Nuclear Magnetic Resonance Determination of Thymine Nearest Neighbor Base Frequency Ratios in Deoxyribonucleic Acid

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Abstract: Nmr spectra at 220 Mcps of single-stranded DNA in neutral aqueous solution exhibit two well-resolved thymine methyl proton resonances that are attributable to the presence of two nonequivalent thymine methyl environments. Analyses of spectra of DNA from different organisms and for different solvent conditions and of thymine-containing deoxyribonucleoside monophosphate dimers indicate that the two magnetic environments depend on whether there is a purine or pyrimidine in the 5′-neighbor position to thymine. Consequently, the intensity ratio of the two resonances provides a measure of the frequency of occurrence of purines *vs.* pyrimidines in this 5′-neighbor position. This ratio was found to vary for DNA's from different species. The results indicate further that, even at 90° in the single-stranded DNA polymer, thymine bases maintain a stacked relationship with respect to purine 5′ neighbors that is similar to that of helical DNA. Incompletely resolved structure of other DNA resonances suggests that multiple environments also exist for other purine and pyrimidine protons.

Earlier,[1] we described the high-resolution proton magnetic resonance (pmr) spectrum at 60 Mcps of single-stranded, calf thymus DNA in neutral aqueous solution. It was noted that the protons of thymine methyl (T-CH_3) groups give rise to two chemically shifted resonances of unequal intensities although in the monomer (TMP) only one resonance is observed.[2] This splitting of 7 cps of the T-CH_3 proton resonance of single-stranded DNA was attributed to the distribution of thymine into two nonequivalent environments, and it was suggested that local magnetic fields arising from ring currents in nearest neighbor bases were responsible for these different shielding environments. In these earlier spectra incomplete resolution of the two T-CH_3 peaks and a poor signal-to-noise level precluded detailed analysis. Pmr spectra of DNA from several species have now been examined with a 220-Mcps spectrometer which provides improved sensitivity and enhanced chemical shifts. The single-stranded forms of DNA obtained by heating the DNA samples in neutral aqueous solution above their "melting" temperatures all exhibit, at 220 Mcps, two well-resolved T-CH_3 proton resonances separated by 28 cps. From examination of these spectra and pmr spectra of deoxyribonucleoside monophosphate dimers containing thymine, we conclude that the lower field member of the pair of T-CH_3 proton resonances arises from thymine residues that have a pyrimidine at the 5′-neighbor position and that the higher field line arises from thymines that have a purine at the 5′-neighbor position. A corollary conclusion is that, even at 90° in the single-stranded polymer, thymine bases maintain a stacked relationship with respect to purine 5′ neighbors that is similar to that in double-stranded helical DNA. The experimental basis for these conclusions is described below.

Experimental Section

Samples of purified DNA of various types were obtained from the following commercial sources: calf thymus (Sigma Chemical Co., Type 1 and CalBiochem, A grade), calf marrow (CalBiochem, A grade), calf spleen (CalBiochem, A grade), and salmon sperm (CalBiochem, A grade). Highly purified samples of C_{16} and T_6 bacteriophage DNA were obtained from Dr. M. A. Jesaitis of Rockefeller University. C_{16} is a Coliphage serologically and genetically related to T-even phage.[3] The $T4_0$ and ϕX-174 DNA samples were prepared in our laboratory. The $T4_0$ Coliphage DNA was isolated by the method of Jesaitis[4] by disrupting the phage with repeated freezing and thawing followed by deproteinization with mixtures of chloroform and octanol. The ϕX-174 DNA was isolated by the method of Sinsheimer.[5]

The following general experimental procedure was followed. DNA was dissolved in neutral D_2O at room temperature to preexchange labile protons and was then lyophilized. A sample of the DNA was prepared for nmr examination by dissolving it in neutral D_2O at room temperature in an nmr sample tube. DNA concentrations of 15–30 mg/ml were used. These samples were examined for pmr at room temperature to determine whether any interfering impurities were present other than residual HDO in the solvent. DNA at this temperature, if it is in the double-stranded, helical form, does not exhibit high-resolution pmr as the resonances are broadened by anisotropic dipole–dipole interactions to such an extent that they are not observable by this technique.[1] The DNA samples were then taken to about 90° where the helical species were melted to the more flexible single-stranded forms, and pmr spectra of these solutions were examined.

