A Proton Magnetic Resonance Study of Polydeoxyriboadenylic Acid

James L. Alderfer[1] and Stanford L. Smith

Contribution from the Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506. *Received December 31, 1970*

Abstract: Proton nmr spectral studies of polydeoxyriboadenylic acid, $d(A)_n$ (and 5′-dAMP) at pH 8.0 as a function of temperature are reported. Chemical shift, area, full width at half maximum (FWHM), and coupling constant data are reported for H-2 and H-8 of the base unit and H-1′ and H-2′,2″ of the sugar. The H-1′–H-2′,2″ coupling constant ($0.5|J_{H\text{-}1'\text{-}H\text{-}2'} + J_{H\text{-}1'\text{-}H\text{-}2''}| = 6.8$ Hz) in the polymer is temperature invariant over the range where it can be observed (52.5–92.5°) suggesting that the deoxyribose ring is either in a fixed conformation or equilibrating rapidly between several conformers. The appearance temperature of H-2 (<5°) is lower than that of H-8 (~10°) and the FWHM of H-8 is greater (several fold) than H-2 suggesting that the bases are in an anti conformation around the glycosyl bond. The polymerization shift (*PS*) *vs.* temperature curves for H-2 and H-1′ and for H-8 and H-2′,2″ are similarly shaped. This requires the bases to be tilted with respect to the helical axis. The polymerization shifts for $d(A)_n$ are larger than those reported for $r(A)_n$ indicating that the bases in $d(A)_n$ are more strongly associated than in $r(A)_n$. Implications of these differences on enzymatic recognition of different types of nucleic acids are discussed.

During the past decade nuclear magnetic resonance (nmr) spectroscopy has been successfully employed to study the conformational structure and electronic aspects of purine and pyrimidine bases, nucleosides, and nucleotides.[2] Application of nmr techniques to nucleic acid polymers has been more limited. Several workers have published proton magnetic resonance data on homogeneous ribonucleic acid polymers.[3-6] McDonald, *et al.*,[4] have shown the chemical shift dependence of several ribopolymers as a function of temperature. Jardetzky[3] made comparisons of monomer and polymer chemical shifts under similar experimental conditions and cited "polymerization shift" values for several polymers. There have also been studies reporting the change in polymer peak area as a function of temperature.[5,6] Polyriboadenylic acid, $r(A)_n$, received some attention in all of these investigations; consequently of all the ribopolymers studied the most data exist for it.

The investigation reported here gives attention to the deoxyribose polymer, polydeoxyriboadenylic acid, $d(A)_n$. (No previous nmr studies of deoxyribose polymers have been conducted because the necessary materials were unavailable.) Considerable effort has been expended by many scientists in an attempt to understand and explain differences in the physicochemical properties of DNA and RNA. To add to this existing knowledge we present here some comparisons and contrasts of $r(A)_n$ and $d(A)_n$ nmr data.

In this study four nmr parameters have been monitored: chemical shift, peak area, full width at half maximum (FWHM), and coupling constant. Because this investigation was especially concerned with the secondary structure $d(A)_n$, the polymer nmr parameters are expressed wherever pertinent relative to a monomer reference, 5′-dAMP, which is devoid of secondary structure.

Using nmr techniques to study polymer secondary structure is made possible by two major characteristics of the polymer system and the nmr experiment. The first is a result of nuclear dipole–dipole interactions. If the polymer molecule or portions of it are sufficiently rigid (*i.e.*, the correlation time, $\tau_c \gg 10^{-4}$ sec) no high-resolution nmr spectrum will be observed. The breakdown of secondary structure can then be followed by the increase in peak area and changes in the FWHM. The second factor is based on the premise that changes in secondary structure can be monitored by concomitant changes in proton chemical shifts after the polymer molecule is sufficiently nonrigid that a high-resolution spectrum can be observed. This assumes that chemical shift changes are primarily the result of conformationally dependent changes in the ring current anisotropy effects of neighboring base units.

Experimental Section

Materials. 9-β-(2′-Deoxy-D-ribofuranosyl)adenine 5′-phosphate diammonium salt (5′-dAMP) obtained from Schwarz Bioresearch, Inc., Orangeburg, N. Y., was used without further purification. Nmr spectra of this compound showed no evidence of extraneous peaks due to proton-containing impurities. Polydeoxyriboadenylic acid, $d(A)_n$, was obtained from the laboratory of Dr. Frederick J. Bollum, Department of Biochemistry, University of Kentucky, Lexington, Ky. It was prepared as previously described.[7] The molecular weight was approximately 160,000 containing about 500 base units. Phosphate salts, K_2HPO_4 and NaH_2PO_4 (analytical grade), and deuterium oxide (99.90% D) from Bio-Rad Laboratories, Richmond, Calif., were used without further purification.

Methods. The polymer, $d(A)_n$, and monomer, 5′-dAMP, solutions were 12 and 10 m*M*, respectively, in phosphate buffer, ionic strength of 0.05 and pH 8.0. The polymer solution was obtained by dialysis against aqueous (D_2O) NaCl, D_2O, and buffer to obtain the final solution. The monomer was dissolved directly in the buffer solution.

(1) Tennessee Eastman Company Fellow, 1968–1969; NIH Predoctoral Fellow, 1969–1970 (NIH 1 FO1 GM41743-01).
(2) P. O. P. Ts'o, M. P. Schweizer, and D. P. Hollis, *Ann. N. Y. Acad. Sci.*, **158**, 256 (1969), and references contained therein.
(3) O. Jardetzky, *Biopolymers Symp.*, **1**, 501 (1965).
(4) C. C. McDonald, W. D. Phillips, and S. Penman, *Science*, **144**, 1234 (1964)
(5) J. P. McTague, V. Ross, and J. H. Gibbs, *Biopolymers*, **2**, 163 (1964).
(6) C. C. McDonald, W. D. Phillips, and J. Penswick, *ibid.*, **3**, 609 (1965).
(7) F. J. Bollum in "Procedures in Nucleic Acid Research," G. L. Cantoni and D. R. Davies, Ed., Harper and Row, New York, N. Y., 1966.

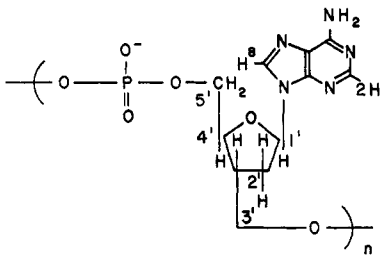

Figure 1. Structure of $d(A)_n$.

The instrument used for this study was a Varian Associates HA-60-IL nuclear magnetic resonance spectrometer equipped with a C-1024 computer of averaged transients, a TMC 220C data output unit, and a V-4343 variable temperature controller. Accessory equipment which was used included: a Hewlett-Packard Model 521 CR frequency counter, a voltage-controlled oscillator (VCO)[8] driven by the C-1024 ramp, a Leeds-Northrup potentiometer, and a copper–constantan thermocouple. The spectrometer was always operated in the frequency sweep mode. RF power levels were adjusted so that saturation did not occur at any temperature and filtering was set to provide minimum signal distortion. Once these instrumental parameters were determined they and all other instrument settings were held constant. Signals from compounds in the reference capillary were examined during and after every run for evidence of instrumental artifacts. If such were found the data from that run were discarded, and the experiment was repeated. Probe temperature was determined using the thermocouple which was checked against an NBS calibrated thermometer yielding temperatures accurate to ±0.2°.

