displays high δ selectivity due to its poor μ receptor affinity. The location observed for this methyl group in model III' is consistent with this proposal.

Conclusions

The use of stereospecific deuteration to yield the *complete* assignment of the ^{1}H NMR spectrum, combined with quantitative interproton distance evaluation from NOESY cross-peak buildup rates and further coupled with distance geometry and energy minimization calculations, provides the best available experimental approach toward the elucidation of the solution conformation of DPDPE. These methods led in the present studies to the identification of one conformer consistent with the NMR data and of significantly lower energy than other allowed conformers. Nonetheless, it is likely that, even for a conformationally restricted peptide such as DPDPE, several similar and perhaps dissimilar conformations are significantly populated and that a weighted average is represented by the NMR spectrum. Such averaging must certainly be present in the more flexible side chains and may account for the observed small discrepancies in intramolecular distances involving side-chain protons between energy-minimized structures and NMR-derived constraints. Since it is quite clear that multiple low-energy conformers can be found that are consistent with experimental observations and display significant differences among themselves, it appears that further efforts to ascertain the solution conformation of DPDPE and, by extrapolation, its active conformation at the δ opioid receptor might best be directed toward uncovering common conformational features that are shared by similar conformationally restricted δ receptor selective peptides but are not found in other structurally related analogues with differing opioid activity. Such comprehensive studies utilizing an approach similar to that described here are in progress in our laboratories.

Acknowledgment. These studies were supported by USPHS Grants DA03910 (H.I.M.), GM37123 (G.M.C.), and GM36184 (R.W.W.) and by Grant DMB-8705006 (G.M.C.) from the National Science Foundation. The General Electric GN500 NMR spectrometer is supported by Grant RR-02415 from the USPHS and by funds from the University of Michigan College of Pharmacy.

Registry No. DPDPE, 88373-73-3; [α-^{2}H]-*p*-anisaldehyde, 19486-71-6; (*S*)-[α-^{2}H]-4-methoxybenzyl alcohol, 124176-47-2; (*R*)-*N*-[deuterio(4-methoxyphenyl)methyl]phthalimide, 124176-48-3; (*R*)-[α-^{2}H]-4-methoxybenzylamine, 124176-49-4; (*R*)-[α-^{2}H]-*N*-(*tert*-butoxycarbonyl)-4-methoxybenzylamine, 124176-50-7; (2*R*)-[2-^{2}H]-*N*-(*tert*-butoxycarbonyl)glycine, 124176-51-8; (2*S*,3*R*)-[3-^{2}H]-phenylalanine, 31262-73-4; (2*S*.3*R*)-[3-^{2}H]-tyrosine, 124176-52-9; (*Z*)-2-benzamido-*p*-methoxy-[3-^{2}H]-cinnamic acid, 39508-47-9; (*Z*)-2-benzamido-[3-^{2}H]-cinnamic acid, 39508-42-4; (*Z*)-2-benzamido-*p*-methoxy-[3-^{2}H]-cinnamic acid azlactone derivative, 39508-46-8; (*Z*)-2-benzamido-[3-^{2}H]-cinnamic acid azlactone derivative, 31348-62-6; hippuric acid, 495-69-2; [α-^{2}H]-benzaldehyde, 3592-47-0; trideuterated 6-(phenoxyacetamido)-(3*S*)-penicillinic acid (*S*)-sulfoxide benzyl ester, 124176-53-0; (2*S*,3*S*)-[4,4,4-^{2}H$_3$]penicillamine, 124176-54-1; trideuterated 6-(phenoxyacetamido)-(3*S*)-penicillinic acid, 124176-55-2; [d$_3$-D-Pen^2,d$_3$-D-Pen^5]enkephalin, 124176-44-9; [[3,3-^{2}H$_2$]Tyr^1,2*R*-[^{2}H]Gly^3]-DPDPE, 124176-45-0; [3*R*-[^{2}H]Phe^4]-DPDPE, 124199-94-6; (3*R*-[^{2}H]-Tyr^1,2*R*-[^{2}H]Gly^3)-DPDPE, 124176-46-1.

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In a Model C:G Base Pair, One Amino Group Rotates and the Other Does Not