Spectra were obtained with a Varian Associates high-resolution pmr spectrometer which operates at a frequency of 220 Mcps. The polarizing field of about 52,000 gauss is furnished by a superconducting solenoid.[6,7] The temperature in the sample zone is established by a stream of nitrogen of controlled but variable temperature and was regulated within ±1° in our experiments. Chemical shifts of the DNA resonances were measured with respect to the methyl proton resonance of sodium 2,2-dimethyl-2-silapentane-5-sulfonate used as an internal reference and are expressed as magnetic field shifts to low field from this reference resonance in units of parts per million (ppm) of the polarizing field. Adequate signal-to-noise ratios for the DNA resonances were obtained by using a computer of average transients (Varian Associates C 1024) to improve the signal-to-noise levels provided by the spectrometer.

Results

As we described previously,[1] single-stranded calf thymus DNA in neutral D_2O at 90° exhibits pmr absorption in five discrete spectral regions: resonances

(1) C. C. McDonald, W. D. Phillips, and S. Penman, *Science*, **144**, 1234 (1964).
(2) The spin–spin coupling of 2 cps between the methyl and H-6 protons of thymine is ignored throughout this paper.
(3) M. A. Jesaitis, *Bacteriol. Proc.*, 45 (1959).
(4) M. A. Jesaitis, *J. Exptl. Med.*, **106**, 233 (1957).
(5) R. L. Sinsheimer, *J. Mol. Biol.*, **1**, 37, 43 (1959).
(6) F. A. Nelson and H. E. Weaver, *Science*, **146**, 223 (1964).
(7) *Chem. Eng. News*, **44**, 46 (Sept. 5, 1966).

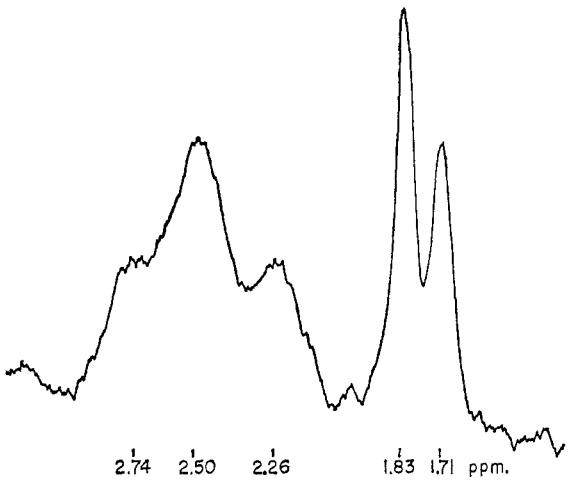

Figure 1. High-field regions of pmr spectrum of calf thymus DNA at 220 Mcps: concentration 20 mg/ml in D_2O, pD 7.0, 93°, 20 spectra averaged in computer of average transients.

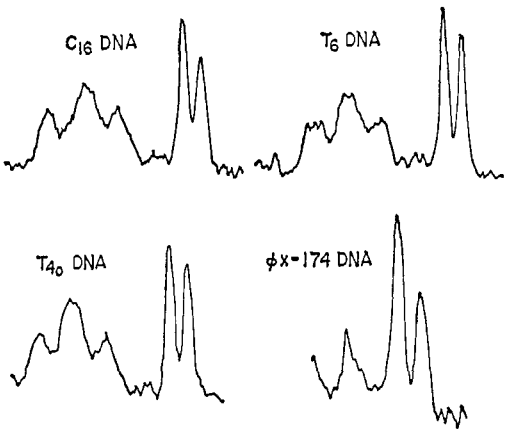

Figure 2. High-field regions of pmr spectra of bacteriophage DNA's at 220 Mcps: C_{16}, 16 mg/ml in D_2O, pD 7, 93°, 30 spectra averaged; T_6, 15.7 mg/ml in D_2O, pD 7, 93°, 30 spectra averaged; $T4_0$, 20 mg/ml D_2O, pD 7, 93°, 30 spectra averaged; ϕX-174, 30 mg/ml in D_2O, pD 7, 91°, 90 spectra averaged.

