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A ^{31}P NMR ANALYSIS OF THE HELIX TO COIL TRANSITION OF NATURAL DNA SAMPLES: EVIDENCE FOR THE EXISTENCE OF DIFFERENT CONFORMATIONAL STATES

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SUMMARY

The helix to coil transitions of calf thymus and salmon sperm DNA were probed by ^{31}P nuclear magnetic resonance spectroscopy. Both the helical and coil forms were observed in the melting region indicating slow exchange between the two forms with an estimated rate of interconversion $\ll 36$ sec^{-1} at 70°C. At least three different signals were also observed at temperatures significantly above the Tm, suggesting three classes of conformational states. One of these classes has a significantly lower T_1 than the other two indicating considerable residual structure in this form. The four only partially resolved signals indicate that the phosphate residues have very similar chemical shift environments. From the experimentally observed small chemical shift differences between the helical and coil forms, it is concluded that the gauche, gauche, conformation predominates in the coil form as has been found for the helix.

INTRODUCTION

Both theoretical (1) and experimental (2) results indicate that the single stranded nucleic acid sugar-phosphate backbone can assume a range of distinct conformations. Although there have only been three studies, to our knowledge, on natural DNA utilizing ^{31}P nmr (3), the technique has been found to be useful for studying conformational transitions in nucleic acids (4). Our initial interest in the method was to supplement our ^{13}C (5) and ^{1}H (6) investigations of ligand-nucleic acid interactions. Prior to undertaking this task, however, it is necessary to have some knowledge of the possible conformational states and the resulting ^{31}P spectra of DNA under a variety of conditions. We report here our investigation of the thermal transition for both calf thymus and salmon sperm sonicated DNA samples by ^{31}P nmr. Evidence for the coexistence of at least four different resonances which indicate four classes of conformational

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states for the DNA backbone in solution at temperatures near the Tm is presented. Even at temperatures considerably above the Tm, we find at least three ^{31}P peaks in the spectra for both calf thymus and salmon sperm DNA.

MATERIALS AND METHODS

Calf thymus and salmon sperm DNA (Worthington Biochemicals) were dissolved in H_2O (0.5M in NaCl) and sonicated using a Heat Systems W-375 sonicator, tuned for maximum output and operating at 10% duty-cycle in the pulse mode for approximately 20 hours (total sonication time of ~2 hours). A gentle flow of N_2 gas was passed through the solution during the duration of the sonication. The temperature, regulated with a Haake thermoregulator and an ethylene-glycol condensor was maintained between 4° and 8°C by chilling the DNA solution in a 40% ethanol/H_2O mixture. Sonication, removal of particulate contamination, precipitation, characterization of DNA and dialysis in standard buffer were performed as described in detail previously (7). Estimation of chainlength by polyacrylamide gel electrophoresis (8) gave a band centered at ~200 base pairs and further extensive sonication did not result in any significant reduction of the DNA molecular weight. DNA obtained this way was further dialysed against nmr buffer (0.01M phosphate, pH=7; $5x10^{-4}$M EDTA) and stored at 4°C. All ^{31}P nmr spectra were taken with a JEOL FX60Q spectrometer. Samples were prepared and concentrated by lyophilizing the DNA stock solution and redissolving it in 99.8% D_2O (Aldrich). 10 mm tube and spectral width of 1000 Hz were routinely used. Temperature of samples was regulated with a JEOL NM5471 Variable Temperature controller and is accurate to within ±1°.

RESULTS AND DISCUSSION

The native form of DNA (low temperatures) has a ^{31}P chemical shift of about 4.3 ± 0.1 ppm, (Figure 1) measured at the center of the broad peak upfield from trimethyl phosphate (TMP). Upon gradual heating a downfield shift of the order of 0.5 ppm is observed (Figure 1). Several features are evident in the spectra shown in Figure 1: (a) a gradual decrease in line width (as the temperature increases) from a width at half height of ~18 Hz at 30°C to ~2 Hz (estimated for the center peak) at 90°C; (b) both the helical and coil forms are observed in the intermediate region, centered near 68°C (where melting occurs), indicating slow exchange between the two forms on the nmr time scale; moreover, from the chemical shift separations between the helical and coil forms a rate of interconversion $\ll 36\ sec^{-1}$ (which corresponds to Peak IV at 70°C) is calculated as an upper limit (Kearns and his coworkers (9) have reported that the rate of disruption of the synthetic poly dA•dT helix must be smaller than 100 sec^{-1}); (c) in the melting region a minimum of four different peaks can be identified

