# Supramolecular Catalysis: Substrate Phosphorylations and Adenosine Triphosphate Synthesis with Acetylphosphate catalysed by a Macrocycle Polyamine

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In aqueous dimethyl sulphoxide solution, the macrocyclic polyamine receptor (**1**) catalyses the phosphorylation by acetylphosphate of phosphate containing substrates R–OP to pyrophosphates R–OPP, *via* the phosphoramidate intermediate (**2**), that acts as a molecular phosphorylating agent, converting for instance ADP into ATP.

Supramolecular reactivity and catalysis represent a major feature of supramolecular systems.[1,2] The design of selective molecular catalysts may reveal important factors contributing to enzymatic catalysis and provide synthetic molecular reagents for specific chemical reactions.[1–4] Phosphoryl transfer processes play vital roles in all living organisms and their study is of special interest.[5–7]

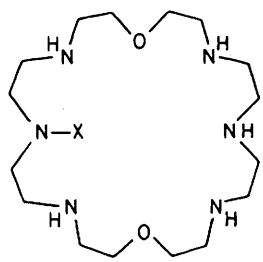
We have shown earlier that protonated macrocyclic polyamines, in particular the [24]-$N_6O_2$ macrocycle (**1**), bind nucleotides and polyphosphates[8] and catalyse their hydrolysis.[9,10] Furthermore, compound (**1**) catalyses the acetylphosphate (AcP, $MeCOOPO_3^{2-}$) breakdown and the synthesis of pyrophosphate (PP) in aqueous solutions.[11] The latter reaction proceeds through the covalent intermediate PN (**2**), which phosphorylates phosphate (P) yielding PP. Thus, (**1**) catalyses both bond breaking (ATP and AcP transformation) and bond making (PP formation) processes.

We now report that when (**1**) is used as catalyst for the solvolysis of AcP in a mixture of DMSO ($Me_2SO$) and water, it mediates the synthesis of PP and triphosphate (PPP) *via* reaction of the phosphoramidate (**2**) with P and PP respectively. More interestingly, in the same solvent system, the solvolysis of AcP in the presence of (**1**) and a phosphoryl acceptor such as adenosine mono- and di-phosphate (AMP, ADP), glucose 1- or 6-phosphate (Glc-1P, Glc-6P), or *p*-nitrophenyl phosphate (PNP) leads to the corresponding pyrophosphate derivatives.

The mechanism and catalysis of the hydrolysis of AcP and related compounds have been extensively studied.[12–15] In the present work, the reaction of AcP in the presence or absence of (**1**) in a mixture of DMSO and $D_2O$ was followed by $^{31}P$ n.m.r. spectroscopy.† Whereas in the absence of (**1**) in DMSO–$D_2O$ (7:3) at pH 7 and 40 °C, the reaction products were acetate and P (as in aqueous solution), three additional species were observed in the presence of (**1**): PP (32%), PPP (23%), and up to 32% of the intermediate PN (**2**) giving a $^{31}P$ n.m.r. signal at 9.55 p.p.m. (Figure 1). These PP, PPP, and PN signals were never detected in the absence of (**1**) under the reaction conditions.

AcP consumption was accelerated by addition of 1 equiv. of (**1**) [by a factor of *ca.* 5 at pH 7 and 40 °C in DMSO–$D_2O$ (7:3)] and was first-order ($k_{obs.}$ 0.070 $min^{-1}$). The kinetic data agree with the reaction taking place predominantly *via* [(**1**)-$nH^+$, $AcP^{2-}$] complexes as the reactive species. The rate of AcP disappearance in the presence of (**1**) was enhanced by increasing the ratio of DMSO to $D_2O$ from zero [$k_{obs.}$ 0.035 $min^{-1}$ in $D_2$–$H_2O$ (1:9), pH 7 and 40 °C][11] to 7:3. At DMSO : $D_2O$ ratios higher than 7:3, no accurate kinetic data could be obtained owing to the low solubility of the reactants. The rate of AcP consumption in the presence of (**1**) in DMSO–$D_2O$ (7:3) at 40 °C was enhanced by increasing the pH of the solution from 3 to 7 ($k_{obs.}$ 0.0054 $min^{-1}$ at pH 3) and showed a maximum at pH ~7. Again, owing to solubility problems, no accurate data were available at pH >7. The amount of PPP formed increased significantly with the ratio of DMSO to $D_2O$, from 5% in a 1:1 mixture to 23% in 7:3. Whereas no PN nor PPP was detected at pH 3 in DMSO : $D_2O$ 7:3 at 40 °C (as in aqueous solution), the percentage of both PN and PPP increased with pH (*ca.* 3% of PPP at pH 5 *vs.* 23% at pH 7).

