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Watson-Crick Base Pairing between Guanosine and Cytidine Studied by ^{13}C Nuclear Magnetic Resonance Spectroscopy

Steffen B. Petersen and Jens J. Led*

Contribution from the Department of Chemical Physics, The H. C. Ørsted Institute, University of Copenhagen, DK-2100 Copenhagen, Denmark. Received January 13, 1981

Abstract: Watson-Crick base pairing in dimethyl sulfoxide/methanol (2/1 v/v) between the nucleosides guanosine and cytidine has been studied by carbon-13 nuclear magnetic resonance (^{13}C NMR) spectroscopy at 67.89 MHz. The equilibrium constant for the base pairing complex has been obtained at two different temperatures by a nonlinear least-squares analysis of the experimental shift data for the base carbons, and the enthalpy of interaction has been found to be ca. -3.8 kcal/mol. The analysis furthermore indicates that while base pairing and changes in the syn/anti conformation ratio are independent processes in the case of guanosine, a change from syn to anti conformation occurs simultaneously with the base pairing in the case of cytidine, in agreement with the Watson-Crick base pairing model. As inferred from the results base pairing alters the polarizabilities of the nucleosides.

Watson-Crick base pairing between mono-, oligo-, and polynucleotides has been studied extensively by various methods in different solvents.¹ The fact that the base pairing consists of a formation of intermolecular hydrogen bonds has led to an intensive use of ^1H NMR spectroscopy^{2,3} in the study of this phenomenon. However, since the protons participating in hydrogen bonding are normally hetero-bound protons subject to chemical exchange, observations of the NMR signals from these protons are often limited as far as temperature range and solvents are concerned. In particular when water is used as a solvent, exchange processes may broaden the resonance lines of the exchangeable protons beyond recognition.^{4,5} For the carbon-bound protons the experimental conditions are less restrictive. However, here the considerably smaller effects caused by adjacent hydrogen bonding groups upon the chemical shifts make these protons less suitable for probing the base interactions.⁴

In water Watson-Crick base pairing between mononucleosides has not been detected by any method, the reason probably being that the mononucleosides are strongly hydrogen bonded to the solvent. Thus it has been suggested^{4,6} that base pairing in aqueous solution has a positive enthalpy of formation. Upfield shifts observed in aqueous solution for the carbon-bound base protons in guanosine (G) and cytidine (C) with increasing concentrations were attributed to ring-current effects due to vertical stacking.^{7,8} For oligo- and polynucleotides in water, the NMR signals from hetero-bound protons have been observed below the melting temperature of the complexes,⁹⁻¹³ showing that here stable hydrogen-bonding complexes are being formed. Above the melting

temperature these signals may disappear^{7,8} as in the case of mononucleosides.

In chloroform and dimethyl sulfoxide (Me_2SO), base pair formation, even between mononucleosides, has been detected by ^1H NMR spectroscopy, while base stacking is absent.^{4,14} These conditions may be attributed to specific interactions with the nucleic acids, as is also indicated by the denaturing character of these solvents.

The above-mentioned limitations, which are associated with the use of ^1H NMR in the studies of hetero-bound protons in nucleosides and nucleotides, do not apply when ^{13}C is used as the observed nuclei. In addition, the chemical shift of ^{13}C is considerably more sensitive to conformational changes than is the chemical shift of ^1H .¹⁵⁻¹⁷ Furthermore, although carbon itself seldom participates in hydrogen bonding,^{14,18,19} the chemical shifts of ^{13}C atoms can be very sensitive to the formation of a hydrogen bond involving adjacent functional groups.²⁰⁻²² Thus, changes in the chemical shifts of the carbonyl group of acetone due to interaction with various solvents have been reported,²⁰ ranging from 2.4 ppm upfield to 37.4 ppm downfield relative to neat acetone. In addition the ^{13}C NMR signals are considerably less affected by exchange of hetero-bound protons on adjacent hydrogen-bonding groups than the signals from the exchangeable protons themselves. Finally, the number of carbon atoms in nucleosides are considerably larger than the number of carbon-bound protons, which may lead to a more detailed picture of the intra- and intermolecular interactions.

