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The Significance of the Aprotic Solvent in Monomer Studies Related to the Primary Subunit Environment in the Macromolecule

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An advantage of aprotic polar solvent systems in the study of monomer interactions relevant to the macromolecular state is demonstrated with the measurement of nucleoside amino proton exchange rates in DMSO/water mixtures. The DMSO/water solvent provides the first unequivocal observation of general acid catalysis of nucleic acid amino proton exchange, which is undetectable in aqueous solution due to the formation of the endocyclic protonated nucleobase. Suppression of nucleobase protonation in the presence of buffer acid is a consequence of anion desolvation in the aprotic solvent. The detected route of general acid catalysis is demonstrated as a consequence of Watson-Crick H-bonding, leading to the implication that amino chemistry is modulated in the helical state to decrease amino proton lifetime in the "closed" macromolecular context of conformational information obtained by hydrogen exchange methods. This useful property of the aprotic solvent can be extended to monomeric studies pertaining to specific local site interactions affecting the function and conformation of proteins and nucleic acids.

KEY WORDS: proton exchange; nucleoside; aprotic solvent; ^{1}H NMR; catalysis; acid-base.

1. INTRODUCTION

In principle, studies of monomeric primary subunits of proteins and nucleic acids (amino acids and nucleosides) should provide sufficient information to account largely for the establishment of conformation and biological function at the macromolecular level. These studies at the monomeric level involve the recognition that the local environment of the primary subunit within the macromolecule is "nonaqueous" or only intermittently aqueous in terms of interactions that establish native conformation. Interesting early examples of these nonaqueous monomeric considerations applied to protein structure are found in the studies of Brandts and Hunt (1967), based on earlier notions presented by Kautzmann (1959) and Tanford (1962), in which the forces promoting protein globular structure can

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be accounted for by summing the individual free energies associated with transferring amino acid side chains from a nonpolar solvent to water. Base stacking interactions that represent the major structural energy of nucleic acid double helices (Gotoh and Takashira, 1981) can be seen at the monomeric level (Tso, 1974) and are based on the concept of "water exclusion," related to the low water solubility of the nucleoside purine or pyrimidine in water (Gordon and Jencks, 1963). The classic observations of Katz and Penman (1966) introduced another nonaqueous property represented by the polar aprotic solvent, which could represent a condition of the interior of a macromolecule where water is excluded, but hydrogen bonding is permitted. With the use of the aprotic solvent (DMSO), solvent competition for monomer-monomer interactions was reduced, allowing for the study of intrinsic specificity in G-C base pair formation (Newmark and Cantor, 1968; Petersen and Led, 1981).

In this study an additional, thus far unappreciated advantage of the polar aprotic solvent is demonstrated in revealing monomeric chemical properties that are relevant to the macromolecule, but hidden in aqueous solution. The use of DMSO/water solvent provides a means to alter proton equilibria that establish species concentrations quite different from those in aqueous solution. An important consequence of this is exemplified here by preliminary data[2] on amino proton exchange of cytidine compounds; a kinetic "window" is provided to detect the presence of a catalytic mechanism that is otherwise obscured by competing aqueous mechanisms. This catalytic route, general acid catalysis, would be the same process of modulating the properties of the amino group within the paired nucleobase in the double helical macromolecule. The relevance of this monomeric system to the study of protein and nucleic acid conformation will be discussed.

