Fluctuations in Nucleic Acid Conformations. 2. Raman Spectroscopic Evidence of Varying Ring Pucker in A-T Polynucleotides

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Abstract: The Raman spectra of double-helical complexes of polydeoxyribonucleotides containing thymidine and adenine have been obtained in aqueous solution at pH 7 and ordinary salt concentrations (0.1–0.2 M NaCl). Both of the double helices, poly(dA)·poly(dT) and poly(dA–dT)·poly(dA–dT), exhibit the normal sugar phosphate Raman vibration of 835 ± 5 cm^{-1} that is characteristic of B-genus DNA. However, at 5 °C or lower the double helices, particularly the poly(dA)·poly(dT), exhibit a measurably strong band at 813–816 cm^{-1} indicative of the C3′-endo furanose ring conformation. At room temperature or slightly above, but far below the double-helical melting point, the 816-cm^{-1} band melts out and only the usual B-genus band remains. Ultraviolet absorption spectra obtained on the same sample show no measurable change in absorption over this temperature range nor is there any measurable change in the intensities or frequencies of the Raman vibrations from the adenine or thymidine rings. From this we conclude that at lower temperatures in aqueous solution there is a repuckering of some of the furanose rings with no change in the base orientation. Poly(dT)·poly(dA)·poly(dT) has also been prepared and shows some Raman bands of anomalous frequency in the sugar–phosphate stretching region. However, these do not fall in the normal range for the A genus and seem to be indicative of differing forms of B-genus DNA.

Nucleic acid polymers with alternating purine–pyrimidine sequences under certain conditions possess secondary structures that differ from the secondary structure of B-DNA.[1-5] In particular poly(dA-dT)·poly(dA-dT) fibers give rise to an eightfold helical structure designated as the "D" form. Recently, the deoxytetramer $(dA\text{-}dT)_2$ has been shown to have alternating C3′-endo–C2′-endo puckered furanose rings by X-ray diffraction studies on single crystals.[5] This finding of furanose conformational heterogeneity has led to the interesting hypothesis that alternating dA-dT polymers in solution will possess alternating C3′-endo–C2′-endo puckered furanose rings.[6] In order to examine this hypothesis in detail we have undertaken a study of the Raman spectra of both poly(dA)·poly(dT) and poly(dA-dT)·poly(dA-dT) as a function of salt concentration and temperature to see if evidence of both furanose ring conformations could be obtained. In this paper, we will not discuss the occurrence of the Z form of alternating poly(dG-dC) polymers under conditions of high salt as the Raman spectra of these materials has already been discussed in detail by Thamann et al.[7]

The original assignments of the Raman marker bands for A-, B-, and C-type DNA[8] has now been verified in several laboratories by a direct comparison of X-ray diffraction patterns and Raman bands from nucleic acid fibers.[9,10] It is now well-known that a sharp intense band in the 805–816-cm^{-1} region is indicative of the A-type conformation while a weaker, more diffuse band at 835 cm^{-1} is characteristic of B-type DNA.[9,10] The conformational sensitivity of these nucleic acid backbone vibrations appear to arise from differences in the A- and B-type puckered furanose rings. A band in the 805–816-cm^{-1} region is associated with the existence of an A-form, C3′-endo puckered ring. The diffuse 835-cm^{-1} band is characteristic of the C2′-endo or B-type furanose conformation. Recent measurements in this laboratory have further confirmed this interpretation by showing that the Raman spectrum of crystals of the dinucleotides GpC and UpA, which contain only the C3′-endo furanose rings, show only the A-genus Raman marker band while the Raman spectrum of crystals of the dinucleotide pTpT, which contains only C2′-endo furanose rings, shows only the B-genus Raman marker band. The conformation of the sugar rings has been established from X-ray diffraction measurements.[11,12] Thus the correlation between Raman marker band frequencies and furanose conformation appears well established.

Though polymers with alternating dA-dT sequences possess secondary structures of a somewhat different nature than that of B-type DNA, the Raman spectrum of double-helical poly(dA-dT) poly(dA-dT) in solution at low salt concentration taken in this laboratory almost 10 years ago showed only the normal B-genus DNA band slightly shifted from 835 to 841 cm^{-1}.[13] The absence of even a weak band in the 805–816-cm^{-1} region indicated that a clearly defined C3′-endo ring conformation is not present at room temperature and ordinary salt conditions. On the other hand, the Raman spectra of poly(dA)·poly(dT) obtained about 6 years ago in this laboratory[14] revealed the simultaneous existence of both C2′-endo and C3′-endo Raman marker bands.

