245. Efficient Molecular Catalysis of ATP-Hydrolysis by Protonated Macrocyclic Polyamines

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(4.X.83)

Summary

Molecular catalysis of ATP-hydrolysis by a number of protonated macrocyclic polyamines **1–9** has been investigated by ^{31}P-NMR spectroscopy, and marked rate enhancements have been obtained. The largest acceleration is produced by the [24]-N_6O_2 macrocycle **1**, and the process displays the following properties: *1.* protonated **1** forms very stable complexes with ATP, as well as with ADP and AMP; *2.* it enhances the rate of ATP-hydrolysis by a factor of 10^3 at pH = 8.5; the rate of hydrolysis is constant over a wide pH-range, from pH = 2.5 to 8.5; *3.* **1** is more efficient than acyclic analogues; *4.* the products of the reaction are orthophosphate (OP) and ADP, which is subsequently hydrolyzed to OP and AMP at a slower rate; *5.* at pH > 6.5, a transient species is detected, which is tentatively identified as a phosphoramidate intermediate, resulting from phosphorylation of the macrocycle **1**; *6.* the reaction presents first-order kinetics and is catalytic. The mechanism of the process is discussed in terms of initial formation of a complex between ATP and protonated **1**, followed by an intracomplex reaction which may involve a combination of nucleophilic or acid catalysis with electrostatic catalysis.

Introduction. – *Molecular catalysis* represents a major feature of the functional properties of supramolecular systems [1]. In particular, the recent development of anion receptor molecules ([2] [3] and references therein), organic ligands which bind organic and inorganic anions, opens the way to the design of molecular catalysts capable of effecting reactions on a bound anionic substrate.

Complexation and transformation of substrates of biological importance are of special interest in this respect. Notably, formation and hydrolysis of adenosine-5′-triphosphate (ATP) occur *via* highly efficient enzymatic reactions catalyzed by the ATPases and play a key role in numerous biological processes: photosynthesis phosphorylation (chloroplast ATPase), oxidative phosphorylation (mitochondrial ATPase), muscle action (myosin ATPase), active transport (Na, K-ATPase, Ca-ATPase) *etc.* There is therefore considerable interest in analyzing the controlling factors and the mechanism of

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these reactions, as well as in discovering non-biological compounds which might catalyze them.

Numerous studies of the mechanisms of ATP-hydrolysis have been performed ([4–7] and references therein). Since metal ions take part in the enzymatic hydrolysis of ATP, its metal-ion-promoted non-enzymatic hydrolysis has been extensively investigated [8–10] and significant rate enhancements have been obtained particularly with coordinated Co(III)-ions [11] [12].

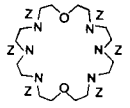
In contrast, very little is known about the catalysis of ATP-hydrolysis by organic molecules, despite the fact that such studies may provide insight into the factors which control ATP-hydrolysis and allow the design of new types of highly efficient artificial molecular catalysts.

Biological polyamines (putrescine, spermidine, spermine) complex nucleotides (AMP, ADP, ATP) [13] but have virtually no effect on the rate of ATP-hydrolysis, whereas the linear pentaethylenehexamine produces a significant rate enhancement [14].

Protonated macrocyclic polyamines have recently been shown to bind strongly AMP, ADP and ATP [2] [15], with affinities comparable to those found in enzyme-substrate complexes. We now report a study of the effect of nine such compounds **1–9** and of linear polyamine **10** on the rate of ATP-hydrolysis.

Results. – Compounds **1–10** have been synthesized earlier [16–18] or were commercially available (see *Exper. Part*). The protonation constants of these molecules have been reported [2] [15] [18] [19].

The *stability constants* of the complexes of protonated forms of **1** with ATP, ADP and AMP have been determined by pH-metric titration (see *Exper. Part*) and are listed in *Table 1*. These values lead to the distribution curves of the various complexes as a



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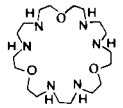
2 Z = CH_3



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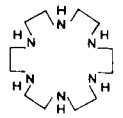

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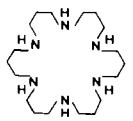

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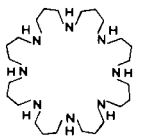

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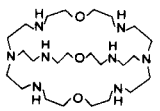

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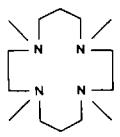

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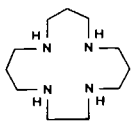

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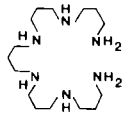

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Table 1. *Stability Constants* (log K_s), *for the Complexes of ATP, ADP and AMP with Protonated Forms of the Macrocyclic Polyamine* **1** [a])

