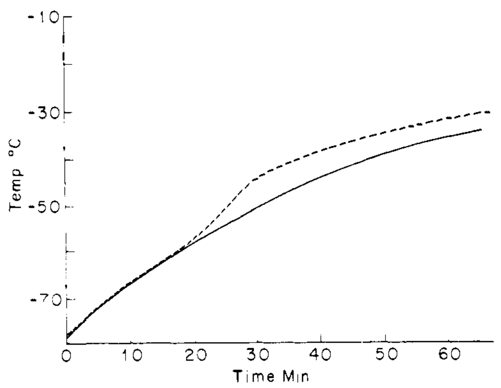

Figure 3. Dynamic calorimeter curves for the rearrangement of *sec*-butyl ion to *tert*-butyl ion: curve a (—) baseline, programmed warming of calorimeter and solvent with or without *tert*-butyl carbonium ion present; curve b (---) reaction curve produced by rearrangement of *sec*-butyl to *tert*-butyl cation and simulated by introduction of varying current through electrical heater.

(Figure 3, curve b) shows a temperature displacement from curve a as a function of the amount of electrical heat added. The apparent heat capacity of calorimeter and contents did not change within the experimental error over the temperature range where the current was supplied to the calibration heater. The same type of temperature displacement vs. time curve would be expected for any exothermic chemical reaction (Figure 3, curve b) which is initiated by increasing the temperature through the range over which the chemical reaction would occur.

When a 0.5 M solution of *sec*-butyl ion was warmed from −80 to −20 °C in the calorimeter, an exothermic reaction occurred to generate a temperature displacement vs. time curve (Figure 3, curve b) concomitant with the formation of *tert*-butyl ion as evidenced by NMR over the same temperature range (−64 to −45 °C). The solution was then cooled to −80 °C and the programmed warming of the calorimeter and contents (now *tert*-butyl ion rather than *sec*-butyl) was repeated. The baseline, (curve a) for warming of the colorimeter and contents in the absence of rearrangement, was thus established as being identical with that of pure solvent. The solution was cooled once again to −80 °C but this time during the programmed warming cycle an increment of electrical heat from the calibration heater was added at an appropriate point so as to simulate the original rearrangement curve; thus the enthalpy corresponding to the difference between the baseline (curve a) and rearrangement (curve b) was established.

The average value for the *sec* → *tert*-butyl rearrangement enthalpy based on six completely independent preparations of *sec*-butyl ion was 14.5 ± 0.5 kcal/mol.[13] The corresponding value of 15–17 kcal/mol for the same process in the gas phase has been determined by mass spectrometric methods.[2,11,12,14] It has also been calculated with extraordinary success by Radom, Pople, and Schleyer.[15] The close correspondence between our observed isomerization energy in solution (ΔH = 14.5 ± 0.5 kcal/mol) and that in the gas phase provides dramatic support for the surmise of Fry, Harris, Bingham, and Schleyer[16] that the degree of electrostatic solvation varies little between similar carbonium ions.[17] Charge-localized ions such as ammonium or alkoxide which are capable of strong hydrogen-bonding or ion-pairing interactions with solvent behave entirely differently.[18,19,20] The remarkable success of the "carbonium ion theory" as applied to aliphatic systems is probably attributable in large part to the relatively small role of specific solvation effects in carbonium ion-like species.

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Conformational Analysis of the Triphosphate Side Chain of Adenosine 5′-Triphosphate. Phosphorus–Proton Nuclear Overhauser Effects

Sir:

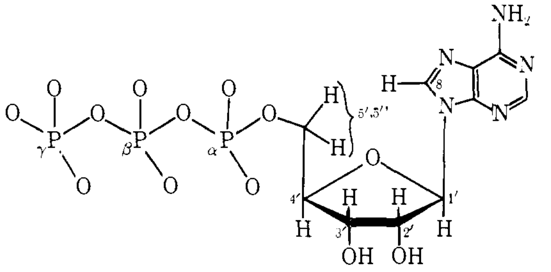
At least six reports have appeared in the preceding 14 years suggesting that phosphorus spin-lattice relaxation is provided, in part, by dipole–dipole interaction with adjacent half-spin nuclei.[1] Given that relaxation mode, it should be possible to observe a phosphorus–proton nuclear Overhauser effect (NOE) of up to 124%.[2] Indeed, Martin and coworkers have verified that expectation.[3] In view of the previous successful application of the proton–proton NOE to