Three 250-Hz segments of each spectrum were signal averaged for 36–81 scans at a sweep rate of 1 Hz/sec. The resulting averaged spectrum was digitized and nmr parameters were determined from the digitized spectrum by a computer program, MULPLOT.[9]

Results

Spectrum and Assignment of Proton Resonances. This study considers only four proton types (Figure 1): H-2 and H-8 of the adenine base and H-1′ and H-2′,2″ of deoxyribose. The assignments of the base protons were made previously[10-12] and can be readily distinguished because H-8 exchanges with deuterated solvent at high temperature.[12] The H-1′ and H-2′,2″ protons were readily identified by their splitting patterns, a triplet and multiplet, respectively, and by their large chemical shift difference. On these bases, the assignments of the spectrum are made as in Figure 2 (aided by higher temperature spectra where the splitting patterns are observable).

Nmr Studies of dAMP. To provide a model for the polymer system, the monomer was studied under conditions similar to those used for the polymer. Studying the chemical shift, area, and FWHM over the 10.0–92.5° temperature range produced significant change in only one parameter, the chemical shift. The two types of sugar protons are essentially temperature independent. H-8 moves upfield and H-2 moves downfield about 4 Hz and 6 Hz, respectively,

(8) Part No. 301-2501-10-1001, M. F. Electronics Corp., New York, N. Y.

(9) MULPLOT is a computer program which processes digitized spectral data producing approximately a 10:1 signal/noise increase over that of the input data. Most important, it abstracts from the experimental spectrum line positions, line widths (FWHM), and areas, thus removing the subjectivity inherent in the manual determination of such data from poorly resolved spectra of a low signal/noise ratio. Unlike similar programs, MULPLOT is specifically written to smooth peaks of varying width with minimum distortion.

(10) S. Matsuura and T. Goto, *Tetrahedron Lett.*, 1499 (1963).

(11) M. P. Schweizer, S. I. Chan, G. K. Helmkamp, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **86**, 696 (1964).

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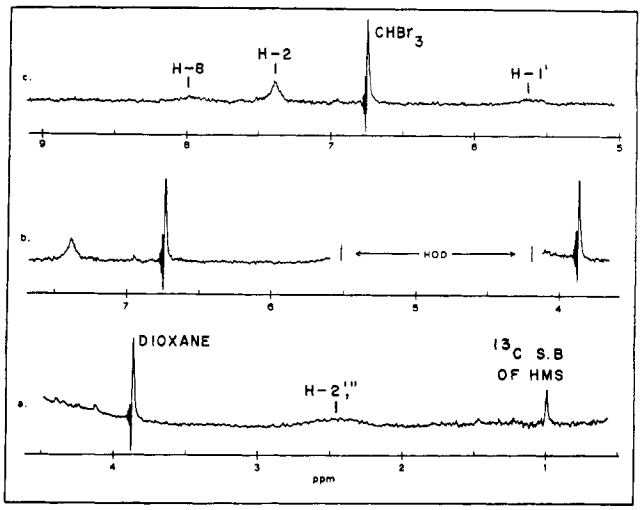

Figure 2. Spectrum of $d(A)_n$ at 25° showing assignment of peaks: a, high field section; b, middle field section; c, low field section.

with increasing temperature. Table I shows these values as a function of temperature relative to HMS and dioxane. There was slight broadening of H-8 and H-2 at 10.0° as seen in Table II, but above this temperature, FWHM and area remained essentially temperature independent. It was possible to monitor the average coupling constant value between H-1′ and H-2′,2″. This is found in Table III and is, within experimental error, temperature independent.

Table I. Chemical Shift Values for 10 m*M* 5′-dAMP at pH 8.0

Proton	Temp, °C	Hertz[b] (HMS)	Hertz (Dioxane)
H-8	10.0	525.53	286.43
	32.0	527.50	285.61
	50.0	528.19	284.21
	70.0	529.17	283.72
	92.5	529.88	282.98
	$CS^a = -0.04265T + 286.75$		
H-2	10.0	505.81	266.71
	32.0	510.73	268.84
	50.0	513.40	269.42
	70.0	516.59	271.15
	92.5	519.28	272.38
	$CS^a = 0.06761T + 266.24$		
H-1′	10.0	403.46	164.41
	32.0	406.91	164.63
	50.0	408.34	164.54
	70.0	409.98	164.54
	92.5	411.68	164.78
	$CS^a = 0.00456T + 164.38$		
H-2′	10.0	175.64	−63.35
	32.0	177.81	−63.97
	50.0	179.88	−63.97
	70.0	181.62	−63.97
	92.5	182.93	−63.97
	$CS^a = 0.00595T - 63.58$		

[a] This equation is derived from a least-squares fit of the experimental data, where T is the temperature in degrees Celsius and CS is the chemical shift relative to dioxane. [b] The capillary used for this study contained HMS, *p*-dichlorobenzene, and CCl_4. As a result, the absolute values for the shifts (relative to external HMS) reported here are different from those reported in Table V. The data in Table V are relative to a capillary containing HMS, bromoform, and CCl_4. This difference is a direct result of the bulk susceptibility difference between the two capillary samples.

Table II. FWHM of H-8 and H-2 in 5′-dAMP

Temperature, °C	H-8	H-2
10.0	4.8	3.3
32.0	3.0	2.4
50.0	3.0	2.1
70.0	2.3	1.5
92.5	*a*	2.1

[a] Could not be accurately determined due to exchange with solvent.

Table III. Deoxyribose H-1′–H-2′,2′′ Coupling Constant in 5′-dAMP

Temperature, °C	*J*, Hz[a]
10.0	6.8
32.0	6.8
50.0	6.9
70.0	6.8
92.5	6.7

[a] $J(\text{Hz}) = |J_{\text{H-1}'-\text{H-2}'} + J_{\text{H-1}'-\text{H-2}''}|/2$.

where $M(\text{H} - \text{X})_{\text{dioxane}}{}^{t°}$ is the chemical shift of monomer proton, H − X, at t° relative to dioxane; $P(\text{H} - \text{X})_{\text{dioxane}}{}^{t°}$ is the chemical shift of polymer proton, H − X, at t° relative to dioxane; and $C(\text{H} - \text{X})$ is a constant.

The necessity for inclusion of $C(\text{H} - \text{X})$ in the equation arises from experimental conditions. A monomer unit in the polymer at pH 8.0 is essentially 3′-, 5′-dADP·Na_2·diester. To make the best measurement of polymerization shift would have been to make a monomer study of this compound. However, this compound was not readily available, so our study was performed on 5′-dAMP·Na_2. While the basic conclusions of this paper are not changed by this difficulty, it seems desirable to simulate the proper reference monomer in so far as possible, recognizing that the assumed additivities described below may not be strictly correct. The constant, $C(\text{H} - \text{X})$, converts our data to the hypothetical diester compound by assuming additivity of substituent effects on chemical shifts.

Table IV. Correction on 5′-AMP·Na_2 to Simulate 3′,5′-ADP·Na_2·$(OMe)_2$[a]

	H − X	H-8	H-2	H-1′	H-2′′
Contribution of 5′-OMe ester					
5′-AMP·(OMe)·Na		8.883	8.717	6.605	
5′-AMP·Na_2		9.055	8.717	6.587	
		−0.172	0.0	0.018	
		(−10.32 Hz)	(0.0 Hz)	(1.08 Hz)	
Contribution of 3′-PO_4					
3′-AMP·Na		8.805	8.697	6.530	
Adenosine		8.820	8.730	6.533	
		−0.015	−0.033	−0.003	
		(−0.90 Hz)	(−1.98 Hz)	(−0.18 Hz)	
Contribution of 3′-PO_4 to H-2′ (from effect of 2′-PO_4 on H-1′)					
2′-AMP					6.610
Adenosine					6.533
					+0.077
					(+4.62 Hz)
Totals for use in eq 1					
	$C(\text{H} - \text{X})$	−11.22	−1.98	+0.90	+4.62

[a] Values from ref 13.

