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Sylvie Nonin, J.-L. Leroy, M. Gueron. Acid-Induced Exchange of the Imino Proton in G.C Pairs. *Nucleic Acids Research*, 1996, 24 (4), pp.586-595. <10.1093/nar/24.4.586>. <hal-02344768>

**HAL Id: hal-02344768**

**<https://hal.science/hal-02344768v1>**

Submitted on 11 Aug 2020

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# Acid-induced exchange of the imino proton in G-C pairs

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Received November 9, 1995; Revised and Accepted January 8, 1996

## ABSTRACT

Acid-induced catalysis of imino proton exchange in G-C pairs of DNA duplexes is surprisingly fast, being nearly as fast as for the isolated nucleoside, despite base-pair dissociation constants in the range of  $10^{-5}$  at neutral or basic pH. It is also observed in terminal G-C pairs of duplexes and in base pairs of drug-DNA complexes. We have measured imino proton exchange in deoxyguanosine and in the duplex d(ATATAGATC-TATAT) as a function of pH. We show that acid-induced exchange can be assigned to proton transfer from N7-protonated guanosine to cytidine in the open state of the pair. This is faster than transfer from neutral guanosine (the process of intrinsic catalysis previously characterized at neutral pH) due to the lower imino proton pK of the protonated form, 7.2 instead of 9.4. Other interpretations are excluded by a study of exchange catalysis by formate and cytidine as exchange catalysts. The cross-over pH between the regimes of pH-independent and acid-induced exchange rates is more basic in the case of base pairs than in the mononucleoside, suggestive of an increase by one to two decades in the dissociation constant of the base pair upon N7 protonation of G. Acid-induced catalysis is much weaker in A-T base pairs, as expected in view of the low pK for protonation of thymidine.

## INTRODUCTION

Imino proton exchange from base pairs at basic and neutral pH is fairly well understood. At basic pH, OH<sup>-</sup> accepts the imino proton from guanosine or thymidine, and the exchange rate is therefore proportional to OH<sup>-</sup> concentration. Below pH 8, this process is slower than acceptance by the imino nitrogen of the opposite nucleoside of the pair, the pH-independent process of 'intrinsic' catalysis (1).

In both cases, proton exchange requires opening of the pair. At basic pH, the ratio of exchange rates in the paired nucleoside and in the monomer is equal to the dissociation constant of the pair, except for a correction factor. In this way, one determines the dissociation constant  $K_{\text{diss}}$ , typically  $10^{-5}$  to  $10^{-6}$  for internal base pairs at room temperature (2). Proton acceptors such as ammonia

may also be used to accelerate exchange, to determine the base-pair dissociation constant and, using high acceptor concentrations, the base-pair lifetime.

Other processes take over below neutral pH. In the guanosine monomer, the proton exchange rate increases in proportion to the proton concentration. This is explained by transfer to water of the imino proton of N7-protonated guanosine, whose imino proton pK is lower than in guanosine: this accelerates the capture by H<sub>2</sub>O (3).

Protonation on N7 is also possible in a paired guanosine (Fig. 3), so that the same process should occur from G-C pairs. As in the case of catalysis by OH<sup>-</sup> at basic pH, the exchange rate would be slower than in the monomer in the ratio of the dissociation constant. However, this is not at all what is observed. There is indeed an acid-induced mechanism, but the exchange rate is nearly the same as in the monomer, i.e. larger than expected by five or six orders of magnitude (2)! In the present work, we show that the acid-induced mechanism, which also affects terminal G-C pairs (4) and G-C pairs of DNA-drug complexes (5,6) is nothing but intrinsic catalysis operating on N7-protonated guanosine. Indeed, the pK of cytidine, the intrinsic proton acceptor, is six units higher than that of the H<sub>2</sub>O acceptor.

As usual, the guanosine monomer provides the benchmark for the analysis of exchange in base pairs. We supplement earlier work (3) by measurements at lower pH and by a study of the effect of formate and cytidine acting as exchange catalysts at acid pH. Next, we examine in detail the instance of paired G in the d(ATATAGATCTATAT) duplex, and, more briefly, other cases, including DNA-drug complexes. In the discussion, we show that the observations are indeed explained by intrinsic catalysis, and not by other mechanisms which we examine.

## MATERIALS AND METHODS

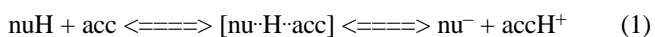
Methods of sample preparation and of NMR were those described previously (4,7).

## PROTON EXCHANGE THEORY (7,8)

### Exchange from mononucleosides

Proton transfer from a nucleoside, nuH, to an acceptor, acc, proceeds *via* the formation, by diffusion-controlled collision of an H-bonded complex [nu...H...acc]:

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The exchange rate is given by:

$$k_{\text{ex,acc}} = q[\text{acc}] (1 + 10^{\text{pK}_{\text{nu}} - \text{pK}_{\text{acc}}})^{-1} \quad (2)$$

where  $[\text{acc}]$  is the concentration of the acceptor, and  $q$  is the rate constant of diffusion-controlled collisions. The last factor in eq 2 is the proton transfer yield, related to the difference between the pKs of the acceptor and of the nucleoside donor.

When water itself is the proton acceptor, the notion of a collision is inappropriate in view of the permanent hydration of the donor, and the product ( $q[\text{acc}]$ ) in eq 2 should be replaced by a rate constant  $R(\text{H}_2\text{O})$ . Still, it is convenient to express this constant in the form ( $q[\text{acc}]$ ). One factor of this product may be chosen arbitrarily, and since the proton must finally be transferred to bulk water, we set  $[\text{acc}] = 55 \text{ M}$  by convention, and we designate  $q_{\text{H}_2\text{O}}$  a pseudo-collision rate. The acceptor pK is that of the  $\text{H}_3\text{O}^+/\text{H}_2\text{O}$  couple,  $-1.7$  (see below).

N7-protonation lowers the pK of the N1 (imino) nitrogen of guanosine, accelerating imino proton exchange (3). The pK [designated  $\text{pK}(\text{N7})$ ] and the enthalpy for N7-protonation of guanosine have been determined by  $^{15}\text{N}$  NMR titration (9). The residence time of the HN7 proton is equal to the diffusion-controlled collision time when pH is equal to  $\text{pK}(\text{N7})$ . This is  $\sim 10^{-11} \cdot 10^{\text{pK}(\text{N7})}$ , or  $\sim 1 \text{ ns}$  for a pK of 2.15 (Table 2), a time short compared to the characteristic NMR times. The observed imino proton exchange rate for isolated guanosine,  $k_i$ , is therefore the average of the rates corresponding to neutral G and N7-protonated G:

$$k_i = (1 - f^+) \Sigma k_{\text{ex,acc}} + f^+ \Sigma k_{\text{ex,acc}}^+ \quad (3)$$

where  $k_{\text{ex,acc}}$  and  $k_{\text{ex,acc}}^+$  are the exchange rates by proton transfer to the acceptor, from neutral G and from N7-protonated G respectively, and where  $f^+$  is the molar fraction of N7-protonated guanosine:

$$f^+ = (1 + 10^{\text{pH} - \text{pK}(\text{N7})})^{-1} \quad (4)$$

The acceptors include  $\text{H}_2\text{O}$ ,  $\text{OH}^-$  and any added acceptors.

