Supramolecular Catalysis: Polyammonium Macrocycles as Enzyme Mimics for Phosphoryl Transfer in ATP Hydrolysis‖

Mir Wais Hosseini,† Jean-Marie Lehn,*,† Kenneth C. Jones,‡ Kathleen E. Plute,‡ Kristin Bowman Mertes,‡ and Mathias P. Mertes*,§

Contribution from the Institut Le Bel, Université Louis Pasteur, 4 rue Blaise-Pascal, F-67000 Strasbourg, France, and the Departments of Chemistry and Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66044. *Received January 26, 1989*

Abstract: The catalytic activity of the polyammonium macrocycle **1** and its substituted derivatives **2-7** has been studied in the hydrolysis of ATP, with an HPLC procedure to follow the reaction course. Substituted compounds showed decreased reaction rates with respect to **1**, pointing to the important role of structural effects in the complexes formed with ATP. The effect of added metal ions and the competitive inhibition by anionic species were investigated. An enzyme kinetic analysis was performed, yielding K_m, V_{max}, and K_i values. The results obtained allow comparison of the features of ATP hydrolysis by **1** and its analogues with those typical of the corresponding enzymes, ATPases. A number of analogies may be discerned between the natural and the artificial catalytic systems.

The application of the fundamental catalytic features of biological transformations in the design of molecular catalysts is of contemporary interest not only for the elucidation of the origin of the efficiency and selectivity in enzymatic catalytic processes but also in the development of artificial enzyme-like catalysts. In this regard macrocyclic polyamines have been found to be efficient supramolecular catalysts. That is, after forming organized molecular associations resulting from the selective binding of a substrate by a receptor molecule, they transform the bound substrate into products.[1] Catalysis by these macrocycles involves (1) recognition and selectivity in the initial substrate binding by the synthetic receptor-bearing reactive groups, (2) transformation of the bound species, and (3) release of the products, to regenerate the catalyst for a new cycle.

It is recognized that macrocyclic and polymacrocyclic polyammonium cations selectively bind a variety of inorganic and organic anions with high affinity.[2] A distinct advantage of these molecules is that their structural features can be specifically designed for selective anion binding, catalysis, and/or transport.[2-4] Recently, the anion binding properties of these receptors have been extended to the study of phosphoryl group transfer from reactive anhydrides such as adenosine triphosphate (ATP). ATP is a stable polyvalent anion at neutral pH and is the substrate for the group of highly efficient enzymes termed ATPases. It was found that the 24-membered ditopic macrocycle $[24]N_6O_2$ (**1**) forms supramolecular complexes by strongly binding ATP and provides significant rate enhancement in its cleavage.[5,6] This paper extends the previous studies[5,6] and examines new analogues of **1** incorporating additional nucleophilic sites on **1** as well as the effects of metal cations on the reaction. That the sequence mimics ATPases was demonstrated by analysis of the reaction by the application of enzyme kinetics, which verified formation of a reactive complex of ATP·**1** (Scheme I).

Results

The effect of macrocycles **1-7** (Figure 1) on the solution structure of ATP was examined by measuring the chemical shift differences in the three respective ^{31}P NMR signals in the presence of the macrocycles (Table I). For compound **1** the largest chemical shift is found for P_β. With the exception of the mercaptoethyl derivative **4** in the monosubstituted series and compound **6** in the disubstituted series the same pattern is noted for all other macrocycles. However, all compounds except **3** showed a significant downfield shift for the terminal phosphate of ATP that is not observed with compound **1**.

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

$$\text{ATP} + \underset{\mathbf{1}}{[24]N_6O_2} \underset{k_{-1}}{\overset{k_1,\,H^+}{\rightleftharpoons}} \text{ATP}\cdot\mathbf{1} \xrightarrow{k_2} \text{ADP} + \mathbf{1} + \text{phosphate}$$

Table I. ^{31}P NMR Chemical Shift Differences for ATP (0.01 M) in the Presence and Absence of 0.01 M Macrocycle at 4 °C and pH 7[a]

macrocycle	chemical shift differences P_α	P_β	P_γ
(**1**) $[24]N_6O_2$[b]	0.0	+0.87	+0.15
(**2**) $[24]N_6O_2(C_2NH_2)$	−0.07	+0.71	+0.72
(**3**) $[24]N_6O_2(C_2OH)$	−0.11	+1.11	−0.09
(**4**) $[24]N_6O_2(C_2SH)$	−0.17	+0.61	+0.81
(**5**) $[24]N_6O_2(C_2NH_2)_2$	+0.16	+0.87	+0.65
(**6**) $[24]N_6O_2(C_2OH)_2$	−0.22	+0.32	+0.67
(**7**) $[24]N_6O_2(C_2SSC_2)$	−0.03	+1.43	+1.09

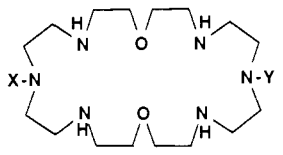
[a] The macrocycles as their hexahydrobromide or hexahydrochloride salt were dissolved in 0.5 mL of solution containing ATP, the pH was adjusted to 7 at 20 °C, and the ^{31}P NMR spectrum was determined at 4 °C unless otherwise noted. Chemical shift differences in ppm are given in relation to the chemical shifts for P_α (−10.56), P_β (−21.55), and P_γ (−6.83) of ATP at pH 7.0 in the absence of macrocycle. Plus represents a downfield shift relative to the standard. [b] Data at 25 °C: Yohannes, P. G.; Plute, K. E.; Mertes, M. P.; Mertes, K. B. *Inorg. Chem.* **1987**, *26*, 1751-1755.

