semiquinone form does not exist in a hydrated form as does the oxidized form. No reasonable simulations could be accomplished if one assumes hydration of $\mathbf{1}_{rad}^{3-}$.

It should be noted that in this study EPR spectra on semiquinone solutions above pH 12 exhibited less spectral resolution than those observed below pH 12. This is in contrast to the apparent high resolution of the spectrum of methoxatin semiquinone at pH 13 in 2 M salicylate.[16] Thus, as in the presence of Li^+ (loc. cit.), the presence of salicylate may alter intermolecular interactions such as semiquinone self-aggregation or aggregation of semiquinone with oxidized or hydroquinone forms. In addition, the presence of such agents probably also serves to alter the water structure. By means of a potentiometric titration, Duine et al.[7] determined an equilibrium constant of 2.54 for the comproportionation of methoxatin at pH 13 in salicylate buffer. This value exceeds our value of K_{pH} at pH 13 with H_2O/HO^- buffer (Table I) by almost 10^3.

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Supramolecular Catalysis in the Hydrolysis of ATP Facilitated by Macrocyclic Polyamines: Mechanistic Studies

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Abstract: Protonated polyaza macrocycles that bind anionic substrates can perform reactions on the bound species and provide information on the various factors contributing to catalysis. They form stable complexes with adenosine triphosphate, adenosine diphosphate, and pyrophosphate, and these supramolecular assemblies catalyze the solvolysis of the bound substrates. ^{31}P NMR was found to be a useful technique both for the kinetic analysis and for the detection of unstable intermediates formed in the reaction. The 24-membered macrocyclic polyamine, 1,4,7,13,16,19-hexaaza-10,22-dioxocyclotetradecane ($[24]N_6O_2$, **1**), catalyzed the hydrolysis of ATP at both pH 3 and 7 with calculated entropies of activation of −11 and −8.7 eu. The reaction is insensitive to ionic strength; however, both sodium and chloride ions depress the k_{obsd} at pH 7. The reaction at neutral pH is characterized by nucleophilic catalysis with the formation of the symmetrical monophosphorylated derivative **2** of macrocycle **1**; a ΔS^* of −26 eu was found for the solvolysis of **2** at pH 7. A series of related 22- to 32-membered rings containing up to 10 groups demonstrated specific structural requirements for effective catalysis of the hydrolytic reactions. Analogues of **1** wherein the oxygen atoms are either replaced by amino groups ($[24]N_8$, **4**) or removed ($[22]N_2C_4$, **6**) enhanced the rate of ATP hydrolysis at pH 3 by a factor of 300. Clear evidence for electrostatic and nucleophilic catalysis coupled with potential sites for acid and base catalysis make such macrocycles fruitful materials for the study of the mechanisms of molecular catalysis in polyphosphate hydrolysis, as well as models for the processes that may occur in enzymes utilizing ATP and related species as substrates or phosphoryl donors.

The design of molecular catalysts may provide invaluable probes for the elucidation of the origin of the efficiency and selectivity in chemical and enzymatic catalytic processes.[1,2] Supramolecular catalysis, catalysis within a supramolecular species, involves initial substrate binding by a receptor molecule bearing reactive groups, followed by transformation of the bound species and, thereafter, release of the products, so as to regenerate the catalyst for a new cycle.[3]

Mechanistic studies exploring the factors that determine the efficiency and selectivity of such catalysts are vital not only to the understanding of the elementary steps of catalytic processes but also to the future development of synthetic reagents designed for specific chemical reactions as well as to the elaboration of model systems capable of revealing factors that contribute to enzymatic catalysis.[1-4]

Two such processes of contemporary interest are studies on phosphoryl group transfer from reactive anhydrides and phosphate ester formation and cleavage in polynucleotides. Prominent in the former group are reactions utilizing the principal biological energy store: adenosine triphosphate (ATP), a stable polyvalent anion at neutral pH that is the substrate for the highly efficient group of enzymes termed ATPases. Of particular interest are the factors that contribute to the 10^{10}-fold difference between the uncatalyzed and the ATPase-catalyzed hydrolysis of ATP.

In the search for catalytic effects extensive studies on the role of the biologically important divalent calcium, manganese, and magnesium cations have been reported.[5] Cobalt(III)–amine complexes induce significant rate enhancements;[6] however, studies with other metals have not found large catalytic effects. Naturally occurring acyclic polyamines putrescine, cadaverine, spermidine, and spermine bind nucleotides[7] but are devoid of catalytic activity on ATP hydrolysis. An increase in the chain length of the

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Table I. Effects of Buffer and Salt on the Observed Rate of ATP Hydrolysis Catalyzed by $[24]N_6O_2$ (**1**) at 70 °C[a]

additions, M			pH 3.6		pH 7.6	
$[24]N_6O_2$ (**1**)	buffer[b]	salt	10^3k_{obsd}, min^{-1}	u[c]	10^3k_{obsd}, min^{-1}	u[c]
–			1.1		0.25	
+			31[d]	0.30	23	0.28
+	0.05				17	0.28
+	0.5				13	0.40
+	0.05	0.25 Me_4NBr	27[e]	0.68	13	0.78[f]
+		0.40 Me_4NBr	29	0.70	14	0.68
+	0.05	0.52 Me_4NBr	27[e]	0.83	11	0.80[g]
+	0.05	0.52 NaBr	26[e]	0.83		
+		0.80 Me_4NBr	26	1.1		
+		0.25 Me_4NBr			12	0.53
+		0.25 LiBr			10	0.53
+		0.25 NaBr			9.3	0.53
+		0.25 Me_4NCl			6.3	0.53
+		0.25 NaCl			3.0	0.53

[a] Solutions containing 0.010 M ATP, 0.015 M **1**, and added salt were examined as described in the Experimental Section. [b] The buffer at pH 7.6 was collidine. [c] Ionic strength was determined with the presumption that both ATP and **1** represented point charges and ignoring complex formation. At pH 3.6 ATP^{3-} and $[24]N_6O_2{\cdot}5H^{5+}$ and at pH 7.6 ATP^{4-} and $[24]N_6O_2{-}4H^{4+}$ were used in the calculations. [d] The same observed rate was determined when the macrocycle concentration was 0.010 M. [e] Piperidine hydrochloride (0.05 M) was present in the solution. [f] The salt concentration at pH 7.6 was 0.30 M. [g] The salt concentration at pH 7.6 was 0.50 M.

polyamines to the pentaethylenehexamine does afford a small rate enhancement.[8]

Supramolecular systems are organized molecular assemblies resulting from the binding of a substrate by a receptor molecule.[3] Polyammonium macrocyclic and polymacrocyclic cations, effective probes for the study of anion coordination chemistry, have been shown to bind selectively a variety of inorganic and organic anions with high affinity,[9] thus forming supramolecular species. The structural features of these anion receptor molecules such as cavity shape and size and number and relative location of neutral or charged coordination sites, as well as nucleophilic or electrophilic groups, can be specifically designed in a ligand so as to fulfill the requirements for selective anion binding, catalysis, or transport.[3,9,10] In a recent study employing polyammonium macrocycles as anion receptor molecules, it was found that the 24-membered ditopic[10] macrocycle $[24]N_6O_2$ (**1**) strongly binds ATP by its two diethylenetriamine subunits, forming a supramolecular complex, and provides a 100-fold rate enhancement in its hydrolysis (Scheme I).[11] This report extends the previous studies and examines the contributions to rate enhancement provided by complex formation, electrostatic catalysis, and nucleophilic catalysis in this model reaction. The results provided guidelines to the design of molecules possessing a specific arrangement of binding and transformation sites that may offer further rate enhancement.

