Coupling of Supramolecular Synthesis of ATP with ATP-consuming Enzyme Systems

Hicham Fenniri and Jean-Marie Lehn*

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

The abiotic ATP-producing system [**1**, AcP, Mg^{2+}, ADP] has been coupled to sets of ATP-consuming enzymes resulting in the production of NADH by a combined artificial–natural enzymatic process.

Extensive work has been directed towards the design of artificial catalysts that would behave as artifical enzymes or enzyme models.[1–3] Supramolecular catalysis, in which reactive receptor molecules bind a substrate and perform a reaction on it, represents a general approach to both abiotic and biomimetic catalysis.[2,3] It would be of interest to develop systems in which artifical and natural enzymatic reagents would be coupled in sequence through their substrate and products. This holds in particular for the bioenergetically crucial species handling adenosine triphosphate (ATP).

† URA 422 of the CNRS.

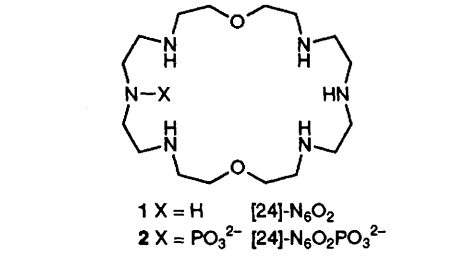
It has been shown that the macrocyclic polyamine **1** is capable of phosphorylating various substrates *via* the key *N*-phosphorylated intermediate **2** that is formed by reaction of **1** with acetylphosphate (AcP). Namely, ATP is produced by phosphorylation of ADP in aqueous Me_2SO[4] or in pure aqueous solution in the presence of Mg^{2+} ions.[5] The data indicated that the latter process, which corresponds to the reaction catalysed by acetate kinase,[6] probably involved a ternary species [**2**, ADP, Mg^{2+}], **3**.

We now report studies in which this ATP-generating system [**1**, AcP, Mg^{2+}, ADP] has been coupled to three ATP-dependent systems of natural enzymes: (*i*) hexokinase (HK),

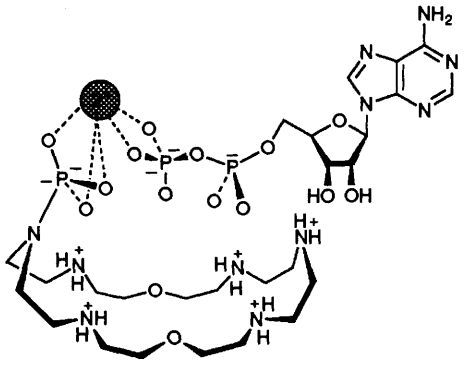
Table 1 NADH (ATP) generation by coupling of the artificial ATP-generating system [**1**, AcP, Mg^{2+}, ADP] with sets of ATP-dependent enzymes

Entry	Enzymes	Yield NAD(P)H (ATP) (%)[a]			
		(*a*) H_2O[b]	(*b*) D_2O[b]	(*c*) Me_2SO–H_2O[c] (2:3)	(*d*) Me_2SO–D_2O[c] (2:3)
1	—[a]	19 (9)[d]	22 (14)[d]	54 (70; 120)[e]	58 (101; 120)[e]
2	HK[a]	41 (40)[c]	53 (54)[c]	134 (203; 271)[e]	148 (250; 267)[e]
3	HK, G-6-PDH[f]	40	56	—	—
4	HK, G-6-PDH,[f] PPase	50	65	—	—
5	HK, G-6-PDH,[f] 6-P-GDH[f]	128[g]	158[g]	—	—
6	HK, G-6-PDH[d,h]	20	35	—	—
7	HK, G-6-PDH,[d,h] PPase	23	49	—	—
8	HK, G-6-PDH,[d,h] 6-P-GDH[d,h]	44[g]	63[g]	—	—
9	PPase	24	27	—	—

