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Exceptional Characteristics of Amino Proton Exchange in Guanosine Compounds†

Bruce McConnell,* Douglas J. Rice, and Francis-Dean A. Uchima

ABSTRACT: Amino ^{1}H NMR line width as a measure of amino proton exchange in guanosine compounds is completely unaffected by the addition of ca. 1 M tris(hydroxymethyl)-aminomethane, imidazole, 2-(*N*-morpholino)ethanesulfonic acid, glycine, or cacodylate, all shown to be effective buffer catalysts in adenosine and cytidine proton exchange. Line broadening, seen only with phosphate and acetate, is established by intermolecular interactions, as well as by amino to water proton exchange. This absence of buffer catalysis of exchange is accounted for by the relatively small implied effect of G(N-7) protonation on amino acidity, based on similar observations with 7-methylguanosine as a model for endocyclic protonation. The requirement for diffusion-controlled proton transfer in buffer catalysis is achieved by nucleobase protonation in adenine and cytosine, but not in guanine.

Buffer catalysis of nucleobase hydrogen exchange has been demonstrated for the amino group of adenosine compounds (McConnell & Seawell, 1972; McConnell, 1974, 1978a; Cross et al., 1975), the amino group of cytosine compounds, and the imino (N-1) group of guanylic acid (McConnell, 1978a). The mechanism of buffer catalysis for the amino protons of adenine and cytosine was shown to involve the exclusive roles of buffer conjugate base as acceptor and the endocyclic protonated nucleobase as amino proton donor; the neutral, unprotonated nucleobase does not contribute to observed buffer-catalyzed exchange and gives up its amino protons only to hydroxyl ion at measurable rates (McConnell, 1974, 1978a). More recent NMR studies have been made (B. McConnell and D. Politowski, unpublished results) that lend support to this mechanism through a comparison of buffer catalysis in amino proton exchange of adenosine, 1-methyladenosine, cytidine, and 3-methylcytidine. As models for endocyclic protonation, the endocyclic methylated nucleobases are about 10^4 more acidic than the unmodified (and unprotonated) compounds at their amino sites, and their amino proton exchange conforms to a relationship between second-order buffer rate constant and buffer dissociation that is expected for diffusion-limited encounters. Thus, buffer-catalyzed exchange in adenine and cytosine is a function of both buffer pK and nucleobase pK, since endocyclic protonation is required to provide a sufficient increase in amino acidity for the observation of proton transfer to a solvent acceptor. This kinetic route was postulated as a mechanism for amino hydrogen exchange in double helical polynucleotides as well (Teitlebaum & Englander, 1975).

Earlier studies showing phosphate-induced broadening of the amino ^{1}H NMR signal in guanylic acid led to the expectation of a similar mechanism, which would provide predictable buffer-induced exchange rates based on the magnitude of the guanine (N-7) pK value (McConnell, 1978b). However, the present study shows that phosphate catalysis is an exception. Other buffers, shown to be effective catalysts of amino proton exchange in adenosine and cytosine, are completely ineffective as catalysts in guanylic acid. An explanation for this lack of buffer catalysis is derived from studies with 7-methylguanosine whose amino proton exchange is equally insensitive to the addition of buffer (with the exception of phosphate). The 50-fold increase in amino acidity that occurs with N-7 methylation of guanine is insufficient to provide diffusion-controlled rates for hydroxyl-catalyzed exchange and is much smaller than the 10^4-fold acidity increase observed with methylation of adenine (N-1) or cytosine (N-3). Thus, guanine amino exchange represents a special case, confirming the mechanism of buffer-catalyzed amino proton exchange in adenosine and cytosine that involves the exclusive kinetic role of the protonated nucleobase at neutral to acidic pH values.

Materials and Methods

The guanosine compound guanosine cyclic 2′,3′-monophosphate (Sigma) was used as supplied, and 7-methylguanosine was prepared from guanosine by the method of

† From the Department of Biochemistry and Biophysics, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii 96822. *Received March 15, 1983.* This work was funded in part by National Science Foundation Grant PCM 77-20154.

