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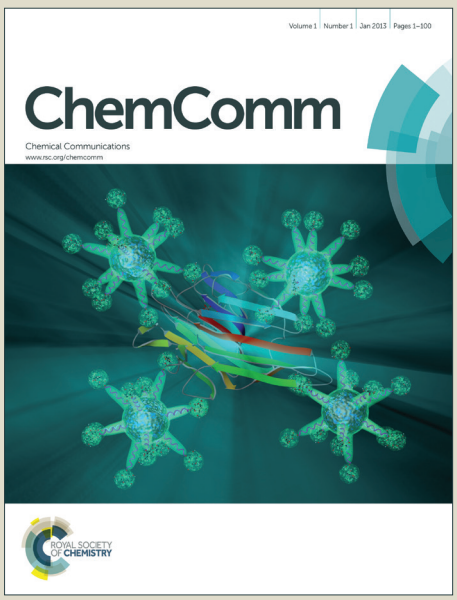

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ATP dephosphorylation can be either enhanced or inhibited by pH-controlled interaction with a dendrimer molecule

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Synthetic polyammonium/polyamine receptors are known to enhance ATP dephosphorylation in solution. ATP interaction with a G-3 poly(ethylene imine) dendrimer shows an unprecedented behaviour, the dendrimer catalyst being able to enhance or inhibit dephosphorylation of the nucleotide depending on the solution pH.

Dendrimers are branched three-dimensional molecules commonly characterized by highly ordered, well defined structures produced by the iterative synthetic routes adopted to grow successive generations (G) of the dendritic architecture around a central core. Their structures can be thought to be characterized by three major parts: the core, the inner shell, and the surface. Lower generation dendrimers are flexible molecules with no appreciable inner regions, while medium sized (G-3 or G-4) have an internal space that is essentially separated from the outer shell of the dendrimer. Very large (G-7 and greater) dendrimers are similar to solid particles, with very dense surfaces due to the crowding of branches in the outer shell.[1] The ability of dendrimer molecules to produce surface and/or inner shell binding, transport and release of a variety of chemical species is encouraging a continuous expansion of their applications in technological areas such as, among others, gene[2] and drug delivery,[3] medicinal chemistry,[4] sensing,[5] advanced materials[6] and catalysis (ref.1c).

We have recently shown that protonated forms of the G-2 poly(ethylene imine) dendrimer L1 (Fig. 1) is able to form stable cation, anion and ion-pair complexes in aqueous solution.[7] When the interaction occurs with the anionic forms of ATP (adenosine 5'-triphosphate), dephosphorylation of this nucleotide to form ADP and phosphate was observed to proceed 2-5 times faster, depending on the solution pH, than the uncatalysed process.[7c] The ability of polyammonium/polyamine receptors to induce catalytic processes, mimicking the behaviour of ATP-ases or kinases, has been observed for receptors of different molecular structures, including macrocyclic, linear and few branched molecules.[8] Although L1 is significantly less efficient than the abiotic receptors producing highest enhancements of ATP cleavage,[9] its catalytic effect falls within the range shown by most of synthetic compounds that are

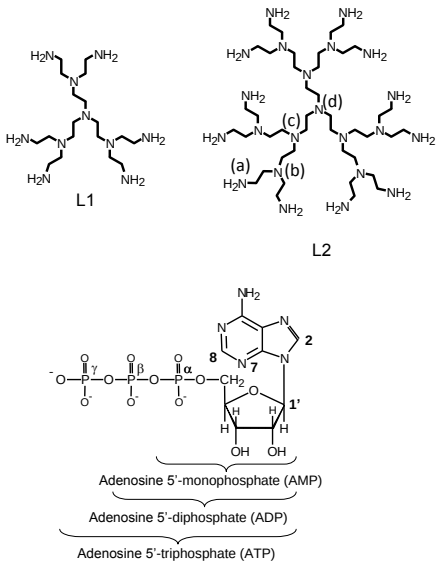

Fig. 1 G-2 (L1) and G3 (L2) poly(ethylene imine) dendrimers and nucleotides.

able to enhance the activation of ATP dephosphorylation.[8,10]

We have now extended this study to the G-3 poly(ethylene imine) dendrimer L2 (Fig. 1),[11] obtained upon addition of a further generation of ethylamino residues to L1, and found an unprecedented behaviour consisting in the possibility of switching the catalytic action of L2 from enhancement to inhibition of ATP cleavage by changing the solution pH.

