Supramolecular Catalysis of Adenosine Triphosphate Synthesis in Aqueous Solution mediated by a Macrocyclic Polyamine and Divalent Metal Cations

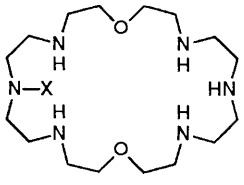
Mir Wais Hosseini and Jean-Marie Lehn

Institut Le Bel, Université Louis Pasteur, 4, rue Blaise Pascal, F-67000 Strasbourg, France

In the presence of the divalent metal cation Mg^{2+} as promoter, macrocycle **1** catalyses the generation of ATP from acetyl phosphate and ADP in dilute aqueous solution at neutral pH.

Supramolecular catalysis, the chemical transformation of a bound substrate, for which complexation and recognition steps are prerequisites, may lead not only to mimics of natural enzymes but also to new catalysts for synthetic applications.[1-5] Whereas the catalysis of bond cleavage reactions has been extensively investigated, the co-catalysis of bond formation reactions still remains a challenge.

In the course of studies on supramolecular catalysis of phosphoryl transfer processes[1,5] it was found that a variety of polyamines, in particular the [24]-N_6O_2 macrocycle **1**, mediate not only ATP hydrolysis[6] but also the phosphorylation of phosphate and of a series of biological phosphate esters to the corresponding pyrophosphates.[7-9] In the presence of phosphoryl donors such as adenosine triphosphate (ATP) or acetyl phosphate (AcP), compound **1**, after complexation of the phosphoryl source, undergoes a phosphorylation reaction leading to the phosphoramidate **2**.[7] This intermediate acts as a genuine molecular phosphorylating reagent *via* phosphoryl transfer to the bound acceptor species,[7,8] in competition with hydrolysis, *i.e.* with phosphorylation of a water molecule. In order to reduce this unproductive reaction and to increase further the amount of active complex, water–organic solvent [such as dimethyl sulphoxide (DMSO)] mixtures were used.[8] In this type of medium (DMSO–H_2O, 7:3) compound **1** indeed promotes the synthesis of biologically important pyrophosphate derivatives; of particular interest was the formation of ATP from ADP and AcP at pH 7 and 40°C.[8] In order to achieve these reactions under physiological conditions (H_2O, pH 7, 37°C) another possibility consisted in the use of divalent metal cations for increasing the amount of the active complex and further to promote the reaction. Indeed, divalent metal ions are known to facilitate phosphoryl transfer processes.[9,10] We report here that in the presence of the biologically important divalent cations Ca^{2+} or Mg^{2+}, compound **1** catalyses the synthesis of ATP from ADP and AcP in aqueous solution at pH 7 and 40°C.



1 X = H [24]-N_6O_2
2 X = PO_3^{2-} [24]-$N_6O_2PO_3^{2-}$

The system used consisted of four components, a phosphoryl donor (AcP), an acceptor (P, PP or ADP), the catalyst **1** and a divalent metal cation (Ca^{2+} or Mg^{2+}); all reactions were run at pH 7 and at 40°C at about 10^{-2} molar concentrations and followed by ^{31}P NMR spectroscopy. Although Ca^{2+} had similar effects as Mg^{2+}, because of precipitation of calcium phosphate only Mg^{2+} was extensively studied. Some results are listed in Table 1. Addition of Mg^{2+} somewhat decreased the rate of disappearance of AcP and of the intermediate **2**.

The addition of Ca^{2+} or Mg^{2+} had a striking effect on ATP formation. Whereas in a mixture of 5 or 10 equiv. of **1**, ADP and AcP, only 7.5 or 10% respectively of ATP was detected (Table 1, runs 3, 8) in the presence of 1 equiv. of Mg^{2+} up to 24.7 and 26.3% respectively of ATP was observed (runs 4, 9). This finding was in agreement with the study of positional isotope exchange (PIX) using ^{18}O labelled ATP.[11] Indeed, whereas in the absence of Ca^{2+}, a PIX value of zero was

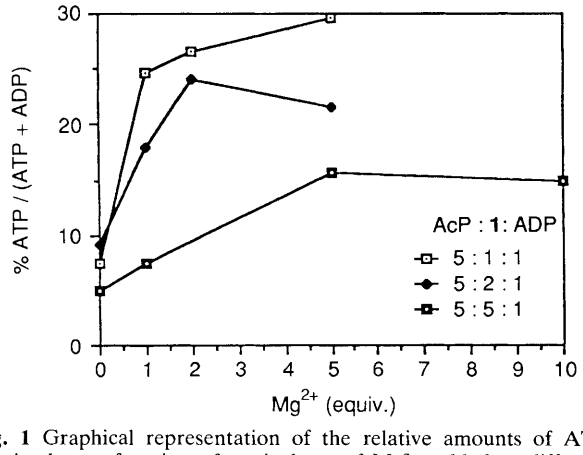
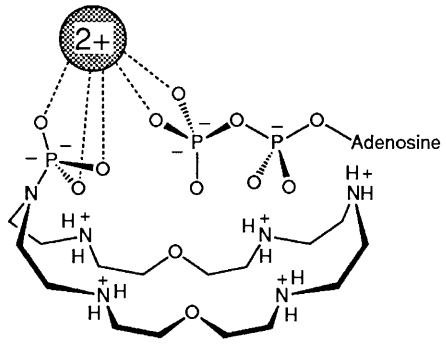