Table I. ATP Phosphorus-Proton Nuclear Overhauser Effects

Irradiation mode	Proton(s) irradiated	% enhancement P[a,b] α	β	γ
Broad band	All	65	17	26
Single frequency	5′,5″	54	26	6.5
	4′	46	22	8.5
	2′,3′	36	7.5	15
	1′	0	0	0
	2	0	0	0
	8	0	0	0

[a] Areas of expanded plots measured by planimetry. Area measurements reproducible to 1%, experimental precision is 3%. [b] Pulsed mode of observation. Observing pulse ca. 40°, pulse delay 11 s, 1000 transients accumulated into 8K, 20 bit memory locations. Resolution less than 2 Hz routinely.

the determination of nucleoside and nucleotide glycosyl torsion angle distributions,[4] there was a potent incentive to develop the phosphorus-proton NOE as a tool for more extensive conformational analyses of nucleotides and oligonucleotides. Thus, presented here is a preliminary conformational analysis of the triphosphate side chain of ATP (I) via the phosphorus-proton NOE.[5]



I

An x-ray analysis of crystalline disodium ATP[6] displays two molecules of ATP, four sodium ions, and six water molecules in the asymmetric unit. The triphosphate side chain is folded in each of the two distinguishable molecules of ATP. The A form typifies the side chain folding so that the γ phosphate is proximate to the furanose oxygen and H-8 while the B form folding places the γ phosphate in the vicinity of H-3′. The glycosyl torsion angle is anti in both forms. Molecular orbital calculations[7] verify the stability of the folded form in the absence of cation influences.

Solution studies of ATP side-chain folding have adopted paramagnetic metals as probes of phosphate geometry.[8,9] Both lanthanide and manganese probes point to side-chain folding corresponding more or less to the A form seen in the crystal.

A deuterium oxide solution of ATP (0.1 M, pD 7.5), that had been freed as well as possible of paramagnetic impurities first by Chelex 100 chromatography then by degassing alternately frozen and thawed solutions at 10^{-5} Torr, was prepared. The NOE experiments were done at 36.44 MHz on a Bruker HX90E spectrometer in the pulsed mode (H^2 lock). Free induction decays were transformed by a Nicolet 1080 computer. Areas under the expanded phosphorus spectra both before and after proton irradiation were determined by planimetry. The data are recorded in Table I and instrument parameters appear as footnotes to the table.

The data in Table I demonstrate that all three phosphorus nuclei of the triphosphate side chain relax to some extent via dipolar interaction with nearby protons. That the Overhauser enhancements are not the theoretical 124% indicates other modes of relaxation. Some additional contributors are: homonuclear phosphorus interaction, residual paramagnetic impurities or entirely different relaxation mechanisms (chemical shift anisotropy and spin rotation are two possibilities). Aggregation will also lead to an overall reduction in the observed Overhauser enhancement (see below). Despite incomplete NOE's it can be concluded that the triphosphate side chain is folded in such a way that the terminal phosphorus interacts with intramolecular protons. This conclusion is based on the broad-band irradiation experiment and follows from the known dependence of the NOE on the inverse sixth power of the internuclear distance as expressed by eq 1,

$$f_i(j) = \rho_{ij}\gamma_j/2\gamma_i R_i \qquad (1)$$

where $f_i(j)$ is the fractional enhancement of nucleus i when nucleus j is irradiated, γ is the nuclear gyromagnetic ratio, ρ_{ij} is the dipole-dipole mediated relaxation rate of i, and R_i is the total relaxation rate of i. The expression for the dipole-dipole interaction is $\rho_{ij} = \gamma_i^2\gamma_j^2\hbar^2\tau_c/r^6$ in which τ_c is the molecular correlation time and r is the distance between i and j. If the side chain were fully extended away from the 5′-carbon, the only protons with which the α, β, and γ phosphorus nuclei would interact would be those attached directly to C-5′ (neglecting intermolecular interactions) and this interaction would be substantially attenuated in the β and γ cases, so much so that, particularly as far as the γ phosphorus is concerned, there would be no observable enhancement.