The catalytic properties of a series of derivatives of $[24]N_6O_2$ (**1**) were examined for their effects on the cleavage of ATP. Loss of ATP was monitored by integration of the respective ^{31}P NMR signals assigned to ATP, ADP, AMP, the intermediate phosphoramidate, and inorganic phosphate during the course of the reaction. The results in Table II show that compound **1** at pH 4 is two to three times more effective than the monosubstituted

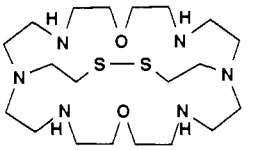
‖ Dedicated to the memory of Mathias P. Mertes.
† Institut Le Bel, Université Louis Pasteur.
‡ Department of Chemistry, University of Kansas.
§ Department of Medicinal Chemistry, University of Kansas.

(1) Lehn, J.-M. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 89-112.
(2) (a) Dietrich, B.; Hosseini, M. W.; Lehn, J.-M.; Sessions, R. B. *J. Am. Chem. Soc.* **1981**, *103*, 1282-1283. (b) Dietrich, B.; Guilhem, J.; Lehn, J.-M.; Pascard, C.; Sonveaux, E. *Helv. Chim. Acta* **1984**, *67*, 91-104, and references cited therein. (c) Peter, F.; Gross, H.; Hosseini, M. W.; Lehn, J.-M.; Sessions, R. B. *J. Chem. Soc., Chem. Commun.* **1981**, 1067-1069. (d) Kimura, E.; Sakonaka, A.; Yatsunami, T.; Kodama, M. *J. Am. Chem. Soc.* **1981**, *103*, 3041-3045. (e) Kimura, E.; Kodama, M.; Yatsunami, T. *J. Am. Chem. Soc.* **1982**, *104*, 3182-3187. (f) Hosseini, M. W.; Lehn, J.-M. *Helv. Chim. Acta* **1986**, *69*, 587-603. (g) Manfrin, N. I.; Sabbatini, N.; Moggi, L.; Balzani, V.; Hosseini, M. W.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1984**, 555-556. (h) Hosseini, M. W.; Lehn, J.-M. *Helv. Chim. Acta* **1988**, *71*, 749-756. (i) Pierre, J. L.; Baret, P. *Bull. Soc. Chim. Fr.* **1983**, *2*, 367-380.
(3) Dietrich, B.; Fyles, T. M.; Hosseini, M. W.; Lehn, J.-M.; Kaye, K. C. *J. Chem. Soc., Chem. Commun.* **1988**, 691-692.
(4) Lehn, J.-M. in *Biomimetic Chemistry*; Yoshida, Z. I.; Ise, N. Eds.; Elsevier: New York, 1983; p 163.
(5) (a) Hosseini, M. W.; Lehn, J.-M.; Mertes, M. P. *Helv. Chim. Acta* **1983**, *66*, 2454-2466; (b) *Helv. Chim. Acta* **1985**, *68*, 818.
(6) Hosseini, M. W.; Lehn, J.-M.; Maggiora, L.; Mertes, K. B.; Mertes, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 537-544.

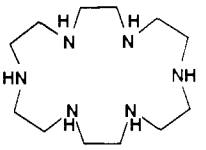
0002-7863/89/1511-6330$01.50/0



1 X = Y = H
2 X = $(CH_2)_2NH_2$; Y = H
3 X = $(CH_2)_2OH$; Y = H
4 X = $(CH_2)_2SH$; Y = H
5 X = Y = $(CH_2)_2NH_2$
6 X = Y = $(CH_2)_2OH$
9 X = H; Y = PO_3^{2-}
10 X = $(CH_2)_2NH_2$; Y = PO_3^{2-}
11 X = $(CH_2)_2OH$; Y = PO_3^{2-}
12 X = $(CH_2)_2SH$; Y = PO_3^{2-}



7



8

Figure 1. Macrocyclic polyamines **1–8** and their proposed reaction products with ATP.

Table II. Observed First-Order Rate Constants of ATP and ADP Dephosphorylation in Equimolar (0.01 M) Concentrations of Polyammonium Macrocycles **1–7** in 10% D_2O/H_2O Solution at 80 °C[a]

macrocycle	$k_{obs} \times 10^3$ (min^{-1}) ATP (pH 4.0)	ATP (pH 7.0)	ADP (pH 7.0)
(**1**) [24]N_6O_2	70[b]	72	22[b]
(**2**) [24]$N_6O_2(C_2NH_2)$	22	36[c]	7.7
(**3**) [24]$N_6O_2(C_2OH)$	34	22[c]	3.2
(**4**) [24]$N_6O_2(C_2SH)$	38	15[c]	2.7
(**5**) [24]$N_6O_2(C_2NH_2)_2$	4.2	7.2	1.4
(**6**) [24]$N_6O_2(C_2OH)_2$	7.8	4.5	1.2
(**7**) [24]$N_6O_2(C_2SSC_2)$	6.5	6.2	1.2

[a] The macrocycles as their hexahydrobromide or hydrochloride salts were dissolved in 0.5 mL of solution containing ATP, and the pH was adjusted to the desired value at 20 °C as described under Experimental Section. The reactions were followed by integration of the respective ^{31}P NMR peaks for starting materials and products on spectra obtained at various time points throughout the study over several half-lives. [b] Data for the reaction run at pH 3.6: Hosseini, M. W.; Lehn, J.-M.; Maggiora, L.; Mertes, K. B.; Mertes, M. P. *J. Am. Chem. Soc.* **1987**, *109*, 537–544. [c] ^{31}P NMR showed a chemical shift at 9.41–9.50 for the phosphorylated derivatives of these macrocycles (P–N). The maximum percentage of the total phosphate compounds, ATP, ADP, AMP, phosphate, and phosphoramidate, in the mixture as determined by the integrals represented by the phosphoramidates was 9.5% for **10**, 8% for **11**, and 5% for **12**.

aminoethyl **2**, hydroxyethyl **3**, and mercaptoethyl **4** derivatives, the latter being the most active. A reversal in the relative activity was noted at pH 7 where the aminoethyl derivative **2** was twice as active as the mercaptoethyl **4** analogue. In all three monosubstituted derivatives **2–4**, a ^{31}P NMR signal was observed in the region of +9.5 ppm during the course of the reaction at pH 7 with the maximum percentage (Table II) during the initial phase of the run. These signals are attributed to the phosphoramidates **10–12** by analogy with the corresponding derivative of **1**, compound **9**, which has been isolated and characterized previously.[7,8] A substantial drop in activity was noted for the disubstituted derivatives **5–7**, which show approximately one-tenth the activity of compound **1**. No evidence for phosphoramidate formation was noted during the course of the reaction with the disubstituted derivatives. In comparison to **1**, a more dramatic decrease in activity was noted for the hydrolysis of ADP in the presence of compounds **2–7**.