Substrate	Ligand		
	1–6 H^+	**1**–5 H^+	**1**–4 H^+
ATP^{4-}	11.00	8.15	4.80
$HATP^{3-}$	7.85		
H_2ATP^{2-}	6.75		
ADP^{3-}	8.30	6.20	3.40
$HADP^{2-}$	5.60		
AMP^{2-}	6.95	5.00	2.85

[a]) $K_s = [\mathbf{1}{-}n\,H^+, H_{4-m}ATP^{m-}]/[\mathbf{1}{-}n\,H^+]\,[H_{4-m}ATP^{m-}]$ and analogous equations for ADP and AMP. Experimental conditions: 1 mM **1**–6 H^+ hexatosylate and 1 mM substrate as Na-salt in aq. solution of 0.01M TsOH and 0.1M TsONa at 25°; titrations performed with 0.1M NaOH; apparatus and procedures as described earlier [17].

function of pH *(Fig. 1)*. The variation of the ^{31}P-NMR shifts of ATP as a function of the ratio R = [**1**]/[ATP] *(Fig. 2)* favors a stoichiometry of 1 : 1 for the complexes present at pH = 3.5. The stability constants of the ATP-complexes of the fully protonated forms of **3**, **5** and **6** are log $K_s \approx 8.5$–9.0 [2]. For the 1 : 1 complex (**7**–6H^+, ATP^{4-}) a value of log $K_s \approx 8.0$ was found [20]. The binding constants and stoichiometries of the complexes of **1**–**7** with ATP, ADP and AMP will be described in more detail in a later publication [20]. A value of log $K_s = 6.4$ has been reported for (**4**–3H^+, ATP^{4-}) [15].

The *rates of ATP-hydrolysis* in presence of polyamines **1**–**10** have been measured by direct observation of the reaction course by ^{31}P-NMR spectroscopy *(Fig. 3)*. The observed first-order rate constants k_{obs} determined from plots of log [ATP] *vs.* time *(cf. Fig. 6)* are listed in *Table 2*. Some data obtained for adenosine-5′-diphosphate (ADP) and for pyrophosphate (PP) have also been added. The results for macrocycle **1** are represented graphically in *Fig. 4*–*7*.

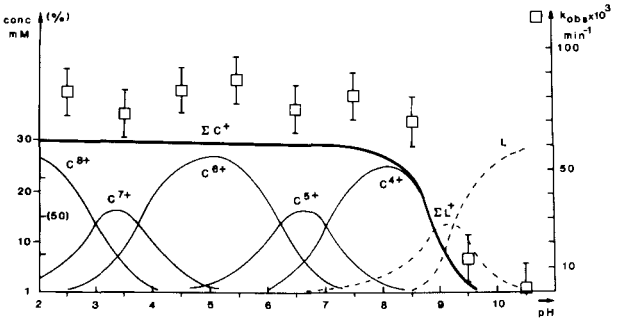


Fig. 1. *Distribution curves of the ATP-complexes formed by protonated forms of the macrocyclic polyamine* **1** *and observed first-order rates of ATP-hydrolysis* (k_{obs}) *in presence of* **1** *as a function of pH.* C^{8+}: (**1**–6H^+, H_2ATP^{2-}); C^{7+}: (**1**–6H^+, $HATP^{3-}$); C^{6+}: (**1**–6H^+, ATP^{4-}); C^{5+}: (**1**–5H^+, ATP^{4-}); C^{4+}: (**1**–4H^+, ATP^{4-}); $\sum C^+$: sum over all complexes present; for clarity only the sum $\sum L^+ = (\mathbf{1}{-}3H^+) + (\mathbf{1}{-}2H^+) + (\mathbf{1}{-}H^+)$ of the uncomplexed protonated forms of **1** which appear at higher pH (> 8) have been represented. The curve L gives the amount of unprotonated macrocycle **1**; the stability constants of the complexes formed by the forms of **1** bearing less than four protons could not be determined (log $K_s < 2$). The curves were calculated for concentrations of 0.03M for both **1** and ATP as in the hydrolysis experiments.

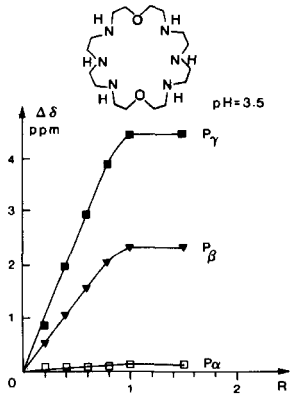


Fig. 2. *^{31}P-NMR shifts ($|\Delta\delta|$) of the α-, β- and γ-phosphate signals of ATP as a function of increasing ligand/substrate ratio $R = L/S = 1/ATP$ at pH = 3.5* (D_2O/H_2O 1 : 9 solution, 0.03M ATP; 20°)

Discussion. – The present results clearly show that macrocyclic polyamines in their protonated forms bind ATP and affect its rate of hydrolysis. Analysis of the data obtained should provide insight into the relationships between structure and activity of these compounds and into the factors which mediate efficient ATP-hydrolysis.