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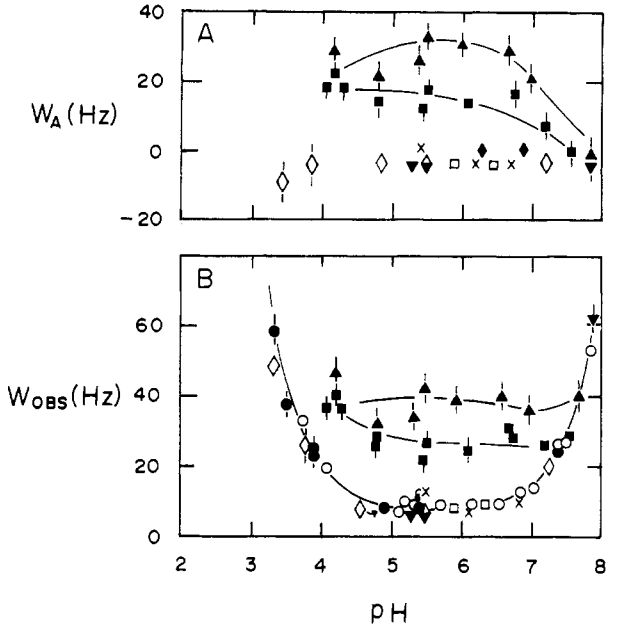

FIGURE 1: pH dependence of amino ^{1}H NMR line width (W_{obsd}) and catalytic line broadening (W_A) in aqueous 2′,3′-cGMP solutions. Data were collected at 27 ± 2 °C at 300 or 360 MHz for 0.03 ± 0.01 M nucleotide without addition (●) and with 0.5 M phosphate (■), 0.75 M phosphate (▲), 0.5 M Tris (◊), 1.0 M imidazole (▼), 0.8 M cacodylate (×), 1.0 M MES (□), and 1.0 M glycine (△). Data obtained at 100 MHz are included for 0.1 M nucleotide (○). Data of (A) were obtained by subtraction of W_{obsd} from the line width in the presence of buffer.

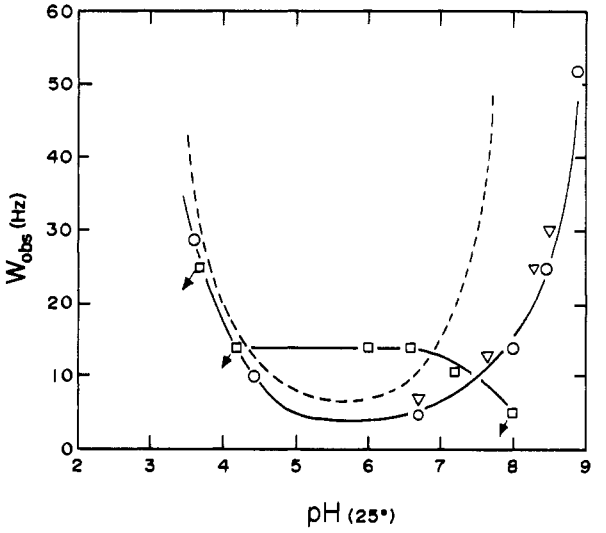

FIGURE 2: pH dependence of guanine amino ^{1}H NMR line width at 4 °C. Observed line widths, W_{obsd}, were obtained at 300 MHz from solutions containing 0.05 M 2′,3′-cGMP, 0.001 M EDTA, and the following additions: 1.0 M $NaClO_4$ (○), 0.5 M sodium phosphate (□), and 1.0 M imidazole (▽). Angled arrows from three phosphate points indicate precipitation at 4 °C in the NMR tube. pH measurements and adjustments were made at 25 °C. The dotted line shows the pH dependence of W_{obsd} at 27 ± 2 °C for comparison.