For example, at high pH (e.g. pH 11), exchange in guanosine proceeds mainly by proton transfer from the neutral nucleoside to the base  $\text{OH}^-$ , a process designated  $\text{N}_{\text{OH}^-}$  (Table 1). Eqs 2 and 3 give:

$$k_i = q_{\text{OH}^-} [\text{OH}^-] = q_{\text{OH}^-} \cdot 10^{\text{pH} - \text{pK}_i} \quad (5)$$

where  $q_{\text{OH}^-}$  is the rate constant of the collision of  $\text{OH}^-$  with the monomer and  $K_i$  is the ionization product of water (Table 2). In contrast, exchange at low pH is mostly by proton transfer from the protonated nucleoside  $\text{HN7G}^+$  to water (the  $\text{P}_{\text{H}_2\text{O}}$  process). By eqs 2–4:

$$k_i = f^+ \cdot q^+_{\text{H}_2\text{O}} \cdot 55 \cdot (1 + 10^{\text{pK}(\text{N1;HN7G}^+) - (-1.7)})^{-1} \quad (6)$$

where  $q^+_{\text{H}_2\text{O}}$  is the pseudo-collision rate constant of water with the protonated nucleoside. Proton transfer from neutral G to water (the  $\text{N}_{\text{H}_2\text{O}}$  process) is significant around neutral pH. The rate is:

$$k_i = q_{\text{H}_2\text{O}} \cdot 55 \cdot (1 + 10^{\text{pK}(\text{N1;G}) - (-1.7)})^{-1} \quad (7)$$

Let us designate by pNB (for neutral and basic) the cross-over between a pH-independent exchange regime and one which is base-controlled. Similarly, pAN will designate the pH for crossover with an acid-controlled regime. If the relevant processes are those of eqs 5 and 7 for pNB, and of eqs 6 and 7 for pAN, one has:

$$\text{pNB} = \text{pK}_i - \text{pK}(\text{N1;G}) + \log(q_{\text{H}_2\text{O}}/q_{\text{OH}^-}) \quad (8)$$

$$\text{pAN} = \text{pK}(\text{N7}) - [\text{pK}(\text{N1;HN7G}^+) - \text{pK}(\text{N1;G})] + \log(q^+_{\text{H}_2\text{O}}/q_{\text{H}_2\text{O}}) \quad (9)$$

The value of pAN may be determined from experiment, and  $\text{pK}(\text{N7})$  and  $\text{pK}(\text{N1;G})$  are tabulated. Assuming that the pseudo-collision rate constant of water is the same for neutral and for N7-protonated guanosine, eq 9 provides a means for the determination of the imino proton pK of N7-protonated guanosine. The values in Table 2 for both G and T have been determined in this manner.

**Table 1.** Nomenclature of the principal imino proton exchange processes operating at different pHs on guanosine and in G-C pairs

	acid pH	neutral pH	basic pH
guanosine	$\text{HN7G}^+/\text{H}_2\text{O}^b$ $\text{P}_{\text{H}_2\text{O}}$	$\text{G}/\text{H}_2\text{O}$ $\text{N}_{\text{H}_2\text{O}}$	$\text{G}/\text{OH}^-$ $\text{N}_{\text{OH}^-}$
G · C pair	$\text{HN7G}^+/\text{cytidine}$ $\text{P}_{\text{C}}$	$\text{G}/\text{cytidine}$ $\text{N}_{\text{C}}$	$\text{G}/\text{OH}^-$ $\text{N}_{\text{OH}^-}$

<sup>a</sup>See Figure 4.

<sup>b</sup>Each process is described as DONOR/ACCEPTOR, and designated by a DONORACCEPTOR symbol, where P stands for N7-protonated guanosine, N for neutral guanosine and C for the cytidine acceptor.

### Imino proton exchange from the open state of a base pair

When the dissociation constant is much less than 1, the kinetics is first order, and the exchange time is:

$$\tau_{\text{ex}} = \tau_0 + (\tau_{\text{ex,acc,open}}/K_{\text{diss}}) \quad (10)$$

where  $\tau_0$  is the base-pair lifetime and  $\tau_{\text{ex,acc,open}}$  is the exchange time (the inverse of the exchange rate  $k_{\text{ex,acc,open}}$ ) from the open pair.

If transfer to a given acceptor proceeds from the open state as in the case of the monomer, the exchange rates in the open state are given by eqs 2 and 3. Any difference between the case of the monomer and that of the open state is summarized by a factor  $\alpha$ :

$$k_{\text{ex,acc,open}} = \alpha k_{\text{ex,acc}} \quad (11)$$

where  $k_{\text{ex,acc}}$  refers to the monomer (eq 2). Similar relations hold for the  $k^+$  rates which apply to protonated G. As previously discussed (10),  $\alpha$  is not strongly catalyst-sensitive, and not too far from unity.

An important contribution to proton exchange is intrinsic catalysis, which involves indirect transfer of the imino proton of guanosine (or thymidine) via a water bridge to the imino position of the cytidine (or adenosine) opposite, in the open pair. As in the case of direct proton transfer to water, intrinsic catalysis does not involve a diffusion-controlled collision, but the collision formalism may still be used. In view of the final transfer of the proton to bulk water, we again use the conventional value, 55 M, for the C (or A) acceptor concentration.

Due to the existence of both neutral and N7-protonated G, the exchange kinetics can be complicated, and it is not even first order in general. However, a first order description should suffice for our NMR measurements, due to one of the following properties: the fast rate of N7-deprotonation, the long characteristic times of the NMR measurements, the fact that in most conditions, one of the two forms of G (neutral or N7-protonated) is responsible for most of the exchange, and the fact that the exchange times in the conditions of measurement are much longer than the base-pair

**Table 2.** Thermodynamic and kinetic parameters of imino proton exchange in mononucleosides (−7; 0; 15; 25°C)<sup>a</sup>

	pK   enthalpy <sup>b</sup> (kJ/mol)		q <sup>c</sup> (10 <sup>9</sup> s <sup>−1</sup> M <sup>−1</sup> )			
			OH <sup>−</sup>	H <sub>2</sub> O	HCO <sub>2</sub> <sup>−</sup>	cytidine
G (N7)	2.32; 2.28; 2.20; 2.15	−8.3 <sup>d</sup>				
G (imino)	9.94; 9.77; 9.44; 9.24	−33.4 <sup>d</sup>	2; #; 7; #	104; #; 193; #	#; #; 0.9; #	#; #; 1.1; #
HN7G <sup>+</sup> (imino)	7.5 <sup>e</sup> ; 7.40; 7.2 <sup>e</sup> ; 7.03	−23		[104; #; 193; #] <sup>e</sup>	#; #; 2.94; #	#; #; 0.73; #
T (O2, O4)	#; #; 0 <sup>e</sup> ; #; #; #					
T (imino)	10.26; 10.1; 9.79; 9.6	−31.3 <sup>d</sup>	2; #; #; 8.5	124; #; #; 172		
NOT <sup>+</sup> (imino)	8.0 <sup>f</sup> ; #; #; #; 7.3 <sup>f</sup>			[124; #; #; 172] <sup>f</sup>		
C(N3)	4.63; 4.53; 4.34; 4.23	−18.8 <sup>d</sup>				
A(N1)	4.03; 3.95; 3.8; 3.7	−15.8 <sup>d</sup>				
OH <sup>−</sup>	16.95; 16.67; 16.05; 15.7	−61 <sup>g</sup>				
H <sub>2</sub> O	−1.7; −1.7; −1.7; −1.7 <sup>h</sup>					
HCO <sub>2</sub> <sup>−</sup>	#; #; #; 3.78 <sup>g</sup> ; #					

<sup>a</sup>Values are given for the four temperatures indicated, the symbol # being used as filler.

<sup>b</sup>Except for the protonated nucleosides, the pK values are either taken from the literature (for some temperatures) or derived from them for use in Table 3, using the enthalpy value which is also from the literature.

<sup>c</sup>The  $q$  values are deduced from the measured exchange time according to eq 2, using the stated pK values, except for the values in brackets, which are explained in Note *e*. In the case of the water acceptor, the use of  $q$  does not imply a diffusion-controlled collision (see text).

<sup>d</sup>Ts'o, P.O.P. (1974) *Basic Principles of Nucleic Acid Chemistry* vol.1, Acad. Press, New York and London, p. 462.