2. MATERIALS AND METHODS

Cytidine and 5-methylcytidine (Sigma) were dried overnight as weighed samples in the vacuum desiccator to remove water and dissolved in freshly opened deuterated (D6) DMSO (Sigma, Gold Label) containing 0.01 M TSP for NMR spectral reference. Aqueous buffers taken to desired acid/base ratios by *p*H adjustment were added to these nucleoside/DMSO mixtures for NMR spectral analysis and amino proton resonance saturation-recovery experiments. These were performed in 5-mm tubes with a Nicloet 300-MHz NMR spectrometer and a 1280 computer for data transformation and reduction. All experiments were performed at 23°C and repeated with different decoupler power settings providing the same rate of recovery after presaturation of the amino ^{1}H resonances (McConnell, 1982). Of the two discrete amino proton resonances observed at this field frequency, the resonances selected for irradiation were the downfield resonance of cytidine and the upfield resonance of 5-methylcytidine, since these represent the non-Watson-Crick protons in very slow rotational exchange with the Watson-Crick proton. Recovery rates were obtained from a single-exponential (nonlinear) least squares fitting function.

[2] The full data and its development are to be submitted for publication elsewhere (B. McConnell and A. Tan, in preparation).

3. RESULTS

First, we define general acid-base catalysis of amino proton exchange in nucleobases with reference to its *p*H dependence, as shown in Fig. 1. General acid-base catalysis for phosphate buffer ($pK = 6.9$) is represented by curve A, showing the sigmoid profile for phosphate as proton donor to the *neutral, unprotonated form* of the nucleobase (general acid catalysis, rate constant $k_A = 85\ M^{-1}\ sec^{-1}$), combined with phosphate as proton acceptor from the *neutral* nucleobase (general base catalysis, $k_B = 5\ M^{-1}\ sec^{-1}$). Curve C represents the expected sigmoid profile for general acid-base catalysis with the values of k_A and k_B reversed. For curves A and C,

$$k\ (sec^{-1}) = B_t[k_A(P) + k_B(1-P)] \tag{1}$$

where B_t is the concentration of total buffer and P is the mole fraction of buffer conjugate acid. As shown in Fig. 1, this relationship is not seen for aqueous cytidine; instead of sigmoid curves, phosphate catalysis goes through a maximum, dropping to very low values above $p\mathrm{H} = pK$ (phosphate) and below $p\mathrm{H} = pK$ (N-3 of cytidine). Typically, for any buffer the entire set of experimental points is well fitted by the relation

$$k\ (sec^{-1}) = P_N k_B[\mathrm{B}] \tag{2}$$

where P_N is the mole fraction of (N-3) protonated cytidine and k_B the rate constant of the buffer conjugate base B (McConnell, 1978). This relation [Eq. (2), curve B]

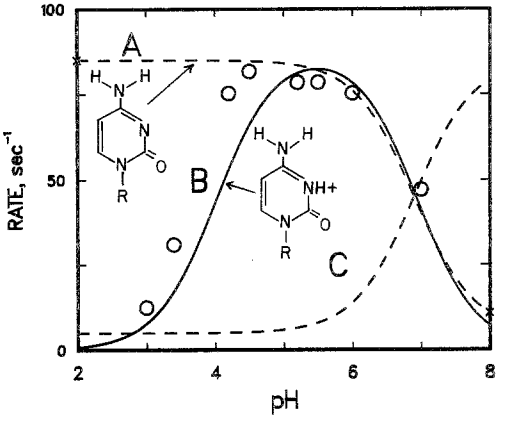

Fig. 1. Phosphate catalysis of amino proton exchange in aqueous cytidine. (O) Experimental amino proton exchange rate increments for 0.1 M 2′,3′-cyclic cytidylic acid resulting from the presence of 0.06 M sodium phosphate. Curves A and C represent general acid-base catalysis by phosphate with extreme rates of 85 and 5 $M^{-1}\ sec^{-1}$ [Eq. (1)] and curve B is based on two mechanisms involving the protonated nucleobase as intermediate in buffer base catalysis [Eq. (2)] or as an inactive intermediate in general acid catalysis [Eq. (3)].