1. Polyprotonated macrocyclic polyamines act as *anion receptor molecules* for a variety of anions, displaying a marked macrocyclic effect of increased binding ability with respect to acyclic polyamines [2] [3] [15]. Thus, *strong complexation* of ATP is achieved by protonated **3**, **5**, **6** [2] [3], **4** [15] as well as by **1**, **2** and **7** (see above) with stability constants in the 10^6-10^{11} range. As a result, ATP-complexes of these compounds are present over a wide pH-range (see for instance *Fig. 1*).

The polyamines **1–10** have widely different protonation constants. The nature, the stoichiometry, the structure, the stability of their complexes depend on their protonation state which itself changes with pH. For a given polyamine/anion pair, the most stable complexes are usually formed between the most highly charged species. ^{31}P-NMR measurements show that at 1 : 1 macrocycle/ATP concentration the complexes have predominantly 1 : 1 stoichiometry. Studies performed with **1**-ATP yield similar limiting chemical shifts for the α-, β- and γ-phosphate resonances of ATP at pH = 3.5, 6.5 and 8.5, indicating that the conformation and the mode of binding of ATP in the complexes present under these conditions, are probably quite similar. In view of the number of species which may be formed and may coexist at different pH-values, a detailed analysis would be required for each macrocyclic polyamine.

Since strongest effects on ATP-hydrolysis have been observed here for the 24-membered macrocycle [24]-N_6O_2, **1**, the present study and discussion will be concerned mainly with this compound. Strong binding of ATP^{4-}, $HATP^{3-}$ or H_2ATP^{2-} by the tetra-, penta- or hexa-protonated forms of **1** *(Table 1)* yields five complexes in the pH-range 2.5–8.5 with the distributions represented in *Fig. 1*.

2. Large rate enhancements of ATP-hydrolysis are observed in presence of macrocyclic polyamines *(Table 2)*. There is, however, a strong dependence on the nature of additive, which may result both from structural factors and protonation state at a given pH.

Table 2. *First-Order Rate Constants* ($k_{obs} \times 10^3$ [min^{-1}]) *and Relative Rates* (k_{rel}) *for the Hydrolysis of ATP, ADP and PP in Presence of the Macrocyclic Polyamines* **1–9**[a])

Macrocycle	pH	ATP	Macrocycle	pH	ATP
None[b])	1.5	19.8	**3**	3.5	22 (13)
	3.5	≈ 1.1, 1.7[b])		7.5	16
	5.4	0.61	**4**	3.5	22 (13)
	7.7	0.26		7.5	18
	8.3	0.12	**5**	3.5	6.2 (3.6)
1	2.5	81		8.5	3.5
	3.5	70[c]) (50)	**6**	6.5	0.45
	4.5	83	**7**[d])	3.5	1.7 (1.0)
	5.5	85 (140)		5.5	1.4
	6.5	75	**8**	3.5	2.9 (1.7)
	7.5	79 (300)	**9**	3.5	2.8 (1.6)
	8.5	68 (570)	**10**	3.5	0.45 (0.3)
	9.5	13.5			
	10.5	1.5			
2	3.5	12 (7)			
	7.5	4.0			

Macrocycle	pH	ADP	Macrocycle	pH	PP
None[e])	5.0	0.60	None[f])	3.5	0.60
				7.5	0.20
1	5.5	16	**1**	3.5	9.7
	6.5	22[g])		7.5	2.2

[a]) In D_2O/H_2O 1:9 at 60°; equimolar concentrations (0.03M) of macrocycle and substrate; macrocycles introduced as hydrochloride salts except for **7** used as hexaperchlorate; observed rates (± 10%) determined from the slope of plots of remaining ATP *vs.* time (min). Relative rates with respect to uncatalyzed ATP-hydrolysis at the same pH are given in parentheses; they are lower limits since the reference values in absence of any additive have been determined at 70° [21].

[b]) Values at 70° from [21] for the NMe_4^+-salt of ATP; for pH = 3.5, the value of *ca.* 1.1 has been obtained by interpolation between those published for pH = 2.7 and 4.0 (1.76 and $0.71 \cdot 10^{-3}$ min^{-1}, 70° [21]), the value of 1.7 at pH = 3.5 has been obtained in the present work for the Na-salt of ATP.

[c]) Value of 80 obtained at pH = 3.5 when 0.5M morpholinoethane-sulfonic acid was used as medium of constant ionic strength.

[d]) Concentration 0.015M.