Scheme I. Macrocycle $[24]N_6O_2$ (**1**) Catalyzed Hydrolysis of ATP to ADP

$$\text{ATP} + \underset{\mathbf{1}}{[24]N_6O_2} \xrightarrow{H^+} \text{ATP}{\cdot}\mathbf{1} \rightarrow \text{ADP}{\cdot}\mathbf{1} + \text{phosphate}$$

Results

The observed rate of ATP hydrolysis at pH 3.6 with a 1:1.5 or 1:1 molar ratio of ATP·$[24]N_6O_2$ (**1**) was 0.031 min^{-1} at 70 °C (Table I). At pH 3.6 the presence of various salts at concentrations ranging between 0.25 and 0.8 M did not change the observed rate of the reaction. However, at pH 7.6 the addition of buffer (collidine) depressed the rate of ATP loss. Although ionic strength did not affect the reaction, specific cation and anion effects were observed. From the results in Table I it is apparent that both sodium ion and chloride ion depressed the rate; the addition of 0.25 M of either of these ions decreased the rate by approximately 25% and 50%, respectively.

Examination of the reaction by varying the ratio of ligand **1**·ATP at pH 3.6 and 70 °C showed a rate saturation effect. For example, at a 1:1 or 1.5:1 ratio a k_{obsd} of 0.031 min^{-1} was calculated. Using a 5 M excess of ligand resulted in a rate of 0.035 min^{-1}, a value not significantly different from that of the equimolar ratio reaction. When the ratio was reversed to a 5-fold excess of ATP, a zero-order reaction was observed for the major part of the reaction. The calculated zero-order rate was 5.8×10^{-5} M L^{-1} min^{-1}; presuming that the maximum concentration of ATP·**1** complex was 0.02 M, the calculated first-order k_{obsd} was 0.029 min^{-1}, essentially the same as that observed with an equimolar ratio.

Evidence for a covalent intermediate arising from a nucleophilic reaction contributing to the overall ATP breakdown had been observed by using ^{31}P NMR at neutral pH.[11] The same intermediate resulting from this reaction also was generated from the reaction of acetyl phosphate and $[24]N_6O_2$ (**1**), and it was shown to be the symmetrical monophosphoramidate **2** by ^{31}P and ^1H NMR experiments.[12] This intermediate may be formed by nucleophilic attack of a central amino group of **1** on the phosphoryl donor. More recent studies monitoring the reaction of ATP and **1** by using ^{31}P NMR showed that the intermediate **2** was the initial product at pH 7.6. The appearance of **2** was concurrent with the formation of ADP and preceded inorganic phosphate formation. The concentration of **2** during the reaction approached a maximum of about 12% of the ATP concentration and diminished rapidly as ATP levels went to zero, indicating that the rate of hydrolysis of the phosphoramidate **2** was faster than its rate of formation from ATP. This point was examined by the preparation of **2** at 40 °C using acetyl phosphate as the phosphoryl donor.[12] When the acetyl phosphate was exhausted, the temperature of the reaction was raised to 70 °C and the time-dependent loss of **2** over several half-lives was observed by ^{31}P NMR. A k_{obsd} of 0.049 min^{-1} was calculated for the breakdown of **2** to give **1** and both pyrophosphate and phosphate. This is 3 times faster than the observed rate of ATP loss that results in the formation of **2**. A similar comparison was made at lower pH. The k_{obsd} for ATP loss catalyzed by **1** at pH 3.6 was 0.000 13 min^{-1} at 22 °C, whereas the k_{obsd} for the hydrolysis of **2** (generated at pH 7) under the

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Table II. Thermodynamic Activation Parameters at 60 °C for the Hydrolysis of 0.010 M ATP and 0.030 M Acetyl Phosphate Catalyzed by Equimolar Concentrations of Macrocycle $[24]N_6O_2$ (**1**) and for the Hydrolysis of the Phosphorylated Macrocycle **2**[a]

substrate (concn)	u	pH	temp, °C	10^3k_{obsd}, min^{-1}	E_a[b]	ΔH^*[b]	ΔG^*[b]	ΔS^*, eu
ATP (0.010 M)	0.30	3.6	21	0.12				
			50	3.7				
			55	6.5				
			60	11	22 300	21 600	25 300	−11
			70	29				
ATP (0.010 M)	0.28	7.6	50	1.8				
			60	6.6	23 400	22 700	25 600	−8.7
			70	15				
acetyl phosphate (0.030 M)	0.48	7.0	40	35				
			60	221	15 700	15 000	23 300	−24.9
			84	840				
phosphoramidate **2** (0.010 M)	0.48	7.0	40	13				
			60	62	16 000	15 300	24 100	−26.4
			84	300				

[a] The activation parameters were calculated from the following relationships: $E_a = \Delta H^* - RT$, $\Delta G^* = -RT \ln kh/Tk_b$, and $\Delta G^* = \Delta H^* - T\Delta S$ where h is Planck's constant, k_b is Boltzmann's constant, and k is the observed rate in s^{-1} for the reaction at a given temperature T. [b] Cal mol^{-1}.

