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Proton Magnetic Resonance Studies of Double Helical Oligonucleotides. The Effect of Base Sequence on the Stability of Deoxydinucleotide Dimers†

Michael A. Young and Thomas R. Krugh*

ABSTRACT: The concentration dependence of the proton magnetic resonance chemical shifts of a series of deoxydinucleotides and deoxydinucleoside monophosphates in neutral H_2O solution has been recorded in the 1–100 m*M* concentration range by the use of pulsed Fourier transform techniques. The self-complementary molecules pdG-dC, dG-dC, pdC-dG, and dC-dG and the complementary mixtures pdG-dG + pdC-dC as well as pdG-dT + pdA-dC interact at low temperatures by the formation of intermolecular hydrogen bonded dimers. Noncomplementary molecules such as pdG-dT, pdT-dG, pdG-dG, pdA-dC, and pdC-dC do not self-associate by the formation of intermolecular hydrogen bonds under the present experimental conditions. The chemical shifts of the amino protons and the base protons are consistent with the interaction of two complementary dinucleotides to form a miniature double helix. An analysis of the chemical shift of the guanine amino proton resonance as a function of dinucleotide concentration has provided approximate dimerization constants. These results show that the stability of the miniature double helices is in the order (pdG-dG)·(pdC-dC) $\gtrsim$ (pdG-dC)·(pdG-dC) > (pdC-dG)·(pdC-dG) > (pdG-dT)·(pdA-dC) which reflects the effect of nucleotide sequence (and composition) on helix stability.

The study of the structure and function of nucleic acids has been an important area of research for the past 100 years. During the last few years a great deal of information has been obtained by a study of the properties of oligonucleotides by a variety of spectroscopic techniques. Nuclear magnetic resonance (NMR) and especially proton magnetic resonance (^{1}H NMR) have been especially useful in providing valuable information on the structure and interactions of mononucleotides, dinucleotides, and oligonucleotides (for a detailed review of the literature, see Ts'o (1974a,b) and Danyluk (1975)). The hydrogen-bonding properties of the nucleotide bases have been studied in *nonaqueous* environments (e.g., Hamlin et al., 1965; Katz and Penman, 1966; Kyogoku et al., 1966; Pitha et al., 1966; Shoup et al., 1966; Newmark and Cantor, 1968) and in the solid state (e.g., see the review by Sobell, 1969). In *aqueous* solutions the stacking interactions of the mononucleotides predominate and it is very difficult to observe base pairing. However, Raszka and Kaplan (1972) observed downfield shifts (<0.15 ppm) of the nucleotide amino resonances in concentrated mixtures of mononucleotides which is evidence for the formation of hydrogen-bonded complexes. We have recently exploited the use of deoxy and ribodinucleotides and dinucleoside monophosphates in the study of drug–nucleic acid complexes and have found that the dinucleotides seem to be good model compounds for DNA and RNA (e.g., Krugh, 1972; Krugh and Neely, 1973; Krugh et al., 1975; Krugh

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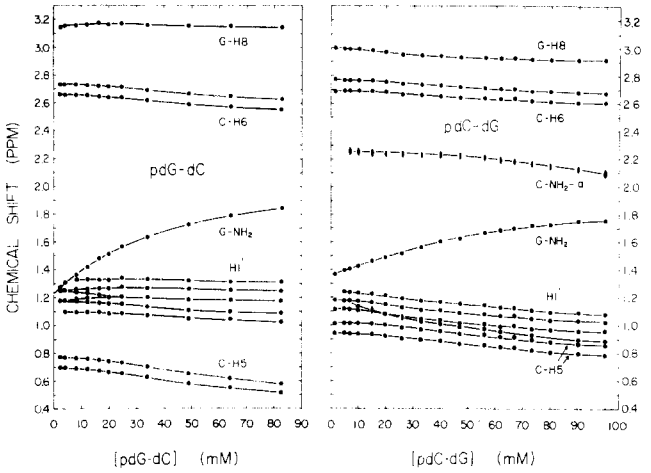

FIGURE 1: The concentration dependence of the proton chemical shifts of pdG-dC (2°C, pH 7.15) and pdC-dG (1°C, pH 7.02) in H_2O solution.