[e]) Calculated from solvolysis data at 80° [36].

[f]) Values at 70° from [37].

[g]) Similar values were observed when ADP was used directly (20 min^{-1}) or when the hydrolysis of ADP resulting from hydrolysis of ATP was followed (22 min^{-1}).

Allowing for the fact that the data for hydrolysis of free ATP have been obtained at 10° higher temperature [21] *(Table 2)*, acceleration factors higher than 50, 13 and 13 are found for **1**, **3** and **4**, respectively, at pH = 3.5. At pH = 8.5 compound **1** produces an *acceleration of the order of* 10^3 or higher, whereas all other macrocycles investigated decrease in activity compared to pH = 3.5. The most efficient compounds contain ethylenediamine units and the least active ones propylenediamine sequences. Indeed, despite their high binding constants with ATP^{4-} the hexamine **5**, the octamine **6** [2] as well as the macrobicyclic polyamine **7** have low or no activity. There seems to be no parallelism

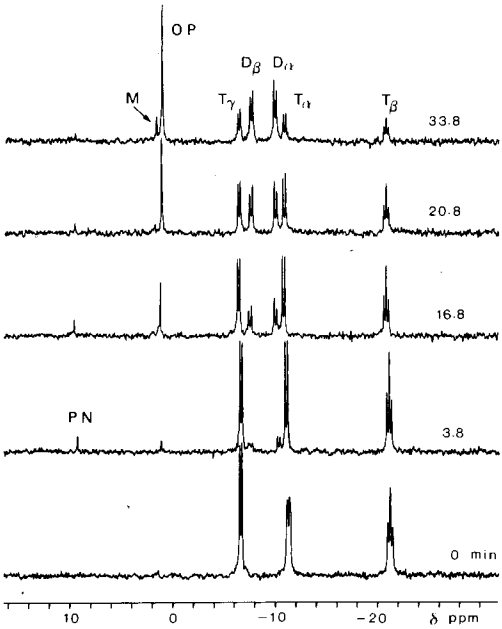


Fig. 3. *Observation of ATP-hydrolysis by ^{31}P-NMR spectroscopy as a function of time.* Proton-decoupled ^{31}P-NMR spectra (at 81 MHz) of 0.03M ATP and 0.03M of the macrocycle **1** at an apparent pH of 7.0 in D_2O/H_2O 1 : 9 at 50° recorded at times indicated (in min), which refer to the time of completion of the acquisitions (250 scans, 3.8 min); t = 0 corresponds to a spectrum taken without heating; the chemical shifts are in ppm relative to external 85% H_3PO_4; the signals are identified by the following symbols: T_α, T_β, T_γ for the α-, β-, γ-phosphate groups of ATP; D_α, D_β for ADP; M for AMP; OP for inorganic phosphate and PN for the intermediate species assumed to be a phosphoramidate derivative of the macrocycle **1**.

between binding strength and rate enhancement. However, the smaller macrocycles **8** and **9** [15] and the acyclic hexamine **10**, which are expected to form much weaker complexes, display little or no activity. It appears that strong complexation of ATP is *required but not sufficient* for enhancing its rate of hydrolysis.

The hydrolysis of ADP is also accelerated by addition of **1**, but to a lesser extent than ATP-hydrolysis, indicating moderate *substrate selectivity*; the rate enhancement is still present but even smaller for PP *(Table 2)*.

3. The *pH-dependence of ATP-hydrolysis* is dramatically affected by addition of **1** *(Fig. 4)*. Whereas the rate of ATP-hydrolysis increases strongly in acidic media below pH ≈ 3 [21], in presence of **1** it is rapid and nearly constant over a wide range, from pH = 2.5 to about 8.5. Above pH = 8.5 it decreases rapidly, with a slope similar to that of the uncatalyzed reaction between pH = 0 and 3. The rate at pH = 8.5 in presence of **1** is comparable to that at pH ≈ 0.5 in the uncatalyzed reaction, indicating that the effect of the macrocycle is equivalent to a change in effective acidity of the reaction conditions *by a factor of about 10^8*.

As seen on *Fig. 1*, the pH-dependence of k_{obs} closely follows the sum $\sum C^+$ over the five complexes which dominate in the 2.5–8.5 pH-range. This indicates that binding is

a prerequisite for catalytic activity, which decreases very rapidly at pH > 8.5 where complexation is much weaker because of lesser protonation of the macrocycle. The lower activity of the other macrocycles **2–7** at higher pH may also arise, at least in part, from a similar decrease in binding ability.

