Magnesium and Calcium Ion Effects on Hydrolysis Rates of Adenosine 5′-Triphosphate

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Rates of hydrolysis of bis(tetramethylammonium), magnesium, and calcium adenosine 5′-triphosphate, $ATPH_2^{2-}[(CH_3)_4N^+]_2$, $MgATPH_2$, and $CaATPH_2$ have been determined at 70 °C in 0.01 M solutions in water and in acetonitrile–water (1:1 v/v) at constant pH in the pH range 0–10. Pseudo-first-order rate constants are obtained from changes in ATP concentration measured by means of liquid chromatography. The behavior of tris- and tetrakis(tetra-*n*-butylammonium) adenosine 5′-triphosphate in acetonitrile-*tert*-butyl alcohol (1:1 v/v) solutions has been studied to aid in the elucidation of mechanisms of nonenzymatic nucleophilic displacements at the P_γ center of ATP. Formation of *tert*-butyl phosphate is taken as the criterion for the presence of metaphosphate, PO_3^-, in the solutions. It is concluded that displacements on the tetraanion, ATP^{4-}, and the trianion, $ATPH^{3-}$, occur via metaphosphate, while displacements on the acid, $ATPH_4$, and the monoanion, $ATPH_3^-$, proceed via intermediates with pentavalent phosphorus (oxyphosphoranes). Reactions of the dianion, $ATPH_2^{2-}$, could involve both metaphosphate and oxyphosphorane intermediates. In the absence of divalent metal ions, the trianion generates metaphosphate faster than the tetraanion by a factor of approximately 18 in water. Magnesium and calcium ions have little or no effect on the rate of formation of metaphosphate from the trianion in water. Magnesium and calcium increase the rate of metaphosphate formation from the tetraanion by factors of approximately 10 and 50, respectively, in water. Rates of hydrolysis at pH <3 are similar in the presence and in the absence of the metal ions. Up to 50 mol of imidazole, pyridine, or quinuclidine/mol of ATP have no significant effect on hydrolysis rates of the tetraanion in water. The conclusions reached in this investigation are not regarded as necessarily applicable to the problem of enzymatic nucleophilic displacements on ATP.

The nonenzymatic hydrolysis of adenosine 5′-triphosphate (ATP) has been the subject of numerous investigations, but major questions pertaining to the mechanism(s) of the reaction remain unsettled.[2] Some of the previous studies have been of a qualitative nature,[3-6] while others have involved kinetic measurements;[7-15] in all cases, the hydrolysis has been carried out in water as the medium.

One of the most interesting questions concerning the hydrolysis of ATP pertains to the effect of metal ions.[7-15] Emphasis in recent studies has been placed on transition-metal-ion effects, although magnesium and calcium are recognized as having the most biological significance.[16]

The present study is concerned with three aspects of the problem of nucleophilic displacements on ATP: (1) determination of the pH–rate profile for the hydrolysis in water and in acetonitrile–water mixtures by utilizing liquid chromatography as the analytical tool; (2) a study of the effect of magnesium and calcium ions on the rate of hydrolysis in both types of media; (3) a qualitative examination of the nature of the products obtained when tetra-*n*-butylammonium salts of ATP are allowed to react in alcohol and in acetonitrile–alcohol solutions. From these studies we have obtained information on the types of mechanisms which operate in displacement reactions at various states of ionization of the ATP molecule, both in the absence and in the presence of the biochemically important magnesium ion.[16]

Experimental Section

Materials. The salts $ATPH_2^{2-}[(CH_3)_4N^+]_2 \cdot H_2O$, $MgATPH_2 \cdot 4H_2O$, and $CaATPH_2 \cdot 2H_2O$ were prepared as described.[17] Bis(tetra-*n*-butylammonium) adenosine 5′-triphosphate, $ATPH_2^{2-}[(n\text{-}C_4H_9)_4N^+]_2 \cdot H_2O$, was prepared as follows. Sigma Chemical Co. grade I $ATPH_2^{2-}Na^+_2 \cdot 3.5H_2O$ (3.69 g, 6.0 mmol) was dissolved in water (ca. 15 mL) and rapidly converted into an aqueous solution of $ATPH_4$ by using a column of BioRad AG50W-X8 resin (H^+ form) at 5 °C. The eluate was collected in a flask containing a methanol solution of tetra-*n*-butylammonium hydroxide (12.0 mmol in 25 mL; Eastman titrant grade) at 0 °C. The solution was evaporated under vacuum at 5 °C, and the salt was dried 18 h at 20 °C (0.5 mm); the neutralization equivalent obtained by titration corresponded to the monohydrate of the salt. Analysis by liquid chromatography showed that the material contained >99% ATP.