Jones & Robins (1963). Sodium phosphate, sodium acetate (Baker), sodium cacodylate, tris(hydroxymethyl)aminomethane (Tris), imidazole, glycine (Sigma), and 2-(*N*-morpholino)ethanesulfonic acid (MES) (Research Organics, Inc.) were used as supplied in the highest purity available. All solutions contained 0.001–0.002 M Na_2EDTA (Sigma) and were pH adjusted with a Beckman Model 4500 digital pH meter and an Ingold combined electrode designed for NMR tubes.

Collection of NMR spectra involved the use of the Varian high-resolution 100-MHz spectrometer and the Nicolet 300-MHz spectrometer,[1] both of the Department of Chemistry, University of Hawaii. Spectra from the latter instrument were obtained by Redfield Fourier-transform (FT) pulse acquisition (Redfield, 1978). Earlier spectra were obtained from the Bruker HXS-360 NMR spectrometer of the Stanford Magnetic Resonance Laboratory, Stanford University.[2] The majority of line width measurements were verified on the Nicolet 1280 computer with Lorentzian line-fitting functions iterated to minimum statistical variance from the digitized data in the spectral region of the amino ^{1}H NMR resonances.

Results

In Figure 1 the pH dependence of line width at 27 °C of the amino ^{1}H NMR resonance is shown for 2′,3′-cGMP alone and in the presence of various buffers previously shown to be effective exchange catalysts of amino proton exchange in adenosine and cytosine compounds (McConnell, 1974, 1978a; McConnell and Politowski, unpublished results). With the exception of phosphate, none of the buffers produce line broadening even at 1 M concentration. This is most apparent in Figure 1A, which shows no specific buffer broadening with Tris, MES, imidazole, glycine, or cacodylate. The phosphate-induced broadening is concentration dependent only in a narrow pH range between pH 4.5 and pH 6.5. Above pH 7, phosphate produces a sharpening of the amino ^{1}H NMR resonance, which may be related to intermolecular association, as evidenced by precipitation at lower temperatures. A pH profile obtained at 4 °C is shown in Figure 2 for data collected in the presence of 0.5 M phosphate and 1 M imidazole and includes a dashed curve representing the 27 °C data for comparison. Precipitation in the presence of phosphate is observed in both line-broadening pH limbs of Figure 2 (pH <5.5 and pH >7) and is associated with zero or negative effects on line width, as opposed to the significant broadening between these pH values. The large difference in line width collected at both temperatures above pH 7 (without buffer) reflects the catalytic role of OH^- in amino proton exchange between the water site and the neutral (unprotonated) guanine nucleobase. The concentration of OH^- is lowered through the effect of decreasing temperature, which decreases K_w from 10^{-14} (at room temperature) to $\sim 10^{-14.8}$ (at 0 °C). This suppression of OH^- catalysis allowed for the observation of imidazole catalysis of amino proton exchange in adenine (McConnell, 1974), but not for guanine, as shown here. The possibility of line-broadening suppression between pH 4.5 and pH 6.5 in response to intermolecular association in the 2′,3′-cGMP–imidazole mixture is ruled out by measurements of constant amino ^{1}H NMR line width in solutions of widely varying concentrations of 2′,3′-cGMP (5–500 mM, not shown).

To examine further the relative contributions of exchange and intermolecular association on amino ^{1}H NMR line width, measurements were taken at several temperatures between pH 5 and pH 6 for 2′,3′-cGMP without buffer and in the presence of phosphate and imidazole (Figure 3). Imidazole has no effect whatever, even at 65 °C, where $-NH_2$ to H_2O exchange in the absence of buffer is clearly the chief broadening factor. However, phosphate broadening exhibits a strong, positive temperature dependence, much greater than that in the ab-

[1] The Nicolet 300-MHz spectrometer was acquired from funds awarded by the National Science Foundation, NSF Grant CHE 81-00240.