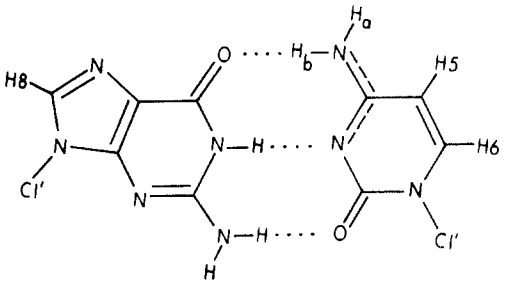

FIGURE 2: The Watson-Crick G·C base pair illustrating the nonequivalent cytosine amino protons.

and Reinhardt, 1975). We have also recently observed that it is relatively straightforward to record proton Fourier transform spectra in H_2O solutions (Mooberry and Krugh, 1975; Krugh and Schaefer, 1975) and in a recent communication we used these techniques to monitor the chemical shift of the guanine 2-amino resonance of pdG-dC in the 3–80 m*M* concentration range. These data showed that the self-complementary deoxydinucleotide pdG-dC forms a hydrogen-bonded dimer in H_2O solution at 2°C, with a dimerization constant of $7.8 \pm 0.7\ M^{-1}$, while the deoxydinucleotide pdG-dT did not show any evidence of intermolecular hydrogen bond formation (Krugh and Young, 1975). Several other workers have also investigated the proton magnetic resonance spectra of oligonucleotides in H_2O solutions (using continuous wave techniques) but in these studies the NH resonance from G·C and A·T base pairs was used to monitor double helix formation. Crothers et al. (1973) studied a mixture of the pentanucleotides d(A-A-C-A-A) and d(T-T-G-T-T) and showed that the guanine NH resonance broadened into the base line, as a result of exchange with the solvent, at a temperature far below the T_m, of the double helix. This phenomenon has also been observed by Patel and Tonelli (1974) in a study of a deoxyhexanucleotide, by Arter et al. (1974) in a study of a ribotetranucleotide, and has been discussed in detail by Borer et al. (1975) in a study of a ribohexanucleotide. Thus, while the NH resonance may provide useful geometrical information on helical nucleic acids and proteins (e.g., Glickson et al., 1969; Kearns et al., 1971), monitoring the N-H resonance will not provide straightforward thermodynamic data. In this paper on the deoxydinucleotides, and in a related paper on the ribodinucleoside monophosphates (Krugh et al., 1975), we will monitor the *amino* resonances of the bases in order to investigate the stability of the miniature double helices formed by the dinucleotides.

Experimental Procedure

All of the deoxydinucleoside mono- and diphosphates were obtained as lyophilized ammonium salts from Collaborative Research Corporation. These compounds contained traces of paramagnetic impurities and solutions were consequently purified by either passage through a 0.5 cm diameter × 8 cm column containing Chelex 100 (Bio-Rad) or by direct treatment with 0.5 ml of a Chelex 100–water slurry followed by filtration. After either treatment, the dinucleosides were recovered through lyophilization and redissolved in doubly distilled H_2O for NMR measurements. Particulate matter was removed by centrifugation or filtering (Millpore Corp., 8 μm pore size). Adjustment of pH was accomplished by addition of small quantities of 1 *N* NaOH or HCl. During the course of the dilution studies the pH of the dinucleoside solutions at 22°C was checked periodically. The dilutions were carried out by addition of weighed portions of H_2O to the NMR sample tube. In this regard, it was noted that the dinucleotides (5′ terminal phosphate group) possessed much better buffering capacity compared to the dinucleoside monophosphates. The concentrations of dinucleoside mono- and diphosphate solutions were determined spectrophotometrically in 1-cm cells using deoxydinucleoside monophosphate molar absorptivities (P. L. Biochemicals Catalog 103) with the exception of pdA-dC (17100 at 260 nm; Collaborative Research, Inc. Lot 494-41A). All reported concentrations are on a dinucleoside basis.

The 100-MHz Fourier transform NMR spectra were recorded with a JEOL PFT-100 NMR spectrometer interfaced with a JEOL EC-100 computer. To minimize the water resonance, the WEFT pulse sequence was employed with the addition of a homogeneity spoiling pulse applied to the Y shim coils for 0.2–0.5 sec during the interval τ between the 180 and 90° pulses. Precise control of τ for optimum solvent resonance elimination was possible by using a pulse adder circuit (Krugh and Schaefer, 1975) in conjunction with the JEOL DP-1 pulse programmer. In cases in which the guanine amino resonances overlapped other spectral features, the SWEFT pulse sequence ($[180°\text{-}\tau_1\text{-}180°\text{-}\tau_2\text{-}90°\text{-T}]_n$) was employed in order to eliminate the resonance that overlapped the amino resonance (Krugh and Schaefer, 1975). Sample temperature was maintained within ±0.5°C during pulse accumulation with a JEOL VT-3 temperature controller and measured by methanol peak separations (Van Geet, 1970). The external proton lock of the JEOL PFT-100 was used. Chemical shifts were measured relative to the solvent water peak with an accuracy of ±0.005 ppm on narrow resonances.