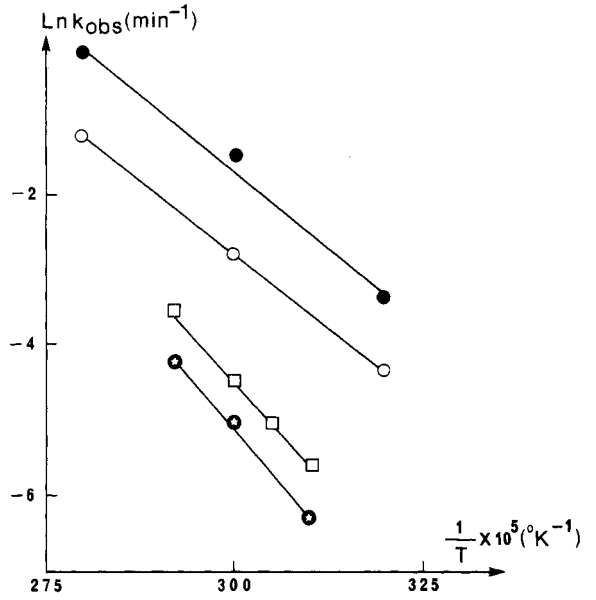


Figure 1. Effect of temperature on the k_{obsd} for the hydrolysis of ATP and acetyl phosphate catalyzed by $[24]N_6O_2$ (**1**) and on the k_{obsd} for the hydrolysis of the 4-phosphoryl derivatives of **1** (**2**); ATP pH 7.6 (☆); ATP pH 3.6 (□); acetyl phosphate pH 7.0 (●); phosphoramidate **2** pH 7 (○).

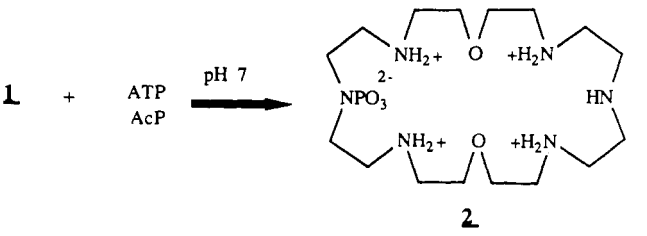
same conditions (pH ~3) was 0.043 min^{-1}, i.e., over 300 times faster.

The effect of temperature on the catalyzed reaction was examined with ATP and macrocycle **1** at pH 3.6 and 7.6 (Figure 1) and gave energies of activation (E_a) of 22.3 and 23.4 kcal mol^{-1}, respectively (Table II). The calculated entropies of activation (ΔS^*) were −11 eu at the lower pH and −8.7 eu at pH 7.6. The same study employing acetyl phosphate and **1** at pH 7 gave an E_a of 15.7 kcal mol^{-1} for acetyl phosphate loss, considerably less than the value obtained for ATP. At the same pH the E_a for the hydrolysis of **2** was 16 kcal mol^{-1}. The free energies of activation (ΔG^*) for all four reactions were in the range 23–26 kcal mol^{-1}. However, both the enthalpy and entropy of activation (ΔH^*, ΔS^*) for the acetyl phosphate and phosphoramide **2** hydrolysis were significantly different from those for ATP hydrolysis.

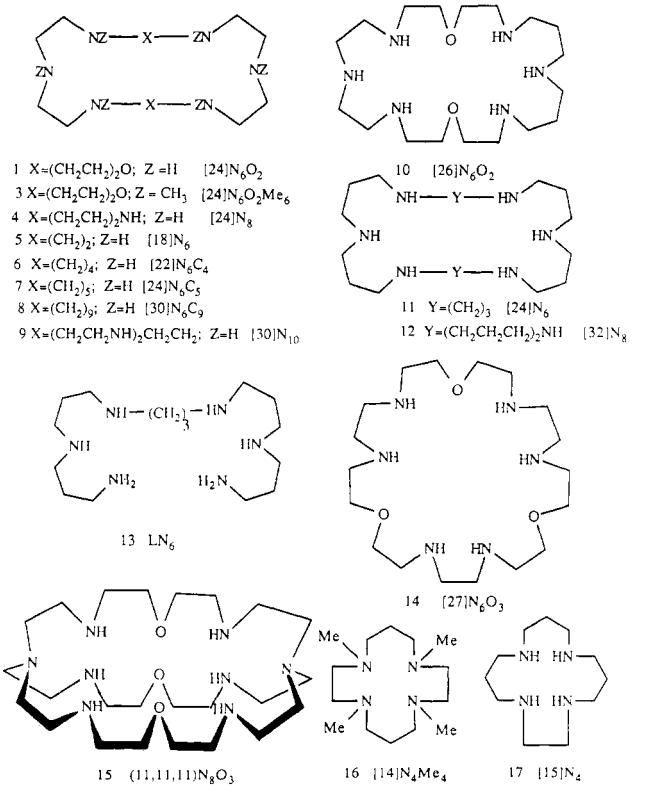
A series of analogues of $[24]N_6O_2$ (**1**) also were examined for catalytic activity (Scheme III). An equimolar ratio of these macrocycles and ATP, ADP, and pyrophosphate were heated to 80 °C, and the loss of substrate was followed by ^{31}P NMR (Table III), giving first-order kinetics in all cases (Figure 2). All of these macrocycles catalyzed ATP hydrolysis at pH 7; however, only compounds **4** and **6** were more effective than **1**. Compounds **4**, **6**, and **7** were more efficient at pH 3 than at pH 7.

All of the compounds examined showed catalytic effects on ADP hydrolysis to give AMP and inorganic phosphate. The only

Scheme II. Phosphoramidate Intermediate **2** Formed in the $[24]N_6O_2$-Catalyzed Hydrolysis of ATP or Acetyl Phosphate (AcP) at Neutral pH



Scheme III. Acyclic, Macrocyclic, and Macrobicyclic Polyamines



unique feature of this reaction was the fact that the rate of the reaction using compound **4** was the inverse of all other studies; the rate at neutral pH exceeded that at pH 3. Pyrophosphate hydrolysis also was catalyzed by the macrocycles examined. Furthermore, enhanced rates compared to **1** were obtained except for compounds **6** at pH 7 and **7** at pH 3.

ATP hydrolysis at pH 7 in the presence of the analogues of **1** also was monitored by ^{31}P NMR for an intermediate similar

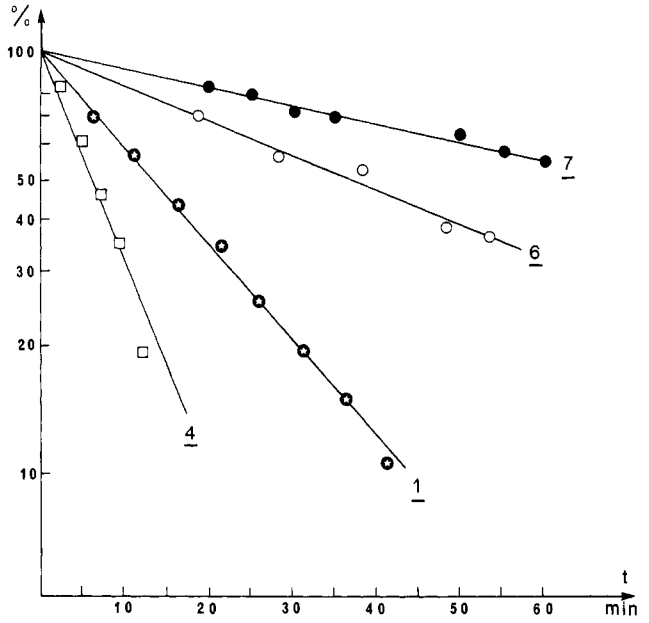


Figure 2. Semilog plot of the percentage of remaining ATP vs. time (min) at 80 °C and pH 7.0 in the presence of equimolar concentrations of macrocycles: [24]N_6C_5, **7** (●); [22]N_6C_4, **6** (○); [24]N_6O_2, **1** (☆); [24]N_8, **4** (□).