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Abstract: Cytosine (C) and guanine (G) form Watson–Crick-type complexes in low-dielectric solvents. Dynamics of complexes between 3',5'-bis(triisopropylsilyl) derivatives of 2'-deoxynucleosides in deuteriochloroform were studied with 300-MHz ^{1}H NMR. We have determined rates of rotation about each amino bond of the C:G base pair. From the temperature dependence of the rates of amino group rotation, rotational activation enthalpies and entropies were calculated with line-shape and time-resolved techniques. For the amino group of G, the rotational activation enthalpy is equal to +10.6 ± 0.3 kcal/mol, and the rotational activation entropy ($\Delta S^{\ddagger}_{rotG}$) is equal to −2.5 ± 1.4 cal/(mol·T). As $\Delta S^{\ddagger}_{rotG}$ is nearly zero, the degree of disorder in the transition state is similar to that of the ground (base paired) state. We propose that rotation of the amino group of G proceeds within the base-paired state. In contrast, for the amino group of C, the rotational activation enthalpy is equal to +18.6 ± 1.3 kcal/mol, and the rotational activation entropy ($\Delta S^{\ddagger}_{rotC}$) is equal to +11.2 ± 3.5 cal/(mol·T). As $\Delta S^{\ddagger}_{rotC}$ is large, the degree of disorder in the transition state is greater than that of the ground state. We propose that rotation of the amino group of C proceeds through a transition state in which the base pair is disrupted. The results suggest that the two amino groups of the C:G base pair rotate via two different mechanisms. The amino group of G rotates within the base-paired state while the amino group of C rotates only during transient base-pair opening.

Although the ground state of DNA is close to the B conformation, DNA is a dynamic molecule which fluctuates between a variety of conformations. Amino groups in DNA rotate, and base pairs flip open and closed. DNA fluctuations are functionally significant, being factors in DNA recognition and reactivity. This paper describes one type of DNA fluctuation: rotation of the two amino groups of the cytosine–guanine base pair (Figure 1A). We have used ^{1}H NMR to study the mechanisms of amino proton exchange in a monomeric model system of DNA.

The properties of nucleic acid monomers have provided considerable insight into those of polymeric nucleic acids. The hydrogen-bonding interactions in the low-dielectric environment of the interior of polymeric nucleic acids[1,2] can be modeled by nucleic

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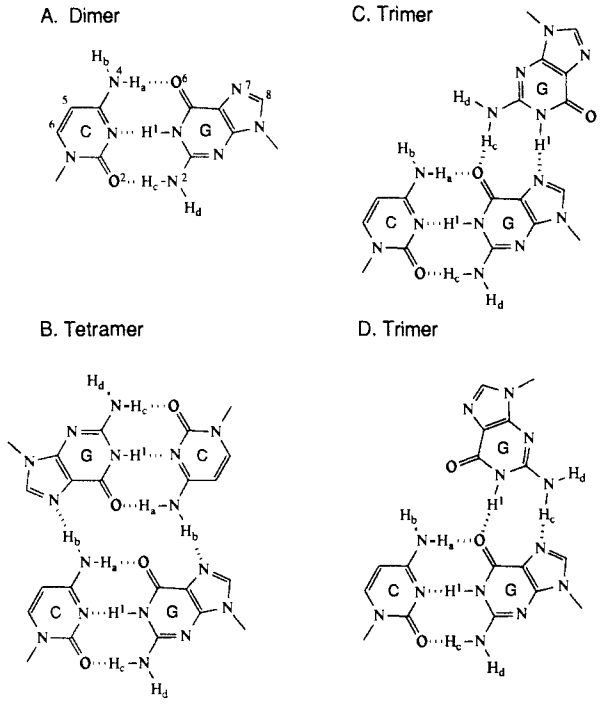

Figure 1. (A) The C:G Watson–Crick base pair; (B) the $(C:G)_2$ tetramer; (C) one possible conformation of the $C:G_2$ trimer; (D) the second possible conformation of the $C:G_2$ trimer.

acid monomers in chloroform solution. Some important structural motifs, such as the Hoogsteen base pair, were first observed in cocrystals of nucleic acid bases.[3] Similarly, in nonaqueous solvents, the Watson–Crick base pair (Figure 1A) forms spontaneously when monomeric guanine (G) is added to cytosine (C).[4-11] We have reported recently that C and G can combine to form more intricate hydrogen-bonded complexes in low-dielectric solution (Figure 1B–D).[12] Monomers of C and G can form both trimers ($C:G_2$) and tetramers $[(C:G)_2]$ in solution.

This paper describes an extension of the monomer model system of DNA to study the rotation of each of the two amino groups of the C:G base pair. We have determined the rates of rotation over a broad range of temperature (118 °C) and obtained the activation enthalpies and entropies. The results allow us to propose mechanisms for amino group rotation for the C:G base pair.

Experimental Section

The 2′-deoxy-3′,5′-bis(triisopropylsilyl) derivatives of guanosine and cytidine were prepared and purified as described elsewhere.[11,13] The products were pure as determined by elemental analysis, thin-layer chromatography, high-performance liquid chromatography, and ^1H NMR. Chloroform-*d* (Stohler) was distilled over P_2O_5 under argon just prior to sample preparation. NMR samples, in 5-mm tubes, were prepared in anhydrous conditions under argon and protected from exposure to atmospheric moisture. The solutions were degassed by bubbling of argon or by at least three freeze/pump/thaw cycles.