Anal. Calcd for $C_{42}H_{86}N_7O_{13}P_3 \cdot H_2O$: C, 50.0; H, 8.7. Found: C, 50.9; H, 9.3.

Tris- and tetrakis(tetra-*n*-butylammonium) adenosine 5′-triphosphates were prepared by the addition of 1 and 2 molar equiv of tetra-*n*-butylammonium hydroxide, respectively, to a methanol solution of $ATPH_2^{2-}[(n\text{-}C_4H_9)_4N^+]_2$ at 20 °C. Evaporation of the solution at 20 °C under vacuum yielded the salts, which were dried for 18 h at 20 °C (0.5 mm). Liquid chromatographic analysis showed that the salts consisted of >99% ATP.

The buffers employed were HCl at pH <2, formate at pH 2.5–4.0, acetate at pH 5.0–6.0, 4-(2-hydroxyethyl)-1-piperazineethanesulfonate (HEPES) at pH 6.7–8.3, and borate at pH 8.0–9.3. All buffer solutions were prepared with salts containing the

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tetramethylammonium cation exclusively, and ionic strength was adjusted to $\mu = 0.2$ with $(CH_3)_4N^+Cl^-$. All buffer materials were Fisher or Baker reagent grade.

Apparatus. The hydrolyses were carried out in glass-stoppered volumetric flasks maintained at 70 ± 0.1 °C in a Colora Ultra-Thermostat. Analyses by liquid chromatography were performed by using a Varian 8500 liquid chromatograph with a Varian variable-wavelength UV monitor as the detector. Peaks were recorded on a Varian A-25 recorder equipped with a disk integrator. Separation of nucleotides was achieved with a Whatman Partisil PXS 10/25 SAX anion-exchange column (25 cm × 4.6 mm i.d.). Isocratic elution with a 0.5 M KH_2PO_4 buffer (pH 4.45) was employed at a flow rate of 1 mL/min. The column temperature was 25 °C.

pH measurements were carried out at 70 °C with a Markson 4XX series combination electrode and an Orion Model 801A digital pH meter standardized with buffers calibrated at this temperature by the National Bureau of Standards.[18]

Kinetic Measurements. A weighed amount of either $ATPH_2^{2-}[(CH_3)_4N^+]_2 \cdot H_2O$, $MgATPH_2 \cdot 4H_2O$, or $CaATPH_2 \cdot 2H_2O$ was dissolved in the thermally equilibrated buffer to give a 0.01 M solution. Aliquots were removed at appropriate intervals, and the reaction was stopped by being cooled to 0 °C. Hydrolysis rates were determined by measuring the decrease in ATP concentration. Since the hydrolysis products, ADP and AMP, have nearly identical UV spectra with that of the reactant ATP, the progress of the reaction was monitored by a liquid chromatographic separation technique. A small aliquot (4.00 μL) was injected, and the eluted components were monitored at 260 nm. The area under each peak was measured with a disk integrator. The pseudo-first-order rate constants were determined from the slope of a plot of ln ($area_t - area_\infty$) vs. time, since the area under the peak is directly related to the ATP concentration. The area at t_∞ was always zero. Plots were linear for at least 3 half-lives. The rate constants were calculated by using a linear least-squares regression analysis. Values of duplicate runs were in agreement to within 5%. The observed rates were invariant with different buffer concentrations.

Several experiments were carried out with the magnesium salt of ATP in the absence of buffers, with the pH maintained approximately constant by addition of either concentrated HCl or $(CH_3)_4N^+OH^-$ solution. The rate constants agreed with those obtained in the presence of buffers within experimental error. The pH values of the reaction mixtures were determined at 70 °C at the beginning and at the end of each reaction. Measurements in solutions which varied in pH beyond ±0.05 units during the course of the reaction were rejected.