Chemical Shift *vs.* Temperature Studies of d(A)$_n$. The raw chemical shift values of the polymer (and monomer) are relative to the external lock signal, HMS. To correct for this external reference, chemical shifts are expressed relative to internal dioxane. Dioxane is not an "inert" internal reference (as is TMS for most organic solvents). It does have the great advantage of eliminating the need for tedious bulk susceptibility corrections. Deviations from ideality are not important in this instance because we are concerned with the differences between monomer and polymer shifts. Any deviations of the dioxane shift arising from hydrogen-bonding, temperature effects, etc. (which are expected to be minor) are canceled during comparison. In addition, the final plotted data are expressed as the "polymerization shift" (*PS*). The general equation for calculating the polymerization shift is

$$PS(\text{H} - \text{X})^{t°} = M(\text{H} - \text{X})_{\text{dioxane}}{}^{t°} - P(\text{H} - \text{X})_{\text{dioxane}}{}^{t°} + C(\text{H} - \text{X}) \quad (1)$$

Secondly, it is assumed that substituting a ribose moiety for a deoxyribose moiety has no major effect on the additivity of chemical shifts.

The first assumption allows corrections on 5′-AMP·Na_2 to simulate 3′-5′-ADP·$(OMe)_2$·Na_2. Table IV makes three types of corrections. The first correction measures the effect of converting the dianion to the monomethylated ester monoanion. The second correction measures the effect of converting the 3′-OH to 3′-PO_4 mono- or dianion. The third correction makes another minor assumption to obtain the effect of a 3′-PO_4 on the H-2′,2′′ protons. It is assumed that the magnitude of the effect of a PO_4 in 2′-AMP on H-1′ will be the same as in 3′-AMP on H-2′,2′′.

The second major assumption simply proposes that corrections calculated in Table IV for 5′-AMP·Na_2 are also valid corrections on 5′-dAMP·Na_2 to simulate the deoxydiphosphate–diester nucleotide at pH 8.0.

Results of polymer shifts relative to dioxane and monomer $+C(\text{H} - \text{X})$ are in Table V and plotted in Figures 3–5. Polymerization shift to a smaller number

Table V. Nmr at 60-MHz Data for $d(A)_n$[a]

Temp, °C	Hertz (dioxane) H-8	H-2	H-1′	H-2′,2″	Area (normalized)[b] H-8	H-2	H-1′	H-2′,2″	FWHM (raw), Hz H-8	H-2	H-1′	H-2′,2″	Hertz (HMS) dioxane
5.0	c	206.3	c	c	c	0.83	c	c	c	5.6	c	c	228.3
7.5	c	206.5	c	c	c	0.79	c	c	c	6.6	c	c	228.9
9.5	243.4	207.6	c	c	0.24	1.02	c	c	9.3	6.4	c	c	228.7
12.5	245.4	207.8	c	c	0.37	0.91	c	c	10.8	4.4	c	c	229.5
15.0	248.1	208.4	c	−86.5	0.57	0.95	c	1.05	16.7	5.2	c	23.1	230.1
17.5	246.6	209.2	c	−86.2	0.40	1.11	c	0.98	6.7	3.9	c	19.4	230.4
20.0	244.9	209.6	c	−86.0	0.50	1.08	c	0.99	7.8	4.2	c	20.0	231.2
22.5	248.8	211.2	c	−85.3	0.44	1.14	c	1.37	5.2	3.7	c	24.1	232.1
25.0	245.8	210.7	101.9	−84.7	0.50	1.12	0.58	1.15	9.0	4.6	12.5	21.7	231.9
27.5	246.8	211.7	104.7	−84.8	0.32	0.98	0.67	1.49	5.5	4.0	12.4	22.3	232.4
30.0	245.7	213.1	105.2	−85.9	0.51	1.21	1.04	1.24	8.7	4.4	15.2	17.2	232.3
32.5	246.8	213.8	106.1	−84.9	0.40	1.05	0.96	1.48	8.6	3.4	16.6	24.4	232.5
35.0	246.3	214.9	106.6	−86.1	0.52	1.15	1.04	1.39	7.6	3.5	13.6	18.5	232.9
37.5	246.7	216.2	108.3	−84.8	0.49	1.00	0.95	1.43	8.3	3.4	14.0	14.3	233.3
40.0	246.0	217.3	109.5	−85.4	0.52	1.01	1.10	1.84	6.1	3.3	14.6	16.1	233.5
42.5	247.7	218.6	110.7	−84.7	0.54	1.03	1.16	1.90	6.2	3.1	13.4	18.6	233.7
45.0	249.6	220.2	111.9	−84.2	0.29	1.14	1.23	1.98	5.5	2.9	11.5	17.7	234.1
47.5	248.2	221.4	112.7	−83.8	0.36	1.23	1.22	2.26	6.1	3.0	8.5	16.9	234.5
50.0	249.0	223.5	114.6	−84.1	0.55	1.19	1.31	2.21	5.4	2.9	9.0	17.7	234.6
52.5	249.7	225.1	115.3	−83.8	0.38	1.20	1.37	2.23	4.5	2.0	7.2	16.1	235.1
55.0	250.0	226.4	116.8	−83.1	c	1.26	1.29	2.73	c	2.3	7.3	16.7	235.3
57.5	250.4	228.2	117.9	−83.2	c	1.17	1.28	2.40	c	1.4	4.1	19.2	235.5
60.0	c	229.7	119.6	−82.0	c	1.30	1.38	2.36	c	1.2	3.5	16.9	235.8
62.5	c	231.0	120.7	−82.6	c	1.12	1.30	2.96	c	1.5	3.6	16.7	236.1
65.0	c	232.1	121.6	−82.3	c	1.37	1.57	2.53	c	1.6	4.2	16.8	236.4
67.5	c	234.0	123.5	−82.1	c	1.34	1.53	3.07	c	1.5	3.7	16.9	236.6
70.0	c	235.6	125.3	−82.0	c	1.31	1.55	3.46	c	1.2	3.8	15.1	236.8
80.0	c	239.2	128.6	−80.2	c	1.36	1.57	2.93	c	1.5	2.5	16.4	237.7
92.5	c	244.3	133.4	−77.6	c	1.31	1.55	3.54	c	1.3	2.6	14.5	238.4

[a] Most of the values reported are the average of several independent observations. Precision (and presumably accuracy) vary with the temperature and how well resolved the particular peak was. Generally, chemical-shift values for sharp peaks such as H-2 or dioxane showed standard deviations of 0.2 Hz. Broad signals such as H-1′ and H-2′,2″ showed standard deviations of 1.0–1.5 Hz. Area and FWHM values showed deviations of 5–10% of the reported value with the larger values, as expected, occurring primarily at low temperatures. [b] Areas are normalized against external bromoform, not to any of the polymer proton areas. [c] Peak not observable.

is toward lower field strength and the inherent monomer shift. The trend of all polymer protons with increasing temperature is to a smaller polymerization shift. The polymerization shift curves are conveniently put into two groups, according to their shape. H-8 and H-2′,2″ form one group and H-2 and H-1′ form another. It should be noted that the total change in polymerization shift for either one of a group is very similar.