represents nucleobase endocyclic protonation with amino proton transfer to the buffer conjugate base. Curve B is also reproduced by the relation

$$k\,(\text{sec}^{-1}) = (1 - P_N)k_A[\text{A}] \tag{3}$$

where k_A is the general acid catalysis rate constant and [A] the concentration of conjugate buffer acid. In this latter case the protonated nucleobase is an inactive form in catalysis by buffer acid, leading to the low-pH decrease in the calculated curve. It is apparent that a fit of the phosphate data of the low-pH side might be obtained by a sum of Eqs. (2) and (3). However, the unequivocal demonstration of the existence of the general acid mechanism would require the elimination of the protonated nucleobase species to demonstrate catalyzed exchange from the neutral, unprotonated pyrimidine. For the protonated nucleobase intermediate, the amino proton exchange rate is three to four orders of magnitude more rapid than the neutral form, since the latter donates amino protons to hydroxyl ion at a reduced rate (McConnell, 1978).

Figures 2-4 demonstrate the sigmoid relationship [Eq. (1)] for cytidine amino proton exchange in DMSO/water mixture as a function of buffer titration. In particular, rate constants for phosphate, acetate, and chloroacetate catalysis exhibit a plateau at low pH and a transition midpoint at the point where $p\text{H} = \text{buffer } pK$, in marked contrast to the typical aqueous profile of Eqs. (2) and (3). It is important to note that the abscissa pH in Figs. 2-4 is the pH value of buffer preadjustment before adding the aqueous buffer to the cytidine-DMSO mixture and not the pH of the DMSO mixture itself. Therefore, the acid/base ratio of the buffer, as monitored in the DMSO mixture by the change in acetate methyl chemical shifts as a function of preadjusted pH, is unchanged after addition of the aqueous buffer to the solvent system. Corresponding phosphate rate constants for 5-methylcytidine are 10-12 times greater than shown in Fig. 2 (not shown), which confirms a real measure of proton exchange in this system. Higher amino proton exchange rates are seen with 5-methylcytidine-guanosine base pairing as well (see below).

Are we observing effects of cytidine N-3 protonation on the amino proton exchange rates to account for the increased catalytic rate constants at low pH within the plateau region? This possibility is rejected by the following observations: First, the positions of the sigmoid curves for the three buffers in DMSO correspond closely

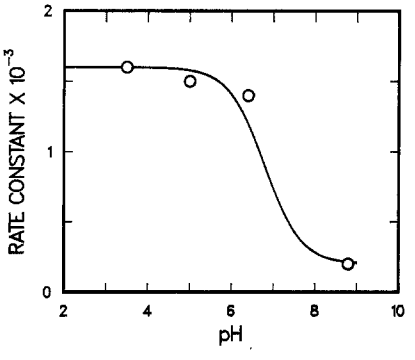

Fig. 2. Phosphate catalysis of cytidine amino proton exchange in DMSO/water. (○) Experimental rate constants obtained by amino ^{1}H resonance saturation recovery and the use of a longitudinal relaxation rate of 3 sec^{-1}. Experimental points were obtained for 0.05 M cytidine D6DMSO plus 4.5 M water. (—) Calculated from the general acid-base function [Eq. (1)] with acid and base rate constants of 1700 and 50, respectively, for a phosphate pK of 6.9. The abscissa "pH" represents that of the aqueous phosphate solutions before addition to the DMSO/cytidine mixture.