Reactions in Acetonitrile–*tert*-Butyl Alcohol (1:1 v/v) Solution. The tetra-*n*-butylammonium salts of ATP^{4-} and $ATPH^{3-}$ were dissolved in the mixed solvent, and the 0.1 M solutions were kept several days at 70 °C to achieve complete reaction. The final solutions were analyzed by ^{31}P NMR spectrometry. Formation of *tert*-butyl phosphate was demonstrated by appearance of a singlet at $\delta(^{31}P)$ −3.2 to high field of 85% H_3PO_4=O and by isolation of the salt $(t\text{-}C_4H_9O)PO_3^{2-}[c\text{-}C_6H_{11}NH_3^+]_2$, mp 192–194 °C (from ethanol) (lit.[19] mp 191–193 °C), by the following procedure. The reaction mixture was evaporated under vacuum. The residue, dissolved in water, was passed through a cation-exchange resin in the Na^+ form. The aqueous solution was evaporated under vacuum, and the residue was analyzed by ^{31}P NMR in D_2O solution. The singlet at $\delta(^{31}P)$ −3.2 was accompanied by an AMP multiplet at +0.8 ppm. The nucleotides were removed from the aqueous solution by treatment with charcoal. The solution was passed through an ion-exchange resin in the H^+ form, and the eluate was added to an excess of cyclohexylamine in water. Evaporation of the solvent and recrystallization of the residue from ethanol gave the amine salt of *tert*-butyl phosphate.

pK_a Measurements. All pK_a's were determined by the procedure of Albert and Serjeant[20] in 0.01 M solutions ($\mu = 0.2$) at 70 °C. The titrant was a 0.100 N solution of tetramethylammonium hydroxide.

Table I. Acid Dissociation Constants of Adenosine 5'-Triphosphate at 70 °C in 0.01 M Solutions ($\mu = 0.2$)[a]

equilibria	solvent	pK_a
$ATPH_2^{2-} \rightleftharpoons ATPH^{3-} + H^+$	water	4.3 ± 0.1
$MgATPH_2 \rightleftharpoons MgATPH^- + H^+$	water	3.9 ± 0.2
$ATPH^{3-} \rightleftharpoons ATP^{4-} + H^+$	water	7.32 ± 0.06
$MgATPH^- \rightleftharpoons MgATP^{2-} + H^+$	water	5.36 ± 0.05
$CaATPH^- \rightleftharpoons CaATP^{2-} + H^+$	water	5.6 ± 0.1
$ATPH^{3-} \rightleftharpoons ATP^{4-} + H^+$	1:1 acetonitrile–water[b]	7.9 ± 0.1
$MgATPH^- \rightleftharpoons MgATP^{2-} + H^+$	1:1 acetonitrile–water	5.78 ± 0.06

[a] Additional cation is $(CH_3)_4N^+$. [b] Mixed solvent is by volume.

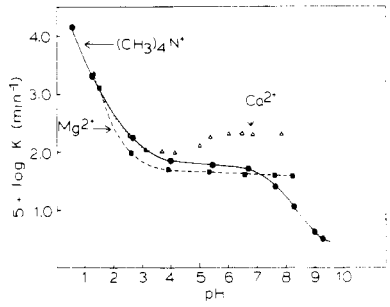

Figure 1. pH–rate profile for hydrolysis of adenosine 5'-triphosphate in 0.01 M aqueous solution ($\mu = 0.2$) at 70 °C.

Results

Effects of Magnesium and Calcium Ions on Acid Dissociation Constants of ATP. pK values measured under the conditions used for the hydrolysis experiments are given in Table I. Magnesium ions increase significantly the acidity of the last ionizable proton of ATP in both solvents, as has already been noted for aqueous solutions.[21,22] The effect of calcium ions on acidity is virtually the same as that of magnesium ions. The acids are only slightly weaker in acetonitrile–water than in water.

In aqueous solution, in the absence of metal ions, the acid ($ATPH_4$) and the monoanion ($ATPH_3^-$) are the only species expected in significant amounts at pH <2. In the pH range 2–6 various mixtures of monoanion, with dianion ($ATPH_2^{2-}$) and trianion ($ATPH^{3-}$) should be present. Only trianion and tetraanion (ATP^{4-}) are expected in the pH range 6–9, with virtually pure tetraanion present at pH >9. In the presence of magnesium or calcium ions, the tetraanion should be present almost exclusively at pH >7, due to the increase in acidity of the last ionizable proton by the metal ions.