Table III. First-Order Rate Constants for the Hydrolysis of 0.030 M ATP, ADP, and Pyrophosphate in the Presence of 0.030 M Macrocyclic Polyamine at 80 °C[a]

macrocyclic polyamine	pH	10^3k_{obsd}, min^{-1} ATP	ADP	pyrophosphate
none	3.6	1[b]	9.3[c]	0.6[d]
	7.6	0.25[e]	7.2[c]	0.2[d]
[24]N_6O_2 (**1**)	3.5	70	32	9.7
	7.0	60 (23)[e]	22[f]	2.2
[24]N_8 (**4**)	3.0	310	20	30
	7.0	120	35	9.6
[22]N_6C_4 (**6**)	3.0	290	40	28
	7.0	22	17	1.2
[24]N_6C_5 (**7**)	3.0	39	3.5	3.9
	7.0	10	4.7	
[30]N_{10} (**9**)	3.0			
	7.0	64		3.0
[26]N_6O_2 (**10**)	3.0			16
	7.0	35		6.3

[a] The macrocyclic polyamine as the hexa-, octa-, or decahydrochloride salt and substrate were dissolved in 10% D_2O/H_2O (1 mL). [b] Value given is for 70 °C and u = 0.2: Ramirez, F.; Marecek, J. F.; Szamosi, J. *J. Org. Chem.* **1980**, *45*, 4748–4752. [c] Values given are pH 3.38 and 7.17 at 95 °C, u = 0.05: Miller, D. L.; Westheimer, F. H. *J. Am. Chem. Soc.* **1966**, *88*, 1507–1511. [d] From the data of Turyn, D.; Baumgartner, E.; Fernandez-Prini, R. *Biophys. Chem.* **1974**, *2*, 269–272. [e] Observed rate at 70 °C. [f] pH 7.5.

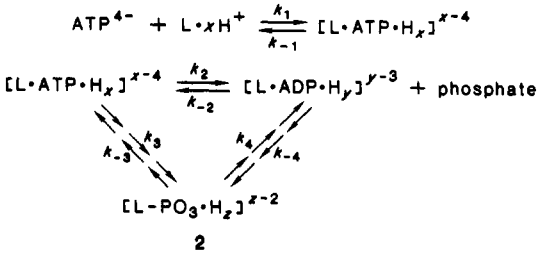
to the phosphoramidate **2**. Only the nitrogen analogue **4** showed a peak at +10 ppm indicative of a phosphoramidate, which never exceeded 5% of the total ATP present during the reaction.

Discussion

The catalysis of ATP hydrolysis by macrocyclic polyammonium receptors resulted in a 100-fold rate enhancement.[11] When the macrocycle [24]N_6O_2 (**1**) was used, two main features were identified that could contribute to this catalytic effect. Firstly, formation of the 1:1 complex ATP·L (Scheme I), was verified by titrimetric analysis and NMR studies.[11] Secondly, both ^{31}P NMR and synthetic results supported the intermediacy of the phosphorylated macrocycle **2** in this reaction at neutral pH (Scheme II).[11,12]

A mechanism for this reaction is outlined in Scheme IV. The major reaction pathway is initiated by formation of the reversible supramolecular 1:1 complex of ATP and [24]N_6O_2 (**1**). The distribution curve calculated from titrimetric analysis showed the

Scheme IV. General Mechanism of Polyammonium Macrocycle Catalyzed Hydrolysis of ATP



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predominant species at pH 3.5 to be $[L{\cdot}ATP{\cdot}H_7]^{3+}$ and $[L{\cdot}ATP{\cdot}H_4]$ at pH 7.5. The stability constants (K_s) for formation of these species were 7×10^7 and 6.3×10^4 M, respectively.[11]

If the observed rate of ATP loss is dependent on the concentration of the L–ATP complex (Scheme IV), then the addition of excess ligand should neither greatly affect the concentration of the complex nor substantially change the rate. At a 1:1, 1.5:1, and a 5:1 ratio of **1**·ATP at 70 °C at pH 3.6 the respective k_{obsd}'s were 0.031, 0.031, and 0.035 min^{-1}, all within experimenal error. Verification of zero-order kinetics when an excess of ATP is used also would confirm the formation of the complex and establish that the reaction is first-order in the concentration of the complex. The calculated first-order rate from the zero-order reaction with a 1:5 ratio of **1** to ATP was the same as that observed with a 1:1 ratio.

pH Dependence. The observed rate constants for ATP hydrolysis in the *lower pH domain* were relatively unaffected by either buffer (excess macrocycle) or ionic strength; a 5-fold increase in the macrocycle at pH 3.6 did not alter the rate. Similarly, both 0.25 and 0.8 M added tetramethylammonium bromide gave the same k_{obsd} at this pH. The absence of a buffer or ionic strength effect in uncatalyzed di- and triphosphate hydrolysis has been noted.[13]

The generally accepted addition–elimination mechanism for ATP hydrolysis at low pH is initiated by nucleophilic attack of water, principally on the γ-phosphate, to form the pentacovalent oxyphosphorane intermediate which leads to the products by elimination of ADP or AMP.[14,15] The uncatalyzed hydrolysis at higher pH is formulated as the elimination of ADP to give metaphosphate, which reacts rapidly with water to give inorganic phosphate.[13,16–18]

The pH–rate profile for the hydrolysis of ATP in the presence of **1** was relatively constant from pH 2.5 to 8.5.[11] There is no compelling reason to think that the mechanism of ATP hydrolysis in the complex at pH 3.5 differs from the accepted addition–elimination mechanism. This is formulated in Scheme IV as the overall conversion of L·ATP to the ultimate products L·ADP and phosphate. The reaction at lower pH values has an observed rate equal to k_2 which encompasses several steps: (1) oxyphosphorane formation via nucleophilic attack by water on the terminal phosphate, facilitated by electrostatic interactions of the developing pentacovalent oxyphosphorane anion with the protonated macrocycle; (2) elimination of ADP from the intermediate, which also could be acid catalyzed by the same proton source, rendering ADP a better leaving group; and (3) rapid equilibration of the ligand–phosphate complex to give the more stable ADP·**1** complex, which will occur since the latter complex is more stable than the former. Therefore, the proposed minimal mechanism for $[L{\cdot}ATP{\cdot}H_7]^{3+}$ breakdown at lower pH as represented by k_2 includes

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these three steps with either of the first two being rate limiting. The same mechanism could be dominant in the pH range 2.5–6.5 because the principal complex is that of the fully protonated receptor.