The effect of metal ions on the catalytic properties of **1** was examined with HPLC in studies employing paramagnetic ions and with both HPLC and NMR for the diamagnetic ions. Mn(II) was the most effective of the metal ions examined, affording a 2.4-fold increase in activity (Table III). Ca(II) and Mg(II) also increased the efficiency of **1** in this reaction. Cu(II), Ni(II), Co(II), Rh(III), and Pt(II) were ineffective, and Cr(III) significantly decreased the rate of ATP cleavage. Only in the studies with Cr(III) did the HPLC pattern change. After the first 15 min of reaction the ATP peak (elution time normally ~12 min) eluted at ~3 min along with AMP; the ADP peak was not affected.

Table III. Effect of Metal Ions on the Observed Rate Constants of ATP Hydrolysis by Polyammonium Macrocycle Receptor Molecules at Equimolar Concentrations (0.005 M) at pH 7 at 70 °C

metal salt	ratio of k_{obs}(metal salt added)/k_{obs}(no salt) **1**[a]	**2**	**3**	**4**	**5**	**6**	**7**
$CaBr_2$	1.4	0.72	1.5	1.5	0.60	1.6	1.6
$MnCl_2$[b]	2.4		1.4		2.0	2.3	
$MgCl_2$	1.3						
$CuCl_2$	1.0						
$NiCl_2$	0.90						
$CoCl_2$	0.60						
$RhCl_3$	0.60						
K_2PtCl_4	0.70						
$CrCl_3$	0.07						

[a] The observed rate at ATP loss for **1** in the absence of metals under these conditions with the HPLC assay was 0.020 min^{-1}. [b] The observed rate of ATP loss in the same reaction with $MnCl_2$ in the absence of macrocycle **1** was 0.0013 min^{-1}.

Analysis of the effect of Ca(II) in the hydrolysis of ATP in the substituted series showed the same relative increase in catalysis for the hydroxyethyl **3** and mercaptoethyl **4** derivatives. The activity of both the mono- and disubstituted aminoethyl derivatives **2** and **5** was decreased by the addition of Ca(II). In contrast, Mn(II) retained its rate enhancement in the presence of the disubstituted aminoethyl derivative **5**. Additional studies on Mn(II), which included the premixing of the metal and ATP prior to the addition of **1** and the use of higher ratios of Mn(II) in the assay, did not affect the rate enhancement.

An HPLC assay[9] was employed in studies on the hydrolysis of ATP at low concentrations since ^{31}P NMR kinetic analysis below 1×10^{-3} M is inaccurate for kinetic analysis. The reaction solutions contained 1×10^{-6} M macrocycle **1** and ATP in concentrations from 5×10^{-6} to 450×10^{-6} M and were examined at 70 °C at pH 7. The initial velocities (Vel) of these reactions were measured and corrected for water hydrolysis with the k_o value of 1.33×10^{-4} min^{-1} according to eq 1. The results were examined by use of the double-reciprocal plot from the Lineweaver–Burk derivation for classical enzyme kinetics in eq 2.

$$\text{Vel} = V_{obs} - k_o[\text{ATP}] \quad (1)$$

$$1/\text{Vel} = \frac{K_m}{V_{max}}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[\text{ATP}]} + \frac{1}{V_{max}} \quad (2)$$

In this derivation V_{max} is the maximal rate at saturating substrate (ATP) concentrations, and K_m is the Michaelis–Menton constant that, in the example studied, should equal $k_{-1} + k_2/k_1$ of Scheme I. The equation also accounts for the decrease in velocity expected for an added inhibitor of the catalyst **1** where [I] is the concentration of the inhibitor and K_i is the inhibition constant for the inhibitor. With compound **1**, the results of the double-reciprocal plot (Figure 2) show the expected kinetics with a V_{max} of 0.064 μmol min^{-1} for loss of ATP determined from the intercept and a K_m of 1×10^{-4} M calculated from the slope.

A similar study was carried out with the macrocycle [18]N_6 (**8**) at a concentration of 5×10^{-6} M and concentrations of ATP ranging from 20×10^{-6} to 500×10^{-6} M. The corrected velocities are listed in Table IV, and the double-reciprocal plot of these results appears in Figure 3. The calculated V_{max} of 0.070 μmol min^{-1} and K_m of 1.2×10^{-4} M were found for ATP breakdown catalyzed by macrocycle **8**.

(7) Hosseini, M. W.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1985**, 1155–1157.
(8) Hosseini, M. W.; Lehn, J.-M. *J. Am. Chem. Soc.* **1987**, *109*, 7047–7058.
(9) Blackburn, G. M.; Thatcher, G. R. J.; Hosseini, M. W.; Lehn, J.-M. *Tetrahedron Lett.* **1987**, *28*, 2779–2782.

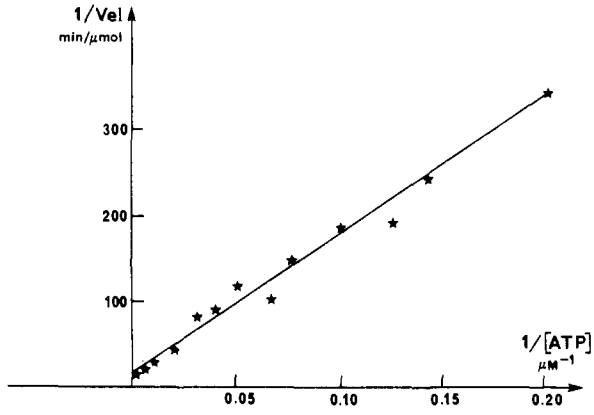


Figure 2. Double-reciprocal plot of velocity (Vel) vs substrate concentration in the reaction of excess ATP with 1×10^{-6} M $[24]N_6O_2$ (**1**) at pH 7 and at 70 °C. Velocities are corrected for water hydrolysis of the substrate ($n = 17$, $r = 0.98$).