In view of the recent interest in the secondary structure of deoxyribonucleic acids with a high dA-dT content we have repeated and extended these earlier Raman results by obtaining the Raman spectra of both poly(dA)·poly(dT) and poly(dA-dT)·poly(dA-dT) at various salt concentrations and temperatures. The triple-helix poly(dT)·poly(dA)·poly(dT) has also been studied to obtain a more complete description of A-T-rich DNA polymers.

Methods and Materials

Single-stranded poly(dA) and poly(dT) as well as double-helical poly(dA)·poly(dT) were purchased from several sources (Sigma Chemical, Calbiochem, Collaborative Research, and P.L. Biochemicals). The double-helical poly(dA)·poly(dT) polymers were used as obtained from the manufacturers. This double helix was also prepared by the methods of LaFleur et al.[15]

Samples of double-helical poly(dA)·poly(dT) from the various sources were prepared for Raman investigation by dissolving a 5 OD unit portion

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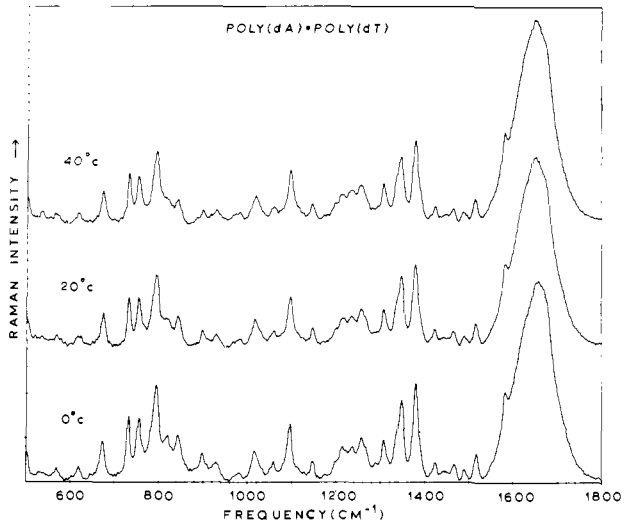

Figure 1. Raman spectra of a pH 7.0 aqueous solution of double-helical poly(dA)·poly(dT) containing 2.7×10^{-2} M polymer phosphate and 0.1 M NaCl; spectrum taken with 300 mW of a 514.5-nm argon laser line.

of the polymer in 30 μL of buffer, which contained either 0.1 M or 0.2 M NaCl solution as well as 0.02 M sodium cacodylate at pH 7.0. All samples of double-helical poly(dA)·poly(dT) were annealed at 40 °C for 5 min prior to Raman investigation. Subsequent to annealing, the samples were allowed to return to room temperature by slow cooling. Equilibration of the samples for Raman investigation at low temperatures was accomplished by slowly cooling the sample contained within a glass capillary tube to the desired temperature at a rate of approximately 0.3 °C per minute.

In order to obtain Raman spectra and ultraviolet absorption spectra on exactly the same sample of poly(dA)·poly(dT), a double-helical sample of 50 OD units was dissolved in 300 μL of 0.1 M NaCl and 0.02 M cacodylate, pH 7, buffer. The Raman spectra and the ultraviolet absorption spectra of this solution were recorded as a function of the temperature. The UV absorption of the polymer at 260 nm was obtained by using special quartz cells with a path length of 0.005 cm. The path length of the cell was calibrated with a 8.4×10^{-3} M solution of adenosine monophosphate. The ultraviolet melting profiles were obtained by monitoring the absorbance at 260 nm as a function of the temperature. The absorbance measurements were made with a Gilford Instruments Model 2000 multiple-sample absorbance recorder attached to a Beckman Model DU recording quartz spectrometer. The apparatus allows for simultaneous measurement of the sample temperature and absorbance. Data points were acquired every 5 s and the temperature was incremented at a rate of 0.6 °C/min. Dilute polymer melting profiles were obtained by using 1-mm path-length cells. Raman spectra of poly(dA)·poly(dT) at temperatures above its double-helical melting point at 72 °C were not obtained because of the occurrence of sample decomposition in the laser beam at temperature above 60 °C.