<sup>e</sup>Dawson, R.M.C., Elliott, D.C., Elliot, C., and Jones, K.M., *Data for Biochemical Research*, Oxford University Press, Ely House W1, 1969, p. 140.

<sup>f</sup>The imino proton pK values for the protonated nucleosides are derived from the measured exchange times at acid pH, using eq 2. For the derivation, the collision rate constant of water for the protonated nucleoside,  $q^+(H_2O)$ , is assumed equal to that for the neutral nucleoside,  $q(H_2O)$ . Using values at two temperatures, one obtains the enthalpy and the pK values quoted for other temperatures.

<sup>g</sup>Values derived from the ionization constant of water at 0 and 24°C, *CRC Handbook of Chemistry and Physics, 50th Edition*, Editor R.C. Weast, The Chemical Rubber Publishing Company, Cleveland, p. D-120.

<sup>h</sup>See 'Proton exchange theory'.

lifetimes. As a result, the exchange rate can be expressed as an average over neutral and N7-protonated species, using averaged values for the base pair lifetime, for the dissociation constant and for the transfer rate in the open state. Labelling the averages with the prime symbol, we have for the base-pair lifetime  $\tau'_0$ :

$$1/\tau'_0 = (1 - f^+)/\tau_0 + f^+/\tau^+_0 \quad (12)$$

Similarly, the open-state lifetime  $\tau'_{\text{open}}$  is given by:

$$1/\tau'_{\text{open}} = (1 - f^+)/\tau_{\text{open}} + f^+/\tau^+_{\text{open}} \quad (13)$$

and:

$$K'_{\text{diss}} = \tau'_{\text{open}}/\tau'_0 \quad (14)$$

Each acceptor, whether external or intrinsic, brings its contribution to the exchange rate from the open state, both for neutral and for N7-protonated G. In analogy with eq 3, the exchange rate  $k'_{\text{ex,open}}$  from the open states is:

$$k'_{\text{ex,open}} = (1 - f^+)(\Sigma k_{\text{ex,acc,open}} + k_{\text{ex,int}}) + f^+(\Sigma k^+_{\text{ex,acc,open}} + k^+_{\text{ex,int}}) \quad (15)$$

where we have singled out the intrinsic catalyst acting on neutral G and on N7-protonated G, and where each contribution is

computed according to eq 2. The pK and  $q$  values of relevant acceptors are given in Table 2.

Lastly, the imino proton exchange time  $\tau_{\text{ex}}$  is given by:

$$\tau_{\text{ex}} \equiv 1/k_{\text{ex}} = \tau'_0 + 1/(K'_{\text{diss}} \cdot k'_{\text{ex,open}}) \quad (16)$$

The solution pH influences  $\tau_{\text{ex}}$  through the concentration of OH<sup>−</sup> and the extent of N7-protonation of G (the factor  $f^+$ , which influences both  $\tau'_0$  and  $K'_{\text{diss}}$ ).

For example, at high pH (e.g. pH 11), exchange proceeds mainly by proton transfer from the neutral species to the base OH<sup>−</sup> (the N<sub>OH</sub><sup>−</sup> process), and the exchange time is much longer than the base-pair lifetime. By eqs 2, 5 and 11, one obtains:

$$1/\tau_{\text{ex}} = K_{\text{diss}} \cdot k_{\text{ex,OH}^-, \text{open}} \quad (17)$$

or:

$$1/\tau_{\text{ex}} = K_{\text{diss}} \cdot \alpha_{\text{OH}^-, \text{open}} \cdot 10^{\text{pH} - \text{pK}_i} \quad (18)$$

where  $\alpha_{\text{OH}^-, \text{open}}$  is the rate constant of the collision of OH<sup>−</sup> with the monomer.

As a second example, in the Abse<sub>n</sub>ce of Added Catalyst, the exchange time of the imino proton of a GC pair at neutral pH is controlled by intrinsic catalysis, i.e. proton transfer to cytidine. In



eq 16, we can neglect both the first term and the contribution of N7-protonated G. Hence:

$$\begin{aligned} 1/\tau_{AAC} &\approx K_{diss} \cdot k_{ex,int} \\ &\approx K_{diss} \cdot (q_{int}^{+} 55) \cdot 10^{pK(N3;C) - pK(N1;G)} \end{aligned} \quad (19)$$

where  $q_{int}$  is the pseudo-collision rate constant corresponding to the conventional 55 M concentration of the cytidine acceptor. As compared to direct transfer to water (eq 7), the pK-dependent term is enhanced a million times by the larger pK of the cytidine acceptor, 4.3 versus -1.7 for water (Table 2).

As a third example, consider the exchange from a GC pair by proton transfer to cytidine from N7-protonated G, whose proportion is given by eq 4. This is expected to be a major exchange process at acid pH. The exchange time  $\tau_{AAC}^{+}$  is a function of  $K_{diss}^{+}$ ,  $q^{+}$  and the imino proton pK of N7-protonated G. In eq 15, the contribution of neutral G is negligible. Instead of eq 19, we have:

$$\begin{aligned} 1/\tau_{AAC}^{+} &\approx f^{+}/\tau_{AAC}^{+} \approx f^{+} K_{diss}^{+} \cdot k_{ex,int}^{+} \\ &\approx f^{+} K_{diss}^{+} \cdot (q_{int}^{+} 55) \cdot 10^{pK(N3;C) - pK(N1;HN7G^{+})} \end{aligned} \quad (20)$$

The pH dependence of this acid-induced process is contained in the fraction  $f^{+}$  of N7-protonated G (eq 4).

Using eqs 18–20, one can determine the pH values pNB and pAN corresponding to the two cross-overs from the pH-independent process to the basic, and to the acidic processes. They are given by eqs 20 and 21, which may be compared with eqs 8 and 9:

$$\begin{aligned} pNB &= pK_i - pK(N1;G) + [pK(N3;C) + \log(55)] \\ &+ \log(q_{int}/(\alpha q_{OH^{-}})) \end{aligned} \quad (21)$$

$$\begin{aligned} pAN &= pK(N7) - [pK(N1;HN7G^{+}) - pK(N1;G)] \\ &+ \log[(q_{int}^{+} K_{diss}^{+})/(q_{int} K_{diss})] \end{aligned} \quad (22)$$

If the exchange rates are controlled by the processes of eqs 18–20, the exchange parameters can be determined from the measured rates. First, one obtains  $\alpha K_{diss}$  by substituting the rate at basic pH in eq 18. Next, substituting in eq 21 the experimental value of pNB (Fig. 4), one obtains the exchange rate ratio  $q_{int}/(\alpha q_{OH^{-}})$ . Lastly, substituting pAN in eq 22, one obtains the ratio  $(q_{int} K_{diss})/(q_{int}^{+} K_{diss}^{+})$  which provides for a comparison of base-pair opening properties of neutral and protonated G. Such procedures were used for computing Table 3. Alternatively, the same parameters can be derived by substitution of directly measured exchange rates in eqs 19 and 20.

### The pKs for protonation of OH<sup>-</sup> and of H<sub>2</sub>O

The transfer of a proton from H<sub>3</sub>O<sup>+</sup> to an acceptor A is written as:



The constant  $K_{ma}(A)$  of the mass action law is given by:

$$K_{ma}(A) = [AH^{+}][H_2O]/([A][H_3O^{+}]) \quad (24)$$

or:

$$[AH^{+}]/[A] = (K_{ma}(A)/[H_2O]) 10^{-pH} \quad (25)$$

whence:

$$pK = \log_{10}(K_{ma}(A)/[H_2O]) \quad (26)$$

When the acceptor is OH<sup>-</sup>, eq 24 becomes:

$$K_{ma}(OH^{-}) = [H_2O]^2/([OH^{-}][H_3O^{+}]) = [H_2O]^2/K_i \quad (27)$$

hence:

$$pK = \log_{10}(K_i/[H_2O]) \quad (28)$$

where  $K_i$  is the ionization constant of water, which is a function of temperature. For instance, at 25°C, the value of  $K_i$  is  $1 \cdot 10^{-14}$ , and the pK is therefore 15.7.