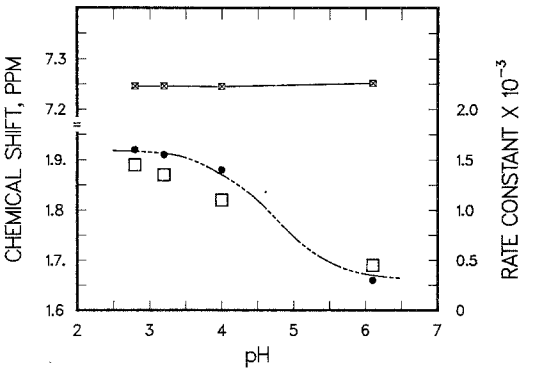

Fig. 3. Acetate catalysis of cytidine amino proton exchange in DMSO/water. Catalytic rate constants (M^{-1} sec^{-1}) were obtained by cytidine amino ^1H resonance saturation recovery in D6 DMSO/4.5 M water, 0.05 M cytidine, and aqueous acetate solutions preadjusted to the desired *p*H before addition. Longitudinal relaxation rates were 3 sec^{-1} in the estimates of (□) acetate rate constants. Chemical shifts of (●) the acetate methyl protons and of (⊠) the downfield resonance of the cytidine amino pair were measured relative to TSP. (- - - -) Calculated from an acetate *pK* of 4.7 using limiting chemical shift values of 1.95 and 1.62 ppm for the acid and base extremes, respectively.

to their dissociation *pK* values, i.e., there is no change in buffer acid/base ratio after introduction of aqueous buffer, which reflects the large suppression of buffer ionization and of associated equilibria needed for protonation of cytidine (N-3). Second, cytidine amino proton chemical shifts sensitive to N-3 protonation (2 ppm) are constant under all conditions of "*p*H," as shown in Figs. 3 and 4, with the exception of the lowest *p*H datum for chloroacetate (Fig. 4). Third, to determine if cytidine amino proton exchange is so highly sensitive to C(N-3) protonation that the amino ^1H chemical shifts of Figs. 3 and 4 are insensitive monitors of exchange according to Eq. (2), aqueous cytidine was preadjusted to varying *p*H and added to DMSO for measurement of amino ^1H chemical shifts and recovery rates after presaturation of their resonances with decoupler pulses (the main procedure for measuring exchange). As shown in Fig. 5, cytidine (N-3) titration is unchanged after addition to DMSO and the typical aqueous profile is seen (curve C, Fig. 1). This profile would conform either to Eq. (2), where [B] is the concentration of neutral cytidine, or to Eq. (3), where [A] is the concentration of (N-3)-protonated cytidine. In either case the amion ^1H chemical shifts measured above *p*H 5.8 (Fig. 5) are associated with small increases in recovery rates, which are insignificant compared to buffer-catalyzed rates corresponding to the same amino chemical shifts (Figs. 3 and 4).

A comparison of general acid catalysis rate constants for phosphate, acetate, and chloroacetate is shown in Table I. A Bronsted relation is seen for the acetates, although the Bronsted slope ($\log k_A/pK$) is quite small, in reflection of limited

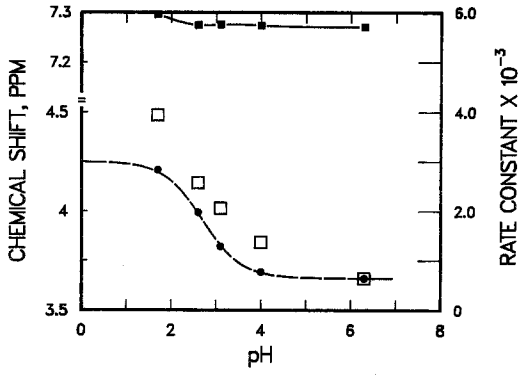

Fig. 4. Chloroacetate catalysis of cytidine amino proton exchange in DMSO/water. (□) Catalytic rate constants (M^{-1} sec^{-1}) were obtained by amino ^1H resonance saturation recovery and a longitudinal relaxation time of 3 sec^{-1} for 0.05 M cytidine in D6 DMSO/4.5 M water containing <0.01 M chloroacetate. Buffer catalyst was preadjusted to the desired *p*H before addition to the DMSO-cytidine mixture. Chemical shifts of (●) the chloroacetate methyl protons and of (■) the downfield resonance of the cytidine amino pair were measured relative to TSP. (- - -) Calculated from a chloroacetate *pK* of 2.8 with acid and base chemical shifts of 4.3 and 3.2 ppm, respectively.