The triple-helical poly(dT)·poly(dA)·poly(dT) was prepared by the method of Arnott et al.[2] and dissolved in 0.18 M NaCl, pH 7.0, at a concentration of about 20 μg/μL.

The Raman spectra were obtained by standard multiscan techniques using a Varian 620i computer. In order to keep the concentration in the range accessible to optical absorption measurements, it was necessary to use somewhat lower concentrations of polynucleotides than is customary for classical Raman measurements. This in turn required a fairly large number of computer-controlled multiscans. Typically, the Raman spectra that required 10–20 multiscans were obtained by using 300 mW of laser power at 514.5 nm from a Spectra Physics 165 argon ion laser. A 1-s data accumulation (photons counted) rate per data point and a 1-cm^{-1} step size between data points were employed.

Results and Discussion

Figure 1 shows the Raman spectrum from 500 to 1800 cm^{-1} of an aqueous solution of double-helical poly(dA)·poly(dT) in 0.1 NaCl at pH 7.0. The small size of the Raman peaks of the double-helical polymer relative to the broad water band at 1660 cm^{-1} is due to the relatively low concentration of the polymer (about 2.7×10^{-2} M mononucleotide or phosphate). At 0 °C there are two pronounced peaks at 816 and 841 cm^{-1} indicative of C3′-endo ring pucker and C2′-endo ring pucker, respectively. When the solution was heated, both Raman marker bands weaken

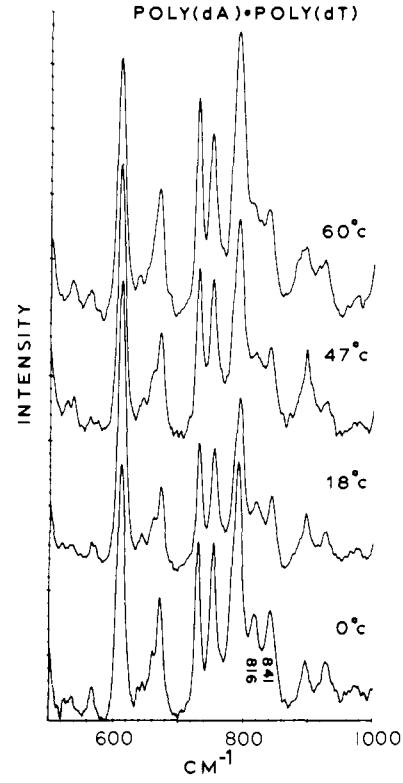

Figure 2. Raman spectra of poly(dA)·poly(dT) (20 mg/mL) in 0.2 M NaCl and 0.02 M sodium cacodylate at pH 7.0 obtained with 300 mW of 514.5-nm light.

considerably, but the C3′-endo marker band at 816 cm^{-1} is almost entirely gone by 40 °C, far below the melting point of the double helix. It is apparent that none of the vibrations in the regions 600–800 cm^{-1} or in the region 1200–1600 cm^{-1} change in either frequency or intensity throughout this 0–40 °C temperature range. These frequency regions contain the base vibrations whose assignments have previously been made by Baret et al.[16] for the polymers and by Lord and Thomas[17] for the monomers.

Detachment of the bases leads to changes in the Raman intensities of the base vibrations, an effect known as Raman hypochromism.[13] The absence of this effect in Figure 1 and the change in the intensities of the Raman bands of the sugar–phosphate backbone indicate that a change in the sugar conformation is occurring with little change in the orientation of the bases. Indeed the cooling of the solution of the poly(dA)·poly(dT) double helix from 40 to 0 °C seems to indicate a repuckering of the furanose rings from the characteristic C2′-endo frequency at 40 °C of 841 cm^{-1} to a mixture of C2′- and C3′-endo ring puckers at 0 °C. The increase in size of both of these Raman marker bands indicates a considerable increased rigidity of the furanose rings at the lower temperature. But particularly striking is the appearance of a moderately strong peak at 816 cm^{-1} that is not usually observed in deoxyribonucleic acids[8-10] and that is definite evidence of a change in the distribution of ring pucker at 0 °C. Experiments on a number of double-helical poly(dA)·poly(dT) samples at salt concentrations from 0.1 to 0.3 M showed the same behavior as seen in Figure 1 except that at higher salt concentrations the C3′-endo marker bands are more pronounced at all temperatures. This is shown in Figure 2 where the Raman spectra of poly(dA)·poly(dT) under equivalent conditions to Figure 1 except that the salt concentration is doubled to 0.2 M are illustrated. Here at 0 °C the 816-cm^{-1} marker band is even more pronounced than in Figure 1. Thus it appears that change in the ionic atmosphere can play a role in the stabilization of the furanose

