Assa-Munt and Kearns 1984).

Further, we tried to identify the base pair exocyclic groups responsible for the temperature-controlled flexibility in poly(dA-dT). poly(dA-dT). In this respect aliphatic substituents in place of the thymine methyl group, which is located

in the double helix major groove, did not much influence the extent of the temperature-induced changes in the CD spectra. In contrast, the changes were totally eliminated in the case of poly(amino^{2}dA-dT) . poly(amino^{2}dA-dT), which differs from poly(dA-dT) . poly(dA-dT) by an extra amino group attached to adenine from the minor groove side. The only temperature-induced changes observed in the CD spectrum of poly(amino^{2}dA-dT) . poly(amino^{2}dA-dT) were those connected with the polynucleotide duplex melting (Fig. 1).

The extra amino group attached to adenine adds a hydrogen bond between the complementary bases and increases the duplex thermal stability (Howard and Miles 1984). However, the inherently low thermal stability of poly(dA-dT) . poly(dA-dT) does not explain its conformational bistability since the temperature-induced changes in the CD spectrum do not become smaller at higher ionic strength stabilizing the duplex (Gennis and Cantor 1972; our data, not shown). Besides the stabilization effect, the extra amino group of amino^{2}dA may dehydrate the double helix minor groove (Drew and Dickerson 1981). A close relationship to hydration of the temperature-induced changes in the CD spectra of poly(dA-dT) . poly(dA-dT) has also been indicated by their dependence on the hydration enthalpy of cations present in the polynucleotide solution (Studdert et al. 1972). Thus it is likely that temperature controls minor groove hydration and that the changing hydration switches between two conformers in poly (dA-dT) . poly(dA-dT).

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