from 7.3 to 8.3 ppm include H-2 and H-8 protons of adenine, H-8 protons of guanine, and H-6 protons of cytosine and thymine; the region from 5.8 to 6.4 ppm includes H-5 protons of cytosine and H-1′ protons of deoxyribose moieties; the region from 4.6 to 5.1 ppm arises from H-3′ protons; the region from 2.1 to 2.8 ppm arises from H-2′ protons; and the two partially resolved T-CH_3 resonances extend from about 1.6 to 1.9 ppm. The H-4′ and H-5′ proton resonances occur in the region from 3.9 to 4.4 ppm and are largely obscured at 90° by the resonance of HDO.

In this study we are concerned mainly with an analysis of the T-CH_3 resonances. A spectrum of the H-2′ and T-CH_3 resonances of calf thymus DNA (Sigma) at 93° is shown in Figure 1. The two T-CH_3 resonances centered at 1.71 and 1.83 ppm are almost completely resolved. The corresponding frequency separation is 28 cps at 220 Mcps as compared with about 7 cps at 60 Mcps, confirming our previous conclusion that a chemical shift and not spin–spin splitting is responsible for the appearance of the two T-CH_3 resonances (chemical shifts expressed in frequency units are linearly dependent on the magnitude of the resonance frequency or the polarizing magnetic field). However, the line widths of these two resonances are considerably greater at 220 Mcps than at 60 Mcps, suggesting that each of the two resolved regions of absorption actually is an envelope of two or more unresolved chemically shifted resonances which also exhibit greater chemical shifts at 220 Mcps and thereby broaden the incompletely resolved envelope. The splitting and relative intensities of the 1.71- and 1.83-ppm T-CH_3 resonances were unaffected by changes in pD of the solution from 6 to 11, although the higher field line was broadened in alkaline solutions. In acidic solutions, the DNA is, of course, rapidly degraded at 90°. It has not been possible to examine the dependence of the DNA resonances on temperature when the solvent is neutral D_2O except in the narrow range from about 85 to 95°. At lower temperatures, the DNA partially reverts to a rigid, helical configuration that results in a broadening of the two T-CH_3 resonances so that they can no longer be resolved. However, by adding dimethyl sulfoxide (10%) to the "melted" DNA solution we were able to obtain well-resolved spectra of the T-CH_3 resonances from 90 down to 50°. The separation and relative intensities of the two T-CH_3 resonances were unchanged over this temperature range.

The intensity ratio of the two T-CH_3 resonances of calf thymus DNA (Sigma) ($I_{1.71}/I_{1.83}$) was measured for Figure 1 and nine other similar spectra, each composed of many individual spectral scans averaged by the computer of average transients. A value of 0.67 ± 0.02 was obtained. Ten similar experiments on calf thymus DNA from another source (CalBiochem) provided identical spectra with an $I_{1.71}/I_{1.83}$ ratio of 0.66 ± 0.02.

Pmr spectra of the T-CH_3 region of DNA from other sources (calf marrow, calf spleen, salmon sperm, and the bacteriophage species C_{16}, $T4_0$, T_6, and ϕX-174) in neutral D_2O at 90° all exhibited two resonances at about the same field positions as those for calf thymus DNA. The intensity ratio, however, did depend on the species from which the DNA was obtained. Spectra of the bacteriophage DNA's showed considerable variation in this respect, and their spectra in the high-field region are shown in Figure 2. Intensity ratios for nine DNA samples are presented in Table I.

Table I. Relative Intensities of DNA T-CH_3 Resonances

DNA source	$I_{1.71}/I_{1.83}$[a]	$\frac{ApT + GpT}{TpT + CpT}$[b]
Calf thymus (Sigma)	0.67	0.77
Calf thymus (CalBiochem)	0.66	
Calf marrow	0.69	
Calf spleen	0.69	
ϕX-174	0.61	0.82
C_{16}	0.75	
Salmon sperm	0.79	0.85
T_6	0.85	0.92
$T4_0$	0.89	0.95

[a] From our analysis, equal to (ApT + GpT)/(TpT + CpT). [b] Calculated from Kornberg, *et al.*[20]

Discussion

The existence of two resonances for T-CH_3 protons in the pmr spectra of single-stranded DNA's in neutral

D_2O at high temperatures implies that there are two magnetically nonequivalent environments for T-CH_3 groups in these polymer molecules. The constancy of the positions and relative intensities of the T-CH_3 resonances for DNA from a single organism regardless of considerable variation of pD, temperature, or solvent environment (addition of dimethyl sulfoxide) indicates that there is no facile exchange between the two environments. This conclusion and the observed species dependence of the intensity ratio of the two T-CH_3 resonances strongly suggest that the two environments depend on the nature of the neighbor bases to thymine in the polymer chain.