Area *vs.* Temperature Studies of $d(A)_n$. The parameter of importance here is the normalized area. This quantity is determined by dividing the raw area of a given peak by the raw area of the external reference, bromoform, for that particular spectrum. As seen in Figure 2, the low- and middle-field sections of the

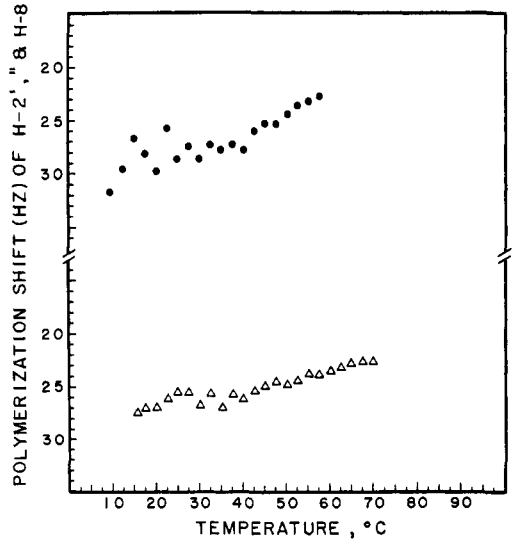

Figure 3. Polymerization shift (Hz) of H-2′,2″ (●) and H-8 (△) *vs.* temperature.

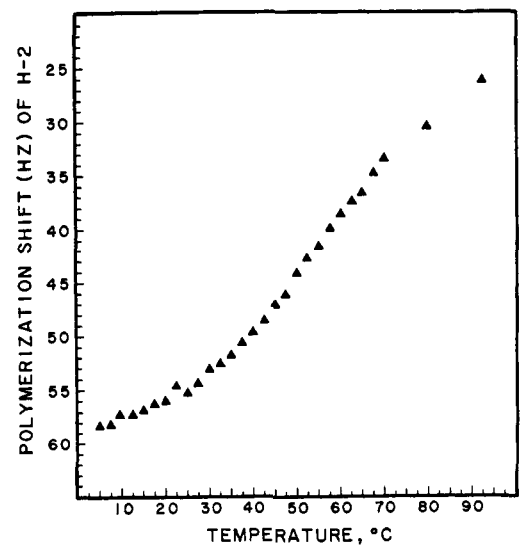

Figure 4. Polymerization shift (Hz) of H-2 *vs.* temperature.

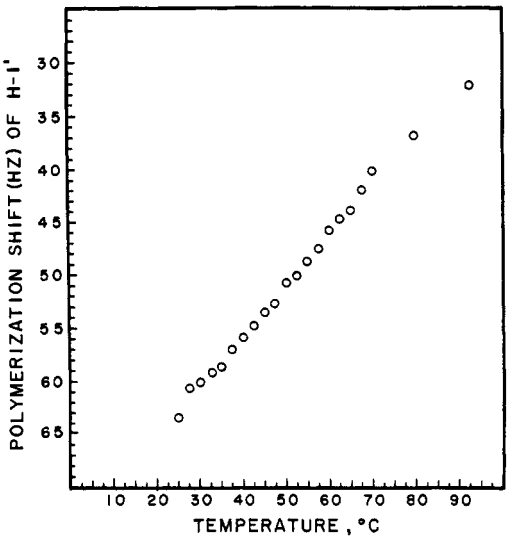

Figure 5. Polymerization shift (Hz) of H-1′ *vs.* temperature.

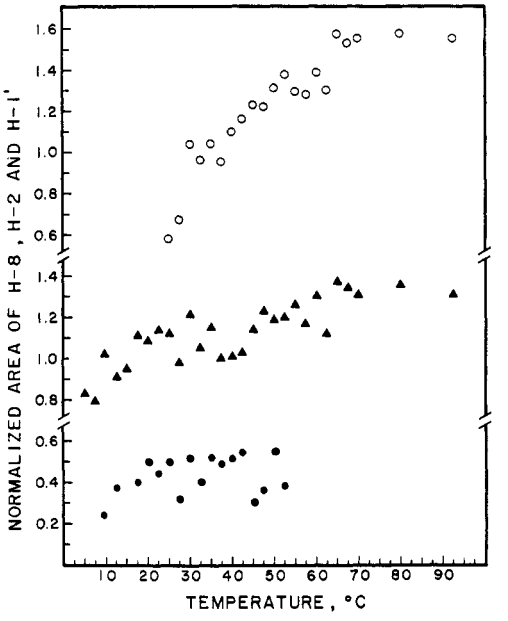

Figure 6. Normalized area of H-8 (○), H-2 (▲), and H-1′ (●) *vs.* temperature.

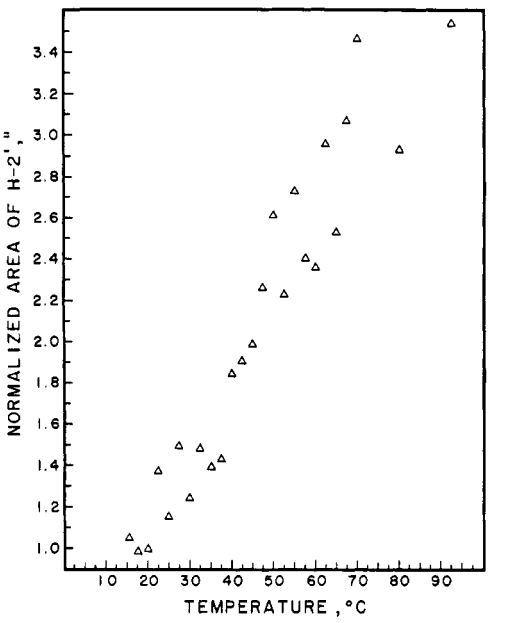

Figure 7. Normalized area of H-2′,2″ *vs.* temperature.

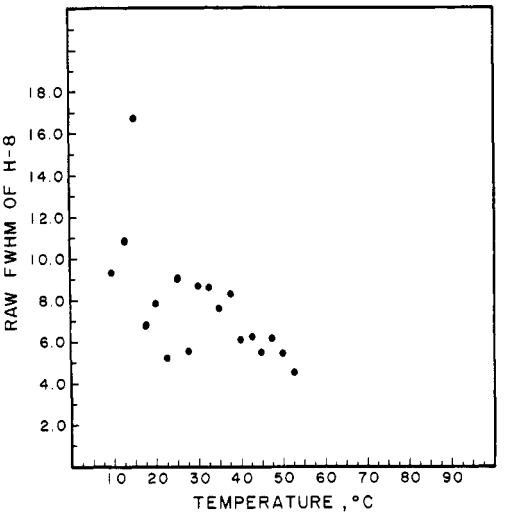

Figure 8. Raw FWHM of H-8 *vs.* temperature.

experimental spectra have the bromoform signal present, but the high-field section does not. Referencing H-2′,2″ to bromoform was accomplished indirectly *via* the dioxane signal which appears in both the mid- and high-field regions using the bromoform:dioxane ratio from the mid-field section at the specified temperature.