Spectra were obtained on a Varian XL-300 spectrometer using 16K double-precision (32 bit) data points over a 5000-Hz spectral width. Modified Hoffman–Forsen experiments[14,15] employed the following acquisition sequence in the interleave mode: (i) 3.0-s selective irradiation; (ii) $90°_x$–$240°_y$–$90°_x$ inversion pulse; (iii) variable delay with selective irradiation gated on; (iv) $90°_x$ observation pulse followed by 1.6-s data acquisition with selective irradiation pulse gated off and return to (i). The 90° and 180° pulse widths were measured at each temperature. A three-parameter exponential fit of at least eight different delay times (iii) was used to determine T_1(app) as defined under Results.

Chloroform-*d* was used as a lock, and the trace nondeuterated chloroform contaminant was used as a reference. Acidified methanol was used for low-temperature calibration, and ethylene glycol was used for high-temperature calibration of the probe. Probe temperatures were reproducible to ±1 °C.

Line-shape calculations using the unmodified Bloch equations were peformed on a Vax 780. Linear least-squares analysis and calculation of rates from line-shape and time-resolved data were performed on a Kaypro 2X using the double-precision (16 bit) mode.

The estimated uncertainties in $\Delta H^{\ddagger}_{rotC}$ and $\Delta S^{\ddagger}_{rotC}$ were obtained (a) by propagation of errors from extreme estimates of various input parameters (such as coalescence temperature or W'') through the rate calculations and Eyring plot least-squares fit or (b) from the 95% uncertainty limits of the slope and intercept of the Eyring plot, whichever was greater. For several reasons, the uncertainties in $\Delta H^{\ddagger}_{rotC}$ and $\Delta S^{\ddagger}_{rotC}$ are greater than the uncertainties of $\Delta H^{\ddagger}_{rotG}$ and $\Delta S^{\ddagger}_{rotG}$. The accessible temperature range was smaller in the case of C amino rotation than for the G amino rotation. The narrower temperature range increased the degree of uncertainty, especially in the extrapolation to $1/T = 0$. Further, it was not possible to achieve temperatures above coalescence, and therefore, the error in estimating the temperature of coalescence was larger.

It is reasonable to have high degree of confidence in the activation parameters because (a) these experiments were performed over a large temperature range, (b) changes in chemical shifts caused by factors other than exchange are negligible compared to the large $\Delta\nu$ (880 or 1213 Hz at 300 MHz), (c) the rates determined by two independent techniques (line-shape and modified Hoffman–Forsen analysis) are in agreement, (d) it was possible to "freeze out" exchange and thus determine W'' with a high degree of certainty, and (e) the unmodified Block equation could be used to accurately reproduce the line widths and overall spectra of exchanging amino protons.

These experiments were conducted under anhydrous conditions with a 30 mM concentration of each nucleoside in chloroform-*d*. The addition of 30 mM water did not affect the results. Further, substitution of the exchangeable protons with 50% or 80% deuterium did not measurably affect rates of rotation, indicating that tunneling was not the mechanism and that transition-state theory provides an appropriate model. Deuteration of 2′-deoxy-3′,5′-bis(triisopropylsilyl)guanosine and 2′-deoxy-3′,5′-bis(triisopropylsilyl)cytidine in which 80% (or 50%) of the exchangeable protons were substituted by deuterium was conducted by equilibrating a solution of nucleosides in methylene chloride with a vast excess of 80% (or 50%) D_2O/H_2O. The D_2O/H_2O was then decanted and the methylene chloride removed by rotary evaporation. The deuterated nucleosides were dried thoroughly under vacuum and protected from exposure to atmospheric moisture. Replacement of hydrogen by deuterium was manifested by a loss in integrated intensity of N–H peaks as compared to the integrated intensity of nonexchangeable protons in ^1H NMR spectra.

Results

This paper describes an ^1H NMR investigation of fluctuations of hydrogen-bonded complexes formed between monomeric C and G. We have previously reported the preparation and utility of 3′,5′-bis(triisopropylsilyl) derivatives of 2′-deoxycytidine and 2′-deoxyguanosine for thermodynamic characterization of base-pairing interactions[11] and for studying $C:G_2$ and $(C:G)_2$ in solution.[12] These nucleoside derivatives provide a sensitive system for studying hydrogen bonding exclusively between the bases. The triisopropylsilyl groups enhance the solubilities of the nucleosides in nonaqueous solvents and prevent the ribose hydroxyls from forming hydrogen bonds. For simplicity of description, the 3′,5′-bis(triisopropylsilyl) derivatives of 2′-deoxyguanosine and 2′-deoxycytidine shall be referred to as G and C, respectively.