When the acceptor is H<sub>2</sub>O,  $K_{ma}$  is equal to 1 by eq 24. The pK of the H<sub>3</sub>O<sup>+</sup>/H<sub>2</sub>O couple is therefore simply  $\log_{10}(1/[H_2O])$ , independent of temperature (Table 2).

## RESULTS

### Imino proton exchange in mononucleosides

*Proton abstraction from deoxyguanosine versus pH.* Except as noted, we focus on the -7°C data for guanosine (Fig. 1, left panel). Three processes can be distinguished (Table 1): (i) At high pH, the exchange rate is proportional to OH<sup>-</sup> concentration. This is assigned to imino proton abstraction from neutral guanosine by OH<sup>-</sup>, the N<sub>OH</sub><sup>-</sup> process. Substituting the observed rate in eq 5, we compute the collision rate constant  $q_{OH^{-}}$ . The result, indicated in Table 2, is independent of the exact value of the imino proton pK, because the latter is much smaller than that of OH<sup>-</sup>: each collision with OH<sup>-</sup> results in abstraction of the imino proton. (ii) Acid-induced catalysis is assigned to imino proton abstraction by water from the fraction of guanosine which is protonated on N7 (3). From the observed acid-induced catalysis, and with the knowledge of the fraction of N7-protonated guanosine, eq 6 provides the value of the product  $q_{H_2O}^{+} 10^{-pK(N1;HN7G^{+})}$ , where  $q_{H_2O}^{+}$  is the pseudo-collision rate constant and the pK is that of the imino proton of N7-protonated G. (iii) The experimental exchange time around pH 5 is shorter than expected from the two processes just mentioned (Fig. 1). This is assigned to a third process, imino proton abstraction by water from neutral guanosine, the N<sub>H<sub>2</sub>O</sub> process. The exchange time corresponding to this process, 84 ms at -7°C, is indicated by a horizontal line in Figure 1. Introducing this value in eq 7, we obtain for the collision parameter the value given in Table 2,  $q_{H_2O} = 104 \cdot 10^9 s^{-1} M^{-1}$ .

Figure 1 also shows the cross-over pH values, pNB and pAN. According to eqs 8 and 9, pNB is determined by the relative values of  $q$  for water and OH<sup>-</sup>, and pAN by the pK for protonation at N7 which is known, and by the relative values of  $q_{H_2O}^{+} 10^{-pK(N1;G)}$  and  $q_{H_2O}^{+} 10^{-pK(N1;HN7G^{+})}$ . The fit gives 2.44 for the log of this ratio. If we assume that the collision factors are equal, this number is the pK difference. In this way we derive  $pK(N1;HN7G^{+}) = 9.94 - 2.44 = 7.50$  (Table 2).

*Proton abstraction from deoxyguanosine by formate and by cytidine (15°C).* Figure 2 shows the effect of formate and cytidine at acid pH. These acceptors were chosen for their pK (pK ≈ 4) which is intermediate between that of N7-protonation of G and that of N1(imino) protonation of N7-protonated guanosine (Fig. 2, Table 2). The unprocessed exchange times are shown in the top panels.

In the left bottom panel, the data are displayed as exchange times for a 1 M concentration of unprotonated formate. The convergence of all the data points on the same curve indicates that it is indeed HCO<sub>2</sub><sup>-</sup>, not HCO<sub>2</sub>H, which is the exchange catalyst. The fit is computed under the assumption that the pH dependence is due entirely to the N7-protonation of G, using the known pK (eq 4, Table 2). The high-pH plateau corresponds to exchange catalysis by formate acting on G (the N<sub>HCO<sub>2</sub>-</sub> process), the low-pH plateau corresponds to exchange catalysis on HN7G<sup>+</sup> (the P<sub>HCO<sub>2</sub>-</sub> process). The fit generates the collision rate factors for formate given in Table 2.

Table 3. Parameters of imino proton exchange in G-C pairs

base	strand	T °C	$\tau_{AAC}^a$ (s)	pAN <sup>b</sup>	pNB <sup>c</sup>	$\alpha K_{diss}^d$ (10 <sup>-6</sup> )	$\log \frac{q_{H_2O} q_{int}^{K^+} K_{diss}^e}{q^+ H_2O q_{int} K_{diss}}$	$\log (q_{int} / (aq_{H_2O}))^f$	
G6	monomer	-7		4.52	5.78				
G6		15		4.42	6.01				
I <sup>g</sup>	G6	ATATAGATCTATAT <sup>h</sup>	15	0.196	4.84	9.8	13	0.42	-2.28
II	G1 <sup>i</sup>		-7	0.023	5.1	7.8	15000	0.58	-4.35
III	G2	CGCGATCGCG <sup>j</sup>	25	0.25	5.5	8.1	270	1.12	-3.52
IV	G3		25	1	6.2	9.3	4	1.82	-2.32
V	G4		25	3	6.5	9.4	1.6	2.12	-2.22
VI	G5	AATTCGATT <sup>k</sup>	15	3	6.2			1.78	
VII	G2	AGCATGCT <sup>l</sup>	15	4.5	6.2	8.3	3	1.78	-3.78
VIII	G2		0	2000	4.8	9.2	0.02	0.32	-2.93
IX	G3	AGGATCCT <sup>m</sup>	0	2200	6.2	9.8	0.002	1.72	-2.33
X	G3*		0	300	6.0	8.6	0.3	1.52	-3.53
XI	G3	AAGCCCTT <sup>n</sup>	0	13000	5.0	8.6	0.15	0.52	-3.53
XII	G5	AAACGTTT <sup>o</sup>	15	700	5.0	9.8	0.002	0.58	-2.28

<sup>a</sup> 1/ $\tau_{AAC}$  is the pH-independent exchange rate due to intrinsic catalysis.  
<sup>b</sup> pAN is the pH for which the rates of exchange *via* the pH-independent pathway and *via* the acid-induced pathway are equal.  
<sup>c</sup> pNB is the pH for which the rates of exchange *via* the pH-independent pathway and *via* OH<sup>-</sup>-catalysis are equal.  
<sup>d</sup> The product  $\alpha K_{diss}$  is the ratio of the rate of OH<sup>-</sup>-catalyzed exchange (or ammonia-catalyzed exchange, in cases II and VI) to that for 2'-3'cGMP (measurements II-V) or for deoxyguanosine (others).  
<sup>e</sup> This is the difference pAN – pAN(monomer), eq 30. The pAN(monomer) values at 0 and 25°C are obtained from those listed by linear intra-and extrapolation vs. 1/T. The collision factors relative to H<sub>2</sub>O are those of the monomer.  
<sup>f</sup> This is the difference pNB – pNB(monomer), eq 29. The pNB(monomer) values at 0 and 25°C are obtained from those listed by linear intra-and extrapolation vs. 1/T. The collision factor relative to H<sub>2</sub>O is that of the monomer.  
<sup>g</sup> The roman numbers correspond to those in Figure 5.  
<sup>h</sup> This work.  
<sup>i</sup> G1<sub>1</sub> is the nucleoside paired with C1, etc.  
<sup>j</sup> Guéron,M., and Leroy,J.-L. (1992) In Eckstein,F., and Lilley,D.M.J. (eds) *Nucleic Acids and Molecular Biology*. Springer Verlag, Berlin, Vol. 6, pp. 1–22.  
<sup>k</sup> Kochoyan,M., thesis, unpublished. Thèse de doctorat de l'Université Paris VII, spécialité Biophysique, 1987; p. 80, p. 110.  
<sup>l</sup> J.-L. Leroy, unpublished data.  
<sup>m</sup> Non-symmetrical complex with chromomycin, which is bound to G3. G3\* designates G3 of the other strand. Gao,X., and Patel,D.J. (1990) *Biochemistry* 29, 10940–10956.  
<sup>n</sup> Complex with chromomycin. Leroy,J.-L., Gao,X., Guéron,M., and Patel,D.J. (1991) *Biochemistry* 30, 5653–5661.  
<sup>o</sup> Complex with echinomycin. Leroy,J.-L., Gao,X., Misra,V., Guéron,M., and Patel,J. (1992) *Biochemistry* 31, 1407–1415.