Fig. 1 Graphical representation of the relative amounts of ATP obtained as a function of equivalents of Mg^{2+} added to different amounts of AcP : **1** : ADP (1 equiv. = 10^{-2} mol dm^{-3}); see also Table 1 and text

Table 1 Generation of ATP, PP and PPP from AcP and ADP catalysed by macrocycle **1** in the absence or presence of Mg^{2+} ions[a]

Run	Equivalents AcP	**1**	ADP	Mg	%ATP/(ATP + ADP)	Conversion (%) W.r.t. **1**	W.r.t. AcP	PP (%) W.r.t. **1**	PPP (%) W.r.t. **1**
1	1	1	1	0	0	16.0	16.0	8.0	0
2	1	1	1	1	9.4	26.8	26.8	8.7	0
3	5	1	1	0	7.5	89.5	17.9	36.5	3.0
4	5	1	1	1	24.7	198.4	39.7	47.1	26.5
5	5	1	1	5	29.7	296.7	59.3	78.0	37.0
6	5	1	2	5	17.7	191.6	38.3	40.6	25.0
7	5	2	1	2	24.0	135.0	54.0	33.0	19.0
8	10	1	1	0	10.0	240.7	24.0	98.7	11.1
9	10	1	1	1	26.3	401.3	40.1	105.0	55.0

[a] In aqueous solution, pH 7, 40°C. Concentration 1 equiv. **1** = 10^{-2} mol dm^{-3} % given as molar proportions. The data correspond to complete AcP consumption; since **1** catalyses the hydrolysis of polyphosphate[6,9] the amounts of ATP, PP and PPP indicated are lower limits to those actually formed; w.r.t.: with respect to.



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obtained during ATP dephosphorylation catalysed by **1**, in its presence the PIX value was *ca.* 6–10%, indicating that ATP was cleaved and regenerated during the reaction.[11] Furthermore, the quantity of ATP formed was found to depend on the M^{2+} : 1 ratio (for either Ca^{2+} or Mg^{2+}), reaching a maximum for about equal amounts of M^{2+} and **1** (Fig. 1); it did not depend on the ratios M^{2+} : ADP or M^{2+} : AcP. P–O–P bond formation from a phosphorimidazolide and a phosphate catalysed by Mn^{2+} and Cd^{2+} has been reported recently.[15]

These results indicated that the reactive species could be a ternary complex (**2**, ADP^{3-}, Mg^{2+}) in which the divalent metal cation bridges the PO_3^{2-} group of **2** and the terminal PO_3^{2-} group of a bound ADP substrate as shown in **3**. The cation could thus promote ADP phosphorylation by helping to keep the reacting species together and/or facilitating the phosphoryl transfer process.

The formation of such a complex **3** was further investigated by ^{31}P NMR spectroscopy. Whereas the ^{31}P chemical shift for **2** in the absence of Mg^{2+} and ADP was at δ 10.08,[7] in their presence, this signal appeared at δ 9.98. Complexation of ADP by protonated **1** induced shifts of 1.18 and 1.82 ppm at pH 7 for the P_α and P_β signals;[12] and P_β signal was further shifted by 0.62 ppm in the presence of both the intermediate **2** and Mg^{2+}, whereas the P_α signal was only slightly affected. In the ternary complex **3**, the phosphoramidate **2** acts as an organic template which, by complexing ADP, brings together both anionic groups (PO_3^{2-} and ADP^{3-}). The divalent metal cation, on the other hand, by bridging the phosphoramidate moiety of **2** and ADP acts as an inorganic template. Bridging by Na^+ or Mg^{2+} cations was also proposed in phosphorylation processes.[10,13] A similar effect might operate in the synthesis of PP from ATP and P on the surface of solid calcium apatite.[14]

The phosphoryl transfer process in such a ternary complex **3** probably involves the proper location of ADP on **2**, held in close proximity to the N–PO_3^{2-} group, with the help of the bridging M^{2+} ion. The latter, together with the ammonium binding sites on **1**, by neutralizing the negative charges on both ADP^{3-} and the N–PO_3^{2-} group, may also facilitate phosphoryl transfer to ADP leading to the formation of ATP.