[2] The Bruker HXS-360 NMR spectrometer, a national facility at Stanford University, was supported by NSF Grant GR 23633 and NIH Grant RR 00711.

Table I: Comparison of Amino Proton Exchange Catalysis in Adenosine, Cytidine, and Guanosine Compounds

log k_{cat} (M^{-1} s^{-1})[a]	buffer	adenosine			cytidine			guanosine		
	pK	H^1[b]	N	m^1	H^3	N	m^3	H^7	N	m^7
hydroxyl	15.8	11	7.6	11	11	7.7	11		8	9.7
phosphate	6.8	5.9	n[c]	6.3	6.3	n	6.1	6.8[e]	n	3.6
imidazole	7.0	6.1	n	6.4	(6.0)	n	(6.0)	n	n	n
Tris	8.2	6.9	n	7.5	6.9	n	7.2	n	n	n
H_3O^+[d]		−6.3		2.8	<−1		<1	−6.3		5.0

[a] Second-order rate constants at 25 ± 2 °C, obtained for adenosine and cytidine compounds from McConnell and Politowski (unpublished results), refer to the concentration of buffer conjugate base, not total buffer (eq 2). Values in parentheses were obtained by interpolation of regression data. [b] H^1, H^3, and H^7 refer to endocyclic protonated species as amino proton donors, where k_A (eq 2) is velocity divided by total nucleobase concentrations. [c] Too low to observe or negligible (n) rates. [d] Hydronium catalysis, derived from $k_{obsd} = k_{H_3O^+}[H_3O^+]$ at low pH, is listed only for comparison of rate increase between pH 4.5 and pH 3. This constant may not be valid for mechanistic purposes, owing to rate plateaus for adenosine compounds below pH 3 (McConnell, 1974; Cross et al., 1975). [e] Value obtained for H^7G is based on the assumption of eq 2, thus far untested for guanosine compounds.

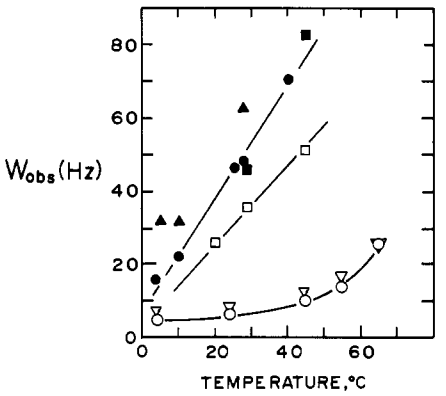

FIGURE 3: Temperature dependence of guanine amino ^1H NMR line width. Amino ^1H NMR resonance obtained at 300 MHz corresponded to 0.05 M 2′,3′-cGMP in 0.001 M EDTA under the following conditions: 1.0 M $NaClO_4$, pH 5.6 (○); 1.0 M imidazole, pH 5.6 (▽); 0.8 M sodium phosphate, pH 5.6 (□); no addition, pH 3.6 (●); 0.25 M sodium phosphate (▲), pH 3.55. Solid squares correspond to solutions of 0.05 M 7-methylguanosine–0.8 M sodium phosphate, pH 5.6.