Rotation about the Amino Bond of Guanine. The two amino protons of G exchange by rotation about the N^2 amino bond. At low temperatures (below −11 °C in a 1:1 mixture of C and G),

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both amino groups of the base pair are in slow rotation on the NMR time scale, and all four amino protons are observable as distinct resonances (Figure 2A; see ref 12 for assignments). Our observation that the amino resonances of G are rotationally distinct at low temperatures is consistent with slow rotation of the amino group of guanosine monophosphate derivatives in aqueous solution.[16] At higher temperatures, the amino group of G is in fast rotation on the NMR time scale, and the two protons are observable as a single coalesced resonance (Figure 2A). The close agreement between observed spectra (Figure 2A) and calculated spectra (Figure 2B) indicates that amino group rotation is the primary determinant of the observed temperature effect on line shape. The formation of $(CG)_2$ tetramers[12] does not appear to affect the line shapes or the rate of rotation about the amino group of G. We have calculated the rates of rotation about the amino bond of base-paired G with two different methods: line-shape analysis[17,18] and Hoffman–Forsen analysis[14] as modified by Mann.[15]

(A) Line-Shape Analysis. With line-shape analysis, the rate of rotation about the amino bond of G (k_g) was calculated over a 108 °C temperature range (from +55 °C to −53 °C). Below −53 °C, rotation of the amino bond of G was frozen out, and amino proton exchange did not make a significant contribution to line shape.

The rate of rotation was determined by eq 1 at temperatures above the coalescence temperature, by eq 2 at the coalescence temperature, and by eq 3 at temperatures below the coalescence temperature.[17,18] Line widths in the absence of exchange (W'') were determined by extrapolating a plot of line width versus temperature to limiting line width. The limiting difference in frequency ($\Delta\nu$) was determined by extrapolating a plot of difference in frequency versus temperature to the limiting difference in frequency. W^* is the observed line width at half-height.

$$2k_g/\pi\Delta\nu = \{W''/\Delta\nu + (W^*/\Delta\nu)[1 + 2(W^*/\Delta\nu)^2 - (W^*/\Delta\nu)^4]^{1/2}\}[(W^*/\Delta\nu)^2 - (W''/\Delta\nu)^2]^{-1} \quad (1)$$

$$k_g = \pi\Delta\nu/\sqrt{2} \quad (2)$$

$$k_g = \pi(W^* - W'') \quad (3)$$

Below the coalescence temperature, two different measurements of k_g are obtained at each temperature (one for each proton). The two measurements, which differ by less than 8% at each temperature, were averaged for the Eyring analysis (see below).

(B) Hoffman–Forsen Analysis. In a small temperature range, it was possible to determine exchange rates by a second, independent technique. Modified Hoffman–Forsen analysis[14,15] provides a technique to measure the rate of polarization transfer between two sites. This technique requires no assumptions regarding $\Delta\nu$ or W''. In general, the rate constant k_A (from A to B) for exchange between sites A and B, is obtained from eq 4.[15]

$$k_A^{-1} = \frac{M_z^A(\infty_A,0_B)}{M_z^A(\infty_A,0_B) - M_z^A(\infty_A,\infty_B)} T_{1A}(\text{app}) \quad (4)$$

In eq 4, $M_z^A(\infty_A,0_B)$ is the area of resonance A when B is not irradiated, $M_z^A(\infty_A,\infty_B)$ is the area of A during steady-state saturation of B, and $T_{1A}(\text{app})$ is the apparent T_1 of A during steady-state saturation of B.

Significant errors in chemical exchange rates are expected from this technique when chemical exchange is not the only contribution to change in area $[M_z^A(\infty_A,0_B) - M_z^A(\infty_A,\infty_B)$, defined here as $\Delta M]$. If exchange is between sites located nearby in space, polarization via dipolar coupling (i.e., the nuclear Overhauser effect) could result in systematic errors in chemical exchange rates as determined by Hoffman–Forsen analysis. The potential for error was large in these experiments as A and B always describe two protons bound to the same nitrogen and are indeed very close in space. Fortunately, with the effect of temperature, we could determine whether chemical exchange or dipolar coupling makes the primary contribution to ΔM. The rate of chemical exchange is expected to increase with temperature while polarization via dipolar coupling is expected to remain constant or decrease with temperature.

Hence, the effect of temperature on ΔM was used to determine whether the predominant contributions were from chemical exchange or from dipolar coupling. The amino protons of C (described below) showed the expected biphasic temperature behavior (Figure 3). In the high-temperature realm (>−30 °C), ΔM for the amino protons of C increased with temperature, indicating that the primary contribution was from chemical exchange. In the low-temperature realm (<−50 °C), ΔM decreased with temperature, indicating that the primary contribution was from dipolar coupling.