The area results of H-8, H-2, H-1′, and H-2′,2″ are listed in Table V and plotted in Figures 6 and 7. There are a few general features which may be observed from these plots. First, at low temperature (10°) H-8 is not observed, while H-2 already has significant area at 5°. Second, throughout the temperature range where data exist for H-8, its curve shape is very similar to that of H-2. Third, the base protons are revealed to a greater extent at lower temperatures than the sugar protons. Fourth, no comparison of appearance temperature (the temperature at which some signal can be detected) can be made between H-1′ and H-2′,2″. H-2′,2″ first appears at ~15°, but due to the overlap with HOD, H-1′ is obscured until 25°. Fifth, H-2 and H-1′ appear to be completely maximized at ~65°, but H-2′,2″ is not.

These observations seem to indicate similarities between different parts of a given moiety—the base or sugar unit. This will be discussed in a later section.

FWHM *vs.* Temperature Studies on d(A)$_n$. Results of raw full width at half maximum are in Table V, and plotted in Figures 8–11. The general trend for all polymer peaks is to sharpen as the temperature increases. Inspection of FWHM *vs.* temperature curves indicates the four proton types cannot be subdivided into groups as were the polymerization shift curves. The peaks for the base protons H-8 and H-2 are significantly different in half width at a given temperature, with H-8 > H-2. Since H-8 is difficult to analyze as low temperatures produce scattered points and is lost due to exchange with solvent above 52.5°, a meaningful slope is difficult to attain. Inspection of the curve for H-8 and that of H-2 below 52.5° suggests a slightly faster narrowing for H-2.

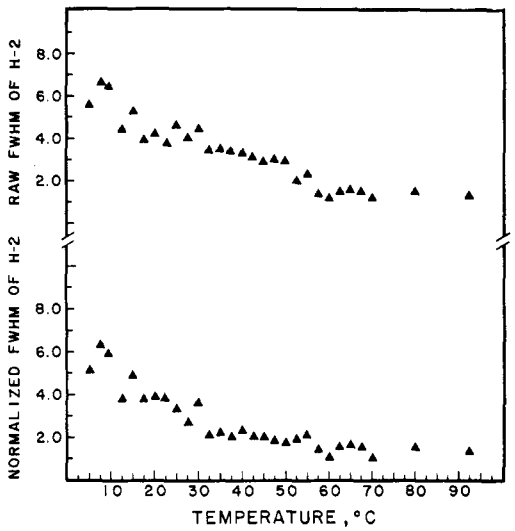

Figure 9. Raw and normalized FWHM of H-2 *vs.* temperature.

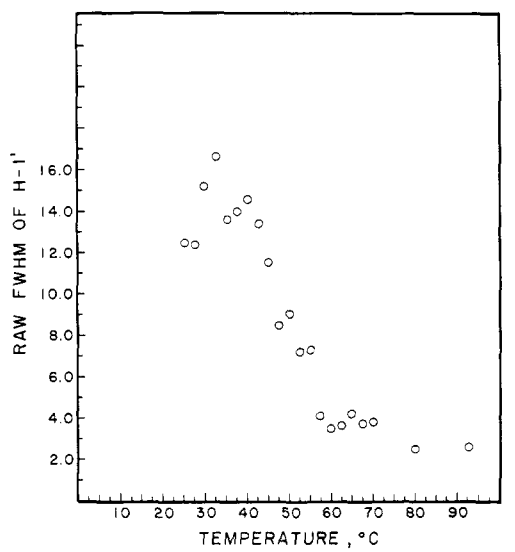

Figure 10. Raw FWHM of H-1′ *vs.* temperature.

FWHM for H-1′ and H-2′,2″ are both much larger than for the base protons. The rate of narrowing from 30 to 55° appears to be somewhat faster for H-1′ than H-2′,2″. An additional feature should be pointed out in the H-1′ curve—a discontinuity exists between 55.0 and 57.5°. Values below 55.0° represent the FWHM of the entire H-1′ signal while values above 57.5° are FWHM for the center peak of the H-1′ triplet. A small plateau exists over 50–80° (H-2′,2″) and 55–70° (H-1′) and then slight narrowing at higher temperatures. The FWHM changes for H-1′ and H-2′,2″ may or may not reflect true line width changes, particularly at lower temperatures. Some or all of the observed changes may reflect small variations in the chemical shift differences and/or coupling constants of the ABX system (*vide infra*).

Coupling Constant Studies on d(A)$_n$. There is only one pair of coupling constants which can be monitored as a function of temperature for this polymer system. The coupling is that observed from H-1′ due to spin–spin coupling to H-2′ and H-2″. This coupling pattern first emerges around 50.0–52.5°. Since H-2′ and H-2″ are not identical, the triplet observed for H-1′ represents the X portion of an ABX system and the spacing of the three lines corresponds to 1/2 $|J_{AB} + J_{BX}|$, in this case the two couplings to the H-2′ protons. It is convenient to refer to this quantity as the H-1′ coupling, H-1′–H-2′,2″ coupling, etc. Over the temperature range that the triplet spacing is observable, within experimental error the "average coupling" is temperature independent, having the value of 7.1 ± 0.3 Hz.

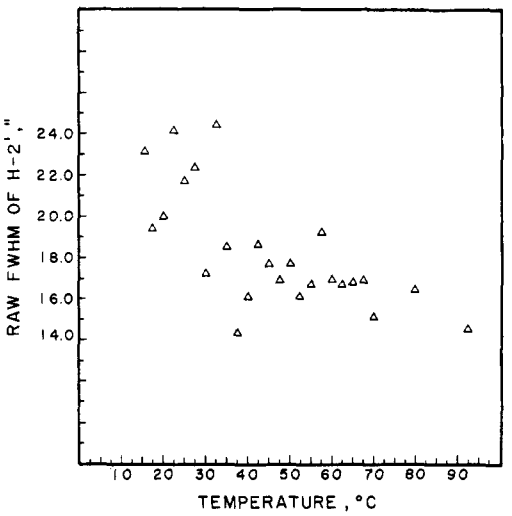

Figure 11. Raw FWHM of H-2′,2″ *vs.* temperature.

Discussion

Monomer Chemical Shift. Brief reference to the experimental design of the monomer system points out some features. Both nmr and vapor pressure osmometry (vpo) results conclusively show that purine nucleoside and nucleotide derivatives associate primarily by vertical stacking of base rings in aqueous solution.[13–16] To minimize the presence of this phenomenon in our monomer system, a concentration of 10 m*M* was chosen.

Since it is generally accepted that most nucleosides and nucleotides have an anti conformation about the glycosyl linkage, 5′-dAMP would also be expected to have the same conformation. Inspection of Table I shows that H-8 (dioxane) moves slightly upfield and H-2 moves slightly downfield with increasing temperatures. The specific effect of the 5′-PO_4 group on H-8, but not on H-2 has been clearly demonstrated by Schweizer, *et al.*[13] The fact that H-8 moves upfield and H-2 does not is consistent with 5′-dAMP being in an anti conformation. At low temperatures H-8 is deshielded by the doubly ionized phosphate, but at higher temperatures, with increased mobility and rotation about the glycosyl bond, on the average less specific deshielding by the phosphate occurs and H-8 moves upfield.

Monomer Area. The fact that the area of monomer peaks shows no appreciable change as a function of

(13) M. P. Schweizer, A. D Broom, P. O. P. Ts'o, and D. P. Hollis, *J. Amer. Chem. Soc.*, **90**, 1042 (1968).
(14) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *ibid.*, **89**, 3612 (1967).
(15) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *ibid.*, **85**, 1289 (1963).
(16) M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, *ibid.*, **87**, 5241 (1965).

temperature is not unexpected. The monomer size permits molecular rotational mobility of sufficient rate throughout the temperature scale to permit a well-resolved spectrum (*i.e.*, $\tau_c \ll 10^{-4}$ sec).