Preliminary single frequency irradiation experiments are reported here to demonstrate the real potential of the phosphorus-proton NOE for conformational analysis. The single frequencies were chosen to correspond approximately to the protons shown in the table. However, it is fairly certain there is substantial multiple resonance irradiation in the 2′,3′,4′,5′ region because these resonances are dispersed over just an 80 Hz range. It is thus the trend in the enhancements as one moves from the 5′ to the 3′ region that permits the qualitative conclusion that the terminal (γ) phosphorus interacts spatially with sugar protons in the 3′,4′ region. From this conclusion it follows that the side chain conformer distribution is characterized by some proportion of the folded form that corresponds roughly to the B form observed in crystalline disodium ATP. The B form is uniquely suggested by the H-3′,4′-γ phosphorus interaction because in the A form these nuclei are too far apart. However, the data do not preclude the A form, detected in solution by paramagnetic probes. The only proton with which the γ phosphorus would be expected to interact in the A form is H-8 of the purine ring (anti glycosyl rotamer) and it is entirely possible under the conditions of these experiments that the actual placement of H-8, as a function of glycosyl torsion angle, is such that no significant interaction would be observed.

ATP is measureably (though not extensively) aggregated under the conditions of these experiments.[10] By analogy with adenosine monophosphate,[11] the aggregation of ATP is probably via a base-base interaction. Thus, it is probable that the sugar moiety and its attached triphosphate would not be conformationally constrained in the aggregate and that, even in the aggregate, the observed NOE's arise from intramolecular contacts.

Extensive aggregation can, however, reduce the overall magnitude of the NOE via an increase in τ_c.[4f,12] This aggregation effect on the NOE was seen when attempts were made to repeat the above experiments on 0.1 M solutions of MgATP. Even though the same precautions were taken to ensure minimization of paramagnetic contaminants, no Overhauser enhancements of the three phosphorus signals could be detected following broad-band irradiation of the proton spectrum. Magnesium is known to enhance ATP aggregation.[13]

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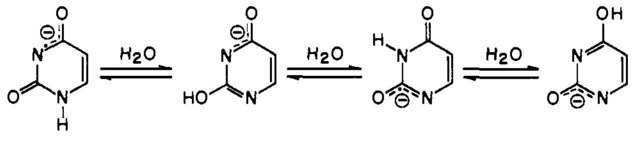
Bifunctional Proton Transfer of the Conjugate Base of Uracil Compared with That of Imidazole in Aqueous Solution[1]

Sir:

Using dynamic NMR techniques,[2] we have observed that the conjugate base of uracil (UH^-, eq 1) undergoes remarkably fast bifunctional proton transfer with water participation, and have studied the reaction mechanism shown in eq 1. In



(U_1H^-) (U_2H^-) (U_3H^-) (U_4H^-)

(1)

^{17}O-enriched water, at pH 8.3–9.3, proton exchange between water molecules at 25° follows the rate law (eq 2).[3]

$$\frac{1}{\tau_W} = \frac{R_W}{2[H_2O]} = 2.1 \times 10^9[OH^-] + \frac{(1.1 \pm 0.2) \times 10^8}{2[H_2O]}[UH^-] \quad (2)$$

The first term in eq 2 is due to hydroxide-catalyzed reaction;[3] the second, with $k = (1.1 \pm 0.2) \times 10^8\ s^{-1}$, involves catalysis by UH^-. k is 300 times greater than the known rate constant k_f for base dissociation (eq 3),[4] and is enormously greater than any plausible rate constant for acid dissociation of UH^-.[5]

$$UH^- + H_2O \underset{k_r}{\overset{k_f}{\rightleftharpoons}} UH_2 + OH^- \quad (3)$$