So far only a few ^{13}C NMR studies on nucleic acid systems have been reported,²³⁻³⁴ while, to our knowledge, only one report

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Table I. ^{13}C Chemical Shifts,^a δ_{inf} , of Guanosine^b and Cytidine at Infinite Dilution and the Slopes, α , Calculated from the Concentration Curves

	δ_{inf} (247)	δ_{inf} (303)	$\Delta\delta_{\text{inf}}^d$	α (247) ^e	α (303) ^e
G2	87.404 ± 0.004	87.307 ± 0.001	0.097	0.162 ± 0.018	0.084 ± 0.009
G4	84.993 ± 0.007	84.849 ± 0.003	0.144	0.115 ± 0.028	0.047 ± 0.015
G5	49.865 ± 0.006	50.267 ± 0.006	-0.402	-0.247 ± 0.019	-0.218 ± 0.022
G6	90.733 ± 0.004	90.610 ± 0.004	0.123	0.676 ± 0.015	0.488 ± 0.016
G8	69.575 ± 0.010	69.506 ± 0.007	0.069	0.745 ± 0.037	0.542 ± 0.029
G1'	19.882 ± 0.013	20.526 ± 0.016	-0.644	0.526 ± 0.047	0.337 ± 0.066
G2'	7.554 ± 0.007	7.556 ± 0.003	-0.002	0.159 ± 0.027	0.080 ± 0.012
G3'	4.096 ± 0.006	4.111 ± 0.004	-0.015	0.273 ± 0.021	0.118 ± 0.016
G4'	18.835 ± 0.010	18.997 ± 0.004	-0.162	0.371 ± 0.040	0.127 ± 0.016
G5'	-5.147 ± 0.015	-4.942 ± 0.004	-0.205	0.222 ± 0.053	0.062 ± 0.021
C2	89.639 ± 0.003	89.584 ± 0.003	0.055	0.278 ± 0.010	0.336 ± 0.010
C4	99.272 ± 0.003	99.346 ± 0.003	0.074	0.050 ± 0.010	0.024 ± 0.010
C5	27.776 ± 0.012	27.672 ± 0.003	0.105	0.560 ± 0.037	0.488 ± 0.012
C6	75.046 ± 0.012	75.142 ± 0.003	-0.096	0.072 ± 0.041	0.063 ± 0.012
C1'	22.668 ± 0.007	23.248 ± 0.004	-0.580	0.009 ± 0.019	-0.024 ± 0.016
C2'	7.942 ± 0.003	7.854 ± 0.003	0.088	0.267 ± 0.009	0.174 ± 0.012
C3'	2.812 ± 0.003	3.058 ± 0.003	-0.246	0.267 ± 0.009	0.108 ± 0.009
C4'	17.515 ± 0.012	17.854 ± 0.001	-0.339	0.286 ± 0.035	0.125 ± 0.004
C5'	6.163 ± 0.015	5.781 ± 0.001	-0.382	0.149 ± 0.041	0.063 ± 0.006

^a In ppm downfield from *p*-dioxane (internal reference). ^b Guanosine carbons are indicated with a G. ^c Cytidine carbons are indicated with a C. ^d $\Delta\delta_{\text{inf}} = \delta_{\text{inf}}(247) - \delta_{\text{inf}}(303)$. ^e In ppm/mol.

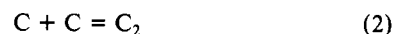
of ^{13}C NMR evidence of base pairing has appeared.³⁰ The purpose of the present study is to explore the potentialities of ^{13}C NMR spectroscopy in yielding information on structures and interactions of mononucleosides in solution. The system chosen for this exploration consists of G and C dissolved in Me_2SO /methanol (2/1 v/v).

Experimental Section

The nucleosides were purchased from Sigma and were used without further purification by dissolving the appropriate amount of nucleosides in 2 mL of a 2/1 (v/v) mixture of Me_2SO and methanol (both analytical grade). Approximately 100 μL of *p*-dioxane (internal reference) was added to each sample. The temperature was measured by using an acetone- d_6 / CCl_4 thermometer³⁵ placed coaxially within the sample. The deuterium resonance of the acetone- d_6 was used as a lock signal. The accuracy of the measured temperature was 2 °C. The spectra were recorded on a Bruker HX 270 spectrometer equipped with a Nicolet 1180 microcomputer. In order to define the frequency domain of the spectra 32768 data points were used. All spectra were recorded by using proton noise decoupling. The employed pulse angle was 30°, while a total delay of 2 s was used between pulses.

Results and Discussion

In solutions containing both G and C, self-associations as well as G-C interactions must be considered. Thus in the analysis of the experimental data the following equilibria are taken into account



Furthermore, an unravelling of the observed shifts in terms of these

two types of interactions necessitates an independent study of the self-associations (eq 1 and 2).