At elevated temperatures loss of area of H-8 occurred. This is a usual occurrence of the imidazole ring proton in purines,[10,11] and purine derivatives,[16,17] due to exchange of the proton with D_2O solvent.

Monomer FWHM. The FWHM of monomer base protons are only slightly temperature dependent as seen in Table II. The FWHM of sugar protons investigated are temperature independent. This slight line broadening of the base protons might be attributed to reduced rotation about the glycosyl linkage at low temperature or a slight amount of intermolecular association. The important feature is that they differ only slightly more than 1 Hz at the most and they are both well resolved. This indicates the base protons of the monomer are not under the influence of the same set of line broadening factors as in the polymer (*vide infra*).

Monomer Coupling Constant. In Table IV, the temperature independence of $J_{H\text{-}1'\text{-}H\text{-}2',2''}$ is seen. These data may be explained by two extremes. First H-1′, H-2′, and H-2″ maintain a rigid conformation relative to each other throughout the entire temperature range studied or second, a rapid equilibrium between various conformations involving H-1′, H-2′, and H-2″ exists. The first situation, a rigid H-1′, H-2″, H-2′ region, cannot be excluded by this data. An interconversion can occur between the C-3′-exo[18] form and the C-2′, C-3′-endo[19] form during which the dihedral angles[20,21] of H-1′, H-2′ and H-1′, H-2″ do not change.

The second possibility, a rapid equilibrium between various conformations, also cannot be excluded by this data. There is evidence supporting a rapidly equilibrating ribose in pseudouridine.[22] The temperature independence of $J_{H\text{-}1'\text{-}H\text{-}2'}$ seems to be a general phenomena observed for nucleosides and nucleotides,[23] although the magnitude of $J_{H\text{-}1'\text{-}H\text{-}2'}$ is base dependent.

Polymer Chemical Shift. The discussion of $d(A)_n$ data can best be made with respect to $r(A)_n$ data where it exists. Different investigators have used different reference compounds, so in Table VI the chemical shift data on $r(A)_n$ from various literature sources are compiled and expressed as polymerization shift along with the polymerization shift data reported here for $d(A)_n$. As indicated in Table VI (last column), at 30° the *PS* for H-8, H-2, and H-1′ of $d(A)_n$ are 0.48, 0.88, and 1.00 ppm, respectively. The absolute *PS* of H-8 in $d(A)_n$ and $r(A)_n$ are quite similar, differing only by 0.06–0.10 ppm. For H-2 and H-1′ the situation is very different. The *PS* of H-2 and H-1′ are 0.36–0.55 and ~0.62 ppm, respectively, larger in $d(A)_n$ than in $r(A)_n$. This large difference in *PS* would seem to reflect a structural difference between $r(A)_n$ and $d(A)_n$. This difference has been monitored by the *PS* which is basically dependent upon ring current anisotropies of the base units. Thus, the data suggest the existence of a different base unit organization in $r(A)_n$ and $d(A)_n$.

The study of $d(A)_n$ has also produced data on H-2′, 2″ of deoxyribose. There are no H-2′ ribo or deoxyribodimer or ribopolymer nmr data presently in print. This situation makes data comparison impossible but comparison within the $d(A)_n$ system is still very useful. The *PS* of H-2′,2″ in $d(A)_n$ at 30° is 26.8 Hz (0.447 ppm) using the hypothetical 3′,5′-dADP·$(OMe)_2$·Na_2 as the reference. This *PS* value is very similar to H-8. The fact that H-8 and H-2′,2″ show very similar *PS*–temperature curves and H-2 and H-1′ show another set of curves, has secondary structural implications. This is due to the fact that *PS* is monitoring base–base interaction which is a function of a polymer secondary structure.

The most general statement which can be made concerning any difference between $r(A)_n$ and $d(A)_n$ is that polymerization shifts indicate a larger interaction between bases in $d(A)_n$ than in $r(A)_n$. Inspection of Corey–Pauling–Koltun molecular models gives some indication of a possible cause for this. The presence of the C-2′ hydroxyl group in $r(A)_n$ appears to provide nonbonded steric interactions to at least two regions. One region is the phosphate group and the second is the furanose ring oxygen one sugar removed in the 3′ direction. The net result of these rotations is to reduce the base–base interactions in $r(A)_n$ relative to those in $d(A)_n$. It is not clear whether the increased interactions in $d(A)_n$ arise from different spacing of the base units or from different degrees or kinds of overlap, between the sequential base units. Either is possible, although the latter are more likely.

The structural implications of *PS* curve similarities are not easily resolved. In the simplest case, two species which experience similar *PS* magnitudes and have the same curve shape are experiencing the same type of anisotropic change as a function temperature. This would lead one to suspect these species were in a close spatial arrangement with respect to each other. A molecular model can be constructed such that H-8 and H-2′,2″ and H-2 and H-1′ are in close spatial arrangement. Such a model requires the H-8 be located immediately above the furanose ring oxygen or that H-8 be located above the H-2″ proton, which puts H-2 in close proximity with H-1′ of the adjacent sugar in the 3′ direction or with H-1′ of the adjacent sugar in the 5′ direction, respectively. This requires a large amount of tilt with respect to the backbone axis. Another possibility is that the species are not located in a close spatial relationship, but the sum of anisotropic effects from different sources makes the same net contribution at different places.

At the present time the data do not distinguish these possibilities. The elucidation of the exact, detailed nature of the base–base interaction must await further study.

Polymer Area. The trend of increasing area of all protons with increasing temperature is generally expected. At low temperatures the polymer is relatively rigid and nonmobile, giving rise to dipole–dipole interactions and reduced area in the high-resolution spec-

(17) R. J. Pugmire, D. M. Grant, R. K. Robins, and G. W. Rhodes, *J. Amer. Chem. Soc.*, **87**, 2225 (1965).

(18) Definition: exo, the atom is on the opposite side of the plane formed by C-1′–O–C-4′ as the C-4′–C-5′ bond.

(19) Definition: endo, the atom is located on the same side of the plane formed by C-1′–O–C-4′ as the C-4′–C-5′ bond.

(20) Definition: the dihedral angle of H_a–C–C–H_b is the angle between the plane defined by the carbon atoms and H_a and H_b, respectively.

(21) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(22) F. E. Hruska, A. A. Grey, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **92**, 214 (1970).

(23) P. O. P. Ts'o, N. S. Kondo, M. P. Schweizer, and D. P. Hollis, *Biochemistry*, **8**, 997 (1969).