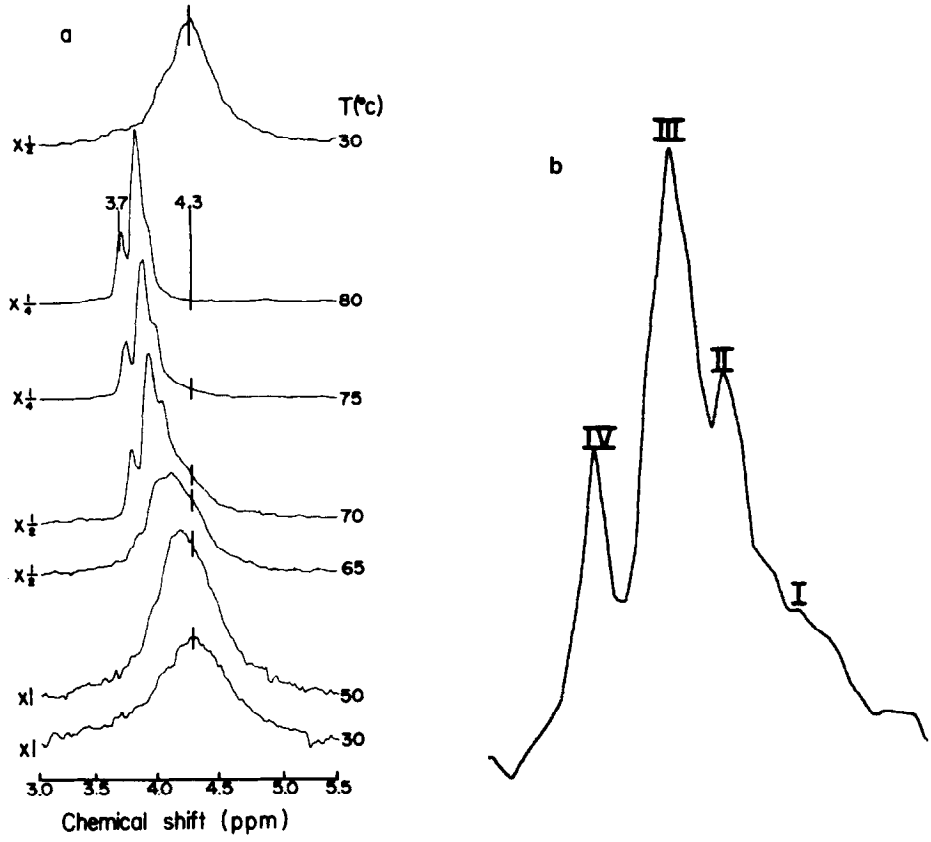

Figure 1 - (a) Representative 24.15 MH_Z Fast Fourier transform ^{31}P spectra of salmon sperm DNA (66 mg/ml, 90° pulse, repetition time (PR) = 5 sec, 4096 data points, 500 scans) at different temperatures. Chemical shifts are relative to TMP. (b) 70°C spectrum of (a) with resolution enhanced by negative exponential filtering (expanded by a factor of 2.4 relative to the spectra in 1(a)).

(Figure 1b); Peak I is assigned to the native form and Peaks II, III and IV to denatured forms; (d) as the temperature increases above the melting transition, Peak I disappears and a redistribution of the intensities of the other peaks occurs; (e) as the temperature is increased from 65° to 90°C, Peaks II, III and IV shift downfield in a roughly parallel fashion by ~0.2 ppm; (f) close examination of the spectra at high temperatures utilizing resolution enhancement techniques (negative exponential filtering)(not shown) suggests that Peak III has a shoulder both on the right and left and Peak IV broadens somewhat, relative to the spectrum at 70°C; (g) when the high temperature sample is cooled to 30°C, however, the signals broaden, coalesce and shift upfield (top spectrum in Figure 1).

The observation of three distinct nmr peaks for the denatured state of DNA is somewhat unexpected. Small nucleotide segments and nucleotide homopolymers have shown a composition and sequence dependence of their ^{31}P chemical shifts (4) but have not shown any tendency to fall into groups with similar chemical shifts. From these published results a continuous envelope of chemical shifts for the sugar phosphate backbone in DNA might be expected in contrast to the results obtained. McDonald *et al* (10) have shown by ^{1}H nmr that there is considerable stacking and structure remaining in denatured DNA. The results in Figure 1 suggest that the coil form may contain at least three conformational classes having different averaged ^{31}P chemical shifts which may be due to variations in (i) P-O bond angles and/or torsional angles, (ii) chemical environment (eg. ring current effects), (iii) residual helical states, and (iv) some combination of the above. Since calf thymus and salmon sperm DNA samples give very similar resonances, there must be some general feature, at least for these two eukaryotic DNA samples, leading to these three ^{31}P resonances. This is being investigated using DNA samples from different sources.

Convincing evidence for the coexistence of significantly different conformational states is shown in Figure 2 from a 180°-τ-90° pulse sequence experiment. In principle, a more ordered form is expected to relax faster due to its restricted dynamic flexibility. The results in Figure 2 show that Peaks II and IV have (in relative terms) the shortest and longest T_1's, respectively, with Peak III having a T_1 close to that of Peak IV (exact T_1 values were not determined because of the extensive peak overlap and the long waiting times required). This suggests then that Peak II represents a class of conformations with considerable structural organization, i.e., the conformations would have the least nongauche character due to some secondary structure which could presumably arise from stacking and/or double stranded helices. Similar conclusions have been reached from ^{31}P magnetic resonance studies of the independently behaving peaks of yeast $tRNA^{Phe}$ (11). What should be emphasized is that in denatured DNA the phosphate groups are in several different discreet states and that a highly structured form remains far above the Tm.