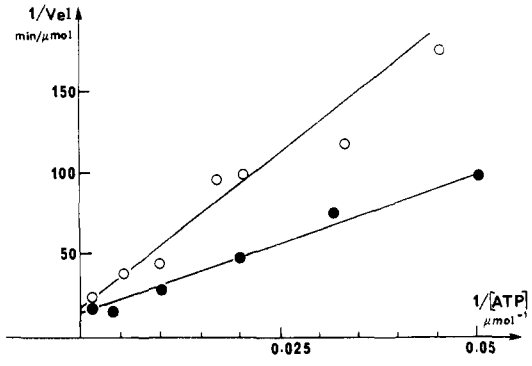


Figure 3. Double-reciprocal plot of velocity (Vel) vs substrate concentration in the reaction of excess ATP with 5×10^{-6} M $[18]N_6$ (**8**) in the absence [(●) $n = 6$, $r = 0.99$] and presence [(○) $n = 7$, $r = 0.97$] of 4×10^{-4} M pentasodium triphosphate at pH 7 and at 70 °C. Velocities are corrected for water hydrolysis of the substrate where $k_o = 0.00013$ min^{-1} with the equation $Vel = Vel_{obs} - k_o[ATP]$.

The study was extended to examine the effect of an inhibitor on the reaction. The inhibitor chosen was triphosphate anion since this polyanionic species should effectively compete with ATP for binding to the catalyst **8**. The results (Table IV, Figure 3) show a decreased velocity of ATP loss in the presence of 4×10^{-4} M triphosphate anion, the intercept yielding $V_{max} = 0.058$ μmol min^{-1}. Calculations using the slope gave an inhibitory constant K_i of 3.3×10^{-4} M.

Discussion

The results of an analysis of anion binding and the catalytic properties among a series of related polyammonium macrocycles have shown that the 24-membered hexaazadioxa macrocycle **1** has unique properties that mimic enzyme-catalyzed phosphoryl-transfer reactions.[6-9] In neutral aqueous solution the tetra- and pentaprotonated forms of compound **1** give high-affinity electrostatic complexes with the tetraanionic form of ATP.[5] The structural properties of the complex formed with **1** apparently favor nucleophilic catalysis, wherein the central neutral amino group in this ditopic receptor displaces ADP by reaction at the terminal phosphate group of the bound substrate. The resulting phosphoramidate derivative of **1** (**9**) hydrolyzes in water to regenerate the catalyst and complete the cycle (Figure 4). Control or regulation, a common feature of enzymatic reactions, also has been demonstrated wherein the addition of calcium ion to the reaction mixture leads to the formation of pyrophosphate.[10] Pyrophosphate synthesis was discovered when acetyl phosphate was reacted with macrocycle **1** in the generation of the phosphoramidate **9**.[7,8] In these reactions, it is postulated that phosphate binding at the second receptor site in the intermediate phosphoramidate **9** is followed by phosphorylation of the bound phosphate to give the product. Recently, it has been shown by positional isotope exchange studies using ^{18}O-labeled ATP that compound **1** catalyzes the backward reaction, *i.e.*, the formation of ATP from ADP and the phosphoramidate **9**, in the presence of Ca^{2+}.[11] Furthermore, in a mixture of DMSO–H_2O, compound **1** also catalyzes the formation of pyrophosphate derivatives of a series of biologically important phosphate-containing substrates. In particular, ATP is obtained from ADP and acetyl phosphate in the absence of Ca^{2+}; the reaction proceeds through the covalent intermediate **9** acting as a molecular phosphorylating reagent.[12]

Table IV. Velocity (Vel) of the Loss of Substrate in the Reaction of Excess ATP with 5×10^{-6} M $[18]N_6$ (**8**) in the Absence and Presence of Sodium Triphosphate at pH 7 and 70 °C[a]

ATP concn (μM)	Vel (μmol min^{-1})[b]	
	no additions	sodium triphosphate present (400 μM)
20	0.013	
25		0.0057
30	0.013	
33		0.0085
50	0.022	
60		0.0089
100	0.033	0.023
200		0.026
250	0.059	
500	0.056	0.042

[a] The reaction solutions were analyzed by quenching aliquots taken at various times throughout the initial course of the reaction, and the loss of substrate and formation of products were measured from the integral values for the reaction components as detected by ultraviolet absorption after HPLC separation as described under Experimental Section. [b] Velocities are corrected for water hydrolysis where $k_o = 0.00013$ min^{-1} with the equation $Vel = Vel_{obs} - k_o[ATP]$.

Studies of the reactions of **1** with acetyl phosphate[7,8] and formyl phosphate[13] revealed another unusual feature of this macrocycle. Although formyl phosphate reacted, as expected, by C–O cleavage leading to formylated macrocycle, acetyl phosphate undergoes P–O cleavage exclusively to give the same phosphoramidate derivative of **1** observed in the reaction with ATP.[7,8] These studies have been extended to model ATP-requiring enzymes that promote carboxylate activation. The addition of both calcium ion and formate to a neutral aqueous solution of **1** and ATP resulted in the formylation of the macrocycle.[14] Labeling studies support the intermediacy of formyl phosphate in the reaction which is proposed to result from the reaction of formate bound at the second receptor site with the phosphoramidate intermediate in a supramolecular assembly.

Catalytic Activity of Analogues of 1. A new series of derivatives of **1** was designed to explore the effect of introducing additional nucleophilic sites, common in catalytically active proteins, on the catalytic properties of the receptors.[15] The three functional groups chosen, amino, hydroxy, and thiol, are analogous to the amino acid residues lysine, serine, and cysteine. Features of interest in the design of these modifications of **1** are enhanced nucleophilic catalysis, additional binding sites that would favor metal ion catalysis, and phosphoryl-transfer reactions that would mimic protein kinases.