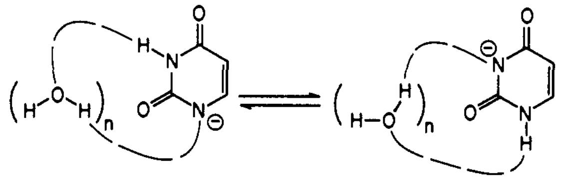
Further consistent evidence was obtained from a kinetic study of NH proton exchange between uracil (UH_2) and water in the pH range 6–8. Assuming that $k \approx 10^8\ s^{-1}$, we may expect that in each reversible cycle $UH_2 \rightleftarrows UH^-$, *both* NH protons of UH_2 exchange with water protons. The experimental rate law for $1/\tau_{NH}$ is given in eq 4.

$$\frac{1}{\tau_{NH}} = \frac{R}{2[UH_2]} = k_r[OH^-] + k_7[UH^-] \quad (4)$$

If both NH protons of UH_2 exchange in each cycle, R must be interpreted as the molar rate of exchange of UH_2; k_r is defined in eq 3, and k_7 is the rate constant for proton transfer between UH_2 and UH^- with water participation.[2] Values obtained at 25° are: $k_r = (1.2 \pm 0.2) \times 10^{10}\ s^{-1}\ M^{-1}$; $k_7 = (2.1 \pm 0.4) \times 10^7\ s^{-1}\ M^{-1}$. k_r is in good agreement with the value, $0.98 \times 10^{10}\ s^{-1}\ M^{-1}$, obtained by relaxation spectrometry at 20°.[4] If only one NH proton of UH_2 were exchanging in each cycle (i.e., if $k < 10^6\ s^{-1}$), the right-hand side of eq 4 would be equal to $2/\tau_{NH}$, and the value inferred for k_r would be inconsistent with that obtained by relaxation spectrometry.

The NH-to-H_2O chemical shift required in the above experiments was found by direct observation in 80% H_2O–20% D_2O at pH 4 to be 6.1 ppm. The NH protons of uracil are downfield from water and form a single featureless resonance, similar to that reported for UH_2 in dimethyl sulfoxide.[6]

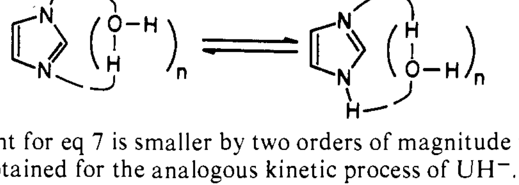
We believe that the high rate constant k represents largely the specific rate of bifunctional proton transfer with water participation in UH^-. This process could be an uninterrupted proton transfer between N_1 and N_3 as in eq 5, or a stepwise water-assisted tautomerization involving the carbonyl oxygen atoms, as in eq 1. To distinguish between these possibilities, we measured proton exchange rates in aqueous solutions of imidazole (Im) in ^{17}O-enriched water at pH 7.0–8.2. The results obtained at 25° are represented by the rate law (eq 6).



(5)

$$\frac{1}{\tau_W} = \frac{R_W}{2[H_2O]} = 1.9 \times 10^9[OH^-] + 2.7 \times 10^9[H_3O^+] + \frac{k}{2[H_2O]}[Im] + \frac{1.3 \times 10^8}{2[H_2O]}[Im][ImH^+] \quad (6)$$

In the present context, only the term proportional to [Im] is of interest. The value obtained for k is $(1.5 \pm 0.5) \times 10^6$. Although k is small compared to the other rate constants, the term accounts for as much as 28% of $1/\tau_W$ in these experiments and is, statistically, highly significant.[8] k is much greater than the known rate constant for base dissociation,[9] and therefore probably represents bifunctional proton transfer with water participation according to eq 7. Note, however, that the rate constant for eq 7 is smaller by two orders of magnitude than that obtained for the analogous kinetic process of UH^-.



(7)

While this work was in progress, Dreyfus et al.[10] reported a kinetic study of the tautomerization of adenine in aqueous solution. For the uncatalyzed process (eq 8), they report k_+