Effects of Magnesium and Calcium Ions on the Hydrolysis of ATP. The kinetic results and the corresponding pH–rate profiles in aqueous solution are given in Table II and Figure 1, respectively. The corresponding data in acetonitrile–water solution are given in Table III and Figure 2, respectively (see paragraph at the end of the paper about supplementary material).

The following effects are noted in the absence of metal ions. (1) The hydrolysis in strongly acid medium (1 N

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Table II. Rates of Hydrolysis of Adenosine 5′-Triphosphate at 70.0 ± 0.1 °C in 0.01 M Aqueous Solutions (μ = 0.2)[a]

pH	10^4k, min^{-1}	$t_{1/2}$
	Cation $(CH_3)_4N^+$	
b	1390.0	5 min
1.45	198.0	35 min
2.66	17.6	6.6 h
3.98	7.13	16.2 h
5.44	6.08	19.0 h
6.69	5.11	22.6 h
7.66	2.65	43.6 h
8.30	1.15	100.5 h
9.03	0.39	298 h
9.30	0.33	352 h
	Cation Mg^{2+}	
1.52	166.0	42 min
2.64	9.29	12.4 h
3.92	4.90	23.6 h
5.36	5.07	22.8 h
6.59	4.15	27.8 h
7.63	3.82	30.2 h
8.25	3.77	30.6 h
	Cation Ca^{2+}	
1.40	173.0	40 min
2.78	13.9	8.3 h
3.28	10.4	11.1 h
3.87	10.4	11.1 h
4.31	9.57	12.1 h
5.19	12.0	9.6 h
5.55	17.9	6.5 h
6.30	21.0	5.5 h
6.67	20.2	5.7 h
7.01	20.0	5.8 h
8.10	20.8	5.6 h
	Cation $(CH_3)_4N^+$ (0.1 M Pyridine)	
8.62	0.76	150.0 h
6.81	5.01	23.0 h
	Cation $(CH_3)_4N^+$ (0.5 M Pyridine)	
8.67	0.83	140.0 h
6.86	4.87	23.7 h
	Cation $(CH_3)_4N^+$ (0.1 M Imidazole)	
8.59	0.72	161.4 h
	Cation $(CH_3)_4N^+$ (0.5 M Imidazole)	
8.70	0.76	152.7 h

[a] $ATPH_2^{2-}[(CH_3)_4N^+]_2$, $MgATPH_2$, and $CaATPH_2$ as starting materials. Buffer contained $(CH_3)_4N^+$ ions exclusively. [b] 1 N HCl.

HCl) is much faster (by a factor of about 4200) than the hydrolysis at pH 9.30 in water. (2) Hydrolysis in the region where the trianion is expected to predominate is faster by factors of about 18 and 12 in water and acetonitrile–water, respectively, as compared to hydrolysis in the region where tetraanion should exist almost exclusively. The faster rate is presumably due to a larger rate constant for the trianion species as well as to its higher concentration (see Discussion). It should be noted that the slope of the pH–rate curve in the pH region above 9 suggests that the curve levels off, as has been observed by previous workers.[11,15]

The shape of the pH–rate curves in the presence of metal ions is consistent with a picture in which the rate constants for the hydrolysis of the magnesium trianion complex ($MgATPH^-$) and the trianion itself ($ATPH^{3-}$) are very similar, while the rate constant for the hydrolysis of the magnesium tetraanion complex ($MgATP^{2-}$) is significantly larger than that of the tetraanion (ATP^{4-}). As a result, the curve describing the pH–rate profile for the magnesium complex levels off in the pH range of 4–8. Magnesium complexation appears to increase the rate constant for the tetraanion by about a factor of 10, without much effect on the rate constant for the trianion.

The curve describing the pH–rate profile for the calcium complex is slightly different in that it reaches a plateau in the pH region of 3–5, slopes upward, and levels off above pH 6. This is attributable to an increase in the rate constant for the tetraanion by approximately a factor of 50 upon calcium complexation, without much effect on the rate constant for the trianion.

Magnesium ions accelerate tetraanion hydrolysis in acetonitrile–water more effectively than in pure water by approximate factors of 60 vs. 10, respectively. Solubility considerations prevented the study of the calcium salt in the mixed-solvent system.

Table II also shows the effect of amines on the hydrolysis of ATP in water, in the absence of metal ions. No significant effect was observed on the hydrolysis rate in the pH range of 7.0–8.7 in the presence of up to 0.5 M pyridine or imidazole.