Potentiometric (pH-metric) titrations performed in water (see the ESI†) showed that tight association occurs between protonated forms of L2 (Table S1, Fig. S1) and the anionic species of ATP, ADP and AMP. An inspection to the equilibrium constants determined for the formation of L2 complexes with these nucleotides (Tables S2-S4) evidences some general characteristics. Nucleotide complexation takes place over all the studied pH range (2.5-11) and involves a large number of ligand protonated species spanning from H_2L2^{2+} to $H_{17}L^{17+}$. The complex stability is very high, even when the ligand is

in low protonation states. Indeed, H_2L2^{2+} affords enough attraction to form anion complexes of considerable stability (e.g. log$K$ = 4.21 for H_2L^{2+} + ATP^{4-} = $[H_2L(ATP)]^{2-}$) while in the case of L1 a minimum of 4+ charge was necessary to form a weaker complex (log$K$ = 3.72 for H_4L1^{4+} + ATP^{4-} = $[H_4L(ATP)]$).[7c] Very likely, the G-3 polyamine-dendrimer L2, with its 12 primary and 10 tertiary amine groups, is able to establish a great number of hydrogen bond contacts with these anionic nucleotides even when it does not hold a great positive charge. Hydrogen bonds are known to play a crucial role in nucleotide-polyammonium receptor interactions.[8,12]

Another interesting aspect of these complex systems is the approximate invariance of stability displayed by complexes with the ligand in high protonation states (Table S2-S4). L2 is a large molecule; its successive protonation stages (Table S1) take place in such a way that the generated ammonium groups are located as far as possible from each other, to minimize the electrostatic repulsion (see the ESI†), leading as a further consequence, to progressive expansion and stiffening of the molecule. Then, in contrast to more preorganized molecules, such as macrocycles for instance, most of the binding sites in the protonated forms of L2 are divergent with respect to the interacting nucleotides. Large ligand dimensions, ligand rigidity and low preorganization make the nucleotide to interact with a sector of the ligand, and therefore the adduct stability is poorly, or not at all affected by ligand protonation occurring far from the bound nucleotide.

On a comparative basis, L2 shows a binding preference for ADP over both AMP and ATP, for given protonation states of ligand and nucleotides, the general trend being ADP > ATP > AMP. That is, L2 performs a selective recognition of ADP, despite the greater negative charge of ATP species should favour the latter. Remarkably, another case of molecular recognition of ADP over ATP was recently reported for a pyrimidine derivative of tren (tris(2-aminoethyl)amine), the G-1 precursor of L2.[13] The binding behaviour of L2 toward the three nucleotides can be evidenced, over the entire pH range, by comparing the effective (conditional) stability constants calculated as a function of pH for these binding events. This is a practical computational method for the analysis of binding selectivity in competitive systems which balances all complex stability constants along with receptor and substrates basicity.[14] Plots of the effective stability constants, as a function of pH, for the nucleotide/L2 systems (Fig. 2) show that the ligand preference for ADP increases above pH 5, while ATP starts being competitive only below pH 9.