Around *neutral pH*, the $[L{\cdot}ATP{\cdot}H_5]^+$ species is predominant. Since it possesses both a nucleophilic site, the free amine function, and protonated sites, it may well be an efficient contributor to ATP cleavage by both acid and nucleophilic catalysis pathways. In fact, $[L{\cdot}ATP{\cdot}H_5]^+$ might be the most efficient species for phosphoryl transfer to the nucleophilic site.

The neutral complex $[L{\cdot}ATP{\cdot}H_4]$ is the principal species present in the *pH range 7–9*. At pH 7.6 ionic effects expressed as a rate decrease were evident. From the preliminary studies it was unclear as to whether this decrease was a result of ionic strength or a specific ion(s) effect. The addition of 0.05 M and then 0.5 M buffer (collidine) gave successive decreases of 25% of the k_{obsd}. However, further increases in salt concentration from 0.4 to 0.8 M did not significantly affect the rate if tetramethylammonium bromide was used. On the other hand, both added sodium ion and chloride ion decreased the k_{obsd} by 25% and 50%, respectively. The same type of effect was found in an electrochemical study of $M(CN)_6^{n-}$ binding by macrocycle **11**, where explanation in terms of competitive binding of chloride and potassium ions was proposed.[19]

Reactions of the Phosphorylated Macrocycle Intermediate. The most interesting observation made between pH 7 and 8.5 with the macrocycle **1** was the formation of an intermediate phosphorylated macrocycle (**2**, Scheme II). The profile of the reaction showed rapid formation of **2** to a maximum level and a gradual decrease in concentration as ATP levels fell. Structure **2** has been confirmed.[12] This reaction is shown in Scheme IV with an overall rate k_3 for formation of **2** and k_4 for its decomposition. Three routes for the formation of **2** can be considered. The first is the *formation of metaphosphate* in the neutral complex $[\mathbf{1}{\cdot}ATP{\cdot}H_4]$ concurrent with the elimination of ADP. Subsequent nucleophilic attack by the central uncharged amino group of **1** in the complex on the metaphosphate would produce **2**. Uncatalyzed phosphate ester[13] and ATP[14,18] hydrolysis at higher pH are believed to proceed in this manner. Arguments against this as the operative mechanism describing k_3 can be made on the following points. First, elimination of metaphosphate is initiated from the oxyanion of ATP; the latter would be neutralized in the complex with the tetraprotonated macrocycle at this pH. Second, the progress of the catalyzed reaction shows the phosphoramide **2** as the initial product that precedes the appearance of phosphate. If metaphosphate were formed, it is reasonable to expect some diffusion to occur and the appearance of some phosphate as the initial product.

The second mechanism possible for pathway k_3 involves *nucleophilic attack* of the central, uncharged amino group of the macrocycle on the terminal phosphate of ATP in the complex. This is appealing primarily because both the initial reactants and the resultant pentacovalent intermediate reside in an overall neutral or +1 complex. Furthermore, anion return from the oxygen to eliminate ADP could be subject to acid catalysis involving an adjacent protonated amino group in the macrocycle. The fact that the pH–rate profile is relatively constant throughout the range 3–8.5 is encouraging in this regard.

A third possibility is intermediate between these discrete routes as described for the amine-catalyzed hydrolysis of phosphate esters.[20] In this case a concerted mechanism also would be accelerated by acid catalysis in the neutral or +1 complex.

Given the finding that a phosphoramidate intermediate is involved in the reaction at neutral pH, the question arises as to the possibility that this pathway also contributes to the reaction at lower pH. For this to occur the rate of breakdown of the intermediate **2** must greatly exceed that of its formation, since **2** was not observed below a pH of 7. That phosphoramidate hydrolysis is faster at low pH is well documented.[21-27] It was found that the rate of breakdown of **2** at pH 3 was 300 times faster than the rate of ATP loss. Thus, although the intermediacy of a phosphoramidate would not be detected in the NMR analysis, it cannot be discounted. An attempt was made to trap such an intermediate using fluoride ion as the nucleophile which should react to form the fluorophosphate,[21] but no distinct ^{31}P NMR signal for this product was observed.

The final step in the mechanism at neutral pH is the *breakdown of the intermediate phosphoramidate* **2**, which is shown in Scheme IV with an overall rate constant k_4. The observed rate of hydrolysis of **2** was 3 times faster than the rate of ATP loss at pH 7. Presumably this takes place by a concerted mechanism whereby water is the attacking reagent.[25-27] However, **2** was hydrolyzed at this pH about 30 times faster than *N*-(*n*-butyl)phosphoramidate.[24] The rate of 0.042 min^{-1} determined at 55 °C is approximately that of the latter at pH 1.5.[24] If the comparison also is made at pH 3 where the rate of hydrolysis of **2** is 0.043 min^{-1} at 22 °C and that of the *n*-butylphosphoramidate is approximately 0.006 min^{-1} at 55 °C, it is apparent that a significant catalytic effect is operative in the hydrolysis of **2**. This would result from neutralization of the charge developing on the terminal phosphate in the transition state by the adjacent protonated amine sites on the macrocycle at the respective pHs. It is interesting to note also that compound **2** has been found to be an effective phosphoryl transfer agent in the formation of pyrophosphate.[12]

Reaction Mechanism and Activation Parameters. The question now arises whether the reaction at pH 7 can be attributed principally to the sequence following k_3 and k_4 in Scheme IV. Other contributions such as acid catalysis can be involved; however, the hydrolysis of **2**, being 3 times faster than ATP breakdown, is clearly not rate limiting.

When used cautiously, comparative analysis of the activation parameters has been helpful in distinguishing the major mechanisms of phosphate ester,[29] acyl phosphate,[30] and pyrophosphate[15] hydrolysis. Both ADP and pyrophosphate hydrolysis in acid show large negative entropies of activation.[15] This is interpreted as support for a binuclear reaction involving the addition of water or another nucleophile to the phosphate in the rate-determining step. In contrast, values of ΔS^* close to zero for the hydrolysis of unactivated monophosphate esters support a monomolecular elimination mechanism consistent with metaphosphate formation.