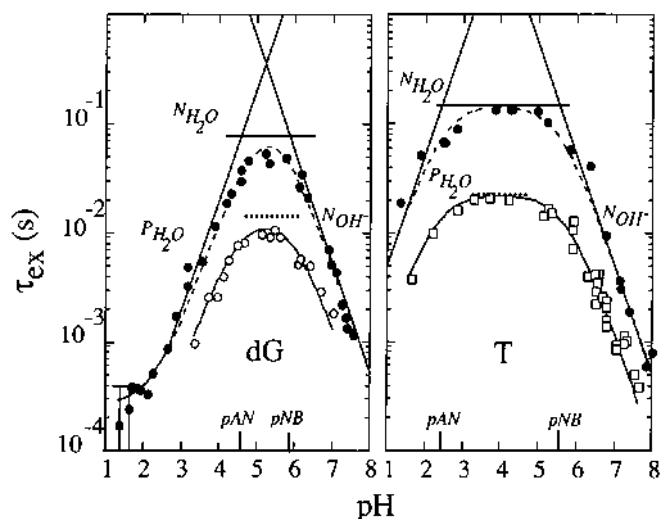
Exchange catalysis by cytidine, presented on the right of Figure 2, is treated similarly.

*Proton abstraction from thymidine (–7°C).* The imino proton exchange measurements for thymidine at –7°C (Fig. 1, right) show that the rate of catalysis by OH<sup>-</sup> is identical to that for deoxyguanosine at the same temperature. In the case of catalysis by H<sub>2</sub>O, the pseudo-collision factor is slightly larger (Table 2). The acid-induced catalysis is assigned to exchange from thymidine protonated on one of O2 or O4, whose pK is ≈ 0 (11). The

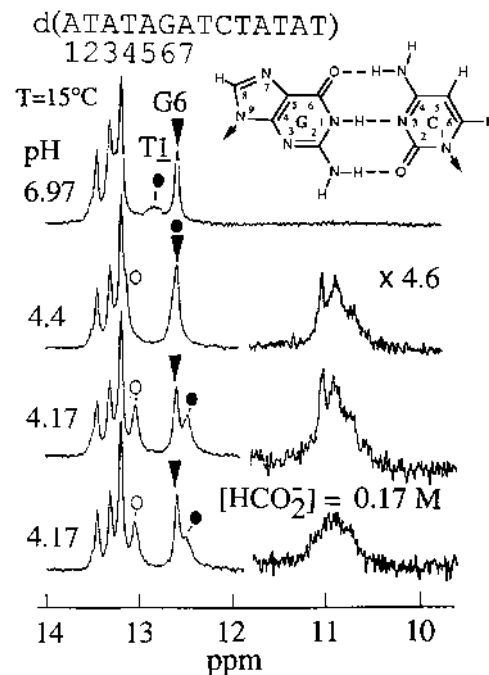
same analysis as for G indicates that the imino proton pK is shifted down by 2.3 units upon O-protonation.

Imino proton exchange in a G-C pair

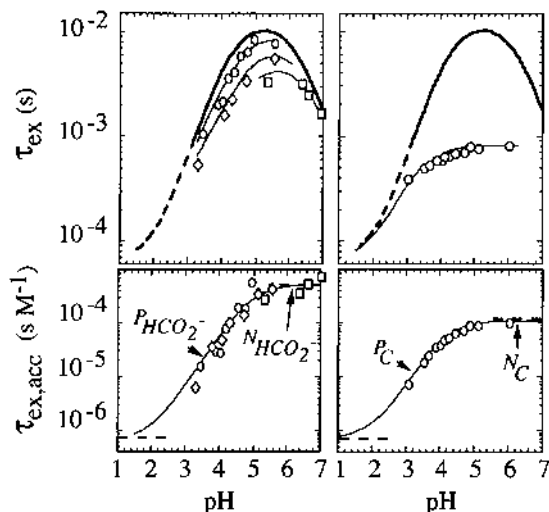
*The d(ATATAGATCTATAT) imino proton spectrum; the effect of formiate.* Figure 3 displays the spectra at different pH values. In the neutral pH spectrum, assigned to the Watson–Crick duplex, the 12.6 p.p.m. peak was assigned to the G6 imino proton by the NOESY cross-peaks with cytidine amino protons (mixing time 250 ms, 15°C, data not shown).



**Figure 1.** Imino proton exchange in deoxyguanosine (left) and thymidine (right) at  $-7^{\circ}\text{C}$  (●). The data are fitted by three processes: a base-catalysed process,  $\text{N}_{\text{OH}^-}$ , and a pH-independent process,  $\text{N}_{\text{H}_2\text{O}}$ , both from the neutral nucleoside donor N; and an acid-stimulated process  $\text{P}_{\text{H}_2\text{O}}$  which corresponds to exchange from the protonated nucleoside donor,  $\text{HN7G}^+$  or  $\text{OT}^+$  respectively. The solid lines associated with each process are computed according to eqs 5–7. The rates of the basic and pH-independent processes are nearly the same for G and T, as expected considering the closeness of the imino proton pKs. The acid-stimulated process is faster for G, because the N7 pK is higher than that of the T oxygen. Hence the similar pNB and dissimilar pAN values. From the data one derives the imino proton pK of the protonated nucleoside, as explained in 'Results'. The open symbols correspond to the exchange times at  $15^{\circ}\text{C}$  for deoxyguanosine and at  $25^{\circ}\text{C}$  for thymidine.



**Figure 3.** Imino proton spectra of d(ATATAGATCTATAT). The neutral pH spectrum (top) is assigned to the Watson-Crick duplex. The weak spectrum around 11 p.p.m. which appears at lower pH is probably due to imino protons of unpaired or Hoogsteen-paired nucleosides from partially dissociated duplexes or from single strands. This spectrum (and also the peak of the terminal T1 of the duplex) is exchange-broadened upon addition of a proton acceptor (formate, 0.17 M), whereas relaxation of the G6 imino proton in the duplex, at 12.6 p.p.m., is not affected.

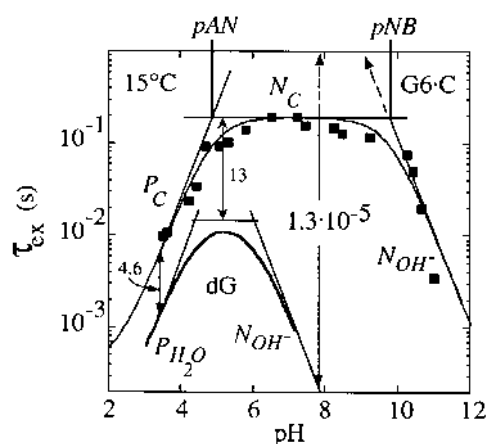


**Figure 2.** Catalysis of dG imino proton exchange by formiate (left) and cytidine (CN3; right) at  $15^{\circ}\text{C}$ . (Upper panels) Exchange times. Left:  $[\text{HCO}_2^-] + [\text{HCO}_2\text{H}] = 10\text{ mM}$  (○),  $30\text{ mM}$  (◆),  $60\text{ mM}$  (□); right:  $[\text{CN3}] + [\text{CN3H}^+] = 123\text{ mM}$  (○). For reference, the thickly traced curve gives the exchange time in the absence of added catalyst at  $15^{\circ}\text{C}$ . (Lower panels) The same data are used to compute the exchange time for an acceptor concentration of 1 M. The acceleration of exchange at low pH is due to exchange from N7-protonated guanine (eq 4). The fits according to eqs 2–4 (solid curves) provide the  $q$  values for formiate and cytidine listed in Table 2.

