When the analytical oversight is corrected, a *new* meaning may be attached to the heat capacity function. Indeed, the meaning of the heat capacity function is contingent entirely upon how we formulate the equilibrium constant for the system. In this way the heat capacity function, either real or anomalous, provides a revealing mechanistic tool.

Nonequivalence of ^{31}P NMR Chemical Shifts of RNA Complexes

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Received March 17, 1981

Phosphorus-31 nuclear magnetic resonance (^{31}P NMR) spectroscopy has been used extensively in studies of nucleic acid constituents.[1-6] Recently ^{31}P NMR has been used to investigate helical complexes of larger oligonucleotides[7] and polynucleotides.[8,9] In the study of $(dG\text{-}dC)_8$[7] and poly(dG-dC),[8] an important feature of the ^{31}P NMR spectrum of the helical complex is the conversion of a single resonance in low salt into two resonances in high salt. The high salt spectrum is consistent with the results of X-ray diffraction studies of oligomers of (dG-dC),[10] which indicate two different phosphate-backbone conformations, one for ..GpC.. and another for ..CpG.. sequences (Z-DNA). Studies of the crystal structure of d-pApTpApT[11] and a variety of existing physical and chemical data on poly(dA-dT) lead to the hypothesis of an alternating B-DNA structure[12] for poly(dA-dT)·poly(dA-dT). In this model the phosphate backbone is different for ..ApT.. and ..TpA.. sequences in the helical complex. This hypothesis has received experimental support from recent ^{31}P NMR studies[9] of poly(dA-dT) where two phosphorus resonances are observed, depending on the temperature and salt concentration. These data support the general contention that base sequence can affect nucleic acid conformation.[13]

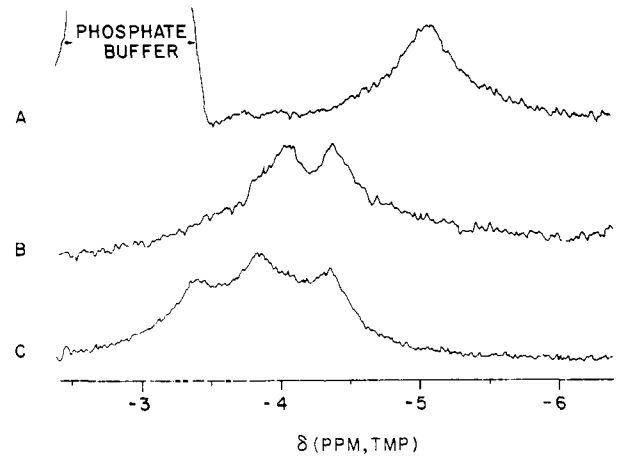

Figure 1. Phosphorus-31 NMR spectra of (A) poly(A^+)·poly(A^+), (B) poly(A)·poly(U), and (C) poly(A)·2poly(U). Conditions are those indicated in Table I.

Table I. P-31 Chemical Shifts[a] of RNA Helical Complexes

complex[b]	chemical shift, ppm
poly(A^+)·poly(A^+)	−5.10
poly(A)·poly(U)[c]	−4.10, −4.45
poly(I)·poly(C)[d]	−4.03,[e] −4.54[f]
poly(I)·poly($C_{12}U$)	−4.03, −4.55
poly(A)·2poly(U)	−3.39,[g] −3.84, −4.36

[a] Maximum intensity position of resonance with respect to internal trimethyl phosphate, 30 °C. [b] Complex is 20-30 mM in base pair, in aqueous solution (20% D_2O) containing 0.003 M EDTA, 0.04 M sodium phosphate and NaCl. The solution pHs are 6.8-7.2 except for poly(A^+)·poly(A^+) (pH 5.6). Concentrations of NaCl are 0.05 M, poly(A^+)·poly(A^+); 0.1 M, poly(A)·poly(U); 0 and 0.6 M, poly(I)·poly(C); 0.6 M, poly(I)·poly($C_{12}U$); 0.4 M, poly(A)·2poly(U). [c] Chemical shifts of noncomplexed poly(A) and poly(U) are −3.97 and −3.85, respectively. [d] Chemical shifts of noncomplexed poly(I) and poly(C) are −3.70 and −4.22, respectively. [e] Complexed phosphates of poly(I) strand. [f] Complexed phosphates of poly(C) strand. [g] Complexed phosphates of poly(U) strand involved in Hoogstein base pair with poly(A).

Variation in the structure of the phosphate backbone is also apparent in fibers of nucleic acids.[14,15] Fibers of RNA homopolynucleotide complexes have been extensively studied by X-ray diffraction to deduce their helical conformations. From these studies it is apparent that phosphate-backbone conformations may be dependent not only on the base sequence but also on the nature of the association of bases through hydrogen bonds in polynucleotide complexes. In the cases of complexes with poly(A), three types of base-base association are observed: poly(A^+)·poly(A^+),[16] poly(A)·poly(U),[17] and poly(A)·2poly(U).[18] The general feature of the phosphate-backbone conformation of the poly(A^+)·poly(A^+) and poly(A)·poly(U) complexes is that of C_2 symmetry with respect to the helical axis of the double-stranded complex (i.e., the conformation of the phosphate backbone of each strand is identical). In contrast, the fiber structure of the poly(A)·2poly(U) complex is deduced as one in which a unique phosphate-backbone conformation exists for each of the three strands.