The thermodynamic parameters for the hydrolysis at pH 3.6 where the predominant complex is $[\mathbf{1}{\cdot}ATP{\cdot}H_7]^{3+}$ show an intermediate negative entropy of activation ($\Delta S^* = -11$ eu) consistent with either nucleophilic attack to give a pentacovalent oxyphoshorane or a concerted mechanism but clearly not supportive of an elimination reaction. The fact that the ΔS^* at pH 7.6 also is negative (−9 eu) could mean that the same general mechanism is operative for the neutral complex $[\mathbf{1}{\cdot}ATP{\cdot}H_4]$.

Di Sabato and Jencks[30] reported a ΔS^* of −3.6 eu for the hydrolysis of the monoanion of acetyl phosphate and a value of +3.7 eu for the dianion hydrolysis, both of which are consistent with a monomolecular elimination mechanism. Kirby and Jencks[29] also note that nucleophilic catalysis in this reaction implies a bimolecular component in the reaction. In contrast, the mechanism of acetyl phenyl phosphate hydrolysis, which cannot eliminate

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metaphosphate, has a ΔS^* of −28.8 eu.[30] In the current study the hydrolysis of acetyl phosphate catalyzed by **1** at pH 7 produced the phosphoramidate **2**.[12] Support for the proposal that a nucleophilic solvolysis reaction occurs rather than elimination–addition is derived from the large negative entropy of activation (ΔS^* = −24.9 eu) for the acetyl phosphate consumption.

A similar value (ΔS^* = −26.4 eu) was calculated for the hydrolysis of the phosphoramidate **2** at pH 7. The magnitude and sign would argue for nucleophilic attack of the reactive center by solvent through either a concerted mechanism or a pentacovalent intermediate. A more discrete interpretation of the significance of the entropy of activation for this reaction is unjustified since Chanley and Feageson[23] reported the uncatalyzed hydrolysis of the protonated and neutral species of phosphoramidic acid has ΔS^* values of −21 and −18 eu in support of a bimolecular reaction. However, phosphoramidic acid monoanion undergoes reaction with solvent, shows nucleophilic catalysis, and has a steric component contributing to the rate of hydrolysis, all of which indicate a bimolecular reaction in spite of the fact that the entropy of activation is near zero (ΔS^* = −1.6[23], −4.8 eu[21]). Benkovic and Sampson[24] favor a concerted mechanism for this reaction rather than formation of a pentacovalent intermediate.

Effect of Receptor Structure on ATP Breakdown. A macrocyclic effect was manifested by the fact that the macrocycle **11** was 14 times more active than the linear analogue **13** in catalyzing ATP hydrolysis.[11] Furthermore, the formation of a complex between ATP and a macrocycle does not assure catalysis.[11] Comparing macrocycle **1** to the macrocycle [32]N_8 (**12**), which shows a 2:1 complex of ATP:**12**, and the macrobicycle bistren (**15**), a 500- and a 70-fold difference for ATP hydrolysis in favor of macrocycle **1** raises two points that address the role of receptor specificity in catalysis: (1) compound **12**, which shows 2:1 ATP:**12** stoichiometry, offers no catalytic advantage and (2) a bicyclic structure such as **15** does not confer a catalytic advantage over the corresponding monocycle **1**.

The 14- and 15-membered tetraaza macrocycles form weaker complexes with ATP[9,11] and are not efficient catalysts. The linear hexamine **13** forms strong complexes with ATP yet is 14 times less active than the corresponding cyclic analogue **11**. The ethylene-bridged analogue of **11**, [18]N_6 (**5**), is over 3 times more active. However, the concentrations of macrocycle and substrate in these studies exceeded the dissociation constants of the complexes; essentially all of the reactants were in the form of the complex.

A comparison of the effect of ring size was evaluated in the propyl-bridged series [24]N_6 (**11**) and [32]N_8 (**12**). Not only was the larger ring macrocycle 15 times less active than **11**, it actually stabilized ATP, showing a rate of hydrolysis lower than for the uncatalyzed reaction.

The hexamethyl analogue of compound **1** was examined and was found to have one-sixth the activity, and no phosphoramidate intermediate was detected. When the arrangement of heteroatoms was changed from the diethylenetriamine sequence in **1** to the ethylenediamine unit (**14**) the rate of ATP hydrolysis decreased significantly.

From the overall evaluation of these results it was clear that the hydrolysis of ATP in the presence of anion receptor molecules was under strong structural control. In order to design more active catalysts, an analysis of the structural factors is necessary. Since the receptor molecule **1** was shown to be the most efficient in the ATP hydrolysis reaction, it was chosen as the model. The macrocyclic polyamines **4** and **6**–**10** could be considered as formally derived from **1**. The octaaza macrocycle **4**, in which amino groups replace the two oxygens in **1**, affords two additional protonation sites. Substitution of both oxygens of **1** by methylene groups (compound **7**) would be expected to have a similar cavity size and perhaps a different solution conformation, but most importantly, it examined the effect of removing the dipolar oxygen sites in the corresponding position in **1**. The receptor **6**, which still possesses a ditopic structure, is a shorter analogue of both the model **1** and its methylene analogue **7**. Compound **10** is a result of an asymmetrical change wherein spacing in one of the two poles of **1** is brought about by substitution of propyl for the ethylene spacing chains between the three nitrogens. The distances between the two poles in the two receptors **1** and **10** should be similar, but they differ by the asymmetry of the charge sites and the local structure of the two poles. Compound **8** represents an increase in the distance between the two binding sites of **1** and **7** by four methylene units.

Compound **9** cannot be considered a structural analogue of the model **1**; however, it was examined to provide information on the cavity size and its effect on catalysis. It is more appropriately an analogue of **4**, which effects an increase in the ring size of **4** by six atoms (24- to 30-membered ring) by insertion of two extra ethylamine units.

Examination of the results of ATP hydrolysis at acid pH (Table III) shows that replacement of the oxygens in **1** by nitrogen (**4**) gives a 3-fold increase in rate. The additional two amino groups provide more protonation sites that could increase the rates by electrostatic or acid catalysis, which would enhance water addition and stabilize the leaving group, ADP. This agrees with the lesser activity shown by the carbon analogue of **1** and **4**, compound **7**. However, the shortened analogue derived by removing the oxygens of **1** or the amino groups of **4** (compound **6**) was 3 times more effective. Since both of the polar atoms were removed, the rate enhancement must be derived from the structural change that results from shortening the distance between the two binding sites.

Analysis of the results *in neutral media* again showed the nitrogen analogue **4** to be more efficient than **1**, most probably because of the additional sites that could provide either acid or base catalysis. Like compound **1**, this macrocycle formed a phosphoramidate intermediate that was observed in the ^{31}P NMR spectra during the reaction.