4. A *macrocyclic effect* on catalytic efficiency is apparent from a comparison of **5** with its linear analogue **10**; a rate increase by a factor of about 15 is observed on cyclization. Furthermore, from the published data [14], the first-order rate constant for ATP-hydrolysis in presence of the linear pentaethylenehexamine is about one-third of that observed for the macrocyclic hexamine **4**. Such effects are probably also operative for other macrocycles studied and may result from the better binding abilities of the macrocycles as well as from the disposition of the sites in the complexes formed.

5. The *course and products* of the reaction have been followed by ^{31}P-NMR spectroscopy, as illustrated in *Fig. 5* for macrocycle **1**. ATP hydrolyzes to OP and to ADP, which thereafter yields AMP and OP. No release of PP by ATP-cleavage between P_α and P_β was observed; its formation would have been detected since its hydrolysis is much slower *(Table 2)* and its ^{31}P-NMR signal is distinct from the others *(Fig. 3)*. These results hold also for the other pH-values and for the other macrocycles.

Although the rate of ATP-hydrolysis in presence of **1** did not change significantly up to pH = 8.5, reactions run between pH = 6.5 and 8.5 revealed the formation of a *transient species* giving a ^{31}P-NMR signal at *ca.* + 10.4 ppm, well downfield from the other signals. After 3.8 min at pH = 7, the reaction medium contained 81% ATP, 21% ADP, 10% OP and 9% of the ^{31}P lost from ATP at + 10.32 ppm *(Fig. 3)*. The latter peak was present only as long as ATP remained and disappeared thereafter; it was not observed at lower pH. A variety of phosphoramidates have ^{31}P-signals in the downfield region from + 3 to + 22 ppm [22–24]. Phosphorylation of **1** with

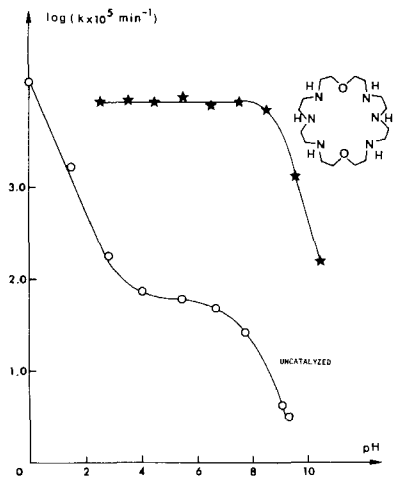


Fig. 4. *pH-Dependence of the rate of ATP-hydrolysis catalyzed by macrocycle* **1** *and uncatalyzed.* Plot of $\log(k_{obs} \times 10^5\ \text{min}^{-1}$ *vs.* pH) for: (★) the hydrolysis of 0.03M ATP macrocyclic polyamine **1** at 60° in D_2O/H_2O 1:9; (○) the uncatalyzed rate of hydrolysis at 70° reported in [21].

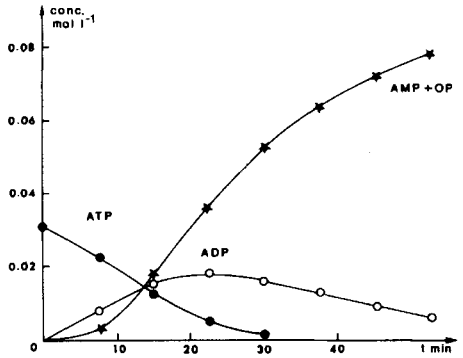


Fig. 5. *Time dependence of the reaction components of ATP-hydrolysis catalyzed by macrocycle* **1**. Plot of the observed molar concentrations of ATP (●), ADP (○), and the sum of AMP and OP (★) *vs.* time in D_2O/H_2O 1:9 solution containing initially 0.03M ATP and 0.03M **1** at 60° and an apparent pH of 7.5.

$(PhO)_2POCl$, followed by base hydrolysis *in situ* (according to the procedure described for the preparation of other phosphoramidates [25]) and adjustement to pH ≈ 7.2, gave a crude reaction mixture displaying ^{31}P-resonances in the + 5 to + 15 ppm region. The transient species observed here may thus be tentatively identified as a *phosphoramidate intermediate* resulting from phosphorylation of an amine function of **1** by ATP. A similar species is formed during the hydrolysis of PP in presence of **1**, since a ^{31}P-NMR signal is also observed at + 10.5 ppm at pH = 7.0, but not at pH = 3.5. Attempts to more fully characterize this intermediate are in progress.