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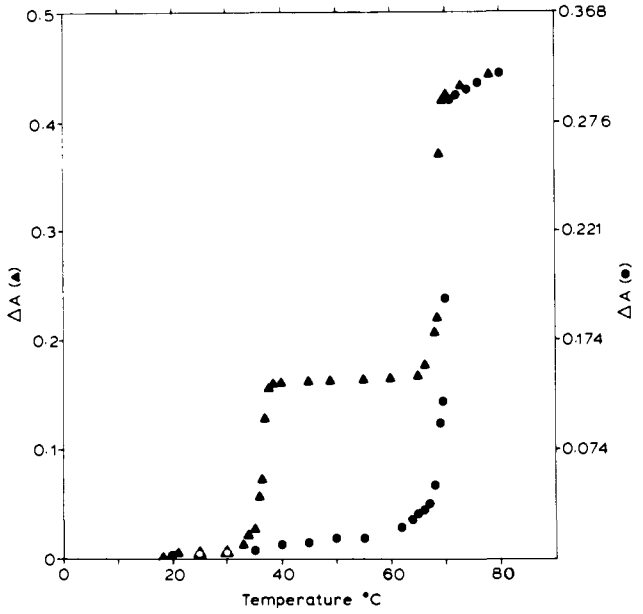

Figure 3. Lower set of filled circles is the change in absorbance at 260 nm with temperature of a double-helical poly(dA)·poly(dT) solution at a concentration of 2.7×10^{-2} M in phosphate. The absorption spectrum of this concentrated solution was taken with a cell of 0.005-cm path length. The upper set of triangular points is the change in absorbance at 260 nm of a solution of the triple helix poly(dT)·poly(dA)·poly(dT) much more dilute (OD at 20 °C = 0.80) at 1-mm path length. NaCl concentration 0.18 M, 260 nm, pH 7.0.

ring pucker, probably due to neutralization of the negative charges.

One potential artifact that must be ruled out in these experiments is the possibility that the double-helical complex has disproportionated into a triple helix of poly(dT)·poly(dA)·poly(dT) and a single-stranded poly(dT). Conceivably the observed Raman changes could be due to the melting of one of the poly(dT) chains from the triple helix. This possibility can be ruled out on the following grounds. First of all, Riley et. al.[18] have demonstrated that at the stoichiometry of our samples (one dA to one dT) only double-stranded helices are formed. Furthermore, earlier studies in this laboratory by Kiser[19] on the triple helix poly(U)·poly(A)·poly(U) have shown that melting off of the one of the poly(U) strands causes large changes to be observed in the intensities of at least some of the Raman bands of the bases.[20] This latter effect is due to the coupling that occurs between the intensities of the base vibrations and the UV absorption due to the phenomenon known as Raman hypochromism.[13] The definitive proof that the changes observed in Figures 1 and 2 are not due to a transition from triple to double helix is obtained from the temperature dependence of the UV absorption of a solution of poly(dA)·poly(dT) using the same conditions for which the Raman spectra were obtained. This curve is shown in Figure 3. Here the UV absorption curve for poly(dA)·poly(dT) under the same salt concentrations as Figure 2 is shown. The polymer concentration was the same as used for Raman samples, 2.9×10^{-2} M phosphate. The only apparent effect of the usually high polymer concentration is the fact that our observed melting point (72 °C) is slightly higher than that reported in the literature[18] for this double helix under identical conditions of added salt (0.2 M NaCl). The absence of any change in the UV absorption definitely rules out the possibility that the changes in the Raman spectra shown in Figure 1 are due to the melting of a triple helix. Analysis of the premelting behavior of calf thymus DNA shows that there is no evidence that A-genus Raman marker bands occur at low temperatures.[14] Thus, it is reasonble to conclude that the occurrence