A mixture of DMSO–$D_2O$ (7:3) containing AcP, (**1**), and a phosphate-containing substrate, *i.e.* AMP, ADP, Glc-1P,



(**1**) X = H
(**2**) X = $PO_3^{2-}$

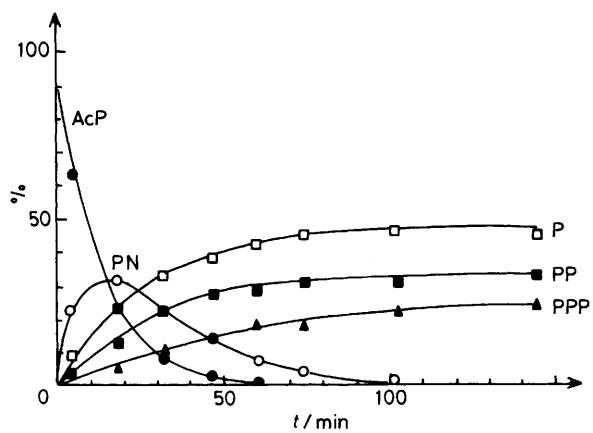



**Figure 1.** Time dependence of the reaction components of AcP solvolysis catalysed by macrocycle (**1**). Plot of the observed percentage of AcP (●), PN (**2**) (○), P(□), PP (■), and PPP (▲) *vs.* time in DMSO–$D_2O$ (7:3) solution containing initially 30 mM AcP and (**1**) at 40 °C and pH 7. % are given in P content (see Table 1, footnote b).

† The AcP reactions were performed on mixed solutions (DMSO–$D_2O$, 2 ml) containing AcP ($Li^+$, $K^+$ salt) and macrocycle (**1**)–6HCl (30 mM each) at various DMSO : $D_2O$ ratios and at various pH values adjusted by addition of 5 M NaOH or HCl solutions and at 40 ± 3 °C. The reaction was followed by $^{31}P$ n.m.r. spectroscopy at 81 MHz with selective proton decoupling. The amounts of the various compounds present in the sample at different times were determined by integration of the $^{31}P$ n.m.r. signals.

**Table 1.** Highest percentage of substrate phosphorylation in DMSO–$D_2O$ (7:3) (pH 7, 40 °C) after consumption of all the AcP and (**2**).[a]

| Acceptor R–OP | PP[b] | PPP[b] | Product R–OPP[b] |
|---|---|---|---|
| None | 30 | 25 | — |
| Glc-1P | 16 | 5 | 12 (Glc-1PP) |
| PNP[c,d] | 31 | <2 | 5 (P-NPP) |
| AMP | 13 | 5 | 8 (ADP) |
| ADP[c] | 6 | <2 | 28 (ATP |

[a] Equimolar mixture of AcP, (**1**), and acceptor R–OP, 30 mM each. [b] The amounts of the products formed are given as the % fraction of the total phosphorus content of the sample; the molar fractions of compounds containing two or three phosphorus atoms are thus 1/2 or 1/3 of the values listed. [c] Equimolar mixture of AcP, (**1**), and acceptor R–OP, 15 mM each. [d] The reaction mixture contained some precipitate.

Glc-6P, or PNP was adjusted to pH 7 and the course of the reaction was followed by $^{31}P$ n.m.r. spectroscopy at 40 °C (Figure 2). After all the AcP and PN (**2**) had been consumed, the reaction mixture contained the phosphorylated substrate in addition to PP and PPP (Table 1). In the absence of (**1**), the mixture of AcP and the substrate did not produce any phosphorylated product.

A detailed analysis of the mechanism of PP formation catalysed by (**1**) in aqueous solution has been performed.[11] On the basis of the results obtained in water and those summarized above, the following sequence of steps may be proposed for the formation of PP, PPP, Glc-1PP, Glc-6PP, PNPP, ADP, and ATP: (a) substrate (AcP) binding by protonated (**1**); (b) phosphoryl transfer within the supramolecular complex giving the covalent intermediate (**2**); (c) binding of the phosphoryl acceptor (R–OP); (d) phosphoryl transfer from (**2**) to R–OP with R–OPP formation; (e) product dissociation and release of the free receptor for a new catalytic cycle.

In order to check the validity of the proposed steps (c) and (d), the following experiments were performed. After isolating the intermediate (**2**) as a white solid from AcP and (**1**) at pH 7 in water, as described previously,[11] it was incubated with 1 equiv. of ADP in DMSO–$D_2O$ (7:3) at pH 7 and 40 °C. 51% of (**2**) was converted into ATP ruling out the direct phosphorylation of ADP to ATP by AcP in the presence of (**1**) and showing clearly the participation of (**2**) in the reaction. The binding of the phosphoryl acceptor by (**2**) is supported by the fact that competitive anions such as thiosulphate decreased significantly the amount of PP (31%) and PPP (<2%). When the phosphoryl acceptor containing a phosphate moiety was added the amounts of PP and PPP were also lowered (Table 1). Furthermore, the phosphorylation of substrates containing both phosphate moieties and hydroxy groups (*i.e.* AMP, ADP, Glc-P), essentially on the phosphate group, as well as the higher conversion of ADP to ATP than AMP to ADP (Table 1), agree also with precomplexation of the phosphoryl acceptor. Further mechanistic studies are in progress.