Results

The proton chemical shifts of pdG-dC and pdC-dG in H_2O solutions are shown in Figure 1 as a function of dinucleotide concentration. The striking feature in both sets of data is the downfield shift of the guanine amino resonance as the dinucleotide concentration is increased. These large downfield shifts are a result of intermolecular hydrogen bond formation (Krugh and Young, 1975, and references therein). The chemical shifts of the base and ribose H(1′)

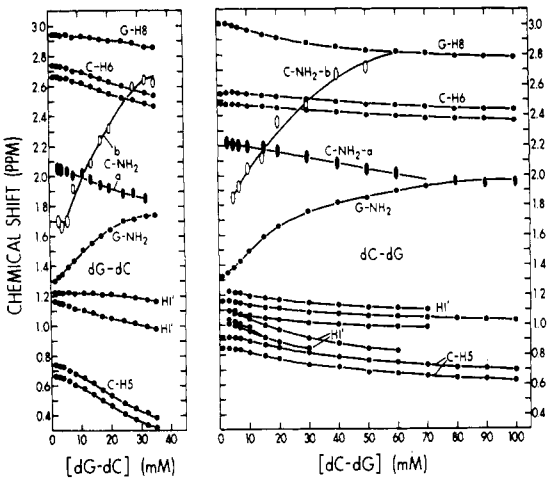

FIGURE 3: The concentration dependence of the proton chemical shifts of dG-dC (2°C, pH 6.85) and dC-dG (1°C, pH 7.05) in H_2O solution. For dG-dC, only the middle peaks of the H(1') triplets are shown.

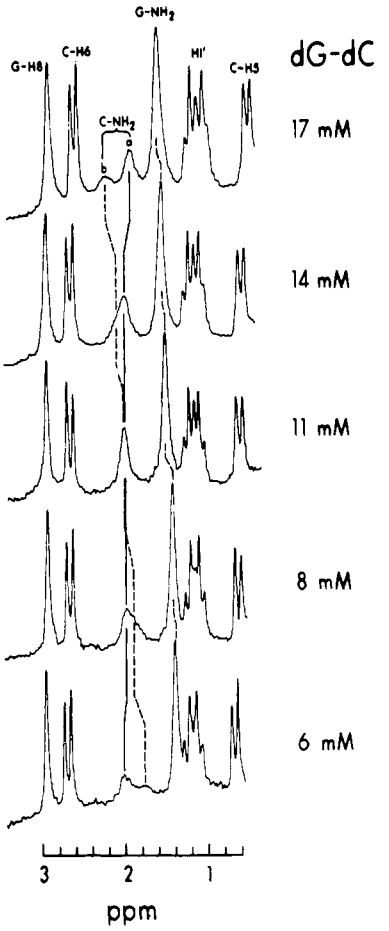

FIGURE 4: The 100-MHz NMR spectra of dG-dC at several concentrations in H_2O solution (2°C, pH 6.85), illustrating the relative chemical shift displacements of the cytosine a and b amino protons and the guanosine amino protons.

protons move *upfield* as a function of increasing nucleotide concentration. For example, in the pdG-dC concentration dependence study (Figure 1, left-hand side) the G-H(8) resonance moves upfield 3 Hz, the C-H(6) resonance moves upfield ~9 Hz, while the C-H(5) moves upfield ~18 Hz over the concentration range studied. It is useful to compare the chemical shifts of 5'-dCMP with the infinite dilution values observed for the deoxydinucleotides. The chemical shift of the C-H(5) proton of 5'-dCMP in H_2O at 0°C is 1.06 ppm. The infinite dilution chemical shift of the C-H(5) proton in pdG-dC is 0.74 ppm, while in pdC-dG the C-H(5) proton is found at 0.97-ppm downfield from the water resonance. The upfield shift of the C-H(5) resonance in going from pdC to either pdC-dG or pdG-dC is consistent with the formation of a dinucleotide in that the ring current of the adjacent guanine base results in the upfield shielding of the C-H(5) proton (e.g., see Ts'o et al., 1969; Kondo et al., 1972). In going from the monomer (dCMP) to a B form of the double helix, we would expect the C-H(5) proton to be shifted much further upfield in a miniature double helix of pdG-dC as compared to a miniature pdC-dG double helix (e.g., see the base stacking patterns of Arnott et al., 1969). The sequence dependent stacking patterns are a fundamental property of a helical structure. The C-H(5) data in Figure 1 (and Figure 3) are consistent with the formation of miniature double helices since the C-H(5) proton of pdG-dC shifts upfield more than the C-H(5) proton of pdC-dG.