In all cases only one resonance peak was observed for each carbon in the proton noise decoupled spectra. This shows that fast exchange between the bonded and the nonbonded states is taking place in the entire temperature range of the present study.

In the following GX and CX denote the carbon atoms in guanosine and cytidine, respectively, X being the carbon number.

A. Concentration Studies. The chemical shifts were measured as a function of the concentration at 247 and 303 K, and the infinite dilution shifts were obtained by extrapolation. The results obtained at 247 K are shown in Figures 1A and 1B. For the concentration and temperature ranges used in this study a linear correlation between the chemical shifts and the concentrations was observed for all carbon atoms. The observed chemical shifts, δ_{obsd} for a nuclei in a molecule taking part in a self-associative reaction are, in the case of fast exchange, given by the weighted average between the chemical shift in the nonbonded state, δ_{inf} , and the chemical shift in the self-associated state, δ_s , where C_M

$$\delta_{\text{obsd}} = \frac{C_M}{C_M + C_s} \delta_{\text{inf}} + \frac{C_s}{C_M + C_s} \delta_s \quad (4)$$

is the molar concentration of the free monomer and C_s is the molar concentration of the bases in the self-associated states. Assuming that $C_s \ll C_M$, eq 4 reduces to

$$\delta_{\text{obsd}} = \delta_{\text{inf}} + \alpha C_M \quad (5)$$

Here $\alpha = 2(\delta_s - \delta_{\text{inf}})K_s$ in the case of dimerization, while K_s is the corresponding equilibrium constant. As shown by the linearities of Figures 1A and 1B this approximation holds in the present case. The parameter values obtained by fitting eq 5 to the experimental shift data are given in Table I together with the extrapolated infinite dilution shifts at 247 and 303 K as well as the difference between these two parameters.

B. Infinite Dilution Shifts. Guanosine. As it appears from Table I, the infinite dilution shifts of G1' and G5 show a considerably larger temperature dependence than the rest of the carbon atoms in guanosine.

These specific and concerted changes should be compared with the results from X-ray analyses³⁶ of purine nucleosides and nucleotides, which show that for the syn conformation in the crystal phase interactions between the O1' atom in the ribose moiety and

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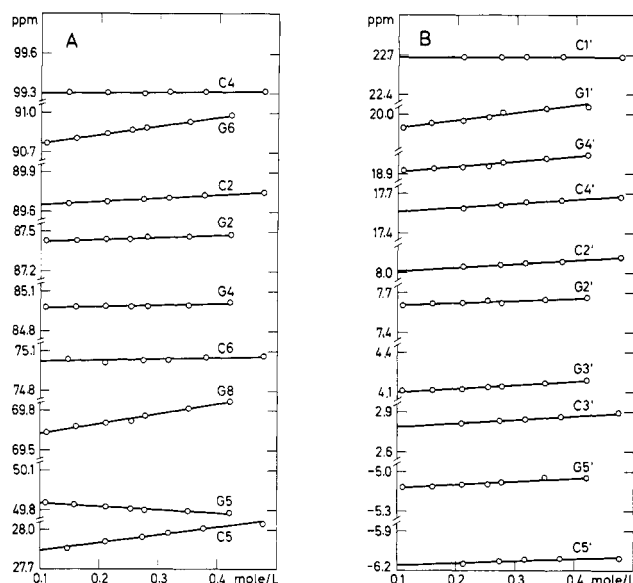


Figure 1. ^{13}C chemical shifts at 67.89 MHz of the base carbons (A) and the ribose carbons (B) of guanosine and cytidine as a function of the nucleoside concentration at 247 K. All shifts are downfield from *p*-dioxane (internal reference).

the adjacent base is an important feature. Thus in the case of 6-thiopurine riboside,³⁶ O1' is in close proximity to the G4–G5 double bond. Also of interest is the suggestion³⁷ that the DNA helix, besides being stabilized by hydrogen bonding, stacking, and ribose–phosphate backbone conformation, also might be stabilized by interactions between the ribose moieties and the π -electron systems of the bases. Furthermore, from circular dichroism studies it has been concluded³⁸ that guanosine in solutions can assume an anti as well as a syn conformation. Thus, guanosine shows a preference for the anti conformation in aqueous solutions, while the tendency to assume the syn conformation is more pronounced in alcoholic solutions. The barrier to internal rotation around the glycosidic bond is of the order of 4 kcal/mol.