At lower pH, two imino proton peaks shift to high field. They are assigned to bases T1 and T2 of the terminal pairs, whose dissociation constant increases due to protonation at the imino position (N1) of adenine (4).

At pH 5 and below, imino proton peaks appear at  $\sim 11$  p.p.m. The integrated intensity is 15% of that of the main spectrum at pH 4.5 and 50% at pH 3.5. They may arise from a frayed duplex or from a single strand. Hoogsteen pairs, in which the N3 imino proton of protonated C is hydrogen-bonded to N7 of G, and in which the G imino proton is exposed to the solvent, could also contribute. We frequently observe such peaks in low-pH spectra of self-complementary oligo-deoxynucleotides. They have also been reported in 200 base-pair fragments of calf thymus DNA (12).

The bottom spectrum shows the effect of a proton acceptor (formiate, 0.17 M) on proton exchange at pH 4.17, a pH where acid-induced exchange is manifest (Fig. 4). There is no discernible change in the line-broadening, nor in the longitudinal relaxation (not shown), of the G6 imino proton in the duplex (at 12.6 p.p.m.). This shows that catalysis of exchange from the pair is  $\geq 400$  times slower than for exchange from the monomer (Fig. 2), as expected if the dissociation constant of the pair is small. In contrast, the effect of formiate on the line width is obvious in the case of the weak spectrum, which corresponds to protons presumably exposed to the solvent, and for the peak, in the duplex spectrum, of the imino proton of the terminal pair A'T1, whose dissociation constant, measured at neutral pH, is large. This point will be discussed below.



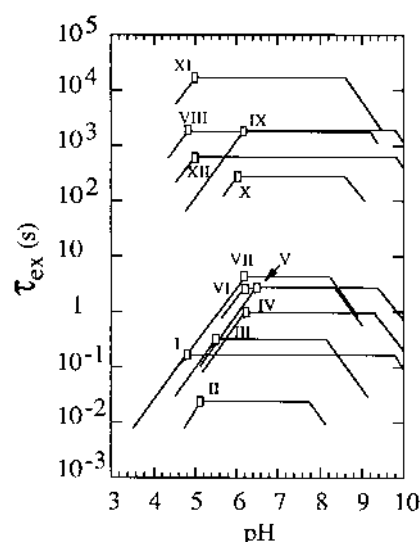
**Figure 4.** Exchange time of the G6 imino proton of the d(ATATAGATCTATAT) duplex (black squares, upper curve) versus pH at 15°C. The exchange times are analyzed in terms of three exchange processes:  $P_C$ ,  $N_C$  and  $N_{OH^-}$  (see text). The rate of hydroxyl catalysis (the  $N_{OH^-}$  process, eq 17) is  $1.3 \cdot 10^{-5}$  times that of the monomer (lower curve, same as in Fig. 1). This determines the apparent dissociation constant  $\alpha K_{diss}$ . The pH-independent process is assigned to concerted transfer *via* a water molecule to cytidine N3 which acts as an intrinsic catalyst ( $N_C$ , eq 19). Acceleration of exchange due to the larger pK of the intrinsic acceptor CN3, combined with slowing down due to the small dissociation constant of the base pair, result in an exchange rate which is only 13 times less than for proton transfer from the monomer to the water acceptor. In the acid-induced exchange process, the acceptor group is again CN3, but transfer takes place from N7-protonated G (the  $P_C$  process, eq 20). The fits (in full line) provide the parameters pAN, pNB, and those derived from them, which are listed in Table 3.

*Proton exchange from the G-C pair of [d(ATATAGATCTATAT)]<sub>2</sub>.* The exchange time of the G6 imino proton versus pH is plotted in Figure 4. At high pH, the straight line with a slope of  $-1$  is characteristic of catalysis by  $OH^-$ . It is displaced vertically by a factor of  $1.3 \cdot 10^{-5}$  from the corresponding line for the monomer: this number is equal to  $\alpha K_{diss}$  according to eq 5 and 18. Around neutral pH, the pH-independent exchange process is only 13 times slower than the  $N_{H_2O}$  process acting in the monomer. Below pH 5, the plus one slope corresponds to an acid-induced process, which, similarly, is only 4.6 times slower than the acid-induced process  $P_{H_2O}$  in the monomer.

The unexpectedly fast exchange at neutral and acid pH is readily explained by intrinsic catalysis which is more efficient than direct transfer to water, due to the larger pK of the intrinsic acceptor, cytidine N3, as discussed in the comparison of eqs 7 and 19. The fit in terms of transfer from G (the  $N_C$  process, eq 19) and from  $HN7G^+$  (the  $P_C$  process, eq 20) is indicated by full lines. It provides the parameters pAN, pNB, and those derived from them, which are listed in Table 3.

*Acid-induced exchange of the G imino proton in other conditions.* We have collected data on acid-induced imino proton exchange in Watson-Crick G-C pairs, including pairs from DNA duplexes (pairs I–VII) and from DNA complexes with chromomycin, a minor groove drug, and with echinomycin, a bis-intercalator (pairs VIII–XII). The parameters relevant for exchange are presented in Table 3, and the exchange times are plotted schematically in Figure 5.

The prominent difference between pairs is the exchange time at neutral pH,  $\tau_{AAC}$ . It varies from  $\sim 20$  ms for a terminal pair to



**Figure 5.** Schematic plot of the rates of the three exchange pathways in various G-C pairs. The straight lines cross at pH values equal to pAN ( $\square$ ) and pNB. Note the small scattering of the pAN values, as compared to the large range, six orders of magnitude, of variation of the neutral pH exchange times. This supports the model where acid-induced exchange occurs from the open state, with a base-pair dissociation constant which is not very different from that at neutral or basic pH. Base pairs are designated by the same roman number as in Table 3; I–VII, free duplexes; VIII–XII, drug-DNA complexes.

$\sim 1$  s for internal pairs of duplexes and to hundreds of seconds and more in the case of the complexes. According to eq 19,  $\tau_{AAC}$  is a function of the inverse of the dissociation constant, which is thus seen to be quite large for the terminal pair and very small for pairs stabilized by complexation. A better estimate of the dissociation constant is obtained from the exchange rate at basic pH (eq 18) or in ammonia, and the conclusion is the same (Table 3).

Despite these large differences, the exchange processes are qualitatively similar. In all cases, imino proton exchange is accelerated at acid and basic pH. The pH-dependence is comparable to that displayed in Figure 4 for the G6 imino proton of the d(ATATAGATCTATAT) duplex. In particular, the dispersion of the pH values for crossover, pAN and pNB, is rather small (spanning  $<2$  pH units in each case) and uncorrelated with the dissociation constant (Table 3, Fig. 5). This will be discussed below.

## DISCUSSION

Proton exchange from G-C pairs at acid pH is nearly as fast as from isolated guanosine. This seems at first in conflict with the well-established result that exchange catalysis by transfer to a *given* proton acceptor occurs in the case of a base pair as it does from the mononucleoside, except for a slowing down by a factor equal to the dissociation constant: this is the case for instance when exchange is catalyzed by  $OH^-$ , as happens at basic pH, or by ammonia etc. When the exchange rate is found to be proportional to  $H^+$  concentration, one could naively expect the same behaviour. But even though  $H^+$  acts in the same way on monomer and open pair (it produces the N7-protonated G from which exchange occurs predominantly), nevertheless the proton acceptor is not the same: for the monomer it is  $H_2O$ , whereas for the open-state nucleoside, it is the complementary cytidine. This intrinsic catalyst is more efficient than  $H_2O$  by a factor whose



logarithm is the difference in pK of the two acceptors, about six units.

