A zinc(II)-based receptor for ATP binding and hydrolysis†

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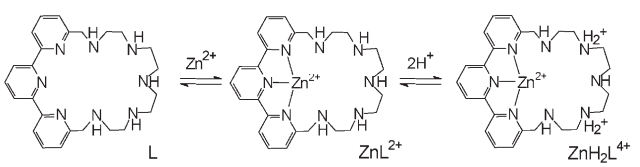
A protonated Zn(II) complex with a terpyridine-containing pentaamine macrocycle catalyses ATP hydrolysis in the presence of a second metal ion, which acts as cofactor assisting the phosphoryl transfer from ATP to an amine group of the receptor.

ATP plays a basic role in the bioenergetics of all living organisms, the center for chemical energy storage and transfer being its triphosphate chain. In Nature ATP hydrolysis is catalyzed by ATPases; a divalent metal cation, generally Mg(II) or Ca(II), is involved in the catalytic mechanism, acting as binding site for ATP or as cofactor, favoring the phosphoryl transfer process.[1–3] However, ATPases which hydrolyze ATP only in the presence of "softer" metal cations (Zn(II), Cd(II) or Pb(II)) are also known.[4] In recent years synthetic hydrolytic systems, from macrocyclic polyammonium cations[5–9] to metal complexes with simple ligands,[10–14] have been developed to elucidate the mechanism of ATP cleavage. Metal complexes generally cleave phosphate monoesters and ATP through activation of the phosphate chain *via* coordination to the metal center(s) and attack of a metal-bound hydroxide on phosphorus. Partially protonated polyamine macrocycles, instead, can hydrolyze ATP[5–9] *via* binding and activation of ATP thanks to the formation of salt bridges between the phosphate chain and the charged ammonium groups and subsequent nucleophilic attack of an amine group at the γ-phosphorus of ATP to give a labile phosphoramidate intermediate. In this case it was observed that the presence of Zn(II) reduces the hydrolytic ability of the receptor.[7]

We have recently reported the synthesis of the terpyridine-containing macrocycle L.[15] In its monometallic Zn(II) complex the metal ion is bound to the terpyridine nitrogens, while the polyamine chain is not involved or weakly involved in metal binding and can easily protonate in aqueous solutions to give $[ZnLH_x]^{2+x}$ complexes (Scheme 1).[15]



Scheme 1

Alternatively, the polyamine chain can be used to bind a second Zn(II) ion, affording dimetallic complexes. The monometallic L complexes are in principle promising binding and/or hydrolytic agents for ATP, since they combine within the same receptor the peculiar features of both metal-based and polyammonium ATP binders. Actually, potentiometric measurements‡ show that ATP is strongly bound to the monometallic complexes with L.

As shown in Fig. 1, ATP binding at alkaline pHs affords the ternary complexes $[ZnL(OH)ATP]^{3-}$ and $[ZnLATP]^{2-}$, while ATP complexes with protonated species of the Zn(II) complex, $[ZnLH_xATP]^{x-2}$, prevail at neutral and acidic pHs. A ^{31}P NMR titration§ shows that the resonances of both the P_γ and P_β phosphate groups shift downfield upon coordination of ATP to the $[ZnLOH]^+$ and $[ZnL]^{2+}$ complexes, probably due to the simultaneous involvement of both phosphate moieties in coordination to Zn(II) (Fig. 1).

The formation of the $[ZnLH_xATP]^{x-2}$ complexes, instead, gives rise only to a further marked downfield shift of the P_γ signal with respect to the corresponding signal of "free ATP", indicating that in the protonated ternary complexes the P_γ phosphate is involved in salt bridges with the positively charged ammonium groups of the ligand and leading us to propose the interaction mode between ATP and the protonated Zn(II) complexes sketched in Scheme 2

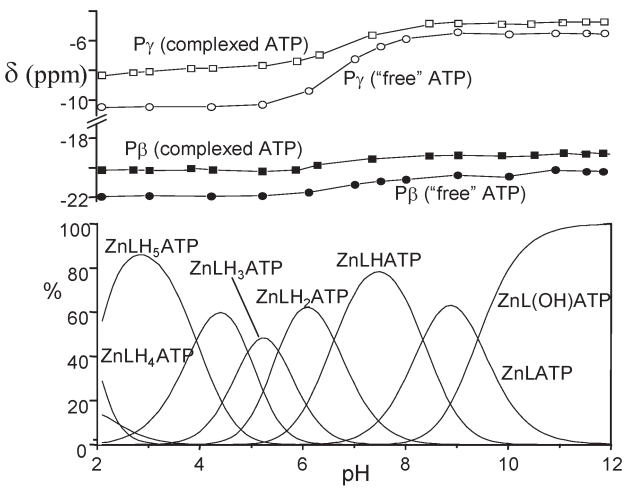


Fig. 1 Distribution diagram of the ternary complexes formed by the Zn(II), L and ATP (1 : 1 : 1 molar ratio) and ^{31}P NMR chemical shift of the signals of the P_γ and P_β phosphate groups of ATP in the presence and in the absence of the Zn(II) complex with L as a function of pH. The chemical shift of the P_α signal does not change in the presence of the Zn(II) complex with L and it has been omitted.