Based on these considerations it seems reasonable to assume that the large and concerted changes of the infinite dilution shifts for G1' and G5 with temperature, which are observed in the present study, are caused, primarily, by a change in conformation. Thus, guanosine, in the solvent used here, shows a preference for the syn conformation with the O1' placed close to the G4–G5 double bond at the low temperature, while at higher temperature the population of the anti conformation increases. Contributions to the change in infinite dilution chemical shift of G5 with temperature, from any intermolecular interactions via N7 or the G6 carbonyl group, seem likely since such interactions also would affect G6 and/or G8 (vide infra), which is not observed. The observed change in the chemical shift for G1' can be directly related to a steric perturbation,^{16,17} which, in the present study, occurs between the G8–H and G1'–H groups.

Cytidine. As shown by the data in Table I the shifts of the ribose carbons in cytidine change, on an average, even more with temperature than those of the guanosine ribose ring. Again, the C1' carbon is the most influenced, indicating a temperature induced change of the conformation of the ribose ring relative to the base moiety, as in the case of guanosine. This is further supported by nuclear Overhauser experiments^{39,40} which indicate that cytidine can assume its syn conformation when dissolved in Me_2SO , as well as its anti conformation.

C. Self-Association. Guanosine. In the case of guanosine the data in Table I show that the chemical shifts of G6 and G8 depend

strongly on the concentration, in contrast to what is observed for the rest of the guanosine carbon atoms. This indicates that N7 and the carbonyl group of G6 are involved in a self-associative process, in close agreement with the two models proposed by Newmark and Cantor⁴ on the basis of ^1H NMR studies. However, neither this proton study nor the ^{13}C data of the present work are able to distinguish between these two models.

The observed concentration dependence of the chemical shift of the G1' carbon atom agrees with a conformational change of the ribose moiety relative to the purine ring, similar to the conclusion drawn on the basis of the temperature dependence of the infinite dilution shifts. However, at 247 K the changes of the chemical shifts of the remaining ribose carbons indicate that more complicated conformational changes of the ribose ring or other types of associations may take place at this temperature.

Cytidine. Also, in the case of cytidine the concentration dependence of the shifts, and in particular the shifts for C2 and C5, show that self-association is taking place. However, in contrast to the observation made for guanosine, the C1' carbon atom of cytidine shows no concentration dependence, indicating that the conformation about the glycosidic bond is unaffected by the self-association.

D. Base Pairing. In order to investigate and interpret the influence of the G–C base pairing upon the chemical shifts of the guanosine and cytidine carbon atoms, two series of measurements were performed.

In the first series, the chemical shifts were measured as a function of temperature for a solution containing both guanosine and cytidine (0.28 M/0.22 M), as well as for solutions containing only one of the two types of nucleosides (Figure 2). As it appears from Figure 2A and 2B the chemical shifts of the base carbon atoms in guanosine are influenced significantly by the presence of cytidine and vice versa. The same holds for the ribose moiety of cytidine (Figure 2D), whereas the shifts of the ribose carbons of guanosine are totally unaffected by the presence of cytidine (Figure 2C).

In the second series of measurements, solutions with a constant total nucleoside concentration but a different G/C ratio were studied at 247 and 303 K. As it appears from Figure 3, the chemical shifts in the mixed solutions vary nonlinearly as a function of the G/C ratio. Knowing the infinite dilution shifts and the self-association shifts, the observed shifts, δ_{obsd} , for the mixed solutions can be unravelled in terms of these two contributions and a shift induced by the base pairing. The latter shift is defined as $\Delta\delta = \delta_{\text{bp}} - \delta_{\text{inf}}$ where δ_{bp} is a shift corresponding to the fully base paired state. Thus the following equation was applied in a nonlinear regression analysis of the observed shift data, assuming that only one type of complex between G and C is being formed.

$$\delta_{\text{obsd}}(i) = \delta_{\text{inf}}(i) \frac{C_0 - C_{\text{bp}}}{C_0} + \delta_{\text{bp}}(i) \frac{C_{\text{bp}}}{C_0} + \alpha(i)(C_0 - C_{\text{bp}}) \quad (6)$$