6. The *reaction kinetics* indicate that ATP-hydrolysis in presence of 1 equiv. of macrocyclic polyamine is first-order for all compounds and pH-values studied *(Fig. 6)*. At a concentration ratio **1**/ATP = 3, a *rate saturation* is observed, in that the rate of hydrolysis (pH = 3.5, $k_{obs} = 0.1\ min^{-1}$) is not significantly greater than at a 1:1 ratio. The high association constants between ATP and the protonated macrocycles imply that

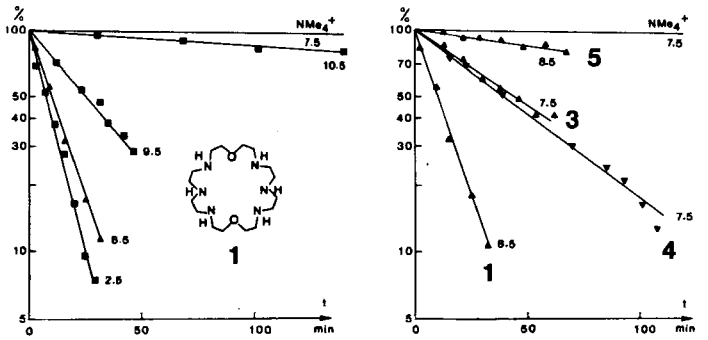


Fig. 6. *Rates of ATP-hydrolysis in presence of macrocycle* **1** *at different pH-values as indicated* (left) *and in presence of different macrocycles at pH-values indicated* (right). Logarithmic plots of the remaining ATP as a function of time; reaction conditions; ATP: 30 mM; macrocycle: 30 mM; 60°; in D_2O/H_2O 1:9, the line 'NMe_4^+' is drawn from literature results on the hydrolysis of the tetramethylammonium salt of ATP [21].

the dominant species in solution are complexes, as clearly shown in *Fig. 1* for compound **1**. Thus, the rate data agree with a reaction course proceeding *via* formation of a complex which is the reactive species, followed by hydrolysis of the bound ATP with first-order kinetics in the concentration of the complex. An analogous behavior was found with linear polyamines [14].

7. That the reaction is a true *catalytic process* is shown by an ATP-hydrolysis experiment at pH = 3.5, in presence of only 0.1 equiv. of **1** *(Fig. 7)*. The change in

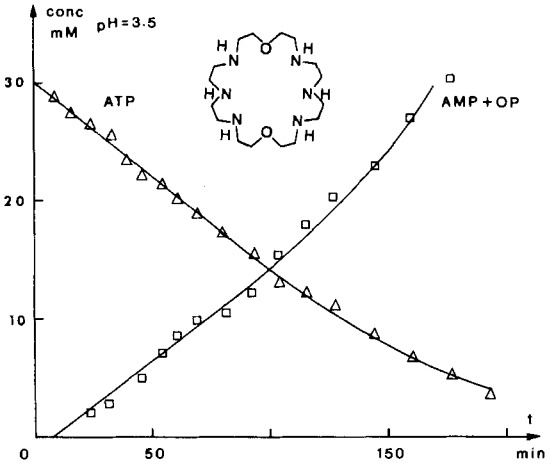


Fig. 7. *Catalytic hydrolysis of ATP by macrocyclic polyamine* **1** *in presence of a tenfold excess of substrate.* Plot of ATP and (AMP + OP) concentrations as a function of time under the following conditions: macrocycle **1**: 3 mM; ATP: 30 mM; pH = 3.5; 60°; in D_2O/H_2O 1:9.

ATP-concentration in the early period is linear in time, with a zero-order rate constant $k'_{obs} = 0.00016$ M $min^{-1} = k_{obs}$ [complex], giving a calculated first-order rate constant $k_{obs} = 0.053\ min^{-1}$, (taking [complex] = [**1**] = 0.003 M) in reasonable agreement with the value of $0.070\ min^{-1}$ observed at this pH under the first-order conditions. The deviation may come from, *inter alia*, another distribution of complexes and some participation of complexes of 2:1 stoichiometry. Furthermore, since the products ADP and OP have appreciably lower binding constants than ATP (see above) this catalytic reaction is also *free from product inhibition* over most of its course. One may note that Co (III)-promoted ATP-hydrolysis requires more than one metal ion per ATP [12].

8. The above results allow to consider *tentative mechanisms* for the molecular catalysis of ATP-hydrolysis by the macrocyclic polyamines.

The lack of correlation between stability of the macrocycle/ATP complexes and efficiency of a given polyamine indicates that, even though complex formation is the first step, catalytic efficiency depends on defined structural requirements of the complex itself, involving protonation pattern, electrostatic and H-bonding effects, spatial arrangement of N-sites, *etc.*

Studies on the non-enzymatic hydrolysis of ATP are consistent with two general mechanisms depending on pH [4–7]. In acidic media the addition of water to the terminal phosphate precedes the elimination of ADP; at higher pH the anionic species may

eliminate ADP to give metaphosphate which subsequently is converted to orthophosphate by addition of water. Furthermore, in presence of acceptors, a third pathway involves phosphorylation of a nucleophile followed by the hydrolysis of the phosphoryl intermediate. Passage through a phosphoenzyme intermediate seems to be the favored path for enzymatic hydrolysis of ATP by ATPases [26]. Since the hydrolysis of ATP in neutral media is estimated to be at least 10^{10} times slower than most of the enzyme-catalyzed reactions, efficient artificial systems must accomodate electrostatic, general acid-general base, and nucleophilic catalysis.