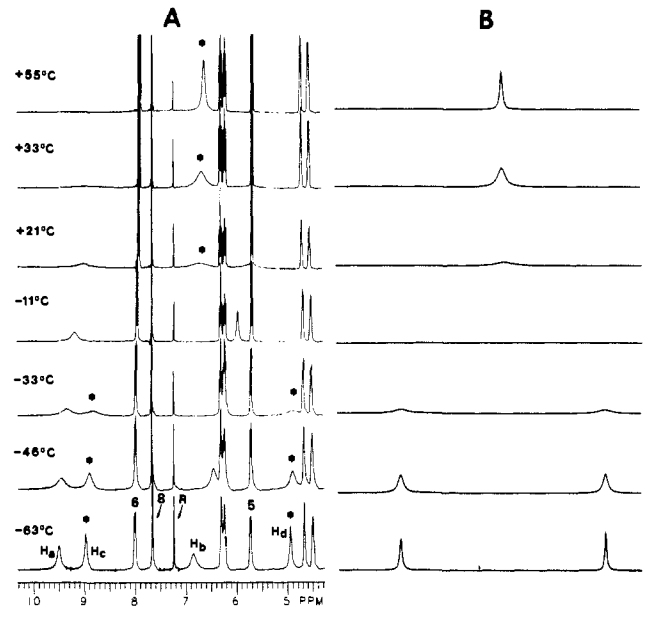

Figure 2. (A) Partial 300-MHz ^1H NMR spectra of 30 mM 2′-deoxy-3′,5′-bis(triisopropylsilyl)guanosine plus 30 mM 2′-deoxy-3′,5′-bis(triisopropylsilyl)cytidine in chloroform-*d*. The asterisks indicate the two exchanging proton resonances; R, at 7.24 ppm, is the nondeuterated chloroform contaminant; the multiplet centered at 6.25 ppm is the resonance at the 1′-protons of both nucleosides. (B) Calculated spectra of H_c and H_d at same temperatures as the experimentally obtained spectra.

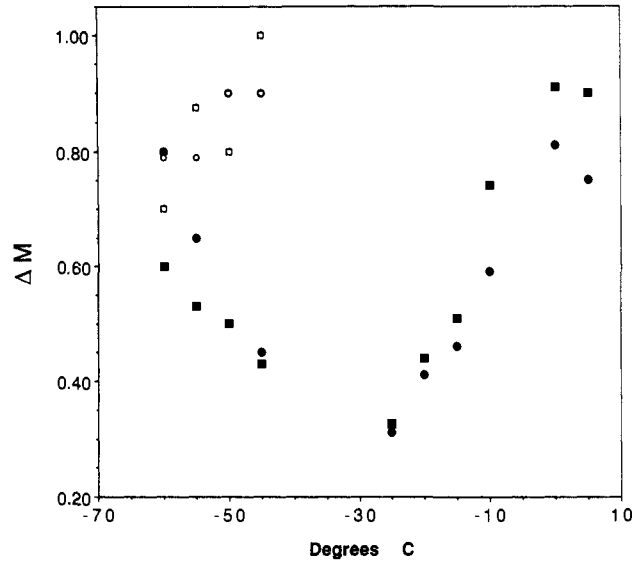

Figure 3. Decrease in intensity of an amino proton resonance during steady-state saturation of the proton bound to the same nitrogen versus temperature. Full circles, H_a; full squares, H_b; hollow circles, H_c; hollow squares, H_d.

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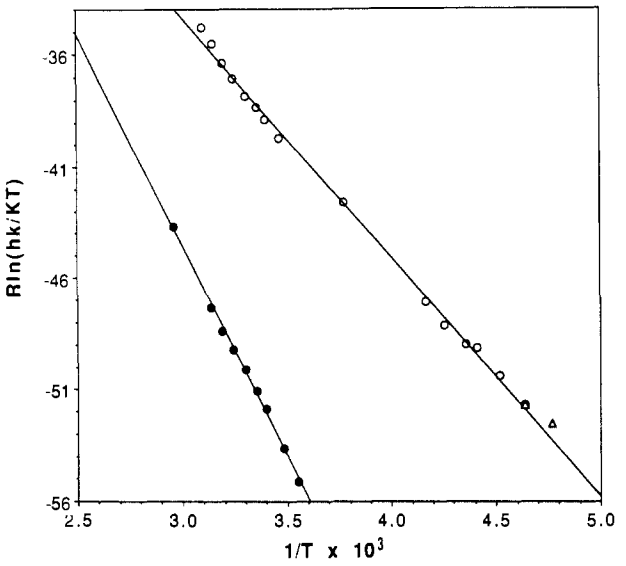

Figure 4. Eyring plots of amino proton exchange obtained from line-shape (circles) and modified Hoffman-Forsen (triangles) analysis: guanine amino proton exchange (hollow); cytosine amino proton exchange (full).

Table I. $\Delta S^{\ddagger}$ and $\Delta H^{\ddagger}$ of Amino Bond Rotation

	guanine[a]	cytosine[a]	(dimethylamino)-cytosine[b]
$\Delta S^{\ddagger}_{rot}$ [cal/(mol·T)]	−2.5 ± 1.4	+11.2 ± 3.5	~0[c]
$\Delta H^{\ddagger}_{rot}$ (kcal/mol)	+10.6 ± 0.3	+18.6 ± 1.3	
$\Delta H^{\ddagger}_{int}$ (kcal/mol)	+8.7 ± 1.4[d]	+12.8 ± 1.5	+16.5 ± 1.5

[a] Under conditions favoring base pairing in chloroform-*d*. [b] (Dimethylamino)-1-(2′,3′,4′,6′-tetraacetyl-D-glucopyranosyl)cytosine in chloroform-*d*.[23] [c] See ref 23 for a discussion of the accuracy of this value. [d] In the closed base pair. [e] In the open state.