Here C_0 is the concentration of the observed nucleoside, C_{bp} is the concentration of the base pair, and $\alpha(i)$ is the slope of the concentration curve for the *i*th nuclei. With use of the expression for the equilibrium constant, K_{bp} , corresponding to eq 3, C_{bp} was calculated from the equation

$$C_{\text{bp}} = 0.5\{(C_T + K_{\text{bp}}^{-1}) - \sqrt{(C_T + K_{\text{bp}}^{-1})^2 - 4C_0(C_T - C_0)}\} \quad (7)$$

where C_T is the total nucleoside concentration. The results obtained by a simultaneous analysis, using eq 5 and 6, of the data of all observable, shifting base carbons in G and C are presented in Table II for both temperatures, while the quality of the fit appears from Figure 3. It is interesting to note that the shifts of C3' and C5' are in close agreement with the K_{bp} obtained from the base carbon shifts. It should be mentioned that the fit to the C5' data shown in Figure 3 could only be obtained after a minor correction of δ_{inf} for this carbon by +0.119 ppm. We are not at the moment able to offer any explanation for this observation. Since a considerable broadening of the cytidine C6 signal prevents

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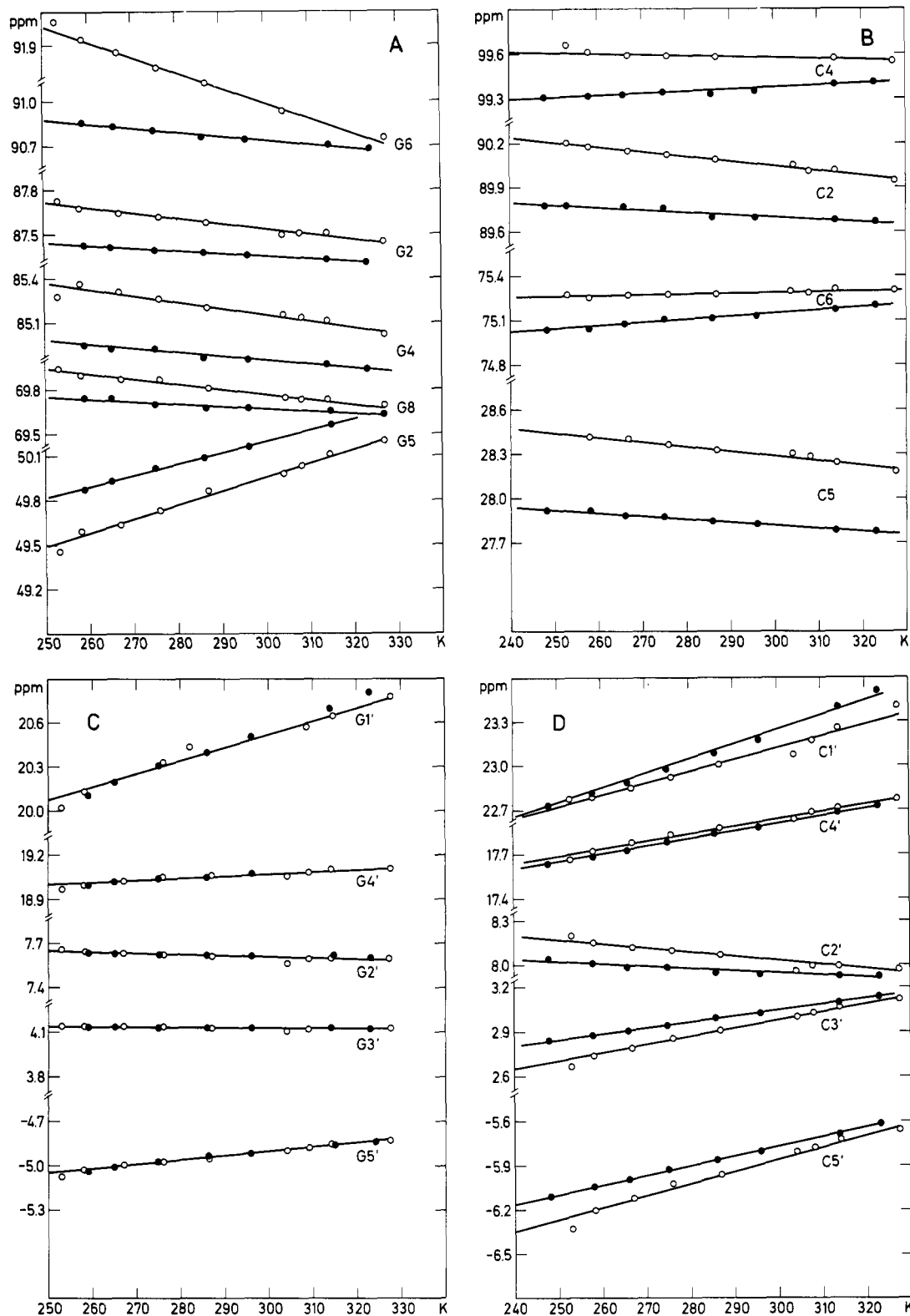