† Electronic supplementary information (ESI) available: experimental details for the potentiometric and ^{31}P NMR experiments. Formation constants of the ATP adducts with L and with its mono- and dimetallic Zn(II) complexes, plots of time-dependence of ATP concentrations in the presence of the Zn–L complex and Zn^{2+} at various pH values, plot of the k_{OBS} values *vs.* Zn^{2+} concentration. See http://www.rsc.org/suppdata/cc/b5/b502229k/

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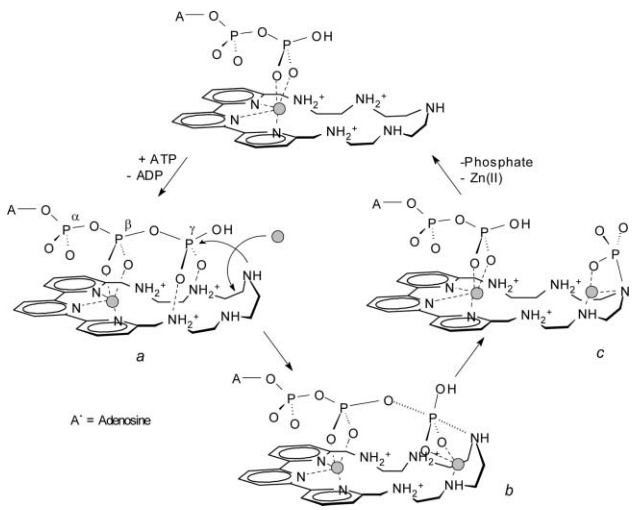
(drawing *a*). At the same time, ^{1}H NMR titrations display an upfield shift of the signals of the adenine protons upon ATP complexation, as generally observed in the case of π-stacking interaction of adenine with an aromatic moiety. The protonated $[ZnLH_x]^{x+2}$ complexes, therefore, behave as multifunctional receptors for ATP, thanks to the simultaneous presence of metal–donors bonds, electrostatic and hydrogen bonding interactions and π-stacking pairing. Actually, the potentiometric study of this system shows that the Zn(II) complexes with L display a markedly higher affinity for ATP than the protonated ligand in the absence of Zn(II), confirming the crucial role of Zn(II) in ATP binding. Ligand L and its monometallic Zn(II) complexes, however, do not show any significant ability in ATP hydrolysis. Addition of an equiv. of Zn(II) ion to solutions containing the monometallic ternary complexes with ATP leads to the formation of dimetallic Zn(II) complexes with the nucleotide (Fig. 2); the second metal is coordinated to the pentaamine chain and therefore this process competes with protonation of the aliphatic amine groups. In consequence, monometallic protonated complexes prevail at acidic pH values even in the presence of a second metal ion, while dimetallic complexes are formed from neutral to alkaline pHs. Once again, the dimetallic complexes do not give ATP hydrolysis. On the contrary, a fast cleavage process is observed by ^{31}P NMR in the acidic pH region,§ where the $[ZnLH_4ATP]^{2+}$ complex and free Zn(II) are simultaneously present (Fig. 2).

The analysis of the time dependence of the ^{31}P NMR spectra recorded at 298.1 K shows that the hydrolytic process leads first to the formation of ADP and a phosphoramidate intermediate (PN), characterized by the typical signal at 10.06 ppm, which is then fast hydrolysed to hydrogen-phosphate (P). As shown in Fig. 2, the rate constants for the process ATP → PN + ADP fit the distribution curve of the tetraprotonated $[ZnLH_4ATP]^{2+}$ species, with a maximum at pH 4 ($k_{OBS} = (3.2 \pm 0.2) \cdot 10^{-2}$ min^{-1}), thus indicating that this complex is indeed the active species. The hydrolysis rate is among the highest observed for ATP dephosphorylation promoted by polyammonium receptors.[6–10] Further complex protonation to give the $[ZnLH_5ATP]^{3+}$ species leads to a complete quenching of the cleavage process. At pH 4, the phosphoramidate intermediate is formed, together with ADP, in the first few minutes up to a relatively high percentage (*ca.* 30%



Scheme 2

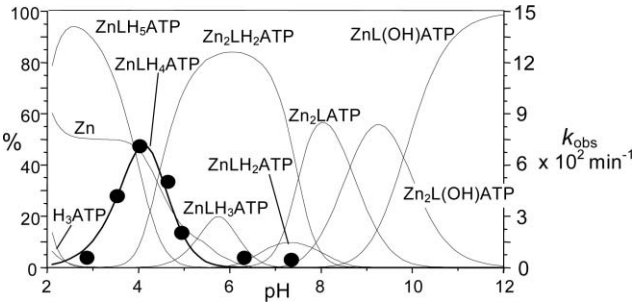


Fig. 2 Distribution diagram for the system Zn(II)/L/ATP in 2 : 1 : 1 molar ratio (—, left *y* axis) and experimental pseudo-first order rate constants (●, right *y* axis) for ATP cleavage at 298.1 K (I = 0.1, $[ZnL] = [Zn^{2+}] = 1.65 \cdot 10^{-2}$ M).

with a 1 : 1 molar ratio between the Zn(II) complex and "free" Zn(II)), compared with other polyammonium macrocycles able to hydrolyze ATP.[5–9]

The hydrolytic process is partially inhibited by ADP coordination (for instance only 70% ATP is cleaved at pH 4), although the stability of the ADP ternary complexes is lower than that of the corresponding ATP complexes. Our complex, however, behaves as a real catalyst, with a turnover number of 13 at pH 4 (298.1 K, $[ZnL] = [Zn^{2+}] = 1 \cdot 10^{-3}$ M, $[ATP] = 2 \cdot 10^{-2}$ M).