Table VI. Polymerization Shift[a] (*PS*) Change of $r(A)_n$ and $d(A)_n$ at 30° with Monomer Reference

Compound	Proton	Reference: 5′-AMP·Na_2	5′-AMP·Na	5′-AMP·(OMe)·Na	3′,5′-ADP·$(OMe)_2$·Na_2
Reference[b]	H-8	9.055	9.857	8.883	8.868
$r(A)_n$	H-8	8.287	8.287	8.287	8.287
	PS	0.768	0.670	0.596	0.581
Reference[b]	H-2	8.717	8.705	8.717	8.684
$r(A)_n$	H-2	8.167	8.167	8.167	8.167
	PS	0.550	0.538	0.550	0.517
Reference[c]	H-8	−117.6	−111.7	−107.3	−106.4
$r(A)_n$	H-8	−74.1	−74.1	−74.1	−74.1
		(43.5)	(37.6)	(33.2)	(32.3)
	PS	0.725	0.627	0.553	0.538
Reference[c]	H-2	−91.6	−90.9	−91.6	−89.6
$r(A)_n$	H-2	−67.8	−67.8	−67.8	−67.8
		(23.8)	(23.1)	(23.8)	(21.8)
	PS	0.397	0.385	0.397	0.364
Reference[c]	H-1′	40.1	40.6	39.0	39.2
$r(A)_n$	H-1′	61.8	61.8	61.8	61.8
		(21.7)	(21.2)	(22.8)	(22.6)
	PS	0.365	0.353	0.380	0.377
Reference	H-8	285.47	279.59	275.15	274.25
$d(A)_n$	H-8	245.67	245.67	245.67	245.67
		(39.80)	(33.92)	(29.48)	(28.58)
	PS	0.663	0.565	0.492	0.477
Reference	H-2	268.27	267.55	268.27	266.29
$d(A)_n$	H-2	213.14	213.14	213.14	213.14
		(55.13)	(54.41)	(55.13)	(53.15)
	PS	0.918	0.906	0.918	0.885
Reference	H-1′	164.48	163.94	165.56	165.38
$d(A)_n$	H-1′	105.21	105.21	105.21	105.21
	PS	0.989	0.979	1.01	1.00

[a] In ppm. [b] From ref 13. [c] From ref 3.

trum. Increasing temperature increases polymer flexibility and mobility, reducing dipole–dipole interactions with a concomitant increase in area of the high-resolution nmr spectrum.

Giving first consideration to appearance temperatures of base protons, Figure 6 shows H-2 is observed at a lower temperature than H-8. At the lowest temperature investigated H-2 was easily observable but H-8 was not. This single piece of data implies the bases are in an anti conformation about the glycosyl bond. A proton in a motionally restricted, crowded, and confined environment would be subject to increased dipole–dipole interactions relative to one in a less restricted and open area. The region above the sugar ("endo" side) is more confined compared to the region away from the sugar (viewing from C-3′ to C-2′) which implies H-8 is above and H-2 is away, or the base is in an anti conformation.

Such an appearance temperature comparison of sugar protons is not possible because the initial appearance of H-1′ results from solvent HOD shifting sufficiently far from H-1′ so as not to obscure it. The appearance temperature at 15.5° of H-2′,2″ after the base protons is consistent with the polymer primary structure and helical secondary structure. Molecular models indicate the bases should have more freedom of movement than the sugar backbone.

The amount of proton area appearance relative to total proton area at low temperature (~35°) is greater for base protons than for sugar protons. This complements appearance temperature data for an earlier mobility of the bases. Over the temperature range 25–65°, H-1′ and H-2′,2″ have about the same rate of appearance. On a per proton basis, this indicates H-2′,2″ is appearing slower than H-1′. This reflects both environmental differences of the H-1′ and H-2′,2″ regions and differences in mobility of these parts of the sugar.

The temperature where the area first maximizes is dependent on the proton type. Because of exchange with HOD solvent by 52.5°, such a maximum for H-8 is not known. H-2 and H-1′ both maximize around 65.0° while H-2′,2″ is still increasing above this temperature. The interpretation of this result is in agreement with the previous observations, implying a reduced mobility of the sugar *vs.* base at a given temperature.

These area results may be compared to those of McTague, *et al.*,[5] for $r(A)_n$. Because experimental conditions (concentration, pH, ionic strength, etc.) are not identical, only a qualitative comparison between this work on $d(A)_n$ and their work on $r(A)_n$ can be made. Their area studies are reported between about 30 and 80°. The rate of H-1′ appearance for $r(A)_n$ is much

faster than this study indicates for $d(A)_n$. At about 40°, H-1′ area of $r(A)_n$ is revealed to the extent of about 90% of the total. For $d(A)_n$ 90% of H-1′ is not revealed until 55–60°. McDonald, *et al.*,[6] also did an area study of $r(A)_n$ over the temperature range 0–80°. Although experimental conditions varied slightly from McTague's work, the results for H-1′ are very similar. At approximately 35°, 90% of the total area is revealed. In both studies the H-1′ protons of ribose are essentially melted at 45–50°, whereas for H-1′ in deoxyribose this occurs around 65–70°.

The area of base protons may also be compared between this work and that of McTague and McDonald. Both of those works do not distinguish between H-2 and H-8 protons, so the H-2 and H-8 area data of this study must be combined for a comparison. The base protons of $d(A)_n$ generally increase in area with increasing temperature until at 52.5°, H-8 is exhanged for deuterium and at ~65°, H-2 area maximizes. This result is in qualitative agreement with $r(A)_n$ data of McTague and in disagreement with McDonald's data. McTague shows an increase in base area with increasing temperature going from 30 to 80°, while McDonald states, "The base protons of poly(A) are fully melted at 20°. . . ."

An extended discussion on the differences of areas and possible implications of these data do not seem warranted in light of the following considerations. The fact that H-8 is exchanged with solvent at elevated temperatures limits the accuracy of these results. The overall precision of these measurements also does not permit a meaningful discussion of these differences. The following generalizations do emerge from the area data. For $d(A)_n$, appearance temperatures implicate an anti conformation for the base unit about the glycosyl bond. The base units appear to be more mobile at a lower temperature than the sugar protons. Among the sugar protons, H-1′ appears more mobile than H-2′,-2″. Comparison of $r(A)_n$ and $d(A)_n$ may be attempted only with respect to H-1′. The data imply that the H-1′ region of $d(A)_n$ is subjected to more broadening mechanisms below ~65° than is $r(A)_n$.

Polymer Full Width at Half Maximum. The decrease in a line width with increasing temperature in a polymer system is generally a result of motional narrowing. To eliminate other possibilities for variations of line width with temperature, values for FWHM of both internal dioxane and external bromoform were determined. Line widths of both compounds are constant throughout the temperature range. Because line broadening can occur from changes of viscosity and inhomogenities of the applied field, these possibilities for polymer broadening are ruled out. Furthermore, the polymer proton most sensitive to this would be H-2. Comparison of plots of normalized FWHM *vs.* temperature and raw FWHM *vs.* temperature in Figure 9 are essentially the same. Thus, the amount of narrowing which occurs is dependent on the local environment and mobility of the protons and not gross sample effects, *e.g.*, viscosity.

The curves of the base protons H-8 and H-2 are significantly different in line width at a given temperature, with H-8 > H-2. Since H-8 is difficult to analyze as low temperatures produce scattered points and is lost due to exchange with solvent above 52.5°, a slope is hard to attain. However, a rough comparison of H-8 with H-2 below 60.0° indicates a slightly faster narrowing for H-2. This is consistent with the area data previously discussed and with their interpretation. The proton in a more confined environment would be potentially subject to more relaxation mechanisms (*e.g.*, spin–spin relaxation and electric quadrupole effects) than one in a less confined, more mobile site. One would again interpret this to imply the base assumes an anti conformation about the glycosyl linkage; on this basis H-8 would be in a restricted area and experimentally be broader than H-2.

FWHM for H-1′ and H-2′,2″ are both much broader than for base protons. It must be realized that at high temperatures in the polymer this is not due only to restricted motion, but also to spin–spin coupling of the deoxyribose ring system. The rate of narrowing between 30 and 55° appears to be somewhat faster for H-1′ than for H-2′,2″. This suggests a preferential mobility of this region over that of the H-2′,2″ region.