Figure 2. ^{13}C chemical shifts at 67.89 MHz of the base carbons (A and B) and the ribose carbons (C and D) of guanosine and cytidine as a function of the temperature. (●) Chemical shift in a solution containing either guanosine or cytidine. (○) Chemical shift in a solution containing both guanosine and cytidine. In all solutions containing guanosine, the guanosine concentration was 0.28 M. In all solutions containing cytidine, the cytidine concentration was 0.22 M. All shifts are downfield relative to *p*-dioxane (internal reference).

a reliable determination of the shift for this carbon at 247 K these data were not included in the regression analysis. Similarly, the shifts for the C1', C2', and C4' ribose carbons were not included due to insignificant changes.

Guanosine. As it appears from the data in Table II and Figure 3, the active part of guanosine in the base pair interaction is the six-membered ring, in agreement with the Watson-Crick model. Again, the shift for G6 is, numerically, almost three times larger

than that for the rest of the carbons in the six-membered ring, in accordance with the close involvement of this carbon atom in one of the hydrogen bondings of the model, while the absence of a base pairing induced shift for G8 (Figure 3) strongly suggests that the interaction occurs at a remote position relative to this carbon.

As is shown in Table II, δ_{bp} for all four carbons in the six-membered ring of guanosine changes with temperature. This

Table II. ^{13}C Chemical Shifts, a δ_{bp} , of Guanosine b and Cytidine c in the Fully Base Paired State (in $\text{Me}_2\text{SO}/\text{MeOH}$ 2/1 (v/v))

	247 K		303 K		$\Delta\delta_{\text{bp}}^e$	$\Delta\delta_{\text{inf}}^f$
	δ_{bp}	$\Delta\delta^d$	δ_{bp}	$\Delta\delta^d$		
G2	87.867 \pm 0.013	0.463	87.779 \pm 0.013	0.471	0.088	0.097
G4	85.734 \pm 0.016	0.741	85.562 \pm 0.015	0.713	0.172	0.144
G5	49.264 \pm 0.016	-0.601	49.634 \pm 0.015	-0.633	-0.370	-0.402
G6	92.757 \pm 0.025	2.024	92.597 \pm 0.027	1.987	0.161	0.123
C2	90.277 \pm 0.016	0.602	90.324 \pm 0.013	0.739	-0.047	0.055
C4	99.738 \pm 0.016	0.465	99.710 \pm 0.012	0.405	0.028	-0.074
C5	28.644 \pm 0.012	0.868	28.650 \pm 0.015	0.978	0.006	0.105
C6	<i>g</i>	<i>g</i>	75.419 \pm 0.013	0.277	<i>g</i>	-0.096
C3'	2.675 \pm 0.016	-0.137	2.912 \pm 0.016	-0.146	-0.237	-0.246
C5'	-6.362 \pm 0.016	-0.318 h	-5.911 \pm 0.016	-0.130	-0.451	-0.382

a In ppm downfield from *p*-dioxane (internal reference). b Guanosine carbons are indicated with a G. c Cytidine carbons are indicated with a C. d $\Delta\delta = \delta_{\text{bp}} - \delta_{\text{inf}}$. e $\Delta\delta_{\text{bp}} = \delta_{\text{bp}}(247\text{ K}) - \delta_{\text{bp}}(303\text{ K})$. f $\Delta\delta_{\text{inf}} = \delta_{\text{inf}}(247\text{ K}) - \delta_{\text{inf}}(303\text{ K})$. g Not obtained. h δ_{inf} slightly corrected, see text.