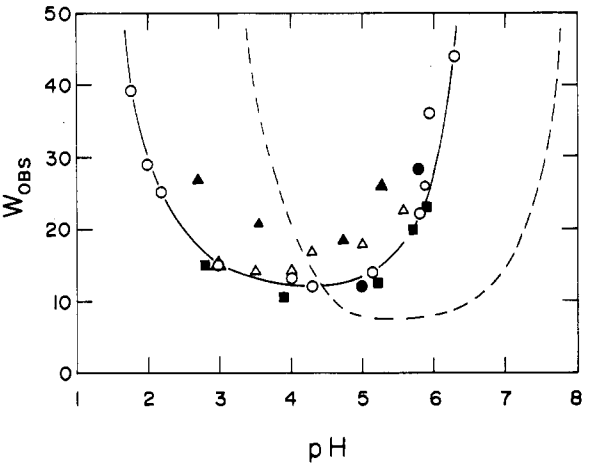

FIGURE 4: 7-Methylguanosine amino ^1H NMR line width vs. pH. Line widths were taken from 100-, 300-, and 360-MHz spectra on 0.05 M solutions of m^7G containing no addition (○), 0.7 M imidazole (■), 0.5 M acetate (△), 0.6 M Tris (●), and 0.5 M phosphate (▲) at 27 ± 2 °C. The dashed line represents the pH profile of 2′,3′-cGMP (unsubstituted guanine), and the alkaline broadening lines for m^7G and 2′,3′-cGMP are calculated from $k_{OH} = 5 \times 10^9$ M^{-1} s^{-1} and 1×10^8 M^{-1} s^{-1}, respectively.

sence of buffer. A strong, positive temperature dependence in line width is observed in the low pH region, where broadening is seen in the absence of buffer. A *negative* temperature dependence of line width, expected for possible association ⇌ dissociation equilibria of intermolecular interactions, is not seen.

It has been shown that methylation of the endocyclic proton binding nitrogen of adenine and cytosine provides a working model for the effects of protonation at the unmodified site and that the effects of methylation and protonation on amino acidity (proton exchange) are large and similar in magnitude (McConnell and Politowski, unpublished results). Data on 7-methylguanosine are shown in Figure 4, in which the dashed line represents the pH profile of unmodified guanine (Figure 1) for comparison. In this case methylation produces a 1.5–1.8 pH displacement of alkaline and acidic line broadening to lower pH values. Fitted rate constants of hydroxyl catalysis for m^7G and 2′,3′-cGMP are $k_{OH} = 5 \times 10^9$ M^{-1} s^{-1} and 1×10^8 M^{-1} s^{-1}, respectively. This represents a change in acidity (k_{OH}) of only 50-fold between neutral, unprotonated guanine and the 7-methyl derivative, much smaller than the 10^4 change observed in adenine and cytosine compounds (Hoo & McConnell, 1979; McConnell and Politowski, unpublished results). Notably, k_{OH} for m^7G is considerably lower than the corresponding constant in m^1A and m^3C amino proton exchange, which is of the diffusion-limited magnitude ($k_{OH} \simeq 10^{11}$ M^{-1} s^{-1}). It is apparent from Figure 4 that the addition of buffers produces the same effects observed with the unmodified nucleobase. Tris and imidazole have no effect, while phosphate broadening is observed, which reflects, once again, catalysis of $-NH_2$ to H_2O exchange, as shown by the strong positive temperature dependence for 7-methylguanosine phosphate mixtures (Figure 3). In addition, acetate broadening appears in the pH region corresponding to the ionization of this buffer. A comparison of catalytic rate constants of amino proton exchange in unsubstituted and endocyclic methylated compounds of adenosine, cytidine, and guanosine is presented in Table I.

The effect of N-7 methylation on the ^1H NMR spectra of guanine nucleobase protons is shown in Figure 5. The large 1.2 ppm downfield shift of the H-8 proton found at 7.95 ppm (DSS) for the unmodified base is not matched by the 0.5 ppm change in chemical shift of the amino proton resonances from 6.34 to 6.83 ppm. Corresponding amino ^1H NMR shift changes with methylation or protonation of adenine or cytosine are over 2 ppm, which reflect the large increase in hydrogen bonding associated with increases in amino acidity.