The Watson–Crick base pairing scheme for a G·C base pair is shown in Figure 2. Only one of the guanine 2-amino protons is involved in hydrogen bonding but since we only observe a single guanine amino resonance, we assume that the guanine amino group is undergoing fast rotation on the NMR time scale. On the other hand, the partial double bond character of the cytosine C(4)–NH_2 bond restricts the amino group rotation which results in the appearance of a separate broad resonance for each of the cytosine amino protons (McConnell and Seawell, 1973; Raszka and Kaplan, 1972; Krugh and Young, 1975). Another consideration in the present experiments is that we have used the 180°-τ-90° pulse sequence to eliminate the water resonance; this may lead to a loss of intensity in the resonances of protons exchanging with the solvent (Krugh and Schaefer, 1975). Experimentally, we were unable to observe the cytosine 4-amino resonances in the pdG-dC spectra at most concentrations, but we were able to observe one of the C-4-amino resonances in the pdC-dG spectra (Figure 1). This resonance moved *upfield* as a function of increasing concentration. We assign this resonance to the C-NH_2-a proton (Figure 2). We have also measured the concentration dependence of the chemical shifts of the deoxydinucleoside monophosphates dG-dC and dC-dG (Figure 3) which lack the terminal phosphate of the deoxydinucleotides. In these two experiments we were able to monitor both of the cytosine amino protons, and, as expected, the C-NH_2-a resonance moves *upfield* while the C-NH_2-b resonance moves *downfield* as a function of increasing nucleotide concentration. The net downfield shift of the C-NH_2-b resonance in these two cases is actually a sum of the large deshielding due to hydrogen bond formation and a small shielding due to the ring current of the neighboring guanine base. The magnitude of the upfield shifts of the C-NH_2-a resonance is consistent with the shielding of this proton by the adjacent guanine ring (for example, note the similarity of the induced shifts of the C-NH_2-a and the C-H(5) protons). From these observations we conclude that the C-NH_2-a proton is not involved in hydrogen bond formation (with another base) in this concentration range.

The spectra in Figure 4 illustrate the broad, low intensity, C-NH_2 resonances in the concentration region where these resonances cross. The G-NH_2 resonance in the dG-dC and dC-dG series moves downfield as the concentration is

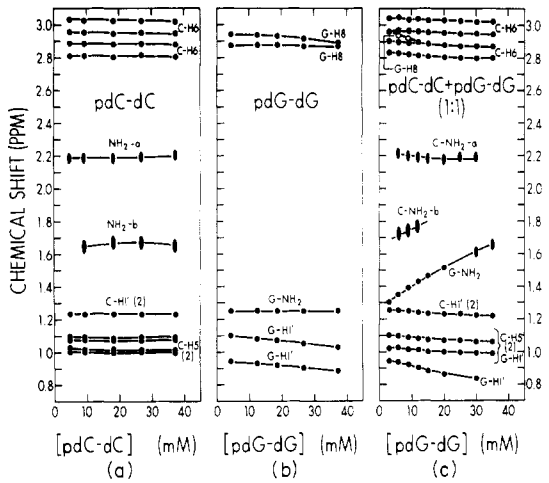

FIGURE 5: The concentration dependence of the chemical shifts of the low-field protons of (a) pdC-dC (1°C, pH 7.10); (b) pdG-dG (1°C, pH 7.30); and (c) the 1:1 mixture of pdC-dC with pdG-dG (1°C, pH 7.10). Only the middle peaks of the H(1′) triplets are shown.

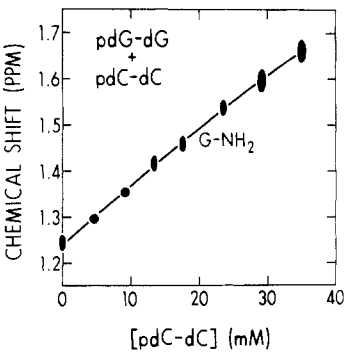

FIGURE 6: Proton chemical shift of the G-NH_2 protons of pdG-dG as a function of the concentration of added pdC-dC. The experiment was performed by the incremental addition of a concentrated (242 mM) solution of pdC-dC to 43 mM pdG-dG. The final concentration of each dinucleotide was 35 mM and this solution was utilized for the concentration study of Figure 5c.

increased. It is an important point to note that because the guanine amino group is undergoing fast rotation the observed downfield shift due to hydrogen bonding is only one-half as large as the actual deshielding due to hydrogen bonding. However, the cytosine amino protons give rise to individual resonances and thus the deshielding due to hydrogen bonding is not averaged. As a result, the observed change in the chemical shift of the C-NH_2-b resonance as a function of concentration is approximately twice as large as that of the G-NH_2 resonance. This observation also supports the formation of Watson–Crick base pairs.