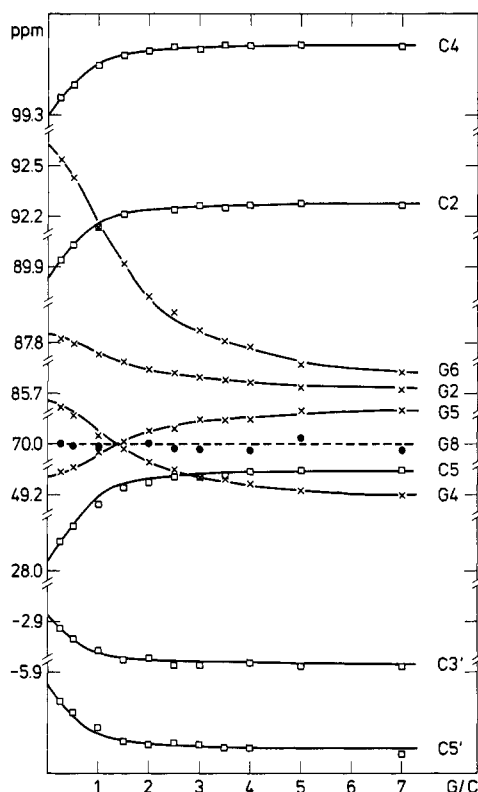


Figure 3. ^{13}C chemical shifts at 67.89 MHz and 247 K of the base carbons of guanosine (X) and cytidine (□) as a function of the molar ratio G/C. Only those of the observable carbons which show substantial chemical shift changes are included. All shifts are downfield from *p*-dioxane (internal reference). The shifts of G8 (●), C3', and C5' were not included in the regression analysis leading to K_B (see text).

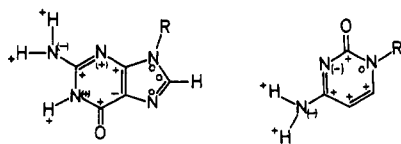


Figure 4. Change in the chemical shifts of the atoms of guanosine and cytidine due to base pair formation. Parentheses indicate estimated shifts. Plus sign indicates downfield shifts.

feature is incompatible with identical structures for the base pair complexes at the two temperatures. However, the data in Table II further show that the temperature dependences of δ_{bp} and δ_{inf} are identical within the experimental errors. As discussed above, the temperature dependence of δ_{inf} can be interpreted in terms of a change in the conformation of the ribose ring relative to the purine moiety. Therefore the similarities in the temperature

dependence of δ_{inf} and δ_{bp} lead to the conclusion that the temperature dependence of the latter is caused by a similar conformational change of the ribose ring, and that this change is independent of the base pairing. This last-mentioned conclusion is directly supported by the complementary information (vide supra and Figure 2) that the shifts of the ribose carbons of guanosine are totally unaffected by the presence of cytidine. Furthermore, the fact that the base pairing induced shifts for any given base carbon in guanosine are practically identical at the two temperatures shows that the nature of the direct interaction in which the base part of guanosine participates, when forming the base pairing complex, remains unchanged within the temperature limits used here.

It is also interesting to note that G5 shows a rather large upfield shift (negative), whereas all other carbons have downfield shifts. The latter trend was also observed for G5 when self-association occurs (vide supra). A similar opposed direction of the chemical shift variations has been reported for the A5 chemical shift, as compared to the rest of the carbon atoms in the adenine ring in a mixture of 9-ethyladenine and 1-cyclohexyluracil. 30

Finally, when the G/C ratio is decreased a downfield shift is observed for G2 (Table II). This is in agreement with the observations made for carbon atoms adjacent to amino groups which participate in hydrogen bonding. 20,41 It should be emphasized, however, that in the present case of nucleosides, hydrogen bond formation also takes place at the neighboring N1 and the carbonyl oxygen of G6, which undoubtedly also affects the chemical shift of G2.

Cytidine. In the case of cytidine, almost identical values of the chemical shifts for the fully base paired state, δ_{bp} , are found at 247 and 303 K, which immediately shows that the complexes formed at these two temperatures are identical. On the other hand, the same close agreement between base pairing induced changes in the chemical shifts of the base carbons at 247 and 303 K, which was observed in the case of guanosine, is not observed for cytidine (Table II). However, the syn conformation, which may be stabilized by an intramolecular hydrogen bond between the hydroxyl group of C5' and the C2 carbonyl group, as was suggested previously, 42 is hampering the base pairing. If this holds, the differences between the base pairing induced shifts obtained at the high and the low temperatures can be explained, qualitatively, on the basis of the temperature dependence of the syn/anti ratio, as in the case in the infinite dilution shifts (vide supra). Thus, at the low temperature a larger fraction of the molecules must be transformed from syn to anti conformation before the base pairing can be established than is the case at the high temperature. Because of a complete exclusion of the syn conformation in the

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fully base paired state, no temperature dependence of the chemical shifts for the base carbons of cytidine in this state is observed. On the other hand, the considerable change with temperature of δ_{bp} for the ribose carbons indicates a change in conformation of the ribose ring with temperature.