Clues to the nature of this neighbor-base-dependent environment for thymine methyl groups were found upon examination of the pmr spectra of 3′–5′ linked deoxyribonucleoside monophosphate dimers containing thymine (TpT, d-TpC, d-TpA, d-TpG, d-CpT, d-ApT, and d-GpT).[8] Spectra of these dimers in neutral D_2O at 20, 50, and 90° will be described in detail elsewhere.[9] Here we are primarily concerned with only the T-CH_3 resonances. In brief, the results were as follows. The T-CH_3 resonance of TMP is a single line located at 1.89 ppm at all three temperatures. All the thymine-containing dimers including TpT exhibit only one T-CH_3 resonance. This resonance occurs from 1.81 to 1.84 ppm at 20° and from 1.86 to 1.89 ppm at 90° for dimers in which thymine is in the 5′-neighbor position (*i.e.*, has a free 5′-hydroxyl group) regardless of the nature of the 3′ neighbor (*i.e.*, for TpT, d-TpC, d-TpA, and d-TpG). The resonance position is similar when thymine has a pyrimidine neighbor on the 5′ side (*i.e.*, for TpT and d-CpT). Thus, the T-CH_3 proton resonance of thymine in these environments corresponds closely to that of TMP and the resonance of DNA at 1.83 ppm. However, as shown in Table II, the T-CH_3 proton resonances of dimers in which thymine has a purine neighbor on the 5′ side (d-ApT and d-GpT) are shifted considerably to higher field values at 20° and move toward the lower resonance fields of the other thymine-containing dimers with increasing temperature.

Table II. Chemical Shifts of T-CH_3 Resonances of d-ApT and d-GpT

	20°	50°	90°
d-ApT	1.61	1.71	1.78
d-GpT	1.68	1.75	1.81

These findings were not unexpected since it is well established that purines and pyrimidines, either as monomers or as components of nucleic acids, tend to form stacked arrays in neutral aqueous solution at low temperatures.[1,10–18] Purine–purine or purine–pyrimidine stacking interactions appear to be stronger than those between pyrimidines.[11] Such stacking interactions involving purines can be manifested in pmr spectra by high-field shifts of resonances of protons near a stacked purine base that are induced by a local magnetic field arising from a ring current in the purine.[1,10,12] The extent of this shift is of course dependent on the magnitude of the ring-current field and on the distance and orientation of the observed proton with respect to the purine ring. Ring-current fields from stacked pyrimidine neighbors are small compared to those from purines.[13] In general, stacked arrays of purine and pyrimidine bases dissociate as temperature is increased. However, when strong stacking interactions are involved and the bases are tied together in polymers, some degree of stacking can persist to quite high temperatures.

In view of the earlier findings just cited, we ascribe the high-field shifts of T-CH_3 proton resonances of d-APT and d-GPT at 20° to nonrandom stacking of the purine and thymine bases in which the favored configuration is one in which the thymine methyl group lies well within the ring-current field of the purine. As temperature is increased, the equilibrium between these stacked forms and forms in which there is a random relationship between the two bases of the dimer is shifted toward the randomized configurations which should provide a T-CH_3 proton resonance at about 1.89 ppm. Apparently, there is a fast exchange between stacked and randomized configurations since a single, narrow, "time-averaged" resonance is observed that includes all participating species. Probably intramolecular stacking also occurs for d-TpA and d-TpG at low temperatures, but the favored configuration is such that the methyl group of thymine is too far from the purine ring to be influenced by the purine ring-current field. (This view is supported by the fact that for d-TpA and to a lesser extent for d-TpG at 20° a substantial shift to high field of thymine 2′ resonances is observed that is temperature dependent whereas the T-CH_3 resonances occur near the normal 1.89-ppm position from 20 to 90°.)[9] Schweizer, *et al.*,[19] have recently reported a rather similar observation in pmr spectra of neutral aqueous solutions of 3′ → 5′ linked diribonucleoside monophosphates in that the H-5 resonance of cytosine of ApC is 12.5 cps to higher field than that of CpA (at 100 Mcps).