The amino group of G rotates at a greater rate than the amino group of C at any given temperature. In the temperature range from −50 to −69 °C, ΔM increased with temperature (Figure 3). It appears likely that at lower temperatures (which are experimentally inaccessible) the plot of ΔM versus T would change slope. However, we could observe only the high-temperature realm of the expected biphasic behavior for the amino protons of G. Above −65 °C, ΔM is clearly in the high-temperature realm, indicating that the primary contribution is from chemical exchange. Thus, it is reasonable to assume that dipolar-induced errors in modified Hoffman-Forsen analysis are not substantial in this temperature range.

The k_g's, as determined by modified Hoffman-Forsen analysis at two temperatures, were consistent with those obtained from line-shape analysis (both are plotted in Figure 4). Modified Hoffman-Forsen analysis was performed at −57 and −63 °C. At temperatures higher than −57 °C, ΔM approached 1, and exchange was too rapid to measure by this technique.

From eq 1-4, k_g has been determined over a broad range of temperature (from −63 to +55 °C). An Eyring plot (Figure 4) of $R \ln (k_g h/KT)$ versus $1/T$ [where R = 1.987 cal/(mol·T), k_g is in Hz, $h = 1.58 \times 10^{-34}$ cal·s, $K = 3.30 \times 10^{-24}$ cal/T, and T is in Kelvin] has been employed to determine activation parameters for rotation about the amino bond of G in the presence of equimolar C in chloroform-*d*. We have found that the activation enthalpy for rotation about the amino bond of base-paired G ($\Delta H^{\ddagger}_{rotG}$) is equal to 10.6 ± 0.3 kcal/mol and the activation entropy for the same process ($\Delta S^{\ddagger}_{rotG}$) is equal to −2.5 ± 1.4 cal/(mol·T) (Table I).

Rotation about the Amino Bond of Cytosine. The two amino protons of C exchange by rotation about the N^4 amino bond. In 1:1 mixtures of C and G, the two amino protons of C appeared as separate resonances at low temperatures (<30 °C) but broadened and disappeared at higher temperatures (Figure 2A). It was not possible to perform satisfactory line-shape analysis on the amino protons of C in 1:1 mixtures due to temperature-dependent formation of $(C:G)_2$ tetramers (Figure 1B). The problem was circumvented by conducting the experiments with 1:2 mixtures of C and G, thus blocking tetramer formation with $C:G_2$ trimers (Figure 1C,D). We have reported previously that trimer formation disrupts the tetramers and prevents the H_b proton of C from hydrogen bonding.[12] When C and G were combined in 1:2 mixtures, the chemical shifts of the amino protons of C were nearly invariant (except for the effects of exchange) with change in temperature, providing a system amenable to line-shape analysis. The assignments for the four amino protons from a 1:2 mixture of C and G are shown in Figure 5A.

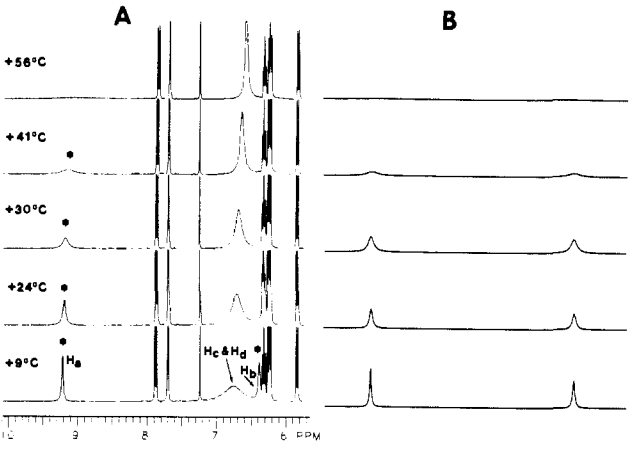

Figure 5. (A) Partial 300-MHz ^{1}H NMR spectra of 60 mM 2′-deoxy-3′,5′-bis(triisopropylsilyl)guanosine plus 30 mM 2′-deoxy-3′,5′-bis(triisopropylsilyl)cytidine in chloroform-*d*. The asterisks indicate the two exchanging proton resonances. (B) Calculated spectra of H_a and H_b resonances at the same temperatures as the experimentally obtained spectra.

With the rates calculated from line-shape analysis, the unmodified Block equation could be used to accurately reproduce the line widths and overall spectra of the exchanging amino protons of C (Figure 5B). The close agreement between observed spectra (Figure 5A) and calculated spectra (Figure 5B) indicates that in these experiments rotation about the amino bond is the primary determinant of the observed temperature effect on line shape.