[a] Yield w.r.t. the amount of macrocycle **1**; for instance 100% means 10 mmol dm^{-3} ATP/NAD(P)H produced when 10 mmol dm^{-3} of **1** is used, assuming all ATP is converted into NAD(P)H; in Entry 1, ATP is determined by an [HK + G-6-PDH] assay; in Entry 2, ATP is determined by a G-6-PDH assay on G-6-P formed by HK; the yield of NAD(P)H also represents the yield of ATP initially produced by [**1**, AcP, Mg^{2+}, ADP]. [b] Conditions (concentrations in mmol dm^{-3}): **1** 10; ADP 10; AcP 50; $MgCl_2$ 80; ethylenediaminetetraacetic acid (EDTA) 30; glucose 100; NAD(P) 10.4; in solvent indicated at 38 °C and pH 7. [c] Conditions as in footnote [b] except $Mg(OSO_2CF_3)_2$ 15, EDTA 5. [d] 10.4 mol dm^{-3} NADP in place of NAD. [e] Same conditions as in footnote [c] with only 5 mol dm^{-3} of **1** (left number) or 2.5 mol dm^{-3} (right number). [f] NAD-dependent enzyme. [g] 2 NAD(P)H molecules produced per molecule of ATP consumed. [h] NADP-dependent enzyme.



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



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that phosphorylates glucose to glucose-6-phosphate (G-6-P); (*ii*) the mixture HK + glucose-6-phosphate dehydrogenase (G-6-PDH), where the latter transforms G-6-P into 6-phosphogluconate (6-P-G) and generates dihydronicotinamide dinucleotide (NADH) from NAD; (*iii*) the three-enzyme mixture HK + G-6-PDH + 6-phosphogluconate dehydrogenase (6-P-GDH), where the latter transforms 6-P-G into ribulose-5-phosphate (R-5-P) $+CO_2$ and yields a second molecule of NADH from NAD. The effect of the replacement of NAD-dependent G-6-PDH and 6-P-GDH by the NADP-dependent enzymes, of the addition of pyrophosphatase (PPase), and of a change in medium to D_2O or to Me_2SO–H_2O, Me_2SO–D_2O (2 : 3) mixtures has also been investigated. Some of the results obtained are listed in Table 1, where the yields of ATP generated by the [**1**, AcP, Mg^{2+}, ADP] system are given as the yields of NADH (or NADPH) produced by consumption of this ATP by the different enzyme systems.

The formation of ATP from ADP by the [**1**, AcP, Mg^{2+}] combination had been studied earlier by ^{31}P NMR observation.[5] An enzymatic test being more accurate than integration of ^{31}P NMR signals, the amounts of ATP generated were determined by following ATP formation *via* spectrophotometric determination of the amount of NADH (λ_{max} = 340 nm, $\varepsilon = 6200\ mol^{-1}\ dm^3\ cm^{-1}$) formed when aliquots of the [**1**, AcP, Mg^{2+}, ADP] solution were treated with [HK + G-6-PDH]. The results confirmed the earlier observations[5] that ATP is indeed produced (Table 1, entry 1), although the yields of ATP determined were somewhat lower.

The second step consisted in adding directly into the [**1**, AcP, Mg^{2+}, ADP] system either HK alone (and then analysing for the G-6-P formed with G-6-PDH) or a [HK + G-6-PDH] mixture and determining NADH. The results (Table 1, entries 2, 3) showed that: (*i*) NADH was obtained, indicating that ATP was indeed formed and therefore that the enzymes were operating properly in the mixture; (*ii*) the quantities of NADH and therefore of ATP initially produced by the [AcP, **1**, Mg^{2+}, ADP] system were much higher than in absence of the enzymes; (*iii*) in the three-enzyme system (entry 5) the production of a double quantity of NADH was expected; the fact that a significantly larger amount was obtained could be attributed to the transformation, by 6-P-GDH, of 6-P-G into 5-R-P; the latter has one less negative charge than the former and may be expected to interfere less with ADP binding by **1** and ATP production. In addition, the rate of ATP formation was much higher in presence than in absence of enzymes, the first-order rate constants being respectively 3.4 and $1.1 \times 10^{-3}\ mm^{-1}$.

Fig. 1 represents schematically the reactions and catalytic cycles involved. The ATP generated from ADP by the artificial protokinase system [**1**, AcP, Mg^{2+}] is immediately consumed by HK to form G-6-P, which is itself used up by G-6-PDH to form NADH from NAD; subsequently G-6-P may be consumed by G-6-PDH with production of a second molecule of NADH.