The low activity of the carbon analogue **7** at pH 3 also is reflected in the reaction of pH 7. Macrocycle **6** presents a strong loss in catalytic effect as a function of pH: while **6** was 4 times more active than **1** at pH 3, at pH 7 it was one-third as active. Changing the ethylene chain in one of the binding sites of **1** to propylene bridges (**10**), increasing the ring size of the effective catalyst **4** by the addition of two ethylenediamine units (compound **9**), or increasing the ring size of the carbon analogue **7** by four additional methylene groups (compound **8**) offered no advantage.

Compounds **4**, **6**, and **7** were examined for their *effect on ADP hydrolysis*. From the results it would appear that **4** and **6** are uniquely adapted to the catalysis of ATP hydrolysis in acid since no unusual effects were noted on ADP hydrolysis. The studies on *pyrophosphate hydrolysis* paralleled the findings for ATP and ADP hydrolysis.

Conclusions that can be drawn from the kinetic and structural studies address the factors that are probably involved in catalysis (see also ref 11). A primary feature, in analogy to enzyme catalysis, is the fact that the active macrocycles form a high-affinity complex with the substrate ATP. It has been proposed that the acid and neutral solution hydrolysis reactions follow a mechanism of addition–elimination or a displacement reaction in preference to a monomolecular elimination of metaphosphate in the first step. Five catalytic factors can contribute to the activity displayed by the present anion receptor macrocycles toward ATP hydrolysis.

1. *An entropic advantage* should be realized if the structure of the complex of macrocycle, substrate, and attacking nucleophile favors a ground state closer to the transition state. The structural factors observed in this study probably contribute to catalysis in this regard. Of interest is the difference in catalytic activity of **6** and **7**. The shortened version **6** of **1** is a very active catalyst in ATP hydrolysis in acid media, whereas replacement of the oxygens of **1** by methylene groups gives **7**, which is 2 and 6 times less active than **1** and **6**, respectively. The increased efficiency of **6** may reflect a structural effect, whereas the decreased activity of **7** may be due to the lower acidity of its ammonium sites in addition to structural factors.

2. *Electrostatic catalysis* is possible in these systems if charge neutralization in the substrate–macrocycle complex allows for

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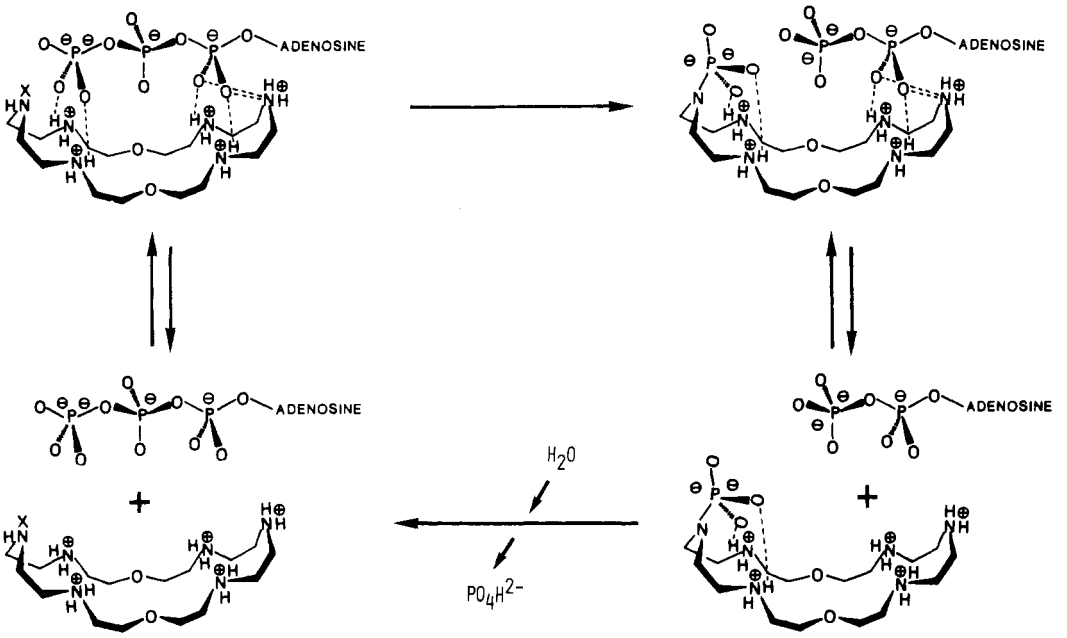

Figure 3. Schematc representation of the catalytic cycle for ATP hydrolysis by the macrocyclic receptor molecule **1** following the nucleophilic pathways. The geometry of the ATP·**1** complex and the binding scheme are hypothetical (see text and ref 11) but compatible with the structures, the dimensions, and the binding-site arrangement of the two partners.

ready approach of the attacking electron-rich nucleophile. Support for this feature comes from the enhanced hydrolysis of ATP in acid and the fact that the complexes are either positively charged or neutral in the pH range where catalysis is observed. Furthermore, electrostatic interactions are larger with the products of either acid catalysis (HPO_2^{4-}, ADP^{3-}) or nucleophilic catalysis (phosphoramidate $\mathbf{2}^{2-}$, ADP^{3-}) since they have higher total charge than the starting ATP^{4-}, at the same pH, after proton equilibration.

3. *Acid catalysis* can be envisioned if the addition–elimination mechanism is operative wherein a protonated site on the macrocycle neutralizes the developing charge on the oxygen in the pentacovalent intermediate. The activation parameters calculated for the reaction agree with such a mechanism. Furthermore, compound **4** with additional protonation sites compared to **1** is 4 times as active. Another expression of acid catalysis is to enhance the leaving-group efficacy of ADP by neutralization of the developing charge on the terminal phosphate. Compound **6** also is a likely candidate for this catalytic feature. More detailed analysis requires information about the structures of the complexes between ATP and the different protonated macrocycles, in particular about the sites of protonation.

4. *General base catalysis* by the amino group could enhance the nucleophilicity of the attacking reagent. This could be provided by the unprotonated amino groups present in all active macrocycles at pH 7.

5. *Nucleophilic catalysis* is clearly indicated by the formation of a phosphoramidate intermediate such as **2**. Although the results available are not conclusive, this may be the primary catalytic mechanism for the breakdown of ATP in its complex with **1**.

Thus, as pointed out previously,[11] facilitation of ATP breakdown by protonated macrocyclic polyamines probably occurs by a combination of *nucleophilic and acid catalysis* with *electrostatic catalysis*. The ditopic coreceptor[10] features of macrocycle **1** and its analogues may be an added advantage, since both diethylenetriamine subunits may participate in binding the ATP substrate and in cleaving it in a push–pull manner.