Discussion

Experiments involving temperature variation were performed to determine whether $-NH_2$ to H_2O exchange was a real factor in establishing NMR line width. The positive temperature dependence of exchange with phosphate, and at low pH in the absence of buffer, indicated that line width is established chiefly by $-NH_2$ to H_2O exchange broadening, in the "slow"

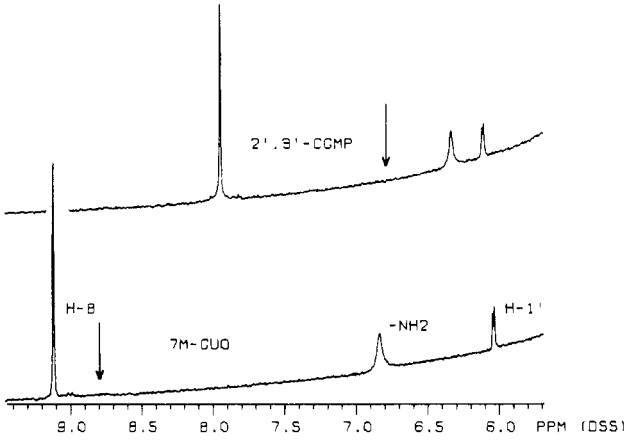

FIGURE 5: Effect of N-7 methylation on 300-MHz ^1H nucleobase spectra of guanosine compounds. Spectra were taken at 25 °C from aqueous solutions containing 0.05 M 2′,3′-cGMP and 0.05 M m^7G at pH 4.9. Small intensities of upfield resonances reflect proximity to the Redfield pulse null position for water suppression. Vertical arrows locate the position of amino ^1H NMR resonances in the corresponding adenosine compounds. The spectral frequency is based on sodium 4,4-dimethyl-4-silapentanesulfonate (DSS).

NMR time frame, where the reciprocal lifetime $1/\tau_{a,b} \ll \delta_a - \delta_b$, and δ_a and δ_b are chemical shifts for the two sites ($-NH_2$ and H_2O). Because extra resonances were not observed between pH 4.5 and pH 6.5, this condition would not be expected to exist in exchange between molecular complexes, and both a field-frequency dependence and a negative temperature dependence would be anticipated for intermolecular interactions involving buffer and guanine compounds. Neither was observed (Figures 1 and 3). However, line width was seen to decrease with increasing activity coefficients outside the pH 4.5–6.5 range, which implicates solute interactions possibly operating to decrease $-NH_2$ to H_2O exchange. It has been shown that self-association of guanosine monophosphate in aqueous solution is slow in the NMR time scale (Pinnavaid et al., 1975). Nevertheless, the comparison of the buffer effects at pH 5–6, i.e., phosphate vs. imidazole, represents mainly a comparison of their relative powers as catalysts in amino proton exchange.

Amino proton exchange in nucleic acids can be expressed in terms of contributions of the neutral, unprotonated nucleobase, N, and its conjugate acid, NH^+, protonated at the weakly basic endocyclic nitrogen (pK_N values are 3.7, 4.0, and 2.1 for adenosine, cytidine, and guanosine compounds, respectively)

$$\text{amino H exchange} \xleftarrow{k_N} N + H^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} NH^+ \xrightarrow{k_{NH^+}} \text{amino H exchange}$$

Given the conditions $k_2 > k_N$ and k_{NH^+} and the essential exchange irreversibility of k_N and k_{NH^+} reactions, the observed increase in rate with the addition of buffer is

$$\pi W_A = k_A\ (s^{-1}) = P_N k_N + P_{NH^+} k_{NH^+} \qquad (1)$$

where P is the mole fraction, k_N and k_{NH^+} are pH-dependent first-order rate constants, and W_A is the observed increase in amino ^1H NMR line width. In the case of adenine and cytosine, $P_N k_N$ is negligible, owing to the observation that fitted values of k_{NH^+} at low pH are large and account for all of the observed broadening at all pH values (and where $P_N = 1$) (McConnell, 1974). In this case

$$k_A = P_{NH^+} k_{NH^+} = \left(\frac{a_{H^+}}{K_N + a_{H^+}}\right) k_B[B] \qquad (2)$$