The formation of PP and PPP is in competition with the hydrolysis of (**2**) and can only occur after some P and thereafter PP have been produced, so that 32% of PP and 23% of PPP correspond to a high conversion of AcP to polyphosphate. On the other hand, phosphorylation of an added acceptor is in competition with both hydrolysis of (**2**) and formation of PP and PPP. Thus, formation of 28% of ATP is remarkable and could even be enhanced when isolated intermediate (**2**) was used (see above).

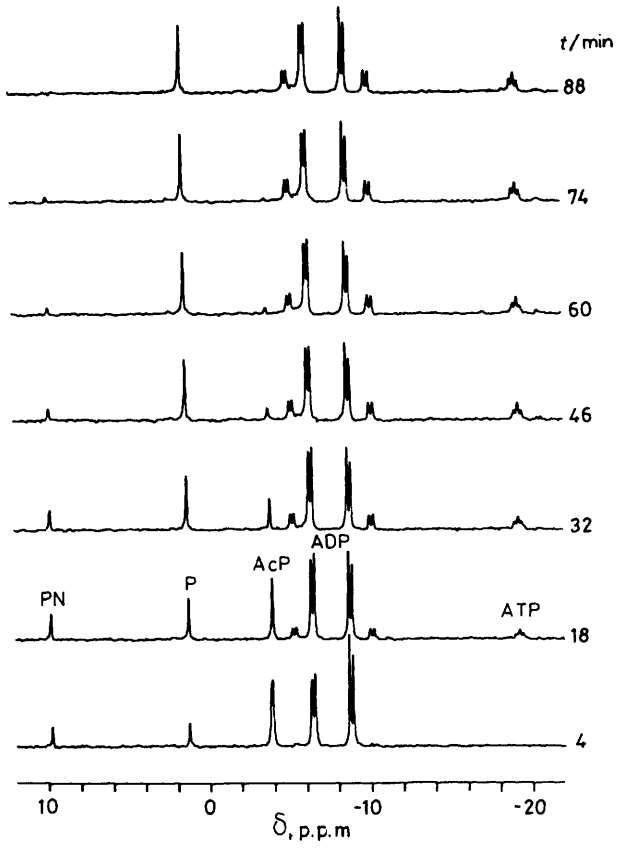



**Figure 2.** Observation of the reaction of AcP in the presence of (**1**) and ADP (30 mM each) by $^{31}P$ n.m.r. spectroscopy (proton decoupled) at 81 MHz as a function of time (DMSO–$D_2O$ (7:1), pH 7, 40 °C). The chemical shifts are given with respect to external 85% $H_3PO_4$.

Reducing the amount of water in the DMSO–$D_2O$ mixture could have two effects: (i) stronger interactions between the charged species should increase the percentage of complexes of the type [(**2**)$n$H$^+$, R–OP] which undergo the second phosphoryl transfer giving a larger amount of product R–OPP; (ii) since the phosphorylation of the acceptor by (**2**) is in competition with phosphorylation of water (PN hydrolysis), decreasing the concentration of water favours the phosphoryl transfer to the acceptor. It has been observed that reducing the reactivity of water, during the uncatalysed hydrolysis of AcP, by addition of 7 M $NaClO_4$, produced *ca.* 9% of PP.[13] On the other hand, solvolysis of concentrated AcP in 87% acetonitrile or dioxane gave 10—14% of PP.[13]‡

Macrocycle (**1**) mediates the phosphoryl transfer from AcP (phosphoryl donor) which plays the role of cofactor to the phosphate moiety of the acceptor. The reaction occurs *via* the phosphorylated macrocycle (**2**) which acts as a molecular phosphorylating agent. In the case of nucleotides, compound (**1**) catalyses both forward (ADP, ATP hydrolysis in water[9]) and, in the presence of AcP as cofactor, the backward

‡ Phosphoramidates have been used as phosphorylating agents, in organic solvents, to produce nucleotide polyphosphates.[16,17] In DMSO, the dismutation of ADP to form ATP and AMP was reported to be dependent on the metal cations.[18] Transphosphorylation between ATP and P yielding PP and ADP was also shown to be catalysed by bivalent metal ions.[19]

reactions (ADP, ATP synthesis in DMSO–$D_2O$), both processes proceeding through (**2**).§

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§ Numerous enzymatic phosphorylations occur *via* a phosphorylated enzyme intermediate.[5] In the case of microsomal glycose-6-phosphatase, the N-3-phosphorylated histidine was isolated.[20] Pyrophosphate dependent acetate kinase catalyses the reaction: AcP + P ⇆ PP + acetate.[21] Enzymatic ATP formation from ADP and phosphoramidate by phosphoramidate–ADP phosphotransferase has been reported.[22]