The much higher yield of ATP may be attributed to the fact that the consumption of ATP by the enzymes as soon as it is formed has several effects; it regenerates ADP and thus maintains a constant ADP level; it removes ATP which would bind to protonated **1** more strongly than ADP and therefore inhibit its own formation; accumulated ATP would itself be back-hydrolysed by **1**;[7] it would also interfere with binding of AcP to **1** and its phosphorylation to **2**. These factors, that all increase the generation of ATP from ADP by **2**, have globally the effect of favouring the ATP formation pathway with respect to the competing hydrolysis reactions of both AcP and **2**.

The lower yield of NADPH (Table 1, entries 6–8) compared to NADH (entries 3–5) may be attributed to the stronger interference of the more highly charged NADP with the

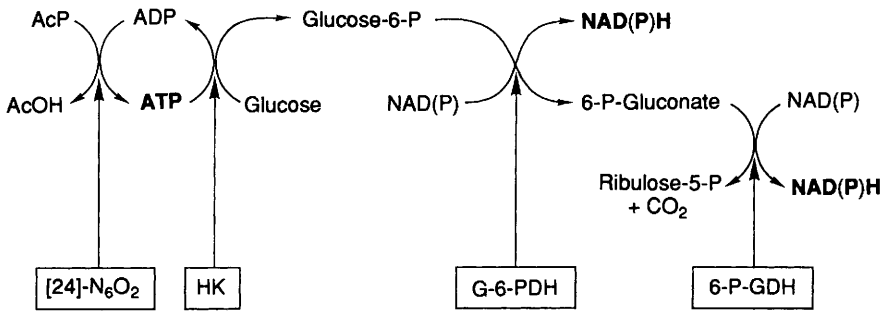

Fig. 1 Sequence of transformations catalysed by the supramolecular ATP-generating system [**1**, AcP, Mg^{2+}, ADP] (**1** = [24]-N_6O_2) and the enzymes hexokinase (HK), glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-P-GDH)

binding of both AcP to **1** and of ADP to **2**. The addition of 6-P-GDH has a larger effect in the case of the NAD (entries 3 and 5) than of the NADP (entries 6 and 8) dependent enzymes; this could be due to the fact that in presence of NADP the inhibitory effect of G-6-P is comparatively less pronounced because of the inhibition by NADP itself.

The marked increase in ATP production in aqueous Me_2SO should result at least in part from a decrease of the hydrolysis reactions of AcP and of **2** that compete with phosphoryl transfer to ADP (see also ref. 4).

The ATP yield and rate of formation were found to increase by a factor of about 1.5 on going from H_2O to D_2O solution. This should be due to proton isotope effects on the sequence of reactions leading to the formation of ATP by the [**1**, AcP, Mg^{2+}, ADP] system. However in view of the complicated series of phosphoryl transfer processes that take place (from AcP to **1** or to water, from **2** to ADP, to phosphate or to water) it is difficult at this stage to determine from which step(s) the isotope effect originates.‡ Whatever the reason, the gross result is that total yields of final NADH above 100% and as high as 250% have been obtained in Me_2SO–H_2O and Me_2SO–D_2O, amounting to a true catalytic process and genuine turnover with respect to the macrocycle **1**.

When PPase was added (Table 1, compare entries 1 and 9, 3 and 4, 6 and 7) more ATP was formed. This may be attributed to the fact that PPase immediately hydrolyses any pyrophosphate (PP) formed by phosphorylation of phosphate by **2**;[11] PP (as well as triphosphate PPP which would be produced by phosphorylation of PP[5]) would be expected to interfere with both phosphorylation of **1** by AcP and of ADP by **2**.

In conclusion, the results described here represent the successful coupling of an artificial enzyme-mimetic process of kinase type[4,5] to a set of natural enzymes. The generation of ATP *via* phosphorylation of ADP by the abiotic system [**1**, AcP, Mg^{2+}] results overall in the generation from AcP of a high yield of NADH as final product.

‡ It has been reported that the hydrolysis of phosphoramidic acid[9] and other phosphoramidate derivatives[10] was about 1.5 times faster in D_2O than in H_2O.

It should be possible to devise other mixed abio/biological systems coupling artifical catalysts to natural ones and capable of producing a product of either biological or practical interest.[8]

Finally, although the artifical ATP-producing system is of low efficiency compared to enzymes, one may wonder whether it would be possible to go a step further and realize its coupling to an ATP-deprived microorganism.

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