It should be noted that under the conditions of this investigation the hydrolysis of ATP in 0.1 N HCl and at higher pH values yields adenosine 5′-diphosphate (ADP) as the major nucleotide product during the first half-life of the reaction. Little adenosine 5′-monophosphate (AMP) is produced during this time. As the ADP concentration increases, the amount of AMP produced slowly increases. In 1 N HCl, besides the expected ADP, there is about 10% of AMP produced even during the first half-life of the reaction. In all cases, the only nucleotide-containing products detected in the reaction by means of liquid chromatography were ADP and AMP.

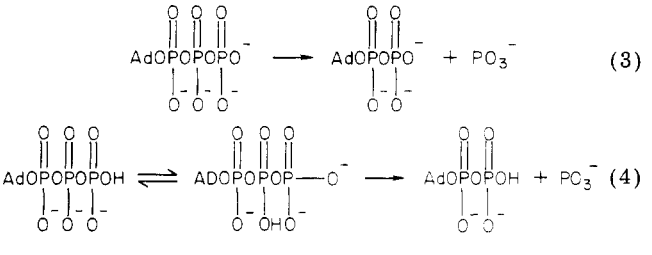
Reactions of ATP with Alcohols. The tetra-*n*-butylammonium salts of ATP are reasonably soluble in alcohols and in mixtures of acetonitrile and alcohols. The behavior of these solutions can be studied by means of ^{31}P NMR spectrometry. By this technique it is observed that a 0.1 M solution of the salt of the trianion in either 2-propanol or in a 1:1 (v/v) mixture of acetonitrile and *tert*-butyl alcohol generates, respectively, 2-propyl phosphate or *tert*-butyl phosphate, plus a mixture of ADP and AMP (eq 1 and 2). An analogous behavior is observed in a 0.1 M solution of the tetraanion.

$$ATPH^{3-}M^+_3 + ROH \rightarrow ROPO_3H^-M^+ + ADPH^{2-}M^+_2 \quad (1)$$

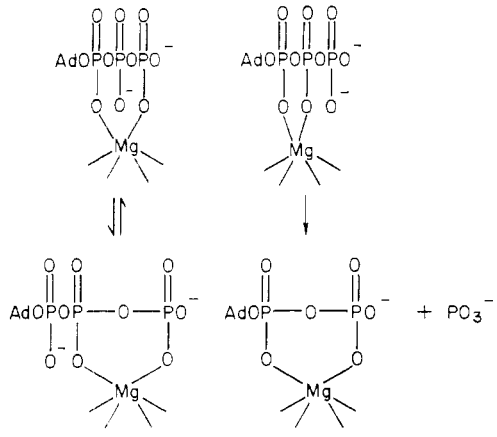
$$ADPH^{2-}M^+_2 + ROH \rightarrow ROPO_3H^-M^+ + AMPH^-M^+ \quad (2)$$

Discussion

The present data support the conclusion that the ATP tetraanion and trianion undergo nucleophilic displacements at the terminal P_γ center by an elimination–addition mechanism in which formation of the monomeric metaphosphate ion is rate limiting. A compelling argument in favor of this mechanism is the ready formation of *tert*-butyl phosphate and of 2-propyl phosphate when the reactions of the tetraanion (ATP^{4-}) and the trianion, ($ATPH^{3-}$) are carried out in the presence of the respective hindered alcohols[23–25] (eq 3 and 4).



Scheme I



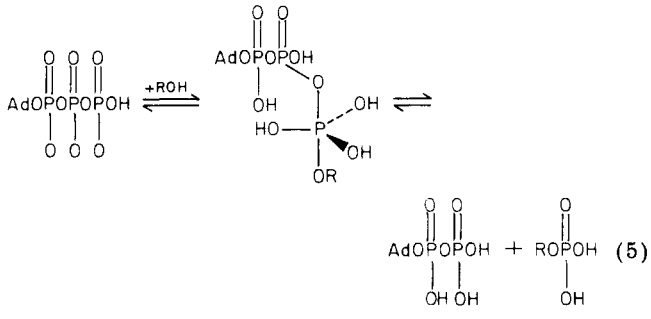
The formation of metaphosphate ion as an intermediate in displacement reactions of species XPO_3^{2-} and XPO_3H^- was suggested 25 years ago[26-30] and has received considerable support from more recent investigations.[23-25,31-33] The proton shift assumed to take place in the trianion allows the more favorable elimination of the metaphosphate anion, rather than elimination of the less stable acid PO_3H.[26,34,35] Although monomeric metaphosphate has not been directly observed in solution, its ready formation in the gas phase has recently been demonstrated by negative-ion chemical ionization mass spectrometry at ~200 °C.[36]