Unfortunately the present system does not allow a determination of the conformational equilibrium around the glycosidic bond through a determination of the nuclear Overhauser enhancement (NOE), primarily because the extreme narrowing condition, $\omega\tau \ll 1$, does not apply. This makes the NOE dependent on several correlation times such as the correlation times for the unassociated guanosine and cytidine, the correlation times of the self-associated dimers and the base pair, as well as the correlation times for the motion of the ribose moieties relative to the bases. In addition the cytidine H5 and H1' as well as the guanosine H8 and cytidine H6 ^1H NMR signals overlap.

Finally it should be noted that the base pairing induced shift obtained for C5 is considerably larger than that obtained for C4, which is adjacent to the hydrogen bonding amino group. This, undoubtedly, is due to a β effect as observed for β -carbons in amino acids.⁴¹

E. Thermodynamic Parameters. From the simultaneous regression analysis of the chemical shift data of all shifting carbons (Figure 3, Table II), the equilibrium constants $K_{247} = 28 \pm 2$ L/mol and $K_{303} = 6.7 \pm 0.2$ L/mol were obtained at 247 and 303 K, respectively. Assuming that the same complex is being formed at the two temperatures, which is amply supported by the analysis (vide supra), an enthalpy of formation for the base pairing complex of $\Delta H = -3.8$ kcal/mol and an entropy of $\Delta S = -8.6$ eu/mol are obtained from these equilibrium constants. These values should be compared with $\Delta H = -5.8$ kcal/mol and $\Delta S = -16$ eu/mol, obtained when using Me_2SO as solvent.⁴ The numerically smaller enthalpy of interaction found in the present study could be due to competing hydrogen bond formation between the bases and the methanol.

F. Induced Polarizabilities. Krugh et al.⁴³ report in a study of guanosine containing dinucleotides that considerably more stacking occurs in hydrogen-bonded dimers than in noncomplementary ribo- and deoxyribodinucleotides, which suggests a co-operative interaction involving hydrogen bonding (to form dimers) and stacking (of dimers). Furthermore, Hanlon⁴⁴ has reported that the stability of the DNA helix depends strongly on the polarizabilities of the bases, i.e., the greater the polarizability the greater the stability, while Bugg³⁶ has concluded on the basis of the results given by Ts'o^{45,46} and Chan⁴⁷ that the degree of self-

association of nucleosides in aqueous solution correlates with the polarizabilities of the bases. On the basis of thermodynamic properties and ^1H NMR data, Ts'o et al.^{45,46} and Chan et al.⁴⁷ have concluded that this self-association of the nucleosides primarily is a vertical stacking involving partial overlap of the base rings. In addition, the interaction leading to stacking appears to be mainly dipole-induced dipole interaction.³⁶

Until recently only ^1H NMR data on the base pair formation have been available. The work presented here together with the work of Iwahashi et al.³⁰ and the ^{15}N study of Ruterjans et al.⁴⁸ make it possible to gain qualitative information on the change in the polarizabilities caused by the base pairing. In Figure 4 the sign of the chemical shift variations due to the hydrogen bond formation is given for each one of the atoms in the rings of cytidine and guanosine. Here the sign of the ^{15}N shift upon base pair formation has been estimated on the basis of ^{15}N titration shift data given by Ruterjans et al.⁴⁸ Since it is normally accepted that the chemical shifts in π -electron systems are correlated with π -electron charge densities,^{25,49-54} the data in Figure 4 indicate that the bond moments and thereby the dipole moments of the bases are altered by the base pairing. This result seems to provide an explanation both of the observations made by Krugh et al.⁴³ and Hanlon⁴⁴ as well as the conclusions made by Bugg,³⁶ in that the base pairing causes a change in the dipole moments of the bases, and thereby a change in the polarizabilities of the bases which in turn leads to the observed increased stacking.

Conclusion

Based on the results obtained in the present study it can be concluded that detailed information on the structure and stability of the base pair complex can be obtained from ^{13}C NMR studies. In addition the results indicate that ^{13}C NMR, in combination with NMR studies of other nuclei, may provide additional information on the mechanisms associated with the stabilization of the DNA double helix.

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