The guanine N_1-H proton also forms a hydrogen bond in the Watson–Crick G·C base pair. However, when guanine is not base paired, this proton rapidly exchanges with the solvent protons and, for example, it is not observable in the ^{1}H NMR spectrum of 5′-dGMP. We have not been able to observe the resonance of the hydrogen bonded guanine NH proton in any of the deoxydinucleotide spectra. This is not surprising in view of the work of Crothers et al. (1973), Arter et al. (1974), and Borer et al. (1975) in which the guanine NH protons broadened into the base line far below the T_m of the oligonucleotide double helix. However, we have observed the guanine NH resonance in a solution of ethidium bromide with CpG where the ethidium bromide acts as a nucleation center and forms a miniature intercalated double helix (Krugh and Reinhardt, 1975).

In the dG-dC and dC-dG experiments the interpretation of the chemical shifts is less straightforward due to both the limited concentration range for dG-dC and the possible aggregation of the deoxydinucleoside monophosphates. The dG-dC was particularly susceptible to aggregation and formed a rigid gel above 40 mM at 2°C and pH 6.85; the solutions also remained quite viscous in the 30–40 mM concentration range under these experimental conditions. There was no visible sign of gel formation in the dC-dG studies even at 100 mM concentration. However, the relatively large chemical shift change of the G-H(8) resonance (22 Hz) may indicate that intermolecular base stacking is more extensive here than in pdC-dG where the G-H(8) resonance moved upfield only 8 Hz. The apparent discontinuity of the G-NH_2 chemical shift vs. concentration curve for dC-dG also seems to indicate that aggregation (other than just dimer formation) is present. There was no evidence of gel formation or nonspecific aggregation in the deoxydinucleotides pdG-dC and pdC-dG over the concentration range studied.

A study of the hydrogen bonding in mixtures of pdG-dG with pdC-dC offers a further demonstration of the specificity of the hydrogen bonding and the role of complementarity. The chemical shift of the guanine 2-amino protons in pdG-dG remains constant in the 4–37 mM concentration range while the other nucleotide protons exhibit small upfield shifts indicative of intermolecular stacking (Figure 5b). The upper concentration limit is due to solubility limitations. The constant G-NH_2 chemical shift clearly shows that there is no appreciable intermolecular hydrogen bonding involving the guanine 2-amino protons in this concentration range. The chemical shift of all the pdC-dC protons are essentially constant over this concentration range (Figure 5a) and thus there is no intermolecular hydrogen bonding of pdC-dC with itself. The concentration dependence of the chemical shift of the G-NH_2 resonance in an equimolar mixture of pdG-dG + pdC-dC (Figure 5c) clearly shows evidence of intermolecular hydrogen bonding involving the guanine 2-amino groups. Unfortunately, the cytosine -NH_2-b resonance was observable in only three of the spectra of Figure 5c. In order to verify the specificity of the hydrogen bonding, an experiment was performed in which aliquots of a concentrated solution of pdC-dC were added to a 42 mM solution of pdG-dG. The chemical shift of the G-NH_2 protons are plotted as a function of the concentration of pdC-dC in Figure 6, where we find that the downfield shift of the G-NH_2 resonance is proportional to the concentration of the pdC-dC, which is consistent with the formation of a hydrogen-bonded dimer.

To test the effect of added salt on the stability of the pdG-dC complex, a dilution study was performed in 1 M NaCl (Figure 7) under similar experimental conditions as employed for the low salt study. The infinite dilution shift of the G-NH_2 resonance of pdG-dC in 1 M NaCl is 0.17 ppm further downfield than the G-NH_2 resonance in a low salt solution, when both samples are referenced to the solvent H_2O peak. There is approximately a 0.1-ppm upfield shift in the reference when 1 M NaCl is added to H_2O, so the net salt effect is to move the infinite dilution shift of the G-NH_2 protons ~0.07 ppm downfield.