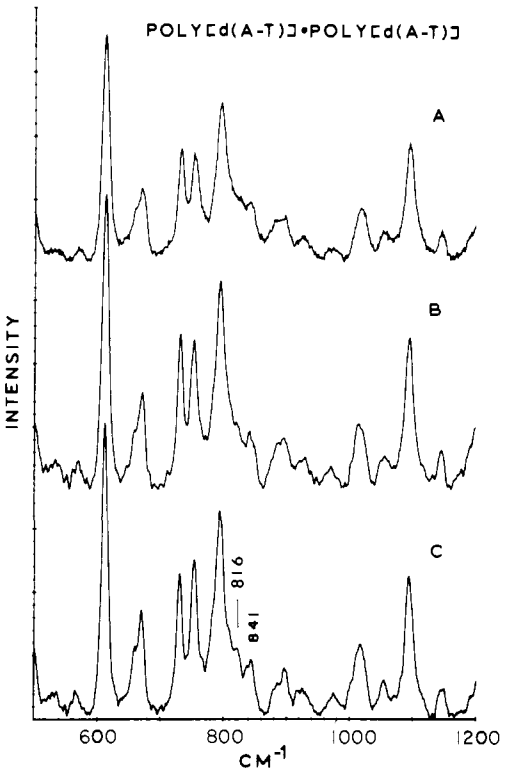

Figure 4. Raman spectra of poly(dA·dT)·poly(dA-dT), 20 mg/mL in 0.02 M sodium cacodylate at pH 7.0 obtained with 300 mW of 514.5-nm light. (A) 0.064 M NaCl and 0.025 sodium cacodylate at 24 °C; (B) 0.5 M NaCl and 20 °C; (C) 0.5 M NaCl and O °C.

of the 813-cm^{-1} band indicative of C3′-endo furanose conformation is due to the specific double-helical base sequence found in poly(dA)·poly(dT).

Recently, potential energy calculations on oligomers of d-(A_m)·d(T_m) have been made by Kollman et al.[21] that indicate that polymers and oligomers of one dA chain and one dT chain would possess a very flexible backbone sugar–phosphate chain. These authors have found that upon energy refinement, some torsional angles and sugar puckers have moved to different local minima. In particular, Figure 7 in their paper shows the repuckering of a sugar in a sequence of d(A_6)·d(T_6). This calculated repuckering may be closely related to the temperature-dependent distribution of furanose pucker that we have seen in poly(dA)-poly(dT).

In Figure 4 the lower trace shows the Raman spectrum of poly(dA-dT)·poly(dA-dT) in 0.5 M NaCl and 0.025 M sodium cacodylate, pH 7.0, at 0 °C. The weak band at 816 cm^{-1} that is observable in the lower spectrum indicates that some C3′-endo conformation must exist at this salt concentration and temperature. A similar band at 816 cm^{-1} is vaguely discernable in the Raman spectrum of poly(dA-dT)·poly(dA-dT) in 0.5 M NaCl at 20 °C, which is displayed as the middle spectrum of Figure 4. The Raman spectrum of poly(dA-dT)·poly(dA-dT) in 0.064 M NaCl at 24 °C (upper spectrum) exhibits only the 841-cm^{-1} band, which confirms the earlier result that only the C2′-endo conformation is clearly defined under these conditions.

It is of interest to examine the sugar–phosphate vibrations of the triple helix poly(dT)·poly(dA)-poly(dT) in order to see what frequencies appear in the 800–850-cm^{-1} region. Figure 5 shows the spectra of the triple helix poly(dT)·poly(dA)·poly(dT) at several temperatures in a 0.18 M NaCl, pH 7.0, solution (20 mg/mL total polymer). One of the poly(dT) chains melts off at 37 °C under the salt conditions employed. The dissociation of one of the poly(dT) strands at this temperature is reflected in the absorption profile presented in Figure 3. The UV absorption profile of the triple-helical material prepared for these Raman