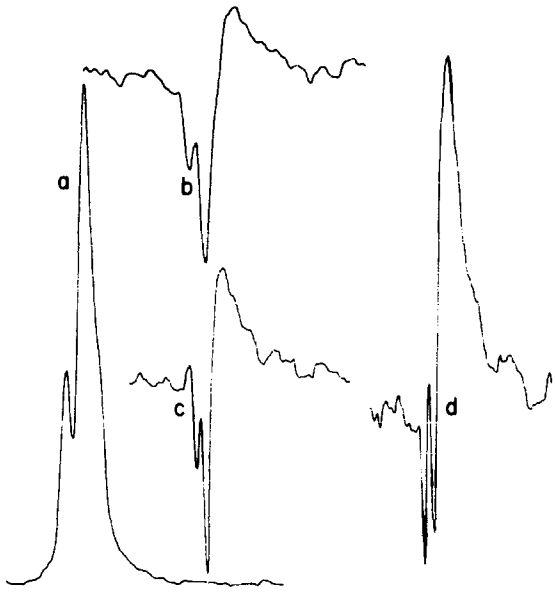

Figure 2 - (a) Spectrum of salmon sperm DNA (16 mg/ml, 80°C, 90° pulse, PR = 16 sec, 153 scans. Note that this spectrum has no appreciable difference in spectral character compared to the higher concentration one at same temperature (Figure 1). (b), (c), and (d) same sample as in (a) but a 180°-τ-90° pulse sequence was applied (phase correction settings were not changed) with τ = 2.42, 2.7 and 2.9 respectively. A .97-Hz broadening function (exponential filter) was applied to each free induction decay before Fourier transformation.

This solution data can be compared to the percentage population of the different conformations of the DNA backbone —gauche, gauche (g,g), gauche, trans (g,t) and trans, trans (t,t) as compiled by Olson (12). The compiled X-ray data show that the (g,g) and (g,t) forms represent about 80% and 20% of the population respectively. Based on the observation that the coil form peaks do not experience large chemical shifts, it is reasonable to assign all the peaks to a predominantly (g,g) form. The small shifts at temperatures above 65°C can then be accounted for by different states, as defined above, which are in rapid (g,g) $\leftrightarrow$ (g,t) equilibria with the (g,t) becoming more accessible at higher temperatures (13).

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REFERENCES

1. Newton, M.D. (1973) J. Am. Chem. Soc. 95, 256-258.

2. Sundaralingam, M. (1969) Biopolymers 7, 821-860.

3.a. Hanlon, S., Glonek, T. and Chan, A. (1976) Biochemistry 15, 3869-3975; b. Yamada, A., Kaneko, H., Akasaka, K. and Hatano, H. (1978) FEBS Lett. 93, 16-18; c. Kallenbach, N.R., Appleby, D.W. and Bradley, C.H. (1978) Nature, 272, 134-138.

4.a. Guéron, M. (1971) FEBS Lett. 19, 264-266; b. Guéron, M. and Shulman, R.G. (1975) Proc. Nat. Acad. Sci. USA 72, 3482-3485; c. Patel, D.J. and Canvel, L. (1976) Proc. Nat. Acad. Sci. USA 73, 674-678; d. Gorenstein, D.G., Findley, J.B., Momii, R.K., Luxon, B.A. and Kar, D. (1976) Biochemistry 15, 3796-3803 and references therein; e. Reinhardt, C.G. and Krugh, T.R. (1977) Biochemistry 16, 2890-2895; f. Davanloo, P., Armitage, I.M. and Crothers, D.M. (1979) Biopolymers 18, 663-680.

5. Davidson, M.W., Griggs, B.G., Lopp, I.G., Boykin, D.W. and Wilson, W.D. (1978) Biochemistry 17, 4220-4225.

6. Davidson, M.W., Griggs, B.G., Lopp, I.G. and Wilson, W.D. (1977) Biochim. Biophys. Acta. 479, 378-390.

7. Davidson, M.W., Griggs, B.G., Boykin, D.W. and Wilson, W.D. (1977) J. Med. Chem. 20, 1117-1122.

8. Maniatis, T., Jeffrey, A. and van deSande, H. (1975) Biochemistry 14, 3787-3794.

9. Early, T.A., Kearns, D.R., Burd, J.F., Larson, J.E. and Wells, R.D. (1977) Biochemistry 16, 541-551.

10. McDonald, C.C., Phillips, W.D. and Lazar, J. (1967) J. Am. Chem. Soc., 89, 4166-4170.

11. Hayashi, F., Akasaka, K. and Hatano, H. (1977) Biopolymers 16, 655-667.

12. Olson, W.K. (1974) Structure and Conformation of Nucleic Acids and Protein-Nucleic Acid Interactions, Sundaralingam, M. and Rao, S.T., Editors, University Park Press, Baltimore, pp. 709-727.

13. Cozzone, P.J. and Jardetzky, O. (1976) Biochemistry 15, 4853-4859.