The elimination–addition mechanism for displacements on the ATP tetraanion also accounts for the increase in the rate of disappearance of ATP caused by magnesium ions at certain pH values. On the assumption that solutions of $MgATP^{2-}$ contain three bidentate complexes of the cation and the polyphosphate chain in relatively rapid equilibrium with each other[17] (Scheme I), the formation of PO_3^- and $MgADP^-$ from one of these complexes should be faster than the formation of PO_3^- and ADP^{3-} from the tetraanion in the absence of this cation perhaps due to negative charge neutralization by Mg^{2+}.

The magnesium ion effect is more significant in the mixed acetonitrile–water solvent than in water, as would be expected if tighter complexes are formed in the more hydrophobic medium. The absence of a significant magnesium ion effect on the rate of disappearance of the trianion complex $MgATPH^-$ vs. $ATPH^{3-}$ is understandable if the enhancement of the rate of formation of PO_3^- by magnesium is offset by interference with the proton shift that seems to be required for metaphosphate formation from the trianion. Conversely, the proton shift leading to the species that generates PO_3^- may not be affected, but the protonation could weaken the binding of magnesium to the polyphosphate chain.

The effects of calcium and magnesium ions on the ^{31}P NMR signals of ATP are very similar.[17] Therefore, the picture given above for the complexation of magnesium with ATP^{4-} can be extended to calcium. In the present hypothesis, the enhanced effect of calcium vs. magnesium on ATP^{4-} hydrolysis suggests that $CaADP^-$ is a better leaving group than $MgADP^-$ during the formation of PO_3^- from the corresponding complexes, $CaATP^{2-}$ and $MgATP^{2-}$, respectively. This could reflect a tighter binding of calcium than magnesium to the pyrophosphate oxyanions.

The large increase in ATP hydrolysis rate at pH <2 is consistent with the operation of the addition–elimination mechanism[37-39] via oxyphosphorane intermediates in displacements of the acid and the monoanion, e.g., eq 5.



The neutralization of the negative charges on the P_γ oxyanions is expected to increase the electrophilicity of this center, presumably with the participation of acid catalysis.

The P_α and P_β centers of ATP have only one ionizable proton, and the present evidence is that such phosphorus centers undergo nucleophilic displacements by the addition–elimination mechanism exclusively. Regardless of the mechanism involved in the hydrolysis, P_α attack by water should generate inorganic pyrophosphate (PP_i) and AMP, which is also the set of products formed in one of the two pathways of P_β attack by water. The second set of possible products of P_β attack are inorganic phosphate (P_i) and ADP, the products also formed in P_γ attack. The isotopic labeling studies that are required to elucidate fully the various pathways in nonenzymatic ATP hydrolysis by the addition–elimination mechanism, i.e., the type of reaction expected at pH <2, have not been described at this time. However, a partial picture can be developed from the following observations.

(a) It is known[7] that the reaction $H_2O + ATP \rightarrow ADP + P_i$ is significantly faster than the reaction $H_2O + ADP \rightarrow AMP + P_i$ in 0.1 N HCl (pH 1.5). This conclusion has been verified in the present study, which suggests a factor of about 4 for the rate advantage in ATP vs. ADP hydrolysis. We observe little AMP formation within the first half-life of the hydrolysis in water at pH 1.45. This result rules out P_α attack and one of the two pathways of P_β attack but is consistent with P_γ attack alone or with an unknown contribution from the second pathway of P_β attack.

(b) In 1 N HCl, the rates of hydrolysis of ATP and ADP become comparable. When the changes in ATP, ADP, and AMP concentrations with time are plotted, one obtains

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curves similar to those expected for consecutive reactions shown in eq 6 when $k_1 \approx k_2$. From these data, and from

$$\mathrm{ATP} \xrightarrow{+H_2O\ (k_1)} \mathrm{ADP} \xrightarrow{+H_2O\ (k_2)} \mathrm{AMP} \qquad (6)$$

the observations that a maximum of 10% of AMP is formed within the first half-life of the hydrolysis in 1 N HCl, it seems unlikely that P_α attack (and one of the P_β attack pathways) could contribute much more than 10% to the hydrolysis of ATP under these conditions of very high acidity.