In conditions which protect H_b from hydrogen bonding (30 mM C plus 60 mM G in chloroform-*d*), the rate of exchange of the amino protons of C (k_c) was determined over a 56 °C temperature range (9 to 65.0 °C, spectra shown in Figure 5A). By use of equations 2 and 3, k_c (substituting k_c for k_g) has been calculated over the temperature range from 9 to 65 °C. Below 5 °C, rotation of the amino bond of C was frozen out, and amino proton exchange does not make a significant contribution to line shape. At temperatures near coalescence, the solvent (chloroform-*d*) vaporizes. Therefore, the coalescence temperature has been estimated as 65 ± 4 °C. An Eyring plot (Figure 4) of $R \ln (k_c h/KT)$ versus $1/T$ indicates that the activation enthalpy for rotation about the amino bond of base-paired C ($\Delta H^{\ddagger}_{rotC}$) is equal to 18.6 ± 1.0 kcal/mol and the activation entropy for the same process ($\Delta S^{\ddagger}_{rotC}$) is equal to 11.2 ± 3.5 cal/(mol·T) (Table I). The activation parameters obtained from the temperature effect on rates of rotation has allowed us to propose mechanisms of rotation (see below).

Discussion

Watson-Crick-type hydrogen-bonded complexes formed between monomeric C and G fluctuate from ground-state conformation. We have measured the rates for amino bond rotation (one type of fluctuation), over a broad temperature range. From the temperature dependence of the rates of rotation, the activation enthalpies and entropies have been determined. The results suggest that the two amino groups in the C:G base pair rotate via two different mechanisms. In the discussion below, we consider probable mechanisms for amino group rotation.

Guanine Amino Group Rotation. The two amino protons of G (H_c and H_d in Figure 1) exchange by rotation about the N^2 amino bond of G. At low temperatures, under conditions in our model

system favoring base pairing, we have observed H_c and H_d as resolved ^1H NMR resonances. This observation is consistent with previous reports that H_c and H_d appear as separate resonances in aqueous solvents on the monomer level[16] and in oligonucleotides.[19-21]

Under experimental conditions which favor base pairing, we have measured the rate of rotation of the amino group of G over a broad temperature range with both line-shape analysis and time-resolved techniques. The temperature dependence of the rate data was used to determine the activation enthalpy ($\Delta H^{\ddagger}_{rotG}$) and activation entropy ($\Delta S^{\ddagger}_{rotG}$) for rotation of the amino group of G (and C, see below). We have determined that $\Delta H^{\ddagger}_{rotG}$ is +10.6 ± 0.3 kcal/mol and $\Delta S^{\ddagger}_{rotG}$ is −2.5 ± 1.4 cal/(mol·T) (Table I).

The small value of $\Delta S^{\ddagger}_{rotG}$ indicates that the transition state for rotation is not disordered relative to the ground state (closed base pair). A mechanism of rotation in which the base pair is not disrupted is consistent with the observed $\Delta S^{\ddagger}_{rotG}$. It would appear that the base pair remains closed and two of the three hydrogen bonds are maintained during the process of rotation of the amino group of G.

From this model of closed rotation of the amino group of G, it is a reasonable approximation to decompose $\Delta H^{\ddagger}_{rotG}$ into the sum of two terms, (a) the intrinsic activation enthalpy of rotation of the amino group of G ($\Delta H^{\ddagger}_{intG}$) and (b) the enthalpy of disruption (i.e., the negative of the enthalpy of formation) of one hydrogen bond ($-\Delta H_{hb}$):

$$\Delta H^{\ddagger}_{rotG} = \Delta H^{\ddagger}_{intG} - \Delta H_{hb}$$

The enthalpy of disruption of one of the hydrogen bonds of the base pair should be approximately equal to (i.e., within 1 kcal/mol) one-third of the total enthalpy of disruption of the three hydrogen bonds of the base pair ($-\Delta H^{\ddagger}_{bp}$). We have previously reported ΔH_{bp} under these experimental conditions to be −5.77 ± 0.20 kcal/mol.[11] Therefore, $\Delta H^{\ddagger}_{hb}$ is estimated to be 1.9 ± 1.1 kcal/mol. From $\Delta H^{\ddagger}_{rotG}$ and $\Delta H^{\ddagger}_{hb}$, a reasonable estimate for $\Delta H^{\ddagger}_{intG}$ is 8.7 ± 1.4 kcal/mol (Table I). In this model, $\Delta H^{\ddagger}_{intG}$ represents the intrinsic contribution to $\Delta H^{\ddagger}_{rotG}$ in the closed, not the open, state. The electronic structure of the bases, and therefore $\Delta H^{\ddagger}_{intG}$, would not be the same in the closed compared with the open state.