Electrostatic catalysis is a prominent factor in the enzymatic reactions as well as in the rate enhancements produced by metal ions in non-enzymatic ATP-hydrolysis [5] [6] [8] [11] [12]. It may also be expected to contribute significantly to the efficiency of macrocyclic polyamines, in view of the large number of charges which they bear when protonated and of the resulting very strong electrostatic binding of ATP. Since the macrocycles are protonated over a wide pH-range, electrostatic catalysis should operate *together* with other catalytic processes and affect the domains and rates of the different mechanistic pathways (as does Mg^{++} for instance [6]).

Acid catalysis may take place in the lower pH-range in combination with electrostatic catalysis, *via* the highly charged complexes formed by the protonated substrates $HATP^{3-}$ and H_2ATP^{2-} with **1**–$6H^+$ *(Fig. 1)*.

The transient species tentatively identified as a *phosphoramidate intermediate* (see above), would be formed by phosphorylation of an unprotonated amine site of the (**1**–$5H^+$, ATP^{4-})- and (**1**–$4H^+$, ATP^{4-})-complexes present in the pH-range of 6.0–9.5 *(Fig. 1)*. Such phosphorylation may result from either *nucleophilic catalysis* involving attack of P_γ by an amine site, or from addition of an amino group to metaphosphate formed by initial elimination of ADP. The intermediate does not accumulate and apparently hydrolyzes to orthophosphate and **1** at a rate that is slightly slower than the rate of the ATP-breakdown. This mechanism may also operate at lower pH, with hydrolysis of the intermediate phosphoramidate considerably faster than the ATP-breakdown so that it is not detected. Formation and fast hydrolysis of phosphoramidates have been reported in different systems involving amine catalysts [23–25] [27] [28]. In particular, accumulation of an intermediate was observed at high pH, and the rate of phosphoramidate hydrolysis was shown to increase with decreasing pH to values which, extrapolated to the present conditions, would be faster than ATP-hydrolysis in presence of **1** [28]. The other macrocycles **2**–**6** may react in a similar fashion, although the intermediate has not been detected, as should be the case when its hydrolysis becomes faster than phosphoryl transfer. The mechanism of the latter reaction has been discussed recently [29].

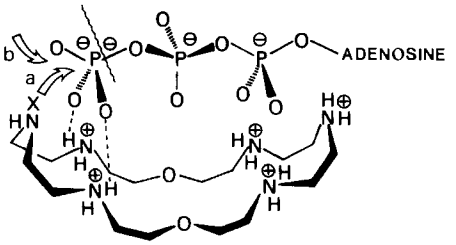
Nucleophilic catalysis of ATP-hydrolysis by the macrocyclic polyamines could therefore involve steps similar to those thought to occur in the enzyme-catalyzed reaction [6] [26]: substrate (ATP) binding by the molecular catalyst C; intra-complex reaction with formation of an intermediate (*N*-phosphorylated macrocycle); product dissociation and regeneration of the catalyst by hydrolysis of the phosphoryl intermediate:

$$\mathrm{ATP} + \mathrm{C} \rightleftharpoons [\mathrm{C.\ ATP}]$$

$$[\mathrm{C.\ ATP}] \rightarrow [\mathrm{C-N(\text{-}Phosphoryl)}] + \mathrm{ADP}$$

$$[\mathrm{C-N(\text{-}Phosphoryl)}] \rightarrow \mathrm{C} + \mathrm{OP}$$

The macrocyclic polyamine **1** possesses the features of a ditopic coreceptor [3] [30] in which both diethylenetriamine subunits may participate in complexing the ATP-substrate and in effecting catalytic ATP-hydrolysis, as schematically represented in the tentative structure **A**, by a combination of *nucleophilic (path a) or acid (path b) catalysis* with *electrostatic catalysis.*



Structure **A** (one possible binding mode of ATP to **1**)

a: X = lone pair, nucleophilic attack
b: X = H^+, water addition

The binding scheme in the complex **A** is not known. The doubly charged terminal $-OP_\gamma O_3^{2-}$-group may be bound to one of the two protonated triamine subunits. Depending on the more or less bent shape of the macrocycle the other subunit could bind either to the P_α- or to the P_β-phosphate group. The (α, γ)-binding scheme would allow the macrocycle to adopt a more extended conformation in which the charged subunits may stay further apart; molecular models indicate that the size and shape of **1** and of ATP (for a crystal structure see [31]) are compatible with such an arrangement. The (β, γ)-binding scheme requires a more compact conformation of **1** than that represented in structure **A**, but provides electrostatic assistance to the departure of the P_β-phosphate group which becomes doubly charged in the product ADP. Interaction of the second triamine unit with both β- and γ-phosphate groups would represent an intermediate case.