In conclusion, the mechanisms of the nonenzymatic hydrolysis of unionized $ATPH_4$ and of three of its ionized species, $ATPH_3^-$, $ATPH^{3-}$, and ATP^{4-}, and the effects of magnesium and calcium ions on the rates of these reactions are reasonably well-known. However, it is still uncertain to what extent the addition–elimination and the elimination–addition mechanisms contribute to nucleophilic displacements on the dianion $ATPH_2^{2-}$. Working in water and in acetonitrile–water mixtures, we have been unable to obtain information bearing on this point.

These conclusions refer to nonenzymatic reactions of ATP in solutions and are not regarded as necessarily applicable to nucleophilic displacements on MgATP and CaATP at the enzymatic active site.

Registry No. $ATPH_2^{2-}[(CH_3)_4N^+]_2$, 75010-81-0; $MgATPH_2$, 1476-84-2; $CaATPH_2$, 71937-66-1; $ATPH_4$, 56-65-5.

Supplementary Material Available: Table III, rates of hydrolysis of ATP in acetonitrile–water; Figure 2, hydrolysis of ATP in 0.01 M acetonitrile–water at 70 °C (2 pages). Ordering information is given on any current masthead page.

Nucleophilic Character of the Alkyl Radicals. 19.[1a] Absolute Rate Constants in the Homolytic Alkylation of Protonated Heteroaromatic Bases by *n*-Butyl and *tert*-Butyl Radicals

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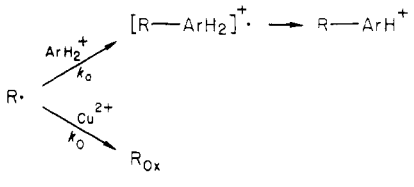
The rate constants for the homolytic alkylation of protonated heteroaromatic bases (quinoline, pyridine, and 4-cyano-, 4-acetyl-, 4-methyl-, and 4-methoxypyridine) by *n*-butyl and *tert*-butyl radicals were measured at 57 °C by competition of the aromatic addition with the alkyl radical oxidation by Cu^{2+} salts (for which the rates are known). With the more activated substrates (quinoline and 4-cyano- and 4-acetylpyridine) the *tert*-butyl radical is significantly more reactive than the *n*-butyl radical, clearly showing that polar effects are more important than steric and enthalpic effects in determining the reaction rates. The reversibility of the alkylation by the *tert*-butyl radical is discussed.

The homolytic substitutions of protonated heteroaromatic bases by nucleophilic carbon-centered radicals are among the most important aromatic substitutions from a synthetic point of view.[1b,c]

Thus, homolytic alkylation can be considered in the heterocyclic series as being as the Friedel–Crafts alkylation is in the homocyclic aromatic series, but without the complications of rearrangement and isomerization of the electrophillic process. The interest is due to the large variety of cheap sources of alkyl radicals which can be used, the good yields, the simple experimental conditions, and the high positional and substrate selectivities. These last characteristics have been ascribed to polar effects; the substrate selectivity increases in the series methyl < primary < secondary < tertiary alkyl radical, in agreement with the expected increasing nucleophilic characer,[2] and a very large ρ value of 5.5 has been determined for *tert*-butylation of 3-substituted pyridines.[3]

However, the much higher selectivity of the *tert*-butyl radical compared with the *n*-butyl radical could not reflect a polar effect. It could be determined either by a lower reactivity of the tertiary alkyl radical, due to unfavorable energetics and steric requirements, in agreement with the reactivity–selectivity relationship, or by the reversibility of the alkyl radical additions to the aromatic rings.

Scheme I



For a better understanding of the polar effect, it was therefore necessary to know the absolute rate constants in the addition of alkyl radicals to aromatic substrates. That is particularly important in view of the fact that the positive ρ values reported for hydrogen abstraction from substituted toluenes by alkyl radicals have been cast into doubt by a recent report,[4] which would exclude a significant polar effect in these reactions.

We report in this paper the absolute rate constants for the addition of *n*-butyl and *tert*-butyl radicals to some protonated heteroaromatic bases.

For primary alkyl radicals the use of the 5-hexenyl radical[5] gave good results; a similar model shows consid-

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