The intrinsic catalyst also operates at neutral pH, where its activity was first detected (1), and where proton exchange (now from the non-protonated G) is again nearly as fast as from the monomer (Fig. 4).

In the present work, we provide evidence for this interpretation of acid-induced catalysis, based on exchange measurements in the monomers and in numerous base pairs. The thesis is that acid-induced exchange of the G imino proton in base pairs is due to intrinsic catalysis of exchange from protonated guanosine, occurring in the open state, like exchange from neutral guanosine at neutral or basic pH, and that furthermore, the properties of the base pair and of the open state are not drastically changed by the N7-protonation of G.

The observations support this thesis, and furthermore they exclude tentative interpretations of acid-induced exchange which do not rely on intrinsic catalysis: exchange from the open state of a pair with a very large dissociation constant, and exchange from a modified structure carrying Hoogsteen-paired G or unpaired G, in which the G imino proton is exposed.

### Acid-induced imino proton exchange in the nucleoside monomers

It has been proposed previously that acid-induced imino proton exchange is due to abstraction by water of the imino proton from N7-protonated guanosine (3), in contrast to abstraction from neutral guanosine which is the process responsible for pH-independent exchange. Since the proton acceptor, H<sub>2</sub>O is the same in acid-induced and in pH-independent exchange, one may assume that the pseudo-collision factor is the same in the two processes. From their relative efficiencies (e.g. the value of pAN), one determines by eq 9 that the pK of the imino proton changes by ~2.3 units upon N7 protonation, leading to the pK values for HN7G<sup>+</sup> listed in Table 2.

If the source of the pH dependence is N7 protonation, the log of the exchange time should be a linear function of pH with slope 1 when the pH is larger than the pK of N7 (~2.3), and should level off at lower pH. A fit of the data to this interpretation is shown by the full line in Figure 1. There is a good fit down to pH 2, and the expected levelling off may be discernible. However the exchange times <pH 2 are too short, due perhaps to experimental error (caused by the large linewidth) or to the influence of other protonated species of G (for instance G protonated on the amino group) on proton exchange.

The marginal acid-induced imino proton exchange in thymidine (Fig. 1) is similarly ascribed to protonation at O2 or O4, which occurs with a pK around 0 (11), as pointed out in the 'Results' section.

The notion that acid-induced exchange corresponds to a change in the properties of the proton donor may be tested with other proton acceptors than water, such as formate and cytidine. The latter is of particular interest since it is the intrinsic acceptor in GC pairs. The effect of these acceptors varies with pH as does the effect of water (Fig. 2), and the simplest explanation is that, like water, they abstract the imino proton of G or of N7-protonated G, depending on pH. The corresponding fits to eqs 2–4 are shown in Figure 2. Their quality supports the model, and notably the features of exchange catalyzed by the acceptor and not by the conjugate acid.

In fitting the data, one must use for consistency the imino proton pK value of HN7G<sup>+</sup> determined in the study of the H<sub>2</sub>O acceptor. The only adjustable parameters are the collision factors of the acceptor with G and with HN7G<sup>+</sup>. The fitting values are given in Table 2. Those for neutral G may be compared to the values compiled for other acceptors (10). The values for HN7G<sup>+</sup> are similar if not identical to those for G (Table 2). The enhancement of the collision factor of formate with protonated G may be due to the negative charge of formate. The reduction in the case of cytidine may be due to lowering of the effective acceptor concentration by trapping of cytidine in C<sup>+</sup>C<sup>+</sup> pairs.

Discounting these considerations, one could determine the imino proton pK in HN7G<sup>+</sup> by the exchange catalysis by formate or cytidine, instead of H<sub>2</sub>O. Assuming in each case the same collision factor for G and for HN7G<sup>+</sup>, the pK values would differ by no more than 0.5 from those derived from catalysis by H<sub>2</sub>O (Table 2). This gives a measure of the uncertainties of the model.

Overall, the experimental results are in agreement with the proposition of McConnell and Politowski. Furthermore they provide arguments against alternative explanations of the acid-induced catalysis which we now consider, and which would assign the pH-dependence of exchange catalysis by formate or cytidine to the conjugate acid, whose concentration is pH-dependent. For instance, N3-protonated cytidine could bind to N7 of guanosine, forming a Hoogsteen pair, in which the G imino proton would remain accessible to attack by H<sub>2</sub>O. This process can be excluded, because its efficiency should level off when the pH is less than the pK of cytidine, ~4.5, contrary to observation (Fig. 2). Furthermore, in the Hoogsteen pair, N7 would be protonated less than one percent of the time (considering the difference between the pK of cytidine and that of N7, Table 2), so that the exchange contribution of cytidine would be <1% of the maximum effect of water. By reference to Figure 1, this corresponds to an exchange time of 5 ms at 15°C, much longer than the exchange time at pH 3 in 123 mM of cytidine, which is 65 μs, as deduced from the right bottom panel in Figure 2.

Another possibility is to combine binding of the conjugate acid to N7 with attack of the imino proton by the acceptor instead of by H<sub>2</sub>O. This leads to a rate which varies as the product of the concentrations of the acceptor and of its conjugate acid, whereas the good fit of the data points to the model proposed above (bottom panels of Fig. 2) shows that the exchange time is independent of the conjugate acid concentration.

### Acid-induced imino proton exchange in G-C pairs

*Interpretation of exchange as from a unique open state.* We now show that base-catalyzed, pH-independent, and acid-induced imino proton exchange from base pairs can all be explained as occurring from the same open state of the pair, with only modest differences between the neutral G-C pair and the HN7G<sup>+</sup>-C pair.

The simplest characterization of the three exchange regimes is by the cross-over parameters pNB and pAN, whose experimental values are given in Table 3. Assuming that the proton acceptors are OH<sup>-</sup> and H<sub>2</sub>O for the monomer, and OH<sup>-</sup> and cytidine for base pairs, we have by the combination of eqs 8 and 21, and of eqs 9 and 22:

$$\text{pNB(pair)} = \text{pNB(guanosine)} + [\text{pK(N3;C)} + \log(55)] + \log(q_{\text{int}}/(\alpha q_{\text{H2O}})) \quad (29)$$

where  $\alpha$  refers to OH<sup>-</sup> catalysis (eq 18); and:

$$pAN = pAN(\text{guanosine}) - \log(q^+_{H_2O}/q_{H_2O}) + \log(q^+_{int}/q_{int}) + \log(K^+_{diss}/K_{diss}) \quad (30)$$

where the  $q$  factors in the first log term are those of the monomer.

The value of  $pNB$  is a measure of the relative efficiencies of attack by  $OH^-$  and of concerted proton transfer, both from the open state of the pair. Concerted transfer could be more sensitive to the structure of the open state, and a change in its rate would shift  $pNB$ . As an example, intrinsic catalysis is much slowed down in the case of terminal pairs, presumably because of wider opening (4), and this shifts  $pNB$  down. Indeed, the smallest  $pNB$  value in Table 3 is for a terminal pair (case II,  $pNB = 7.8$ ).

With respect to the monomer,  $pNB$  is shifted by about +6 units (the terms in brackets) due to the  $pK$  difference between the proton acceptors cytidine and water. The log term, which involves the ratio of the pseudo-collision factors for transfer to cytidine in the open state and for transfer to water in the monomer, also contributes to the shift. We have no independent estimate for this ratio. The experimental values of  $pNB$ , ranging from 7.8 to 9.8, correspond to values of the log term between -4.35 and -2.22 (Table 3, last column).