The compilation of the data in Figure 7 provides a comparative summary of the guanine amino proton chemical shifts. We have also included the data for pdG-dT and

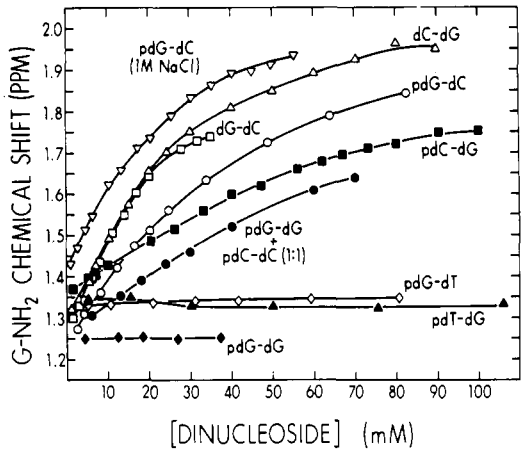

FIGURE 7: The concentration dependence of the guanine 2-amino protons of several deoxydinucleotides. The conditions were pdT-dG (1°C, pH 7.05); pdG-dT (2°C, pH 7.15); pdG-dC in 1 *M* NaCl (2°C, pH 7.00). The conditions for the other nucleotides were listed in the previous figures.

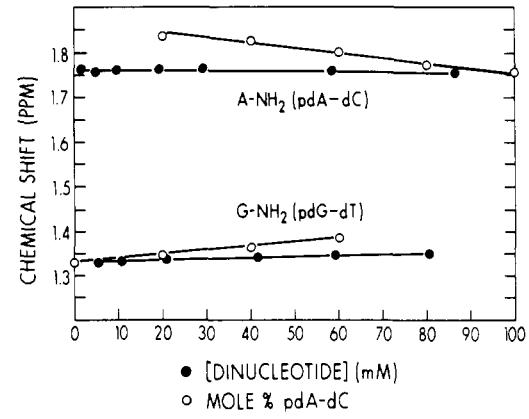

FIGURE 8: The concentration dependence of the chemical shifts of the guanine 2-amino protons in pdG-dT and the adenine 6-amino protons in pdA-dC. The solid circles give the data for the individual nucleotide solutions while the open circles are for a continuous variation experiment in which the total dinucleotide concentration was always 50 m*M* (1°C, pH 7.10).

pdT-dG to show that the G-NH_2 resonance in these compounds is not a function of concentration. The other proton resonances of both pdT-pG and pdG-dT change less than 5 Hz over this concentration range, which implies that there is little nonspecific intermolecular stacking of these noncomplementary deoxydinucleotides. On the other hand, mixtures of pdG-dT with pdA-dC, which are complementary in the Watson–Crick sense, do interact through the formation of hydrogen bonds involving both the guanine 2-amino protons and the adenine 6-amino protons (Figure 8). These data also show that the interaction between the complementary dinucleotides pdG-dT + pdA-dC is much weaker than the self-association of pdG-dC. This is thus a clear-cut example of the influence of base composition on the stability of the miniature double helix just as the base composition influences the stability of double-helical DNA polymers (e.g., Marmur and Doty, 1959, 1962).

Equilibrium Constant Calculations. Equilibrium constants were calculated from the concentration dependence of the guanine 2-amino chemical shifts. We will assume that the principal equilibrium involves the formation of dimers between complementary dinucleotides. For the self-complementary dinucleotides the equilibrium is

$$2N \overset{K}{\rightleftharpoons} N_2 \quad (1)$$

for which the appropriate expression is

$$\delta_{obsd} = \delta_M + (\delta_D - \delta_M)\left(\frac{4K[N_0] + 1 - \sqrt{(8K[N_0] + 1}}{4K[N_0]}\right) \quad (2)$$

where δ_{obsd} is the observed chemical shift, δ_M is the chemical shift of the protons in the monomer state; δ_D is the chemical shift of the protons in the dimer, and $[N_0]$ is the stoichiometric concentration of the nucleotide. For the complementary mixture of dinucleotides we have

$$A + B \overset{K}{\rightleftharpoons} AB \quad (3)$$

for which the appropriate expression is

$$\delta_{obsd} = \delta_M + (\delta_D - \delta_M)\,([1 + K([A_0] + [B_0]) - \sqrt{1 + 2K([A_0] + [B_0]) + K^2([A_0] - [B_0])^2]}/2K[B_0]) \quad (4)$$