In an alternate mode of binding, where the triphosphate group of ATP is oriented *parallel* to the triamine sequences of **1** (90° rotation from the relative orientation shown in **A**), all three phosphate groups could interact with the ammonium sites, allowing again the two subunits to cooperate in catalysis, but in a different fashion from **A**.

Detailed structural data are required to distinguish between these various possibilities and to establish the nature of the complexes formed. Work in progress should help to elucidate the pathways of ATP-hydrolysis catalyzed by macrocyclic polyamines, which may involve a combination of the mechanisms and of the different complex species considered above.

9. A number of extensions of the present work may be envisaged to increase catalytic power and substrate selectivity, to analyze structure-activity relationships and to derive enzyme models. For instance polyamines of other structural types may be investigated. Fitting macrocyclic polyamines like **1** with side chains bearing reactive groups (such as amine, carboxylic acid or imidazole for instance) may further improve their efficiency. The introduction of rigid groups may increase substrate selectivity.

Since ATP-hydrolysis by Co(III) has revealed that the active species involves two or three metal ions [11] [12] dinuclear macrocyclic metal complexes of suitable geometry [32] might provide entries into the design of efficient dinuclear *metallocatalysts.*

Conclusion. – The present study extends our earlier work on *supramolecular catalysis* (catalysis within a supramolecular complex) to anionic substrates. Binding and transformation of other anionic species of chemical or biochemical interest may be considered [33]. Thus, molecular catalysts for the hydrolysis of phosphate esters might, in addition to their properties as chemical reagents and as enzyme models, provide entries to the directed cleavage of nuclei acids, related for instance to the splitting of tRNA by Pb(II)-ions [34] or to the 'ribozyme' self-splicing RNA-process [35]. Finally, a better understanding of the factors involved in phosphate or polyphosphate cleavage should be of help for the design of molecular catalysts capable of effecting phosphorylation, bond-making reactions.

Experimental Part

Compounds. The synthesis of the macrocyclic compounds has been described: **1** [16]; **3**, **5**, **6** and **10** [17]; **7** [18]; **4** is commercially available from *Aldrich Co.* as its trisulfate salt and has been converted to its hexahydrochloride by passage over an anion exchange column; **8** and **9** are commercially available from *Strem Chemicals, Inc.* and were converted into their hydrochloride forms; **2** has been obtained as a viscous colorless liquid, by permethylation of **1** by the *Eschweiler-Clarke* reaction (71% yield) and presents spectral and analytical properties in agreement with its structure. Na-salts of ATP and ADP were obtained from *Boehringer*, Mannheim. All other chemicals used were high-purity commercial reagents.

Methods. *^{31}P-NMR spectra* were recorded at 81.015 MHz on a *Bruker SY 200* spectrometer; the chemical shifts are (+, down field) from 85% H_3PO_4 as external standard; probe temp. was regulated by the variable temp. accessory at 60 ± 3°.

Protonation constants (pK_a) and *stability constants* (K_s) were determined by computer analysis of pH-metric titration curves following the procedures used earlier ([17] and references therein). The stoichiometry of ATP-complexes was studied by following ^{31}P-NMR shifts as a function of macrocycle/ATP ratio (see *Fig. 2*).

Kinetic studies were performed by following the time evolution of the proton-decoupled ^{31}P-FT-NMR spectra (100–500 acquisitions) of substrate + macrocycle mixtures. Since most ^{31}P-NMR signals of ATP, ADP, AMP, pyrophosphate (PP) and orthophosphate (OP) are distinct, ATP-hydrolysis can be monitored conveniently and accurately (± 10%) by following the changes in concentration of the various species with time by signal integration of: P_β in ATP; P_α and P_β in ADP; sum of P_α in AMP and of OP since these signals often overlap; depending on the sample, P_α of AMP and OP may give different signals, usually at pH > 7.0. The method was accurate for reactions having half-lifes > 5 min. The NMR samples (2 ml in 10 mm o.d. tubes) contained 0.03M ATP (or ADP, or PP) and 0.03M macrocycle in H_2O/D_2O 9:1 adjusted to the desired pH at 25° with 5M NaOH, HCl or $HClO_4$. The change in pH during the reaction was usually < 0.3, which should not affect significantly the rates, since they are constant over a wide pH-range.

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