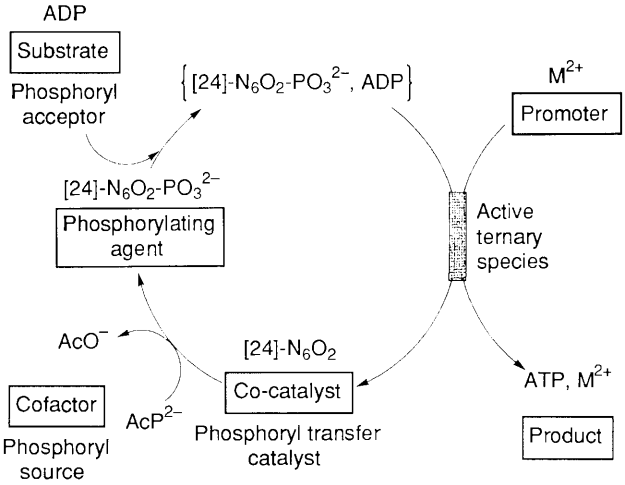

Fig. 2 Catalytic cycle for ATP generation from ADP using AcP as phosphoryl source, macrocycle **1** as catalytic unit and Mg^{2+} as promoter

The effects of the relative amounts of **1**, ADP and AcP on ATP formation in the presence or absence of Mg^{2+} were investigated (Table 1).

Increasing the ratio of **1** to ADP above 1 : 1 or 2 : 1 caused a decrease in amount of ATP formed with or without Mg^{2+} present (Table 1, runs 4 and 7). Since an equimolar mixture of AcP and **1** produced about 40% **2**,[7] excess of unphosphorylated **1**, by binding ADP, is expected to decrease the amounts of (**2**, ADP) complex and therefore the yield of ATP.

Similarly, the amount of ATP formed decreased significantly when the ratio of ADP to **1** was increased from 1 : 1 to 2 : 1 (Table 1, runs 4 and 6). This may also be explained in terms of competition between ADP and AcP for **1**. Since ADP^{3-} is more strongly bound by **1** than AcP^{2-}, displacement of the latter lowers the amount of intermediate **2** produced and therefore also of ATP. Indeed, whereas for a ratio of ADP to 1 of 2 : 1, up to 58% of **2** was detected, only 39 and 28% was formed for 2 : 1 and 5 : 1 ratios respectively.

On the other hand, increasing the relative amount AcP/**1** = z from 1 to 10 in equimolar mixtures of **1**, ADP and Mg^{2+} gave a marked increase in ATP formation from z = 1 to 5 and then a levelling off for z = 10 (Table 1, runs 2, 4 and 9). Since a 1/1 AcP–**1** mixture yielded 43[13] and 39% of **2** in the absence or

presence of Mg^{2+} respectively, a five-fold excess of AcP may lead to almost complete phosphorylation of **1**, so that only a small increase in **2** may be expected for a larger excess of AcP. Furthermore, this saturation behaviour agrees with **2** being the phosphorylating reagent.

In addition to ATP, PP and PPP were also formed in the reactions studied. The amounts of PP and PPP increased markedly when Mg^{2+} was present. They also increased with the AcP to **1** ratio, reaching 105% PP and 55% PPP for AcP : 1 = 10 : **1** in the presence of Mg^{2+}. These observations may be rationalized in terms of competition of P, PP and ADP for **2**. Mg^{2+} and Ca^{2+} have been shown to promote the formation of small amounts of PP during ATP hydrolysis in the presence of **1**.[9]

In order to increase further the amount of ATP, we also studied the effect of Mg^{2+}, Ca^{2+} and **1** in a mixture of DMSO and H_2O.[8] In a 7:3 mixture, the reaction of equimolar amounts of AcP, **1** and ADP gave *ca.* 9.3% of ATP.[8] With a five-fold excess of AcP in a 5:5 DMSO–H_2O mixture, under the same conditions, up to 31.4% of ATP was formed. This amount was further increased to 42% by addition of 1 equiv. of Mg^{2+} (with respect to **1**). Although Ca^{2+} had similar effects, owing to the precipitation of calcium phosphate and pyrophosphate accurate data could not be obtained. For the same reasons, attempts to increase the ratio of DMSO to H_2O were unsuccessful.

Based on the results presented here, one may propose the following catalytic cycle for ATP synthesis (Fig. 2): the macrocycle **1** after complexing the phosphoryl source (AcP) is phosphorylated to the phosphoramidate **2**; the binding of the phosphoryl acceptor ADP to **2** followed by the divalent metal cation leads to the ternary complex (**2**, ADP^{3-}, M^{2+}); finally, within this supramolecular species, a second phosphoryl transfer reaction from **2** to ADP takes place leading to the synthesis of ATP.

In summary, in such a system, AcP is the phosphoryl source, ADP the acceptor, the metal cation the promoter and the macrocycle **1** the co-catalyst bringing the species together and performing the transfer.

The present reaction, in addition to being an attractive procedure for ATP generation in aqueous solution, may be extended to the synthesis of other oligophosphorylated compounds. It could also be used for preparing labelled ATP by using ^{32}P labelled AcP.

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