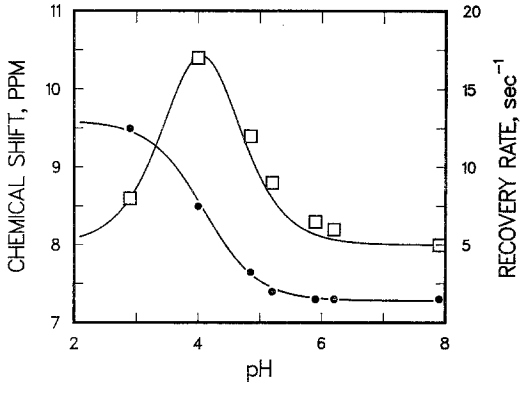

Fig. 5. The effect of cytidine pretitration on amino proton exchange in DMSO/water. Aqueous 0.65 M cytidine preadjusted to various *p*H values (abscissa) was added to D6 DMSO to a final concentration of 0.05 M. Final water content was 22.4 mole% (4.5 M). (●) Chemical shift and (□) recovery rates (right ordinate) of the non-Watson-Crick cytidine amino ^1H resonance. (—) Fitted functions using a cytidine *pK* of 4.1, limiting chemical shifts of 9.64 ppm (acid) and 7.31 ppm (base), and a rate constant of 300 M^{-1} sec^{-1} for k_B in Eq. (2), where B is cytidine N-3.

Table I. Rate Constants of General Acid-Base Catalysis of Cytidine Amino Proton Exchange in DMSO/Water[a]

	Rate constant (M^{-1} sec^{-1})	
Buffer catalyst	Acid, k_A	Base,[b] k_B
Phosphate	1700	<50
Acetate	1600	<50
Chloroacetate	3100	<50

[a] Rate constants obtained by dividing the observed exchange rate measured at 23°C by the concentration of total buffer were those representing the low- and high-*p*H buffer species. These rate constants depend on water content, which was 22.4 mole% in this case.

[b] Exchange rates for the high-*p*H form of buffer were not significantly greater than the corresponding rates in the absence of buffer.

proton transfer in the transition state for exchange catalysis in this system (Kresge, 1975). Values for general base catalysis k_B are questionable and may be too low to measure accurately by NMR saturation recovery, since small variations in longitudinal relaxation may dominate the recovery process.

All the above experiments represent data from a DMSO/water mixture, in which the water content was 4 M or 22.4 mole%. The effect of water content on general acid-base catalysis in DMSO is shown in Figs. 6 and 7. While k_B (too low to measure) is largely unaffected, k_A is influenced by water content. The Bronsted slope mentioned above depends on water content, since this effect is largest in chloroacetate catalysis. The effect of water on exchange rates in the absence of buffer is shown in Fig. 7, where it is seen that there is no increased exchange at the water content used in the buffer catalysis experiments. Instead, there is an abrupt stimulation of exchange beginning at 40 mole% with a maximum slope in the region of 66 mole% (see Section 4). The increased rates above 40 mole% reflect in part C(N-3) protonation as well as the formation of a new H bond between water and cytidine N-3, but not the role of water as an acceptor of the amino protons

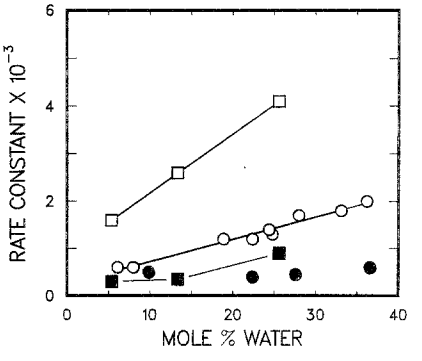

Fig. 6. General acid-base rate constants as a function of water concentration for cytidine amino proton exchange in DMSO. Rate constants for (○, ●) acetate and (□, ■) chloroacetate were obtained by amino ^1H resonance saturation-recovery, using a longitudinal relaxation of 3 sec^{-1}. Open and closed symbols are rate constants obtained at lowest (acid form) and highest *p*H (base form), respectively.