It thus appears that the T-CH_3 resonance of DNA at 1.71 ppm arises from thymine bases that are in a stacked relationship with neighbor purine bases such that the thymine methyl groups are in the ring-current fields of the purine rings. Furthermore, all these thymine–purine geometrical relationships that are effective in shifting the T-CH_3 resonance are maintained at 90° since there is no change in position or intensity of this resonance as temperature is decreased. We strongly suspect that the favored stacking arrangement of thy-

(8) The conventional designation is used wherein the first moiety has a free 5′-hydroxyl group and the second has a free 3′-hydroxyl group.
(9) C. C. McDonald, L. R. Melby, W. D. Phillips, and D. R. Strobach, in preparation.
(10) O. Jardetzky, *Biopolymers Symp.*, 1, 501 (1963).
(11) P. O. P. Ts'o, I. S. Melvin, and A. C. Olsen, *J. Am. Chem. Soc.*, **85**, 1289 (1963).
(12) S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *ibid.*, **86**, 4182 (1964).
(13) M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, *ibid.*, **87**, 5241 (1965).
(14) D. N. Holcomb and I. Tinoco, Jr., *Biopolymers*, **3**, 121 (1965), and references therein.
(15) J. Brahms, A. M. Michelson, and K. E. Van Holde, *J. Mol. Biol.*, **15**, 467 (1966).
(16) M. Leng and G. Felsenfeld, *ibid.*, **15**, 455 (1966).
(17) S. I. Chan, B. W. Bangerter, and H. H. Peter, *Proc. Natl. Acad. Sci. U. S.*, **55**, 720 (1966).
(18) C. C. McDonald and W. D. Phillips, "Proceedings of the Second International Conference on Magnetic Resonance in Biology," Pergamon Press Inc., New York, N. Y., 1967.
(19) M. P. Schweizer, D. P. Hollis, and P. O. P. Ts'o, Abstracts, 9th Annual Meeting of the Biophysical Society, San Francisco, Calif., Feb 24–26, 1965, p 16.

mine with neighbor purines in single-stranded DNA is similar to that in the deoxyribonucleoside monophosphate dimers and, therefore, that the DNA resonance at 1.71 ppm arises only from methyl protons of thymine bases that have a purine at the 5′-neighbor position. This assignment is supported by inspection of the geometrical relationships of stacking of thymine in an ApTpA sequence in a model of the structure of double-stranded helical DNA (Figure 3). Here, the methyl group of thymine is quite close to the adenine ring on the 5′ side and quite remote from the adenine ring at the 3′-neighbor position. To summarize our hypothesis, in single-stranded DNA at 90° in neutral aqueous solution, thymine bases retain a stacked configuration with purine bases on the 5′-neighbor side that is similar to the configuration in helical DNA. The intensity ratio $I_{1.71}/I_{1.83}$ then provides a measure of the ratio of purine to pyrimidine bases at the 5′-neighbor position of thymine bases in DNA. Whether stacked relationships of other bases are retained at 90° cannot be determined from the T-CH_3 resonances, but, as discussed below, multiple resonances in other resonance regions of DNA suggest that such is the case. We suggested above that the breadths of the T-CH_3 resonances at 1.83 and 1.71 ppm indicated that these lines were envelopes of unresolved resonances. Minor perturbations of the T-CH_3 resonance positions may well occur that depend on which particular purine or pyrimidine base is the 5′ neighbor and also on the identity of the 3′ neighbors.