Studies of the binding of ATP by these macrocycles are based on the ^{31}P NMR chemical shift changes in ATP induced by complex formation and comparison of the observed shifts with

(10) (a) Yohannes, P. G.; Mertes, M. P.; Mertes, K. B. *J. Am. Chem. Soc.* **1985**, *107*, 8288–8289; (b) Yohannes, P. G.; Plute, K. E.; Mertes, M. P.; Mertes, K. B. *Inorg. Chem.* **1987**, *26*, 1751–1755.

(11) Bethell, R. C.; Lowe, G.; Hosseini, M. W.; Lehn, J.-M. *Bioorg. Chem.*, in press.

(12) Hosseini, M. W.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.* **1988**, 397–399.

(13) (a) Jiang, Z.; Chalabi, P.; Mertes, K. B.; Jahansouz, H.; Himes, R. H.; Mertes, M. P. *Bioorg. Chem.*, in press. (b) Jahansouz, H.; Mertes, K. B.; Mertes, M. P.; Himes, R. H. *Bioorg. Chem.*, in press.

(14) Jahansouz, H.; Jiang, Z.; Himes, R. H.; Mertes, M. P.; Mertes, K. B. *J. Am. Chem. Soc.* **1989**, *111*, 1409–1413.

(15) Hosseini, M. W.; Lehn, J.-M.; Duff, S. R.; Gu, K.; Mertes, M. P. *J. Org. Chem.* **1987**, *52*, 1662–1666.

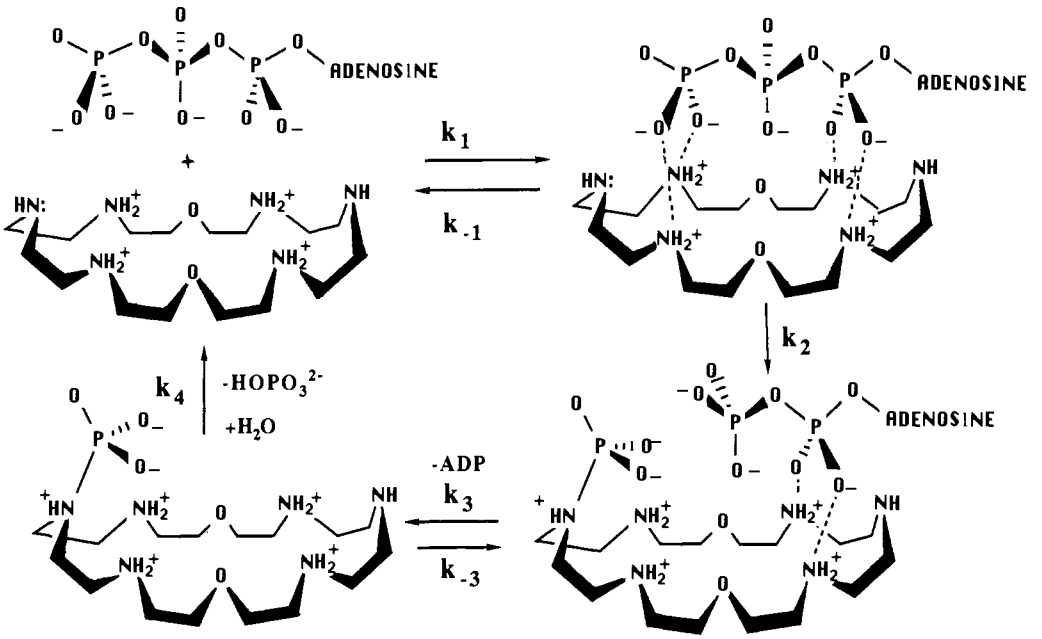


Figure 4. Schematic representation of the sequence of reactions for ATP dephosphorylation catalyzed by macrocycle **1** (k_3 and $k_4 > k_2$). The geometries of the complexes are hypothetical but compatible with potential structures of the species involved.

those found with **1**.[16] Chemical shifts in such systems are sensitive to both the O–P–O bond angles and the torsional angles in phosphate esters and anhydrides.[17] Alteration of the solution structure of ATP by the addition of **1** causes a dramatic shift on the P_β and minimal effects on the P_α and P_γ signals (Table I). This P_β shift also is observed in the entire series, compounds **2–7**. However, the monothiol derivative **4**, the monoamino derivative **2**, and the three disubstituted derivatives **5–7** all show additional alteration in the structure of ATP as reflected by the P_γ shift.

Analysis of the catalytic properties of these macrocycles (Table II) at pH 4 and 7 where the penta- and tetraprotonated species of **1** predominate[5,16] shows that the monosubstituted derivatives retain significant catalytic efficiency. The aminoethyl macrocycle **2** is more active at pH 7 than at pH 4. In all three monosubstituted derivatives **2–4** clear evidence for the presence of the intermediate phosphorylated macrocycle was obtained from the ^{31}P NMR resonance at 9.5 ppm. The percentage of total phosphorus-containing products represented by this P–N resonance was maximal during the early course of the reaction and varied from 9.5% for **2** to 5% for the reaction with **4**. Therefore, the monosubstituted macrocycles follow more or less the same reaction course as **1** in that nucleophilic catalysis is a prominent feature of the reaction. Although the catalytic efficiency of these analogues is reduced (compound **2** is half as active as **1**), the number of reaction sites also is reduced if the primary nucleophile is the central secondary amine. Given this consideration, both the aminoethyl and hydroxyethyl derivatives retain essentially the same activity as **1**. The same type of behavior was observed for another monosubstituted derivative of **1** bearing a side arm attached to an acridine moiety. In addition, although the efficiency of this compound in both ADP and ATP reactions was reduced, a better selectivity of catalysis for ATP over ADP was observed.[18]

Although the disubstituted derivatives promote ATP breakdown 40–70 times faster than water hydrolysis, they have one-tenth the activity of **1** at both pH 4 and pH 7. The central (unprotonated) secondary amino group clearly is a reactive nucleophilic site in compounds **1–4**. The chemical shifts induced in the ^{31}P NMR signals of ATP by **1** suggest that the structure of the triphosphate portion of the molecule has undergone significant alteration of the O–P_β–O angle. All three of the monosubstituted derivatives share this property. However, the least catalytically active member of the monosubstituted derivatives, compound **4**, has a significant structural effect at both the central and the terminal phosphate that apparently does not favor catalysis.