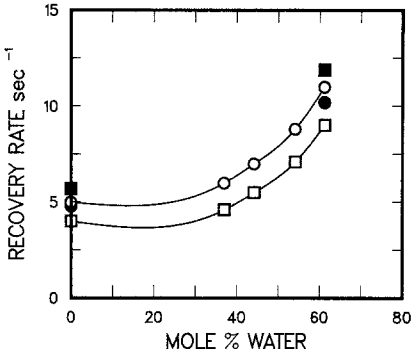

Fig. 7. Cytidine amino ^{1}H recovery rates as a function of water concentration in DMSO. Saturation-recovery measurements were obtained in D6 DMSO containing either (□) 0.05 M cytidine, (○) 0.05 M 5-methylcytidine, (■) 0.25 M cytidine, (●) or 0.25 M 5-methylcytidine after sequential water additions of <0.1 of the total volume.

(McConnell and Politowski, 1984). A new H bond at C(N-3) must be formed with the addition of buffer acids as well, since proton equilibria are suppressed in this solvent system. Thus, the C(N-3) H-bonded form of cytidine (as exists in the macromolecule) may exchange more rapidly than the non-H-bonded form. This possibility was examined as a function of G-C base pairing in DMSO.

To see the effect of G-C Watson-Crick base pairing on cytidine amino proton exchange, amino ^{1}H recovery rates were measured at low, decreasing C/G ratios to maximize the fraction of cytidine in complex with guanosine. For the analysis we assume rapid equilibrium averaging of the free (slower exchange) and bound (faster exchange) states of cytidine,

$$k_{av}(\text{sec}^{-1}) = P_{cf}(k_{cf} - k_{cb}) + k_{cb}$$

where P_{cf} is the mole fraction of free cytidine, and k_{cf} and k_{cb} are the amino proton exchange rate constants for the free and bound forms, respectively. Values of P_{cf} were obtained by iterative solution to a quadratic equation for G-C base pairing using the measured constants of Newmark and Cantor (1968) for G-C and cytidine self-association. Raw recovery data, obtained with the assumption that T_1 is the same for the free and bound forms (recovery rate $= 1/T_1 + k_{av}$), is plotted in Fig. 8 as a function of guanosine concentration. Observed recovery rates conform to that

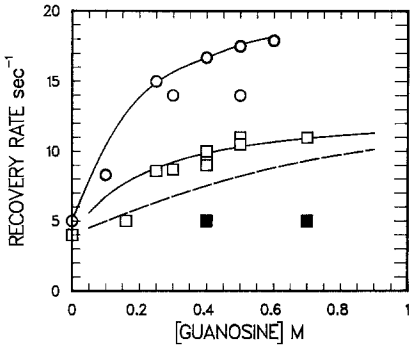

Fig. 8. Cytidine amino ^{1}H saturation-recovery rates in DMSO as a function of added guanosine. Rates for (□) 0.05 M cytidine, (○) 0.05 M 5-methylcytidine, and (■) 0.4 M cytidine were obtained at 25°C in D6 DMSO, independent of water concentration. (—) Calculated from C+G=CG Watson-Crick equilibria using K(association) = 5.1, including terms for cytidine self-association (see text), longitudinal relaxation rate of 3 sec^{-1}, and exchange rates for free and bound cytidine of 2 and 10 sec^{-1}, respectively. Rates for free and bound 5-methylcytidine are 3 and 20 sec^{-1}, respectively.