If the frequency of occurrence of the various possible 5′ neighbors of thymine in double-stranded DNA were random, the purine–pyrimidine ratio at the 5′ position (and $I_{1.71}/I_{1.83}$) would be unity. That such nearest neighbor frequencies are not random but species dependent has been shown by the elegant work of Kornberg and co-workers.[20] For the DNA samples that were examined in this study, $I_{1.71}/I_{1.83}$ ratios presented in Table I show that the frequency of occurrence of pyrimidines exceeds that of purines at the 5′-neighbor position to thymine in each case. For calf and salmon DNA, the thymine nearest neighbor frequencies reported here represent averages of the values of the various, different-component DNA molecular types. In addition, for all the species having double-stranded DNA, the unique values of the complementary strands are averaged in the measured frequency ratios. It has been established for the double-stranded DNA of the virus SP-8 that one strand is purine rich and the other is pyrimidine rich.[21] Presumably, the same is true to a greater or lesser extent for most double-stranded DNA's and one would expect the (ApT + GpT)/(CpT + TpT) ratio to be greater for the purine-rich strand than for the pyrimidine-rich complementary strand. The virus ϕX-174 provides a single-stranded DNA molecule of known base composition.[5] A random distribution of these bases would yield a value for (ApT + GpT)/(CpT + TpT) of 0.966 whereas the measured value is 0.61.

In agreement with our hypothesis for the assignment of the T-CH_3 resonances, the $I_{1.71}/I_{1.83}$ ratio is constant

(20) J. Josse, A. D. Kaiser, and A. Kornberg, *J. Biol. Chem.*, **236**, 864 (1961); M. N. Swartz, T. A. Trautner, and A. Kornberg, *ibid.*, **237**, 1961 (1962).

(21) J. Marmur, C. M. Greenspan, E. Palecek, J. Levine, and M. Mandel, *Symp. Quant. Biol.*, **28**, 191 (1963).

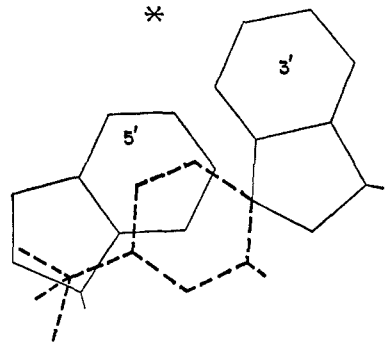

Figure 3. Representation of stacking relationships of bases of a d-ApTpA sequence in helical DNA as viewed parallel to the helix axis (*). The 5′- and 3′-purines are positioned, respectively, above and below the plane of the thymine.

for DNA from different cell types of a single species but varies with different species. Some of the DNA samples that we examined were from species studied by Kornberg, *et al.*,[20] and comparisons can be made between values of the purine:pyrimidine frequency ratio at the 5′-neighbor position to thymine determined by these two very different methods. From their nearest neighbor frequency data, ratios of (ApT + GpT)/(CpT + TpT) have been calculated and are shown in Table I. It can be seen that there is qualitative but not quantitative agreement between the two methods. Our data indicate in general a considerably greater departure from randomness in favor of pyrimidines over purines at the thymine 5′-neighbor position. It would appear that the nmr determination of this ratio is the more direct and should be less subject to error. Nevertheless, there are potential pitfalls. If a small component of intensity from the nearby H-2′ resonances underlies the T-CH_3 resonance at 1.83 ppm, the "measured" purine:pyrimidine ratios would be smaller than the true values. We believe this possibility to be unlikely but as yet have failed to completely eliminate it.

Indications of residual conformational structures in single-stranded DNA at 90° are also to be found in the incompletely resolved multiple resonances of other resonance regions in the pmr spectra of DNA. In the H-2′ region from 2.1 to 2.8 ppm that is shown for several DNA species in Figures 1 and 2, the principal resonance absorption consists of three broad resonances at about 2.26, 2.50, and 2.74 ppm. Pmr spectra of the monomers for the same conditions show that the two H-2′ protons of purine monomers are not equivalent, the resonances occurring at 2.56 and 2.75 ppm for d-AMP and at 2.48 and 2.69 ppm for d-GMP. For the pyrimidine monomers the resonances of the H-2′ protons are coincident, occurring at 2.29 ppm for d-CMP and at 2.31 ppm for TMP. Although the positions of the DNA H-2′ resonances correspond rather closely to those of the monomers, the relative intensities at 2.26, 2.50, and 2.75 ppm would be expected, in the absence of perturbations, to be 2:1:1. This intensity relationship is not found and, in addition, we have observed large shifts from the monomer resonance positions for some of the H-2′ resonances of deoxyribonucleoside monophosphate dimers. Here again, we believe that ring-current shifts accompanying stacking interactions are responsible both for the shifts of the dimer resonances and for the unexplained intensity