While the monosubstituted derivatives retain one recognized reaction site analogous to **1**, all of the disubstituted derivatives have tertiary amines in these positions. Tertiary amines such as trimethylamine do react with acetyl phosphate by P–O cleavage; however, the product is unstable.[19] Although steric factors in the disubstituted analogues may alter the structure to a less active form, the reaction of the central tertiary amine with the terminal phosphate of ATP leading to a trialkyl phosphoramidate would be unlikely because of steric hindrance, the substantially altered overall charge on the resulting complex, and the instability of the expected product. Therefore, the low catalytic activity of the disubstituted analogues **5–7** confirms the importance of the central secondary amino group in **1** and in the monosubstituted derivatives as depicted in Figure 4.

Effect of Metal Ions. The universal requirement for metal ions in enzyme-catalyzed phosphoryl-transfer reactions involving ATP prompted a study of the effects of added metal ions on the reactions of **1** with ATP. Previous results using NMR for kinetic analysis prohibited the study of paramagnetic ions. On the basis of the report by Blackburn et al.,[9] an HPLC separation was used for analysis of the time-dependent decrease in ATP concentration catalyzed by macrocycles and metal cations. Confirmation of the methodology was obtained with calcium and magnesium ions in conjunction with **1**, which had been studied previously with NMR techniques.[10] Of the metal ions studied (Table III) manganese(II) and chromium(III) ions are the exception. The former more than doubled the rate of ATP hydrolysis catalyzed by **1**, whereas chromium(III) greatly reduced the catalytic effect of **1**. Additional studies with manganese(II) involving premixing of the metal ion with ATP and the use of up to a 3-equiv excess of metal ion did not alter the observed rate increase. Extension of the studies with **1** to the effects of calcium(II) on the reaction of the analogues with ATP showed the same rate enhancement with all macrocycles with the exception of the amino derivatives **2** and **5**. The findings that the rate enhancement persisted at the same level with the remaining macrocycles and that manganese(II) retained its

(16) Hosseini, M. W.; Lehn, J.-M. *Helv. Chim. Acta* **1987**, *70*, 1312–1319.
(17) (a) Gorenstein, D. G. *J. Am. Chem. Soc.* **1975**, *97*, 898–900. (b) Gorenstein, D. G.; Kar, D. *Biochem. Biophys. Res. Commun.* **1975**, *65*, 1073–1080.
(18) Hosseini, M. W.; Blacker, A. J.; Lehn, J.-M. *J. Chem. Soc., Chem. Commun.*, **1988**, 596–598.
(19) (a) Di Sabato, G.; Jencks, W. P. *J. Am. Chem. Soc.* **1961**, *83*, 4400–4405. (b) Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* **1965**, *87*, 3199–3208.

catalytic advantage in the presence of the amino-substituted analogues suggest that the role of calcium(II) and manganese(II) is to order ATP in a more reactive structure. One alternative explanation is that these cations assist in the displacement of the leaving group, ADP, by electrostatic catalysis.[10b]

A clue to the reason for the 15-fold decrease in the reaction rate on the addition of chromium(III) was obtained from the HPLC profile of the reaction. The initial HPLC separation ($t \sim 20$ s after mixing) showed normal retention times for the three nucleotides ATP, ADP, and AMP. However, after 15 min the ATP peak now eluted with AMP. Given that the separation is charge dependent, a stable Cr(III)·ATP complex with a reduced overall charge is reasonable. Chromium(III) is recognized as being substitutionally inert, which may affect the nucleophilic attack and subsequent release of the terminal phosphate if ATP is coordinated to the chromium ion. Furthermore, this complex may be expected to associate less well with **1** and may have greatly reduced reactivity. The inertness of Cr(III)·ATP complexes is recognized in their use as chirality probes.[20]

Enzyme Kinetic Analysis. If, as previously proposed, compound **1** is a synthetic catalyst that models enzymatic reactions and in particular ATPase enzymes, the application of enzyme kinetics to the study of this reaction should provide verification and additional information on complex formation and the mechanism of the reaction. The initial velocity (Vel) of the reaction of excess substrate (ATP) with a 1 μM solution of the catalyst **1** showed steady-state kinetics. This finding confirms the preassociation of the substrate and catalyst in a reactive Michaelis-type complex. The results plotted in Figure 2 with eq 2 yield a calculated maximum velocity (V_{max}) of 0.064 μmol min^{-1}. The K_m for the reaction is 1×10^{-4} M. This value approximates the normal K_m for ATPase substrates.

The reported dissociation constant for the complex **1**·ATP is 1.6×10^{-5} M and 7.1×10^{-9} M for the tetra- and pentaprotonated forms of **1**, respectively.[5] The fraction of each complex depends on the pH and is close to 1/1 at pH 7 under the conditions used. The K_m for the reaction is not a true dissociation constant but rather a value equal to the ratio of the loss of complex by dissociation and reaction ($k_{-1} + k_2$, Scheme I) to complex formation (k_1, Scheme I). One may also note that whereas two reactive, unprotonated sites are present in the complex of ATP with **1**–4H$^+$, only one such site is left in **1**–5H$^+$.