where a_{H^+} is hydrogen ion activity, K_N is the acid dissociation constant of the nucleobase site, and k_B is the fitted second-order rate constant for proton transfer from NH^+ to the buffer conjugate base, B, of the acid–base pair, $BH^+ \rightleftharpoons B + H^+$. "Eigen" plots of k_B, i.e., log k_B vs. buffer pK (pK_B), are linear with positive unit slope until $pK_B \leq pK_D$, where pK_D is a measure of the amino pK of NH^+ (McConnell and Politowski, unpublished results). This conformance indicates diffusion-controlled rates for the formation of the donor–acceptor encounter complex (Eigen, 1964; Crooks, 1975). The implication is that no buffer catalysis would be seen, were it not for the transformation of the nucleobase amino from a poor proton donor to a "normal" acid as a result of endocyclic protonation. Accordingly, in a nucleobase system associated with significantly lower inductive effects of nucleobase protonation, one would expect to see no buffer catalysis under the premise that $P_N k_N \ll P_{NH^+} k_{NH^+}$. This is exactly what is observed here in studies on guanosine; N-methylation has relatively little effect on guanine amino acidity and, with the exception of phosphate and acetate, no buffer catalysis is observed with either the methylated or unsubstituted nucleobase. It is reasonable to assume a weak effect of guanine N-7 protonation, in view of the similarity of methylation and protonation in their effects on amino acidity in adenosine and cytidine (McConnell and Politowski, unpublished results). Thus, the general lack of buffer catalysis in guanine is consistent with the mechanism of eq 2, which excludes for all nucleobase systems the unprotonated nucleobase, N, as a kinetically active species providing observable buffer-catalyzed exchange rates by this method. This is borne out further by the observations that, in the absence of buffer, guanine amino proton exchange at pH 5–6 is much slower than that of adenine and cytosine, while k_{OH} is comparable for the guanine neutral base, N (McConnell, 1974, 1978).

The exceptions, the catalytic effects of phosphate (and acetate), might be accounted for in regard to the size of $k_{OH} = 5 \times 10^9\ M^{-1}\ s^{-1}$ for m^7G, which appears to be low but marginally close to the diffusion-controlled rate (10^{10}–$10^{11}\ M^{-1}\ s^{-1}$). In this case, amino acidity could become stringently ion selective for proton transfer, sufficient for phosphate or acetate anions, but insufficient for the neutral conjugate bases of amine buffers. If we make the untested assumption that catalysis by these anionic bases involves the same mechanism (eq 2), rate constants (k_B) estimated for phosphate ($pK_B = 6.8$) and acetate ($pK_B = 4.7$) are $6.6 \times 10^6\ M^{-1}\ s^{-1}$ (phosphate/2′,3′-cGMP, $pK_N = 2.1$), $4 \times 10^3\ M^{-1}\ s^{-1}$ (phosphate/m^7G), and $62\ M^{-1}\ s^{-1}$ (acetate/m^7G). Thus, the effects of endocyclic protonation and methylation would not be the same in guanine amino exchange with respect to phosphate; the amino of H^7-2′,3′-cGMP would be $\sim 10^3$-fold more acidic than that of m^7G. This effect could reflect additional, unknown exchange routes in 2′,3′-cGMP and could be exclusive to the phosphate dianion. For m^7G the ratio of k_B for phosphate and acetate (64) is in fair agreement with the ratio of their dissociation constants (125).

In view of the results, the increased line broadening observed at pH <4.5 (Figure 1) cannot be attributed to the exchange mechanism of eq 2. The possibility of the mechanism of eq 2, in which water (the conjugate base of the $H_3O^+ \rightleftharpoons H_2O + H^+$ equilibrium) might act as proton acceptor with N-7-protonated G as amino proton donor, is not encouraged by a number of observations. Low pH broadening occurs in the same pH region for adenosine and guanosine compounds, in spite of the large differences in their endocyclic pK values. It has been noted by McConnell and Politowski (unpublished

results) that this mechanism (eq 2) does not account for exchange at pH <4 in adenosine compounds, since the resulting values of k_B in eq 2 are at variance by two orders of magnitude with those expected from buffer data. These workers showed that consistent kinetic parameters are obtained only for cytidine compounds, whose exchange at pH <4 is quite slow, producing virtually no pH broadening at pH <2. In addition, low pH broadening is seen for both m^7G (Figure 4) and m^1A (McConnell and Politowski, unpublished results), which suggests direct exocyclic amino protonation as opposed to endocyclic protonation as a chief mechanism for purine amino exchange at low pH. The formation of an *amino*-protonated intermediate is consistent with the *decrease* in low pH exchange upon endocyclic methylation (Figure 4) since this substitution would decrease the basicity of $-NH_2$.