If the exchange parameters of the  $HN7G^+C$  and  $G-C$  pairs were the same, all the log terms in eq 30 would be zero, and  $pAN$  would be the same as for the monomer,  $\sim 4.4$ . (In the representation of Figure 4, this would correspond to equal vertical distances between the horizontals and between the lines of slope 1 on the acid side). In fact, the average  $pAN$  in Table 3 is  $\sim 1.2$  units above that of the monomer, indicating some change in properties of the base pair (open or closed) upon  $G$  protonation. The increase in  $pAN$  could be due to an equal increase of the logarithm of the dissociation constant when  $G$  is protonated, which could be rationalized if base-pair opening brings the  $N7$  group near to a phosphate group, a conformation that could be stabilized in the case of  $HN7G^+$  by the proton charge and/or by H-bonding, direct or through a water molecule. Opening towards the major groove of B-DNA does indeed bring  $GN7$  closer to the 3'-oxygen of the 5'-neighbor of the same strand.

More important than the origin of the  $pAN$  shift is its relatively small value and variation,  $<2$  units across a collection of base pairs whose dissociation constant spans seven orders of magnitude (Table 3). The range of  $pAN$ , a function of the properties of two different base pairs,  $HN7G^+C$  and  $G-C$ , is in fact smaller than that of  $pNB$ , a parameter which compares processes involving the single pair  $G-C$ . This small range suggests that exchange takes place from an open state of the pair with similar properties across the entire pH range. If instead the acid-induced process occurred in the closed state, or in an open state unrelated to the open state responsible for the pH-independent process,  $pAN$  would be expected to be strongly affected by the latter's dissociation constant, and this is contrary to observation.

*Acid-induced proton exchange without intrinsic catalysis.* In this section, we examine tentative explanations of efficient acid-induced catalysis which are not based on intrinsic catalysis.

Since the acid-induced exchange rate is comparable to that of the monomer, one explanation would be that the base-pair dissociation constant is very large when  $G$  is protonated, for instance 1/4.6 in the case of the pair of Figure 4.

There are three arguments against this mechanism. One is provided by the case of terminal pairs, such as case II (Table 3, Fig. 5) in which acid-induced exchange is even faster than that of the monomer: this requires a more efficient catalyst than  $H_2O$ , as

in a similar observation which provided one of the original arguments for intrinsic catalysis at neutral pH (1). A second argument is that changing to a large dissociation constant should change the chemical shift, as observed for terminal base pairs upon heating or upon going to basic pH (4). In the duplex spectra in Figure 3, there is a large shift of the T1 imino proton peak at acid pH, and of T2 as well, which we ascribe to the increase in the rather large dissociation constant, upon protonation of the complementary adenosine. But the G6 peak does not move. The third argument, based on the studies of the external catalysts formate and cytidine, is the strongest. It is simply that, as stated in the 'Results' section, these catalysts are  $\geq 400$  times less efficient in the duplex than in the monomer. This is incompatible with a dissociation constant of 0.1 or larger for the  $HN7G^+C$  pair. But it is exactly what is expected if the dissociation constant is  $<1/400$ , as it is indeed at neutral or basic pH.

Another mechanism of acid-induced exchange is suggested by the imino proton spectrum which develops upfield in DNA at acid pH (e.g. in Fig. 3), a spectrum compatible with the presence of  $G-C^+$  Hoogsteen pairs. In such a pair, the  $G$  imino proton exchanges quickly because it is exposed to the  $H_2O$  acceptor. If the structure which harbors the Hoogsteen pair is in sufficiently fast exchange with the duplex, this will contribute to proton exchange as measured on the duplex spectrum. It was tentatively proposed in a previous publication (10) that this could explain acid-induced exchange.

However, this process is not efficient enough. As explained in the discussion of proton exchange from the monomer,  $GN7$  would be protonated  $<1\%$  of the time in the Hoogsteen pair. At pH 4.17, with a relative population of at most 20% (Fig. 3), the Hoogsteen-bearing structure will provide an exchange rate which is 2/1000 times that of the fully protonated monomer, which is  $\sim 10^4 s^{-1}$ , according to Figure 1. This leads to a rate of  $20 s^{-1}$ , or an exchange time of 50 ms, comparable to 30 ms observed (Fig. 4). But this rate would be achieved only if exchange between the two forms corresponding to the two spectra is fast enough. Again, the formate experiment proves decisive: broadening of the upfield spectrum by formate (lowest spectrum in Fig. 3) is not passed on to the low-field (duplex) spectrum, which is unchanged even in its longitudinal relaxation.

Exchange transferred from the imino proton of unpaired  $HN7G^+$  is excluded by similar arguments.

*Application to NOE measurements.* Both the theory and the observations show that efficient acid-induced exchange of the imino proton with water is a general feature of  $G-C$  pairs. Since such exchange may affect NOE transfer, one should choose for NOE measurements a pH at which the rate is small. Table 3 and Figures 4 and 5 show that in the case of base-paired imino protons, pH 7 is optimum in all cases, and is in some cases better than pH 5.2, the pH for slowest imino proton exchange from the monomer.

*The case of A-T pairs.* In A-T pairs, exchange of the imino proton proceeds as for  $G-C$  pairs at basic and neutral pH, but acid-induced exchange has not been characterized (2). This is understandable, since the pH below which it would be visible should be two units less than for  $G-C$  pairs (compare the  $pKs$  in Table 2, and the  $pAN$  values in Fig. 1). In such conditions, the interpretation of accelerated exchange is dubious because of extensive duplex dissociation.

## CONCLUSION

We have shown that the general and surprisingly efficient acid-induced exchange of the guanosine imino proton in Watson–Crick pairs is caused by intrinsic catalysis in the open state of the  $\text{HN7G}^+\text{C}$  pair, a pair whose dissociation constant is moderately enhanced by the protonation of G. Together with the case of Watson–Crick A·T and G·C pairs at neutral pH in the absence of external catalysts, and the case of  $\text{C}\cdot\text{C}^+$  pairs (13), this is a third situation of general occurrence where intrinsic catalysis provides the fastest exchange mechanism, by far.

## REFERENCES

- Guéron, M., Kochoyan, M. and Leroy, J.-L. (1987) *Nature* **328**, 89–92.
- Guéron, M., and Leroy, J.-L. (1992) In Eckstein, F. and Lilley, D.M.J. (eds) *Nucleic Acids and Molecular Biology*. Springer Verlag, Berlin, Vol. 6, pp. 1–22.
- McConnell, B. and Politowski, D. (1984) *Biophysical Chemistry* **20**, 135–148.
- Nonin, S., Leroy J.-L., and Guéron, M. (1995) *Biochemistry* **34**, 10652–10659.
- Leroy, J.-L., Gao, X., Guéron, M., and Patel, D. J. (1991) *Biochemistry* **30**, 5653–5661.
- Leroy, J.-L., Gao, X., Misra, V., Guéron, M., and Patel, D. J. (1992) *Biochemistry* **31**, 1407–1415.
- Guéron, M., and Leroy, J.-L. In James, T. D. (ed.) *Methods in Enzymology*, Vol. 261. Academic Press, San Diego. In the press.
- Eigen, M. (1964). *Angew. Chem. Int. ed. Engl.* **3**, 1–19.
- Buchner, P., Maurer, W., and Ruterjans, H. (1978) *J. Magn. Reson.* **29**, 45–63.
- Guéron, M., Charretier, E., Hagerhorst, J., Kochoyan, M., Leroy, J.-L., and Morailon, A. (1989) In Sarma, R.H., and Sarma, M.H. (eds.) *Structure and Methods, Proceedings of the Sixth Conversation in Biomolecular Stereodynamics*. Adenine Press, NY, Vol. 2, pp. 113–137.
- Dawson, R. M. C., Elliott, D. C., Elliot, C., Jones, K. M. (1969) *Data for Biochemical Research*. Oxford University Press, Ely House, W1, p. 140.
- Banville, D. L., Marzilli, L. G., and Wilson, W. D. (1986) *Biochemistry* **25**, 7393–7401.
- Leroy J.-L., Gehring, K., Kettani, A., and Guéron, M. (1993) *Biochemistry* **32**, 6019–6031.