Again a knowledge of the structure of the supramolecular species formed between ATP and protonated **1** would be of prime importance for a deeper understanding of the process. Assuming that ATP must be bound in such a way that the central (low pK_a) amino group of the macrocyclic receptor (rather than a solvent molecule) be positioned for attack on the terminal phosphate, the catalytic cycle for the nucleophilic pathway may be schematically represented as shown in Figure 3. Such a structure for the complex is compatible with molecular dimensions and conformations. In addition to this α,γ-phosphate binding scheme, α,β-binding may also be envisaged, depending on the shape of the macrocycle (see also ref 11). Attempts at isolating and characterizing such a complex are being pursued.

Conclusion

Complementing and extending earlier work,[11] the present study has provided further insight into the catalysis of ATP hydrolysis provided by binding to the protonated macrocyclic polyamines. In particular, structural factors and activation parameters indicate that several catalytic effects contribute differentially as a function of pH. Analysis of such supramolecular processes helps to understand the factors that determine the efficiency and selectivity of catalytic reactions and to dissect the various mechanistic contributions. Of particular interest in the present case are (1) the detection of a nucleophilic pathway which involves a phosphorylated intermediate capable of transferring a phosphoryl group to an acceptor molecule,[12] (2) the operation of different catalytic contributions to the mechanism of ATP hydrolysis, and (3) the analogy to the overall transformation performed by the ATP hydrolyzing enzymes. With respect to the latter feature, the macrocyclic catalysts described here and earlier[11] may be considered as functional mimics of ATPases.[3a]

Experimental Section

Materials. The syntheses of most of the polyammonium compounds have been described. Compound **4**,[32,33] **6**, and **7**[34] were prepared by modification of published procedures wherein cesium carbonate was used in the cyclization step and hydrobromic acid in acetic acid in the presence of phenol was used in the final deprotection step. The syntheses of **8**, **9**, and **10** will be published.[35] Compounds **1**,[36] **3**,[11] **11**, **12**, **13**, **14**[37] and **15**[38] were described. Compound **5** is commercially available from Aldrich as

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its trisulfate salt and has been converted to its hexahydrochloride by using an anion-exchange resin. Compounds **16** and **17** are available from Strem Chemicals, Inc., and were converted to their hydrochloride salts. The sodium salts of ATP and ADP and the lithium-potassium salt of acetyl phosphate were obtained from Boehringer Mannheim. All other chemicals used were high-purity commercial products.

Methods. ^{31}P NMR spectra were recorded at 81 MHz on a Bruker SY 200 or at 121.42 MHz on a Varian XL300; chemical shifts in ppm are relative (+, downfield) to an external reference of 85% H_3PO_4. Probe temperature was regulated by a variable-temperature accessory. The use of low decoupler power for heteronuclear decoupling at the reported concentrations of reagents and salts in 5-mm NMR tubes did not result in apparent temperature variations. This contrasts with previous studies wherein, using a 2-mL sample in a 10-mm tube, the temperature was not accurately maintained by the variable-temperature control.[11b]

The solution pH was recorded at 22 or 25 °C with a Metrohm 636 titrimeter; adjustments to the desired pH of 1- or 2-mL samples containing the ligand and substrate were made by using ~5 NaOH or HCl. The buffer used in this study was collidine (pK_a 7.32) at pH 7.6.

Kinetic studies were performed by following the time-dependent change in the integrals from the resolved ^{31}P NMR signals of P_α, P_β, and P_γ of ATP and the peaks for inorganic phosphate, pyrophosphate, acetyl phosphate, and phosphoryl derivatives of the macrocycles **1** and **4**. Calibration curves were employed when the integral ratios were not equal because of variaions in the ^{31}P relaxation times. By this method of analysis the calculated standard deviation for the observed rates was 6%.

In a typical experiment, a 1.0- or 2.0-mL solution containing 0.010 or 0.030 M ATP, the polyamine as its hexahydrochloride or hydrobromide salts (0.010, 0.015, or 0.030 M), and, when indicated, added buffer or salts in 10% D_2O/H_2O were placed in the NMR probe in 5- or 10-mm tubes at the temperature indicated. By the use of a kinetic program an adequate number of acquisitions were accumulated for each sequential spectrum over a period of several half-lives.

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Registry No. 1, 43090-52-4; **2**, 105763-00-6; **4**, 297-11-0; **6**, 58512-71-3; **7**, 56187-15-6; **9**, 862-28-2; **10**, 105763-01-7; ATP, 56-65-5; ADP, 58-64-0; acetyl phosphate lithium potassium salt, 94249-01-1; pyrophosphate, 14000-31-8.

Diabatic Surfaces for Two-Bond Addition Reactions. The Role of Resonance Interaction

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Abstract: Diabatic surfaces for two-bond cycloaddition reactions are examined in terms of a diabatic surface analysis which includes the computation of the resonance interaction between the reactant-like and product-like diabatic surfaces. A qualitative analysis and rigorous numerical computations are presented for a concerted synchronous mechanism (a two-bond process), a concerted asynchronous mechanism (a concerted one-bond process), and the first step of a two-step mechanism (a nonconcerted one-bond process) for both "allowed" and "forbidden" processes. The results illustrate that the resonance interaction is the dominant factor which controls the mechanistic preference between two-bond and one-bond processes. For a Woodward-Hoffmann forbidden process, the magnitude of the resonance interaction is found to be much smaller for the (forbidden) synchronous process than for the one-bond process; this leads to the expected preference for the one-bond process. For a Woodward-Hoffmann allowed process in the comparison of a concerted two-bond mechanism and the first step of a two-step mechanism, it is found that magnitude of the resonance interaction at the transition structure geometry can lead to a preference for the concerted process.

1. Introduction

In a recent paper, Dewar[1] has examined, qualitatively, the mechanisms of two-bond reactions by using the Evans-Polanyi[2]/Evans-Warhurst[3] diabatic surface model. As a result of this analysis, Dewar was able to derive the rule that *synchronous multibond mechanisms are normally prohibited*. Furthermore, exceptions to this rule were to be expected[1,2] when the transition state for the synchronous multibond mechanism is strongly stabilized by the *resonance interaction* between reactant-like and product-like diabatic surfaces. Dewar[1] has suggested that this situation is most likely to occur for Woodward-Hoffmann *allowed* reactions. Our objective in this paper is to examine this conjecture in some detail for the particular case of two-bond additions. We shall proceed first in a similar fashion to Dewar[1] with qualitative arguments and then present the results obtained with a quantum mechanical implementation of the same model for a few examples.

Recently we have developed[4,5] a method for the quantitative analysis of potential energy surfaces in terms of diabatic surface methods, first proposed by Evans et al.[2,3] This analysis has been applied successfully to our MC-SCF transition structure computations on the 1,2- and 1,3-sigmatropic shift in propene,[6] the cycloaddition of two ethylenes,[7,8] and the 1,3-dipolar cycloaddition

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