predicted by calculation and confirm qualitatively that an expected decrease in recovery with increased cytidine concentration is realized (closed squares in Fig. 8). That resonance recovery data are a valid measure of proton exchange is borne out by the use of 5-methylcytidine as control, the amino protons of which exchange more rapidly than those of cytidine. Kinetic parameters for fitting the recovery data for G-C base pairing are as follows: For cytidine, $k_{cf} = 2\ \text{sec}^{-1}$, $k_{cb} = 10\ \text{sec}^{-1}$, and $K(\text{association}) = 5\ \text{M}^{-1}$. For 5-methylcytidine, $k_{cf} = 3\ \text{sec}^{-1}$, $k_{cb} = 20\ \text{sec}^{-1}$, and $K(\text{association}) = 20\ \text{M}^{-1}$. In all cases, $1/T_1 = 3\ \text{sec}^{-1}$. While the association constant for cytidine is taken from published values, the constant for 5-methylcytidine was a fitted value with consideration given to pyrimidine self-association (B. McConnell and A. Tan, in preparation).

4. DISCUSSION

The issue of the nonaqueous environment in monomeric studies is of particular importance to the study of protein and nucleic acid structure by the method of hydrogen exchange. Attenuation of exchange rates in macromolecules provides information on the forward and reverse rates of structural "opening" and "closing" reactions that establish access of solvent or catalyst to the encrypted exchange site (Englander and Kallenbach, 1983). Quantitative information derived from these measurements hinges entirely upon an estimate or prediction of the "intrinsic" exchange rates of the sites under observation. These estimates are derived either from *a priori* use of questionable exchange parameters, e.g., "diffusion encounter" rate constants (Hilbers and Patel, 1975), or from studies of monomeric exchange behavior in aqueous solution (McConnell and Politowski, 1984). The chief weaknesses of the aqueous monomeric studies are the assumptions that the exchange ("open") environment in the macromolecule is "aqueous" and that exchange cannot occur from the "closed" state. While these considerations apply generally, a uniquely clear example of the consequences of these two assumptions is found in monomeric studies of nucleic acid proton exchange at the amino sites (which are similar chemically to the peptide nitrogen protons of proteins). Aqueous studies have shown that exchange of adenine and cytosine exocyclic amino protons is influenced by interactions at the endocyclic basic nitrogen, notably A(N-1) or C(N-3) protonation in the monomeric state (McConnell and Politowski, 1984), in the double helical state (Teitlebaum and Englander, 1975), and in short, self-complementary double helices (McConnell, 1984). As shown above, in aqueous buffer-catalyzed exchange the rate data are consistent with the two mechanisms: (1) amino proton abstraction by buffer base from the endocyclic-protonated nucleobase or (2) buffer acid proton donation to the unprotonated nucleobase species (general acid catalysis). This second route could be initiated by hydrogen bond formation between the unprotonated nucleobase and protonated buffer species, as would be the situation in the Watson-Crick base pair, leading to exchange from the "closed" helical state in the macromolecule. Although the interpretation of aqueous general acid catalysis has been made in a similar system (Cross *et al.*, 1975), its unequivocal demonstration is prevented by the impossibility of avoiding extensive nucleobase protonation in the presence of sufficient amounts of protonated buffer species for formation of a

(hydrogen-bonded) complex. In DMSO/water mixtures it is apparent that buffer conjugate acid can exist without significant protonation of the basic endocyclic site in cytidine compounds, providing for the first demonstration of general acid catalysis and increased proton exchange with Watson-Crick base pairing. This latter exchange route may account for the observation of "general base catalysis" in closed helices (Leroy *et al.*, 1985).