Figure 4. Low-field regions of pmr spectrum of calf thymus DNA at 220 Mcps: concentration 30 mg/ml in D_2O, pD 7, 93°, 50 spectra averaged.

distribution of the DNA H-2′ resonance region. Additional investigation of oligonucleotide spectra will be necessary to completely elucidate these interactions.

Finally, the two lowest field resonance absorption regions of calf thymus DNA are shown in Figure 4. The resonance absorption from 5.8 to 6.4 ppm which comprises H-5 protons of cytosine and H-1′ protons shows evidence of structure, but the individual resonances are not sufficiently resolved to permit detailed analysis. From examination of corresponding pmr spectra of monomers and dimers, we have tentatively assigned the partially resolved resonances in the 7.3 to 8.3 ppm region as indicated in Figure 4. The H-8 proton positions of purine bases deuterate at a significant rate in neutral D_2O at 90°[22,23] and, therefore, are probably not exhibiting their full intensity in this spectrum (particularly H-8 of guanine which is more labile than H-8 of adenine). Three or possibly four partially resolved resonances can be seen in the region assigned to the H-6 proton of thymine that presumably reflect different environments for this proton in residual conformational structures of the polymer molecule. However, the intensity in this region is too great to be accounted for from H-6 protons of thymine alone, and we conclude that shifted components of the lower field resonances (for example, from H-6 of cytosine) also contribute intensity in this region. Thus, it appears that if the resonances in this region could be more clearly resolved, it might be possible to obtain additional nearest neighbor base frequency information for thymine and perhaps other bases. Work is in progress to attempt to achieve this required degree of resolution.

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(22) F. J. Bullock and O. Jardetzky, *J. Org. Chem.*, **29**, 1988 (1964).
(23) C. C. McDonald, W. D. Phillips, and J. Penswick, *Biopolymers*, **3**, 609 (1965).

Hypochromism Accompanying Purine–Pyrimidine Association Interactions

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Abstract: Ultraviolet absorption studies on binary mixtures of model nucleoside derivatives of adenine (A), uracil (U), guanine (G), cytosine (C), and inosine (I) in chloroform solution are reported. Specific band hypochromic or hyperchromic effects are found only in solutions containing the complementary pairs A + U, G + C, and I + C. Comparison of the present results with those of infrared studies shows that hypochromic effects in these systems are due to the formation of hydrogen-bonded complexes between the bases (base pairing). It is therefore demonstrated that parallel stacking of the bases as occurs in undenatured DNA is not the only condition for hypochromism in $\pi^* \leftarrow \pi$ transitions of the bases.

Hypochromism in polynucleotides, which is the decrease in absorbance per chromophore in the polymer compared to that of the monomer, has been attributed by Tinoco[2,3] and Rhodes[4] to dipole–dipole interaction between transition moments in neighboring oscillators.[5] This has been termed an off-resonance interaction since the interacting dipoles originate from different electronic transitions. Large hypochromic effects are predicted for this model[3] when the base

(1) This research was completed while the author was a National Institutes of Health Predoctoral Fellow (1964–1967) and constitutes part of a Ph.D. thesis submitted to the Department of Chemistry, Massachusetts Institute of Technology, Feb 1967. Inquiries may be addressed to the Department of Biophysics, King's College, London.
(2) I. Tinoco, Jr., *J. Chem. Phys.*, **33**, 1332 (1960); **34**, 1087 (1961); *J. Am. Chem. Soc.*, **82**, 4785 (1960).
(3) H. DeVoe and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 500, 518 (1962).
(4) W. Rhodes, *J. Am. Chem. Soc.*, **83**, 3609 (1961).
(5) Some authors, including those cited, use the term "dispersion force interaction" to refer to dipole–dipole interactions of various orders. It appears preferable at present to restrict the term "dispersion force" to that which leads to the R^{-6} term in the potential energy.