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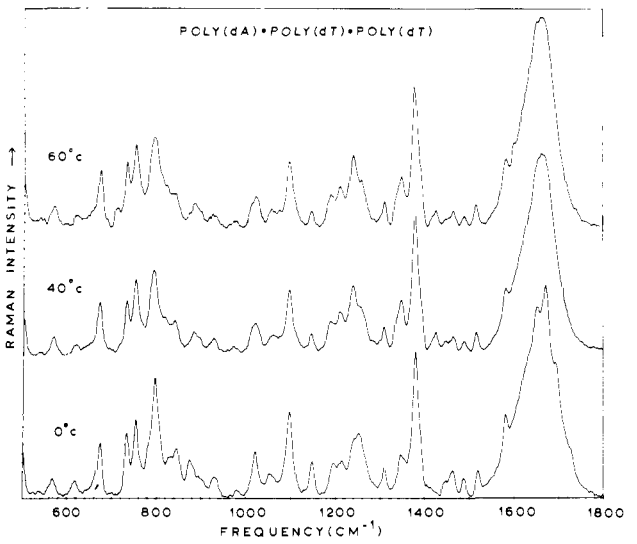

Figure 5. Raman Spectra of poly(dT)·poly(dA)·poly(dT) (30 mg/mL) in 0.1 M NaCl at pH 7.0 obtained with 300 mW of 514.5-nm light.

studies exhibits the classical two-step absorption profile associated with a triple-helical form. Consequently, the three Raman spectra contained in Figure 5 represent different structural states of the dT·dA·dT polymer. The spectrum at 0 °C corresponds to a triple helix. The other two spectra represent a mixture of double-helical poly(dA)·poly(dT) and single-stranded poly(dT). Several changes are observed in the Raman spectra upon dissociation of the single poly(dT) strand from the triple helix. The two most pronounced changes that are observed in the base vibrations are the very large increase in the 1240-cm^{-1} peak and the 1424-cm^{-1} peak. The 1424-cm^{-1} peak is most probably an adenine vibration that is not observed in the triple helix apparently due to interaction of the adenine with the second thymine. However upon the melting off of the poly(dT) strand, the band at 1424 cm^{-1} appears. The 1240-cm^{-1} band is a thymine band that increases greatly in intensity when ordinary DNA is melted.[14] Consequently, it is not surprising to see a similar large increase upon dissociation of the poly(dT) strand. Since Figure 1 shows no such base vibrational changes in the 0–40 °C temperature range, we conclude again that poly(dT) is not melted from poly(dA) under the conditions employed.

The bottom spectrum in Figure 5 shows no peaks in the 800–825-cm^{-1} region that could be evidence for C3′-endo ring conformation. Indeed there is a valley, i.e., a minimum in the scattered Raman intensity at 820 cm^{-1}. However, in the 0 °C Raman spectrum there are two Raman bands at 830 and 845 cm^{-1} that could be indicative of two different C2′-endo structures in the triple helix. Upon melting of the triple helix to double helix, we see the two weak bands at 816 and 840 cm^{-1} characteristic of the poly(dA)·poly(dT) double helix at 40 °C in this salt concentration. The spectrum at 60 °C shows no measurable change over the 40 °C spectrum, but the quality of the spectrum is poorer and the signal to noise ratio is worse.

The question arises as to whether poly(dG-dC)·poly(dG-dC) and poly(dG)·poly(dC) double helices may also show the C3′-endo ring pucker at low temperatures. Our studies show conclusively that poly(dG-dC) gives a strong 833-cm^{-1} band at 0 °C and 0.1–0.2 M salt with no evidence whatever for a band in the 805–820-cm^{-1} region. Studies on poly(dG)·poly(dC) are under way and must be made with careful controls because of the possibility of disproportionation, aggregation of poly(dG), etc. However, our initial results show no 814-cm^{-1} band at low temperature but a weak shoulder in the 810–820-cm^{-1} range. This could be indicative of a C3′-endo ring pucker, but it appears to be at a much lower level than in poly(dA)·poly(dT) where a sharp strong identifiable band at 816 cm^{-1} is easily observed.

In conclusion we may state that poly(dA)·poly(dT) shows repuckering of the furanose rings at low temperatures from the typical double-strand B-form DNA to a mixture of C2′-endo and C3′-endo ring puckers. This change appears to occur with no change in base orientation. A similar but much smaller effect appears in poly(dA-dT)·poly(dA-dT). It is possible that A-T-rich regions in DNA use this possibility of furanose flexibility for recognition sites by proteins.

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Registry No. Poly(dA)·poly(dT), 24939-09-1; poly(dA-dT), 26966-61-0; poly(dT)·poly(dA)·poly(dT), 30177-40-3.