Experiments carried out on solutions containing the monometallic Zn(II) complex with L at acidic pHs and increasing amount of Zn(II) also showed the formation of increasing percentages of phosphoramidate intermediate (Fig. 3).

The rate of ATP dephosphorylation to give PN and ADP displays a pseudo first-order dependence on "free" Zn(II) concentration, giving rise to an overall second-order kinetics ($v = k[ZnLH_4ATP^{2+}][Zn^{2+}]$), with a rate constant of 4.2 ± 0.2 M^{-1} min^{-1} at 298.1 K and pH = 4.

These data are in accord with a mechanism involving first coordination of $ATPH^{3-}$ (ATP is mainly in its monoprotonated form at pH 4) to the $[ZnLH_3]^{5+}$ complex and then metal-assisted nucleophilic attack of an unprotonated amine function to give the PN intermediate (*c* in Scheme 2).

The fact that ATP cleavage takes place only in the presence of a "second" Zn(II) ion with second order kinetics suggests that the transition state could be stabilized by this metal ion (*b* in Scheme 2), probably through coordination of the metal to the unprotonated

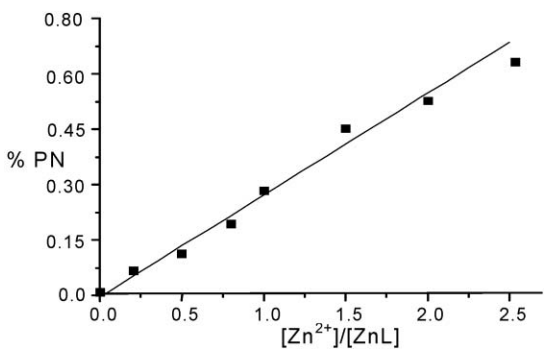


Fig. 3 Percentages of PN intermediate formed in the presence of increasing amounts of $[Zn^{2+}]$ at pH 4 and T = 298 K. The relative concentration is based on the initial concentration of ATP.

amine groups of the macrocycle and to the γ-phosphate of ATP, leading to a higher activation of the γ-phosphorus to the nucleophilic attack. At the same time, the PN intermediate could be stabilized thanks to coordination to the metal, accounting for the observed relatively high percentage of PN formed during the cleavage process. In such a way, the second Zn(II) ion could "assist" the transfer of the γ-phosphate from ATP to an amine group of the macrocycle. The quenching effect observed with the formation of the $[ZnL(ATP)H_5]^{3+}$ complex can be reasonably attributed to protonation of an amine group of the macrocycle, which results in no more being available for the nucleophilic attack.

Finally, the formed ADP is replaced by ATP in the ternary complex, thanks to the higher stability of the ATP complexes with respect to the ADP ones.

The present system, therefore, represents a unique case of ATP dephosphorylation promoted by the simultaneous action of a metal complex, which is used essentially for substrate anchoring, and of a second metal, which probably acts as cofactor, assisting the phosphoryl transfer from ATP to an amine group of the receptor.

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Notes and references

‡ The formation constants of ATP with L and its complexes were obtained by means of potentiometric measurements in 0.1 M Me_4NCl at 298.1 K by using the method and procedure described in reference 9.

§ ^{31}P NMR experiments were carried out on a 400 MHz instrument at 298 K. In the ^{31}P NMR titrations, HCl and NMe_4OH were used to adjust the pH values. The pH was calculated from the measured pD values by using the eqn: pH = pD − 0.40. The rate of ATP cleavage in the presence of the zinc complex with L was measured by an initial slope method monitoring the decrease of the ATP resonances. Potassium hydrogen phthalate (pH 2.7–4.5), MES (pH 4.5–6.5) and MOPS (pH 6.5–8.5) buffers were used (50 mM). In a typical experiment, ATP, the zinc complexes with L and $ZnCl_2$ were mixed in D_2O solution at the appropriate pH and stored at 298.1 ± 0.1 K. The reaction mixture was sampled by drawing a 0.8 ml portion of solution generally every 2–5 minutes. To quench the hydrolytic effect, each sample was immediately refrigerated at 278 K and its pH was adjusted to 7 (in these conditions ATP hydrolysis is negligible for several hours). No ATP hydrolysis was observed in the absence of added $ZnCl_2$. ^{31}P NMR spectra were then recorded for each sample, determining the percentages of each species in solution.

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