Another feature should be pointed out in the H-1′ curve—a discontinuity exists between 55.0 and 57.5°. The values below 57.5° represent the FWHM of the entire H-1′ peak while values above 55.0° are from the center peak of the H-1′ triplet. A small plateau exists over ~50–80° (H-2′,2″) and 55–70°C (H-1′) and then slight narrowing at higher temperatures.

Temperatures at which the plateaus begin correspond fairly well with the maximum area points of H-2, H-1′, and H-2′,2″. This is not unexpected since both parameters will level off in the limit of motional narrowing. It is of considerable importance that there is correspondence between area and FWHM. Smith, *et al.*,[24] have shown that increasing area as a function of temperature is not necessarily a monitor of a melting secondary structure, but may be monitoring dissociation of salt induced aggregates. For this to be the case, it was necessary for salt concentration to exceed 0.75 *M* NaCl, at a polynucleotide concentration of 100 mg/ml at pH 6.6. The salt concentration and polymer concentration of the $d(A)_n$ system studied here are far below this so aggregation is not likely.

Polymer Coupling Constant. Of the proton types observed in the polymer, only H-1′ and H-2′,2″ are bonded closely enough to show observable spin–spin coupling. While this is necessary, it is not a sufficient condition to observe a coupling constant because of line broadening due to dipole–dipole interactions. A rather abrupt change in shape is observed at about 50.0–52.5°. Up to this temperature H-1′ is broad and lacks detail, but at 52.5° the triplet of H-1′ is easily seen. Above this temperature until 80.0°, the three peaks of the triplet progressively sharpen.

The rather abrupt appearance of the coupling in H-1′ might lead one to anticipate a corresponding change in another H-1′ parameter. This does not seem to be the case with the possible exception of the area. There is a slight plateau in the area plot from ~50.0 to 60.0° but considering the error limits of area measurement, the significance of this feature is doubtful.

It is of much interest to observe the value of the average coupling constant, $1/2|J_{\text{H-1′–H-2′}} + J_{\text{H-1′–H-2″}}|$ as a function of temperature. Throughout the temperature range where this coupling is observable it is in-

(24) I. C. P. Smith, T. Yamane, and R. G. Shulman, *Science*, **159**, 1360 (1968).

variant (7.1 Hz) within experimental error. This result is to be contrasted to results of Hruska, *et al.*,[25] for rAprA.

They report a strong temperature dependence of $J_{H\text{-}1'\text{-}H\text{-}2'}$ in both the 3′ and 5′ portions of the dimer. For both riboses this coupling varies ~2.4 Hz over the temperature range 4–71°. They have also reported the temperature independence of $J_{H\text{-}1'\text{-}H\text{-}2'}$ in both 3′-AMP and 5′-AMP. The $J_{H\text{-}1'\text{-}H\text{-}2'}$ in the dimer tend toward the respective monomer values at the high temperature limit. They attribute the changes in $J_{H\text{-}1'\text{-}H\text{-}2'}$ of the riboses to a conformational change occurring during the intramolecular destacking of the base rings of the dimer. Because a variation of $J_{H\text{-}1'\text{-}H\text{-}2',2''}$ is not observed in $d(A)_n$ as a function of temperature, two possible situations may be considered as previously explained for 5′-dAMP. Either the H-1′, H-2′,2″ region is fixed throughout the temperature range 52.5–92.5° or by 52.5° an equilibrium of conformations exist such that $J_{H\text{-}1'\text{-}H\text{-}2',2''}$ is an average value of them all.

Although the H-2′,2″ region is rather featureless at low temperatures, some detail becomes apparent at high temperatures. The detailed structure does not make nearly such an abrupt appearance as H-1′ and it occurs at a much higher temperature. This can be due to several factors. It is possible that the H-2′,2″ region is experiencing constraints in addition to those experienced by the H-1′ region. The presence of the phosphate group at the 3′ carbon may be responsible for this. Another factor may be the coupling of the H-1′ proton. This results in more and closer spaced lines which makes resolution more difficult. The latter possibility would seem to account for the rather continuous increase in resolution with temperature.

Conclusions

The conclusions of this study on $d(A)_n$ are conveniently separated into three areas that deal with the sugar unit conformation, sugar–base conformation, and base–base interactions. Information on the deoxyribose moiety comes from all of the four nmr parameters studied—chemical shift, area, FWHM, and coupling constant. The results which can be directly related to sugar conformation come from the "coupling constant," $J_{H\text{-}1'\text{-}H\text{-}2',2''}$. It has been shown that this spacing is invariant with temperature throughout the measurable temperature range (52.5–92.5°). In light of the fact that $J_{H\text{-}1'\text{-}H\text{-}2',2''}$ of the monomer is temperature independent from 10.0 to 92.5° and is essentially the same value as the polymer, and that the temperature-dependent ribodimer couplings tended toward the ribomonomer at high temperature, the rapidly equilibrating region about H-1′, H-2′,2″ seems to serve as the simplest explanation.

Another conclusion on $d(A)_n$ structure can be made with respect to the sugar–base conformation. The evidence for a preferred conformation about the glycosyl bond comes from two sources, the area and FWHM. Sufficient data have been published on the work of ribomonomers and ribodimers to conclude in those cases that the base assumes an anti conformation. A similar conclusion was reached by Holcomb, *et al.*,[26] for $r(A)_n$. The deoxyribose monomer data of this study also indicate an anti conformational preference. In $d(A)_n$ the increased H-8 *vs.* H-2 line width and lower area appearance temperature for H-2 both support the idea the bases are in an anti conformation about the glycosyl bond.

Consideration of the base–base interactions provides some conclusions to be made on the base conformations yielding such an interaction. There were several general trends which emerged from the polymerization shift data of $d(A)_n$. The shapes of the *PS vs.* temperature curves for H-8, H-2, H-1′, and H-2′,2″ fall into two groups according to the shape of the curve. H-8 and H-2′,2″ form one group and H-2 and H-1′ form another group. As stated in the Discussion, this can be explained by two different geometric situations which yield similar results. Either the protons of a group are in a spatially close relationship and thus experience similar ring current anisotropies or they are in different spatial volumes but the sums of anisotropies at their respective locations are equivalent. The fortuitous anisotropic equivalence at two different sites seems unlikely. The former situation requires that the bases must be tilted with respect to the backbone axis. Such a situation has been eluded to by Bush and Scheraga[27] in their CD study of $d(A)_n$.

A comparison of this work on $d(A)_n$ with that previously published for $r(A)_n$ reveals some important conclusions. It appears there is a stronger interaction between the bases in $d(A)_n$ than in $r(A)_n$. Even though the nmr data in print for $r(A)_n$ are very limited, it is clear that around 30° the polymerization shift is significantly larger (twofold) for $d(A)_n$ relative to $r(A)_n$. This can be most easily explained by attributing the increased *PS* to an increased ring-current effect in $d(A)_n$ resulting from either a different relative orientation or a closer stacking of the bases.

The possibility that a difference in the stacking arrangement exists in $d(A)_n$ and $r(A)_n$ has important implications in molecular biology. The differences in the roles of RNA and DNA in a living system are very important ones. It is of prime importance that in the replication and transcription processes, the enzyme performing the process is able to recognize the difference between RNA and DNA. It is possible that an enzyme's active site is designed so the execution of a process is dependent on the proper base–base organization of the polymer, *i.e.*, the secondary structure difference is the basis for recognition. In this way, for example, a DNA duplicating enzyme could not use RNA as a template for making another polymer because the enzyme and RNA could not "fit" together and properly associate for the reaction to occur.

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