where $[B_0]$ is the concentration of the nucleotide with the amino protons whose chemical shift is being measured. A nonlinear least-squares regression analysis program was used to obtain the values of K, δ_M, and δ_D. The input data sets consisted of the observed chemical shifts and the stoichiometric dinucleotide concentrations plus the estimated errors in each observable. The equilibrium constants and limiting chemical shifts calculated from the dilution data for pdG-dC, pdC-dG, and the mixture pdG-dG + pdC-dC are given in Table I. The curves in Figure 9 show the excellent agreement between the observed and calculated chemical shifts (for the parameters given in Table I) and provide solid support for the interpretation of the data in terms of a dimerization equilibrium. The uncertainties listed with the calculated parameters (Table I) are calculated standard deviations based upon the quality of the input data. The infinite dilution shifts, δ_M, are well defined in the present experiments but the chemical shifts of the dimer, δ_D, are less well defined because we are unable to obtain experimental data at high concentrations. Even if solubility were not a problem, the nonspecific intermolecular base stacking would influence the data at high concentrations of dinucleotide. However, we feel that the present data provide reliable estimates of the dimerization constants. For example, it is well known that the presence of salt stabilizes the double helix and this is clearly evident in the data for pdG-dC where the dimerization constant goes from 7.8 to 13.4 M^{-1} upon the addition of 1 *M* NaCl. The data are also clear cut in that pdG-dC has a larger dimerization constant than pdC-dG (7.8 M^{-1} vs. 3.0 M^{-1}) which thus illustrates the effect of sequence on the stability of a miniature double helix. pdG-dG + pdC-dC forms the strongest complex and thus the order of stability of the dimers is (pdG-dG)·(pdC-dC) ≳ (pdG-dC)·(pdG-dC) > (pdC-dG)·(pdC-dG) > (pdG-dT)·(pdA-dC).

Discussion

The data described herein clearly demonstrate that complementary deoxydinucleotides (and deoxydinucleoside monophosphates) form hydrogen-bonded dimers in neutral aqueous solutions at low temperatures. At ambient temperatures (~25°C) there is no evidence of any appreciable concentration of hydrogen-bonded dimers in a 25 m*M* solution of pdG-dC, from which we estimate that the dimerization

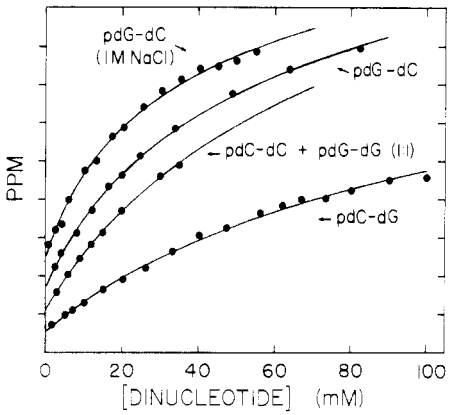

FIGURE 9: A comparison between the experimental G-NH_2 chemical shifts and the calculated dimerization curves using the parameters in Table I. The excellent agreement supports the interpretation of these data in terms of a dimerization equilibrium. Each division of the ordinate is 0.10 ppm; the curves have been offset on the ordinate in order to prevent overlap.

Table I: Calculated Parameters Assuming a Dimerization Equilibrium.

	δ_M (ppm)	δ_D (ppm)	K (l./mol)
pdG-dC (2°C)	1.22 ± 0.01	2.71 ± 0.06	7.8 ± 0.7
pdG-dC (2°C, 1 *M* NaCl)	1.39 ± 0.01	2.63 ± 0.07	13.4 ± 2.0
pdC-dG (2°C)	1.35 ± 0.01	2.76 ± 0.11	3.0 ± 0.5
pdG-dG + pdC-dC (1°C)	1.26 ± 0.01	3.18 ± 0.16	9.1 ± 1.1

constant at 25°C is $<2\ M^{-1}$. Thus it would be difficult to monitor the formation of a miniature double helix in neutral aqueous solution at 25°C. The large change in the dimerization constant over this temperature range is a reliable indication that the ΔH of dimerization is large and negative ($\Delta H \simeq -10$ kcal), as expected (e.g., see Borer et al., 1974). It should be possible to measure the dimerization constant as a function of temperature to extract the interesting thermodynamic data, at least over the 0–15°C temperature range. However, the interpretation of this quantitative data will be limited by the inescapable problem of the concomitant nonspecific aggregation, especially base stacking, at high nucleotide concentration (e.g., see Ts'o, 1974a,b). In the deoxydinucleotides this nonspecific aggregation does not seem to be an important problem because, within experimental error, the guanine amino resonance dilution curves are well represented by a dimerization equation (Figure 9). Even the noncomplementary deoxydinucleotides pdT-dG, pdG-dT, or pdA-dC do not appear to extensively aggregate in the concentration range used in this study. We caution that the dimerization constants may be less reliable than the calculated standard deviations indicate. However, they do appear to be reliable enough to allow an ordering of the dinucleotides in terms of the stability of dimer formation: (pdG-dG)·(pdC-dC) $\gtrsim$ (pdG-dC)·(pdG-dC) > (pdC-dG)·(pdC-dG). It is interesting to note this is the exact ordering that Borer et al. (1974) would predict on the basis of their experiments with *ribo* oligonucleotides.