In the same manner the 18-membered hexaaza macrocycle **8** was examined. While a catalyst promoting ATP cleavage, this compound is five times less active than compound **1**.[5,6] Given this difference, the initial velocities of the reaction of compound **8** at 5×10^{-6} M in the presence of (20–500) $\times 10^{-6}$ M ATP were measured by the HPLC procedure. The results in Table IV report the initial velocities corrected for water hydrolysis. Examination of the double-reciprocal plot in Figure 3 shows the approach to maximum velocity (intercept) at high substrate concentrations. The calculated V_{max} for the reaction (0.070 μmol min^{-1}) is approximately the same as that found for compound **1**. However, the use of 5-fold greater concentration of **8** gives a turnover number or k_{cat} of 0.014 min^{-1} (V_{max}/[**8**]), five times less than that of **1**. The calculated K_m for the reaction was 1.2×10^{-4} M, approximately the same as that found for compound **1**.

Competitive inhibition of enzymatic reactions by an analogue of the substrate that has affinity for the reaction site of the enzyme will decrease the velocity of substrate consumption according to the derivation in eq 2. Furthermore, if the binding of substrate and inhibitor occurs at the same site, the V_{max} of the inhibited and uninhibited reactions will be the same. Triphosphate anion was chosen because it should approximate the affinity of ATP binding by the catalyst and the lack of UV absorbance precludes interference with the method of analysis. The results of this study (Table IV, Figure 3) verify the expected pattern for enzyme inhibition kinetics. From eq 2 the derived V_{max} was 0.058 μmol min^{-1} for ATP loss and the K_i for triphosphate anion was 3.3×10^{-4} M. The former is reasonably close to that found for the uninhibited reaction and suggests competitive inhibition of the reaction by triphosphate anion.

A comparative analysis of the effectiveness of **1** as an ATPase mimic can be informative. Given the concentration of the catalyst, the turnover for **1** in this reaction is 0.064 min^{-1}. Allowing for reaction temperature differences, this is 10^5 times slower than the average turnover of 1000 min^{-1} for enzymes (ATPase = 3.2×10^4 min^{-1}).[21] An alternative analysis of enzymatic reactions is commonly extended to the determination of the apparent second-order rate constant, which in this case is the ratio of k_{cat}/K_m. For the reaction of compound **1** with ATP this ratio is 6.4×10^2 L mol^{-1} min^{-1}. The corresponding value for a typical ATPase with a K_m of 1×10^{-4} M and a k_{cat} of 3.2×10^4 min^{-1} is 3.2×10^8 L mol^{-1} min^{-1}, a difference of 10^6. While this difference offers considerable latitude in improving the efficiency of **1**, it is remarkable that such simple systems exert the degree of catalytic aptitude observed considering the vast differences in size and complexity of ATPase and related phosphoryl-transferring enzymes compared to **1**.

Conclusions. The results of the study of modifications of macrocycle **1** by the addition of pendant functionalities reinforce previous findings that the structural features of **1** are uniquely suited for the catalysis of phosphoryl-transfer reactions using ATP as the substrate. That the aminoethyl and hydroxyethyl derivatives of **1** (**2** and **3**) retain essentially the same activity in neutral solution confirms the previous identification of the central secondary amine as the primary reaction site. The decrease in activity of compounds **4**–**7** may be related to structural changes reflected in the ^{31}P NMR spectra of the substrate. The catalytic advantage offered by manganese(II) in the ternary reaction of **1**, ATP, and metal cation mimics that found previously for Ca(II).[10] Finally, the ability to utilize enzyme kinetics in the analysis of the chemistry of **1** and ATP indicates that these compounds can be considered as bonafide enzyme mimics.

The following observations on the properties of **1** and related macrocycles can be compared to enzymatic reactions. (1) The reactions described in this and previous studies are run in neutral aqueous media. (2) Compound **1** binds anions primarily through electrostatic interactions and hydrogen bonding. (3) The observed binding is selective and depends both on structure and on charge density. (4) The affinity constants of **1** for polyanionic species mirror those found for substrates in enzymatic reactions. (5) Metal ion catalysis is observed using common ions also present in biological transformations. (6) Regulation by the addition of a "third effector", Ca(II), alters the course of the reaction. (7) The reaction is dependent on a Michaelis-like complex of substrate and catalyst. (8) Nucleophilic or covalent along with electrostatic catalytic effects take place and model ATPase enzymes that undergo phosphorylation as an intermediate in the reaction. (9) The intermediate product of the reaction of **1** with ATP is active in the reaction with a second substrate; in the present case phosphate and carboxylate anions are viable substrates giving pyrophosphate and carboxyl phosphate, again modeling two other common enzymatic reactions. (10) The reaction is subject to competitive inhibition by substrate analogues. (11) In the absence of a second substrate, the intermediate phosphoramidate of **1** is rapidly hydrolyzed by water to regenerate the catalyst for another cycle.

Given these parallels, catalytically active polyammonium macrocycles such as those described in this work can be considered as "protoenzymes", primitive, but encouraging, attempts to mimic the catalytic features of naturally occurring enzymes by the design and synthesis of potentially useful artificial catalysts.

Experimental Section

Materials. The syntheses of the polyammonium compounds **1**[22] and **2**–**7**[15] have been described. Compound **8** is commercially available from Aldrich as its trisulfate salt and was converted to its hexahydrochloride

(20) Cleland, W. W. *Methods Enzymol.* **1982**, *87*, 159–179.

(21) Walsh, C. F. *Enzymatic Reaction Mechanism*; W. H. Freeman: San Francisco, CA, 1979; p 33, 186.

(22) Comarmond, J.; Plumerē, P.; Lehn, J.-M.; Agnus, Y.; Louis, R.; Weiss, R.; Kahn, O.; Morgenstern-Badarau, I. *J. Am. Chem. Soc.* **1982**, *104*, 6330–6340.

with an anion-exchange resin. Pentasodium polytriphosphate was a product of Sigma Chemical Co. The sodium salts of ATP and ADP were obtained from Boehringer Mannheim. All other chemicals used were high-purity commercial products.

Methods. (A) NMR Kinetic Analysis. ^{31}P NMR spectra were recorded 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.