Cytosine Amino Group Rotation. In analogy with rotation of the amino group of G, the two amino protons of C (H_a and H_b in Figure 1) rotate about the N^4 amino bond of C. We have observed H_a and H_b of base-paired C as resolved resonances. This observation is consistent with previous reports that H_a and H_b appear as separate resonances in the C:G base pair formed between monomers[7] and in oligonucleotides.[19-22]

For the present study, the rate of rotation of the amino group of C was measured over a broad temperature range under experimental conditions in which C is involved in Watson–Crick-type interactions with G. The C:G_2 trimer[11,12] was employed to prevent interference from non-Watson–Crick-type hydrogen bonding of the H_b proton of C (Figure 1). The temperature dependence of the rate data was used to determine activation parameters ($\Delta H^{\ddagger}_{rotC}$ and $\Delta S^{\ddagger}_{rotC}$) for C amino rotation. We have determined that $\Delta H^{\ddagger}_{rotC}$ is +18.6 ± 1.3 kcal/mol and $\Delta S^{\ddagger}_{rotC}$ is +11.2 ± 3.5 cal/(mol·T) (Table I).

Compared to the $\Delta S^{\ddagger}_{rotG}$ [−2.5 ± 1.4 cal/(mol·T)] and $\Delta S^{\ddagger}_{rot}$ of (dimethylamino)cytosine (Table I), $\Delta S^{\ddagger}_{rotC}$ measured here is positive and large in magnitude. Further, the sign and magnitude of $\Delta S^{\ddagger}_{rotC}$ are in the range expected for a mechanism involving disruption of the base pair (see Kyogoku et al.[9] for an estimate of the entropy of base pairing under similar conditions). Therefore, it appears that rotation of the amino group of C does not occur within the base pair. We propose a mechanism of rotation that

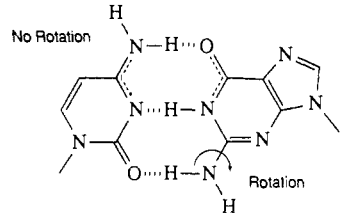

Figure 6. Schematic diagram of the proposed electronic structure of the C:G base pair.

proceeds only via the transient disordered state of base-pair disruption.

From this model of open base pair rotation of the amino group of C, it is a reasonable approximation to decompose $\Delta H^{\ddagger}_{rotC}$ into the sum of two terms, (a) the intrinsic activation enthalpy of rotation of the amino group ($\Delta H^{\ddagger}_{intC}$, in the open, not closed, state) plus (b) the enthalpy of disruption (i.e., the negative of the enthalpy of formation) of the base pair ($-\Delta H_{bp}$):

$$\Delta H^{\ddagger}_{rotC} = \Delta H^{\ddagger}_{intC} - \Delta H_{bp}$$

From the value of $\Delta H^{\ddagger}_{rotC}$ reported here (18.6 ± 1.3 kcal/mol) and our previous experimental determination of ΔH_{bp} (−5.77 ± 0.20 kcal/mol, see ref 11), $\Delta H^{\ddagger}_{intC}$ is estimated to be 12.8 ± 1.5 kcal/mol (Table I). This estimation for $\Delta H^{\ddagger}_{intC}$ is consistent with a report by Becker and co-workers, who found that (in the same solvent employed here) $\Delta H^{\ddagger}$ for rotation of the amino group of (dimethylamino)cytosine is 16.5 ± 1.5 kcal/mol (Table I). Their value for (dimethylamino)cytosine is slightly greater than that reported here for C. Dimethylation of the amino nitrogen would be expected to increase the double bond character of the amino bond and thus increase the internal hindrance to rotation.

In summary, it appears that in this monomer model system of DNA the amino group of G rotates within the closed base pair while the amino group of C rotates only during transient base pair disruption. The large hindrance to rotation of the amino group of C in the base pair reported here is consistent with the relatively high acidity of H_b in the base pair.[16] Both the high acidity of H_b and the high hindrance to rotation of the amino group of C are expected if the O^6–H_a hydrogen bond and N3–H^1 hydrogen bond are electronically coupled as shown schematically in Figure 6. The double bond character of the amino bond of C is significantly increased by base pairing while the amino bond of G, which is not coupled, is relatively unaffected. The amino bond of C would also be expected to exhibit significant double bond character in trimers and tetramers (Figure 1). Lowdin's proposal of simultaneous tautomerization within the base pair[24] provides an alternative model to account for both the high acidity of H_b and the high hindrance to rotation of the amino group of C. However, we have concluded from an infrared spectroscopic search for rare tautomers in the C:G base pair (MacPhail, R. A.; Williams, L. D.; Jones, D. A.; Shaw, B. R., unpublished results) that the low probability of formation of rare tautomers precludes Lowdin's proposal as the basis of these observed properties of the C:G base pair.

Our results from a monomer model system are consistent with the observation by Mirau and Kearns[22] that, in certain conformations of DNA (Z-DNA) at least, the amino group of C does not appear to rotate in the closed state. However, rotation of the amino group of G takes place within the closed state in DNA.[19,20]

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