All of the chemical shift data are consistent with the formation of miniature double helical complexes between complementary deoxydinucleotides in which Watson-Crick base pairs are formed. However, we will refrain from a detailed discussion of the chemical shifts because of the possible influence of nonspecific base stacking which limits the extraction of geometric data from the analysis of the induced chemical shifts.

A comparison of the pdG-dG + pdC-dC and pdG-dT + pdA-dC data clearly shows that the stability of the dimer is significantly reduced by changing one G·C base pair to an A·T base pair. Thus we would not expect to observe any appreciable dimerization if both G·C base pairs are changed to A·T base pairs, to give pdA-dT or pdT-dA. We have also obtained similar results for a related study of the ribodinucleoside monophosphates (Krugh et al., 1975a). The present experiments continue to demonstrate the utility of the di- and oligonucleotides as models for DNA and RNA, both by themselves, and in the study of drug–nucleic acid complexes (e.g., see Krugh et al., 1975b; Krugh and Reinhardt, 1975, and references therein). Finally, the development of the present techniques provides a convenient means of monitoring hydrogen bonding in a variety of systems.

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Conformation and Interaction of Short Nucleic Acid Double-Stranded Helices. I. Proton Magnetic Resonance Studies on the Nonexchangeable Protons of Ribosyl ApApGpCpUpU†

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ABSTRACT: ^1H nuclear magnetic resonance (NMR) spectra of a self-complementary ribosyl hexanucleotide, A_2GCU_2, are investigated as a function of temperature and ionic strength in D_2O. Seventeen nonexchangeable base and ribose-$H_{1'}$ resonances are resolved, and unequivocally assigned by a systematic comparison with the spectra of a series of oligonucleotide fragments of the A_2GCU_2 sequence varying in chain length from 2 to 5. Changes in the chemical shifts of the 17 protons from the hexamer as well as the six $H_{1'}$-$H_{2'}$ coupling constants are followed throughout a thermally induced helix-coil transition. These δ vs. T and J vs. T (°C) profiles indicate that the transition is not totally cooperative and that substantial populations of partially bonded structures must exist at intermediate temperatures, with the central G·C region being most stable. Transitions in chemical shift for protons in the same base pair exhibit considerable differences in their T_m values as the data reflect both thermodynamic and local magnetic field effects in the structural transition, which are not readily separable. However, an average of the T_m values agrees well with the value predicted from studies of the thermally induced transition made by optical methods. The values of $J_{1'-2'}$ for all six residues become very small (<1.5 Hz) at low temperatures indicating that $C_{3'}$-endo is the most heavily populated furanose conformation in the helix. The δ values of protons in the duplex were compared with those calculated from the ring current magnetic anisotropies of nearest and next-nearest neighboring bases using the geometrical parameters of the A′-RNA and B-DNA models. The δ values of the base protons in the duplex calculated assuming the A′-RNA geometry agree (±~0.1 ppm) with the observed values much more accurately than those calculated on the basis of B-DNA geometry. The measured δ values of the $H_{1'}$ are not accurately predicted from either model. The synthesis of 35 mg of A_2GCU_2 using primer-dependent polynucleotide phosphorylase is described in detail with extensive discussion in the microfilm edition.

In recent years extensive studies have been made of the physical properties of oligoribonucleotides of defined sequence which are capable of double helix formation. The investigations dealt with the structure of these molecules in solution as well as the thermodynamics and kinetics of their helix-coil transition. The pioneering studies of Martin, Uhlenbeck, and Doty (Martin et al., 1971; Uhlenbeck et al., 1971) provided thermodynamic information from the hypochromicity as a function of temperature for several double helical oligomers. These papers also outlined the enzymatic procedure for the "block copolymerization" of such oligonucleotides, whose sequences were designed to favor the formation of perfect double helices. This obviated the problems of triplex formation and other states of high agregation encountered by previous workers.

Subsequent studies have substantially extended the understanding of the structure and stability of these oligomer helices in solution. Borer et al. (1974b) have reported thermodynamic parameters for 19 RNA duplexes of chain length 6–14 based on their hypochromicity as a function of

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