The solution pH was recorded at 22 or 25 °C with a Radiometer pH meter; adjustments to the desired pH of 0.5-mL samples containing the ligand and substrate were made with ~5 M NaOH or HCl. 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 peaks for inorganic phosphate and the phosphoryl derivatives of the macrocycles **1–4**. Calibration curves were employed when the integral ratios were not equal because of variations 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 0.5-mL solution containing 0.010 or 0.030 M ATP and the polyamine as its hexahydrochloride or hexahydrobromide salt (0.010, 0.015, or 0.030 M) in 10% D_2O/H_2O was placed in the NMR probe in a 5-mm tube at the temperature indicated. By the use of an automated program, an adequate number of acquisitions were accumulated for each sequential spectrum over a period of several half-lives.

(B) HPLC Kinetic Analysis. A Waters Model 501 high-performance liquid chromatograph together with Waters Model 481 absorbance detector and Model 740 data analyzer was used in these studies. Samples were injected on a silica column containing amine groups (Waters Bondpak-NH_2) which, in the reverse phase of operation, gives an ion-exchange-based separation. The mobile phase was a mixture of 15% acetonitrile and 85% 0.05 M ammonium phosphate at pH 4.5.

Aqueous solutions of the substrates, macrocycles, and inhibitor if present in concentrations of 1 μM or more each of substrates at 70 °C with pH adjusted as described in the NMR studies were used. Samples were analyzed by first quenching 20-μL aliquots of the reaction mixture by addition to 40 μL of the mobile phase adjusted to pH 10.5 prior to injection. Resolution of AMP, ADP, ATP, and ATP analogues afforded integral values used in the determination of the concentrations of the individual adenine-containing species at each time point. Analysis was based on multiple samples accumulated over several half-lives with the exception of the studies using excess ATP, where initial velocities were obtained from multiple samples taken during the initial phase of the reaction.

Acknowledgment. This work was supported by a grant from the National Institute of General Medical Sciences (GM 33922) at the University of Kansas and by the Centre National de la Recherche Scientifique (CNRS) at the Université Louis Pasteur.

Stereodifferentiating Complexation of Diastereomeric Cyclic Depsipeptides by Alkali Ions

Horst Kunz* and Hans-Georg Lerchen

Contribution from the Institut für Organische Chemie, Universität Mainz, D-6500 Mainz, Federal Republic of Germany. *Received August 2, 1988*

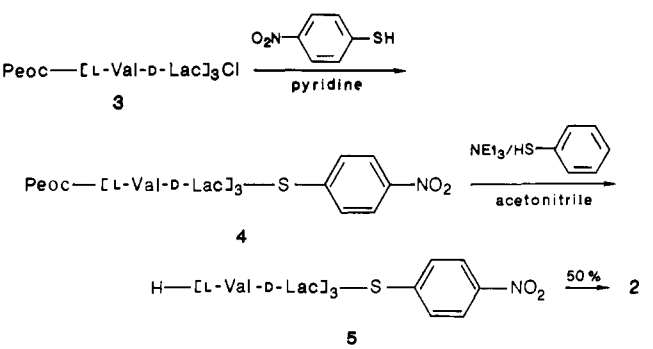
Abstract: The all-L-configurated cyclohexadepsipeptide cyclo[L-Val-L-Lac]$_3$ (**1**) and its diastereomer cyclo[L-Val-D-Lac]$_3$ (**2**) surprisingly show completely different complexing abilities toward alkali cations. As is demonstrated by ^1H and ^7Li NMR spectroscopy, **1** does not form complexes with alkali ions. In contrast, the diastereomer **2** shows marked complex formation with Li^+, Na^+, and K^+ ions. This yes–no diastereoselectivity of alkali ions toward the diastereomeric cyclodepsipeptides **1** and **2** may be of general interest for understanding selective biological ion transport and actions of ion-mediated biological catalysis.

Complexation of chiral molecules plays a decisive role in biological processes, e.g., in the construction of enzyme active sites or in the selective ion transport via chiral carriers or channels. Cyclic depsipeptides consisting of alternating sequences of α-amino acids and α-hydroxy acids, e.g., valinomycin[1] and the enniatines,[2] show strong antibiotic effects, which obviously are closely related to their complexing abilities toward cations.[3] The complexing properties of these antibiotics and of their synthetic analogues depend upon ring size and structure of the constituents and are also sensitive to configurational changes.[4]

We here report on a yes–no decision in the complexation of alkali ions by diastereomeric cyclohexadepsipeptides consisting of either L-valine and L-lactic acid (c-LL) **1** or L-valine and D-lactic acid (c-LD) **2**.

cyclo[L-Val-L-Lac]$_3$ **1** cyclo[L-Val-D-Lac]$_3$ **2**

Scheme I



The synthesis of the formerly unaccessible all-L-configurated cyclodepsipeptide **1** with exclusively non-N-methylated amino acid residues was accomplished by applying the *N*-(2-phosphonio)-ethoxycarbonyl- (Peoc-) protected linear depsipeptide chlorides.[5] Analogously, the D-lactic acid containing diastereomer **2** can more easily be cyclized starting from the Peoc-hexadepsipeptide chloride **3**. It is reacted first with *p*-nitrothiophenol in pyridine. The

(1) Brockmann, H.; Schmidt-Kastner, G. *Chem. Ber.* **1955**, *88*, 57.
(2) Plattner, P. A.; Nager, U.; Boller, A. *Helv. Chim. Acta* **1948**, *31*, 594.
(3) Shemyakin, M. M.; Ovchinnikov, Y. A.; Ivanov, V. T.; Kiriushkin, A. A.; Shdanov, G. L.; Ryabova, I. D. *Experientia* **1963**, *19*, 566.
(4) For a review, see: Ovchinnikov, Y. A.; Ivanov, V. T. In *International Review of Science: Organic Chemistry, Series 2*; Rydon, H. N., Ed.; Butterworth: London, 1976; Vol. 6, p 219.
(5) Kunz, H.; Lerchen, H.-G. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 808.

0002-7863/89/1511-6335$01.50/0