Some new implications of these results on guanine can be mentioned with respect to the mechanism of exchange in polynucleotides. First, the exchange mechanism postulated for amino protons in polynucleotides (Teitlebaum & Englander, 1975) is not general for all nucleobases. Endocyclic protonation does not provide a general buffer-catalyzed exchange route for the guanine amino protons, whose rates would be catalyzed chiefly by hydroxyl ion involving the neutral, unprotonated base. Therefore, the large effect of imidazole on all exchange classes of DNA may require interactions of a special type, e.g., intercalation (McConnell & von Hippel, 1970). Second, the large difference in effect of buffers on amino exchange in adenine, cytosine, and guanine should provide a means for the assignment of amino ^1H NMR resonance in aqueous self-complementary oligonucleotides [see, for example, Patel (1977)]. Third, an inverse relationship, conceived between amino H-bond strength and helical stability, was based on a notion that base pairing in helix formation might require weak hydrogen bonding with competing water molecules (McConnell, 1978b). On this basis the expected increase in amino acidity following guanine N-7 protonation was postulated as a mechanism for in vivo destabilization of G-C-rich regions in DNA. This speculated mechanism does not appear to be supported by these results, which show that the increase in guanine amino acidity with N-7 protonation is much smaller than expected. Weak amino to water hydrogen bonding might not be altered greatly in guanine through N-7 interactions, which is borne out by the comparison of the ^1H NMR spectra of 2′,3′-cGMP and m^7G (Figure 5).

Registry No. 2′,3′-cGMP, 634-02-6; 7-methylguanosine, 20244-86-4; phosphate, 14265-44-2; acetate, 64-19-7.

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Sequence-Specific Recognition of Deoxyribonucleic Acid. Chemical Synthesis and Nuclear Magnetic Resonance Assignment of the Imino Protons of λ O_R3 Operator Deoxyribonucleic Acid†

Shan-Ho Chou, Dennis R. Hare, David E. Wemmer, and Brian R. Reid*

ABSTRACT: Using solid-phase phosphite triester methods, we have synthesized both strands of the phage λ O_R3 DNA sequence, reannealed them, and studied the native operator duplex by high-resolution NMR at 500 MHz. At 7 °C the imino protons of the two terminal base pairs at each end have disappeared from the spectrum by exchange broadening. The 13 detectable imino resonances have been assigned to their respective base pairs in the duplex by using sequential nearest-neighbor NOE connectivity methods described previously. In cases where two imino protons overlap in the spectrum, spin diffusion was used to drive the cross-saturation further afield in order to produce second-order next-nearest-neighbor effects. The results show that the imino connectivity method can be used to unambiguously assign the imino proton spectrum of operator DNAs containing one to two full turns of the helix.

The recognition of short stretches of specific DNA sequences such as operators and promoters is of central importance in the control of gene expression. Operators consist of approximately 20 base pairs at the proximal end of genes or operons and are specifically recognized and bound by repressor proteins; several bacterial and viral operators have been characterized with respect to sequence and the binding affinity of their corresponding repressors (Bourgeois & Pfahl, 1976).

The right operator locus O_R of bacteriophage λ is a par-

† From the Chemistry Department and Biochemistry Department, University of Washington, Seattle, Washington 98195. *Received March 22, 1983.* This work was supported by instrumentation grants from the Murdock Foundation, the National Science Foundation (PCM80-18053), and the National Institutes of Health (GM2874-O1S1).

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