It is apparent from this study that the exchange route of Eq. (2) is virtually eliminated in DMSO/water mixtures containing both cytidine dissolved as the neutral base and buffer added as pretitrated aqueous solution. The suppression of reaction routes involving a protonated intermediate has been observed by Wolford (1964) in the hydrolysis of acetal in DMSO/water and has been correlated with the high extent of proton solvation from a positively charged conjugate acid. This rate attenuation was reversed at a specific mole fraction of DMSO (33 mole%), which corresponds to a DMSO/water ratio that is associated with the concomitant reversal of a number of solvent physical properties, indicating a profound change in solvent structure in this range. It can be seen from Fig. 7 that this same region (67 mole% water) approximates the DMSO/water ratio where amino proton exchange in the absence of buffer increases most abruptly with increasing water content. With this transition the normal aqueous water equilibria are restored, which provide for the formation of the highly reactive C(N-3) protonated intermediate of cytidine. On this basis we can conclude that the marked stimulation of general acid catalysis by water at concentrations well below 40 mole% (Fig. 6) reveals a direct involvement of water necessary for the catalytic process without its mediation for the process of base protonation. A second well-studied property of DMSO and aprotic solvents in general is the suppression of anion solvation, which accounts for $\sim 10^6$-fold increases in bimolecular reaction rates and for suppression of acid dissociation of carboxylates (Parker, 1969). For example, the acetate pK is 11.6 in DMSO (Ritchie, 1969). The implication of these DMSO properties demonstrated here is that above a DMSO/water ratio of 1/2 an aqueous buffer titrated to a given acic/base ratio can be added without a change in buffer species and without proton transfer to a second base (cytidine or 5-methylcytidine) present in solution. Thus, a mechanistic "window" is provided for the study of catalytic routes for reactions important in the macromolecule, but unseen in aqueous monomer studies. The extension of the aprotic solvent system to examine peptide interactions may be of similar value.

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REFERENCES

Brandts, J. F., and Hunt, L. (1967). *J. Am. Chem. Soc.* **89**, 4826.
Cross, D. G., Brown, A., and Fisher, H. F. (1975). *Biochemistry* **14**, 2747.
Englander, S. W., and Kallenbach, N. R. (1983). *Q. Rev. Biophys.* **16**, 521.

Gordon, J. A., and Jencks, W. P. (1963). *Biochemistry* **2**, 47.
Gotoh, O., and Takashira, Y. (1981). *Biopolymers* **20**, 1033.
Hilbers, C. W., and Patel, D. J. (1975). *Biochemistry* **14**, 1656.
Katz, L., and Penman, S. (1966). *J. Mol. Biol.* **15**, 220.
Kauzmann, W. (1959). *Adv. Protein Chem.* **14**, 1.
Kresge, J. A. (1975). In *Proton Transfer Reactions* (Caldin, E. F., and Gold, V., eds.), Wiley, New York, pp. 179–199.
Leroy, J.-L., Broseta, D., and Gueron, M. (1985). *J. Mol. Biol.* **184**, 165.
McConnell, B. (1978). In *11th Jerusalem Symposium on Quantum Chemistry and Biochemistry* (Pullman, B., ed.), Reidel, Dordrecht, p. 147.
McConnell, B. (1982). *J. Am. Chem. Soc.* **104**, 1723.
McConnell, B. (1984). *J. Biomol. Struct. Dynam.* **1**, 1407.
McConnell, B., and Politowski, D. (1984). *Biophys. Chem.* **20**, 135.
Newmark, R. A., and Cantor, C. R. (1968). *J. Am. Chem. Soc.* **90**, 5010.
Parker, A. J. (1969). *Chem. Rev.* **69**, 1.
Petersen, S. B., and Led, J. J. (1981). *J. Am. Chem. Soc.* **103**, 5308.
Ritchie, C. D. (1969). *J. Am. Chem. Soc.* **91**, 6749.
Tanford, C. (1962). *J. Am. Chem. Soc.* **84**, 4240.
Teitlebaum, H., and Englander, S. W. (1975). *J. Mol. Biol.* **92**, 79.
Ts'o, P. O. P. (1974). In *Basic Principles of Nucleic Acid Chemistry* (Ts'o, P. O. P., ed.), Academic Press, New York, Vol. I, pp. 453–584.
Wolford, R. K. (1964). *J. Phys. Chem.* **68**, 3392.