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We have studied these three polyribonucleotide complexes[19] in aqueous solution by ^{31}P NMR spectroscopy (80.6 MHz) above (spectra not shown) and below the thermal denaturation temperature (T_m) (Figure 1) of the helical complex. Above the T_m, the spectral lines are generally sharp ($\Delta\nu_{1/2} < 2$ Hz) and consist of one or two resonances of expected intensity,[22] reflecting the homopolymer stoichiometry of the respective complexes. Below the T_m (Figure 1), the resonances are somewhat broader ($\Delta\nu_{1/2} \sim 20$–50 Hz), presumably reflecting effects due to reduced correlation times of the phosphates of the base-paired complexes. Additional features of the spectra of these complexes are as follows: (a) poly(A^+)·poly(A^+) has *one* phosphorus resonance;[23] (b) poly(A)·poly(U) has *two* resonances of approximately equal intensity; (c) poly(A)·2poly(U) has *three* resonances, each of approximately equal intensity. The chemical shift positions of the resonances of these and additional polynucleotide complexes are listed in Table I. An unanticipated feature of these spectra is the observation of multiple resonances in three of the double-stranded helical complexes [poly(A)·poly(U), poly(I)·poly(C), and poly(I)·poly(C_{12}U)].[25] Plausible explanations for the observation of more than one resonance are (1) two equal populations of helical complexes having different phosphate-backbone conformations, each having C_2 symmetry with an exchange rate slow on the NMR time scale, (2) a single helical complex having C_2 symmetry but containing local phosphate magnetic anisotropy[27] (e.g., due to solution environment[30]), and (3) a single helical complex where the two strands have different phosphate-backbone conformations. Various studies of these complexes (effects of temperature, ionic strength, RNA–DNA hybrid complexes) do not support the first explanation[32] and provide tentative assignments[33] of some of the base-paired resonances in Table I. The second and third explanations are both considered possible contributors to the multiple resonances observed. Our collective results from these complexes in various salts[35] of poly(A)·2poly(U) at conditions where the triple-to-double-stranded conversion occurs, and the observed three resonance pattern of poly(A)·2poly(U) anticipated from the unique strand conformations of the fiber,[18a] strongly suggest that the third explanation (strands with different phosphate-backbone conformations) is a reasonable contributor to the multiple resonances observed in these complexes. Additional experiments are necessary to unequivocally assign the resonances to specific strands and to determine the relative contributions of mechanisms 2 and 3 to the observed chemical shifts. However, irrespective of the explanation of the resonance multiplicity, these results illustrate the utility of the ^{31}P NMR technique for probing the phosphate backbone of individual strands in helical complexes.

Acknowledgment. We acknowledge the National Science Foundation and National Institutes of Health (CA-25438) for support in purchasing the NMR spectrometer and Franklyn Wissler for performing the ring-current calculations.

(19) Polynucleotide complexes were prepared as previously described.[20] These complexes were sonicated by using a Branson Sonifier (Model W185; equipped with a tapered micro tip) at 0 °C under nitrogen atmosphere. Integrity of the complex was determined by using thermal denaturation profile (UV spectroscopy) before and after sonication, and after sonication the complex had a broad molecular weight distribution as determined from polyacrylamide gel electrophoresis (7.5%) in 40 mM Tris acetate (pH 8.0). The size was generally greater than 150 base pairs, using the Hae III restriction fragments of PM2 DNA as comparative size markers.[21]

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(33) (a) The poly(I)·poly(C) helical resonances are assigned by comparison to poly(dI)·poly(C) (−4.27 and −4.66 ppm) by assuming the two resonances most similar in chemical shift are those from the RNA strand. (b) In poly(A)·2poly(U), the Hoogstein base-paired poly(U) strand (lowest field resonance) was assigned by comparison of the poly(A)·poly(U) and poly(A)·2poly(U) spectra and by a temperature study of poly(A)·2poly(U) at low salt where the 3 → 2 + 1 stranded conversion[34] occurs. At this condition the lowest field resonance disappeared and reappeared as a sharp line with a chemical shift expected for free poly(U), while the intensity of the other two higher-field resonances remained constant.

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Reaction between HO_2· and Chlorine in Aqueous Solution

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Received May 1, 1981

Reactions in aqueous solution containing ferrous sulfate are among the best understood of radiolytic reaction mechanisms, but hitherto unsuspected reactions must be taking place when large radiation doses are applied instantaneously in the presence of added chloride.[1] We have recently found indications that yields under these conditions might be explicable if HO_2· radicals reduce Cl_2.[2] This communication reports independent confirmation of this reaction, obtained in a system designed to demonstrate it.

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