Analogous studies performed for the interaction of L2 with PO_4^{3-}, $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$, which are the inorganic portions of AMP, ADP and ATP, respectively, showed that the binding features of the phosphate anions are consistent with those of the corresponding nucleotides, including some disadvantageous binding of $P_3O_{10}^{5-}$ in a limited region above pH 10, but below pH 10 the trend of binding stability expected on the basis of electrostatic considerations ($P_3O_{10}^{5-}$ > $P_2O_7^{4-}$ > PO_4^{3-}) is invariably observed (Fig. S2). Accordingly, we can conclude that electrostatic and hydrogen bond interactions between the protonated forms of L2 and the phosphate chains of the nucleotides are the driving forces of these association processes, while the adenosine residue furnishes a significant regulation of the nucleotide-ligand interaction, determining the binding selectivity.

^{31}P NMR spectra recorded on ATP solutions at pH 3, 6 and 9, in the absence and in the presence of L2 showed a downfield shift of the ^{31}P NMR signals of ATP, in particular of P_β and P_γ, determined by interaction of the nucleotide with the positively charged ligand (Fig. S3). At pH 9, only P_β shows a significant shift, P_α and P_γ being modestly affected, while greater coordination induced shifts are observed at lower pH. Accordingly, modelling calculations (see the ESI†) performed for the $[H_9L2(ATP)]^{5+}$ and $[H_{15}L2(H_2ATP)]^{13+}$ adducts, which are main species at pH 9 and 3 (Fig. S4), respectively, showed that, under an implicit simulation of the aqueous environment, the nucleotide forms a greater number of salt-bridge interactions with the more charged ligand form (Fig. 3).

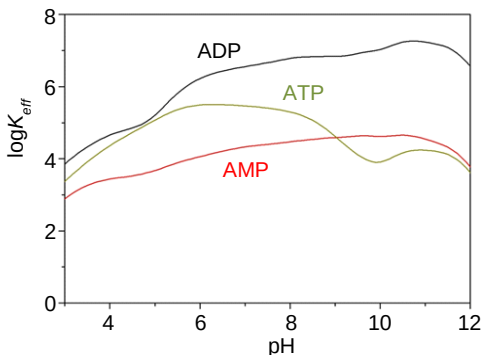

Fig. 2 Logarithms of the effective stability constants (K_{eff}) for the interaction of L2 with ATP, ADP and AMP calculated as a function of pH.

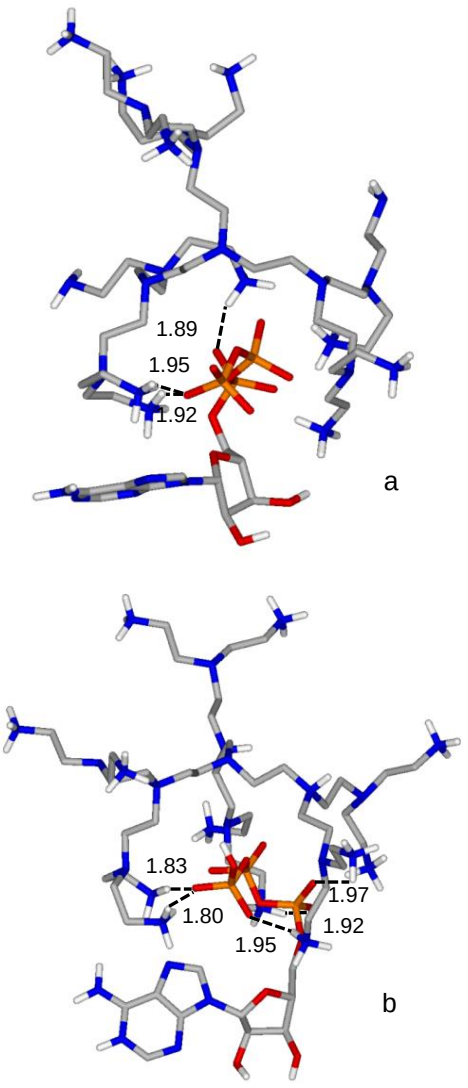

Fig. 3 Minimum energy structures calculated for the adducts $[H_9L(ATP)]^{5+}$ (a) and $[H_{15}L(H_2ATP)]^{13+}$ (b). Hydrogen bond distances are in Å. Only contacts shorter than 2.0Å are shown.

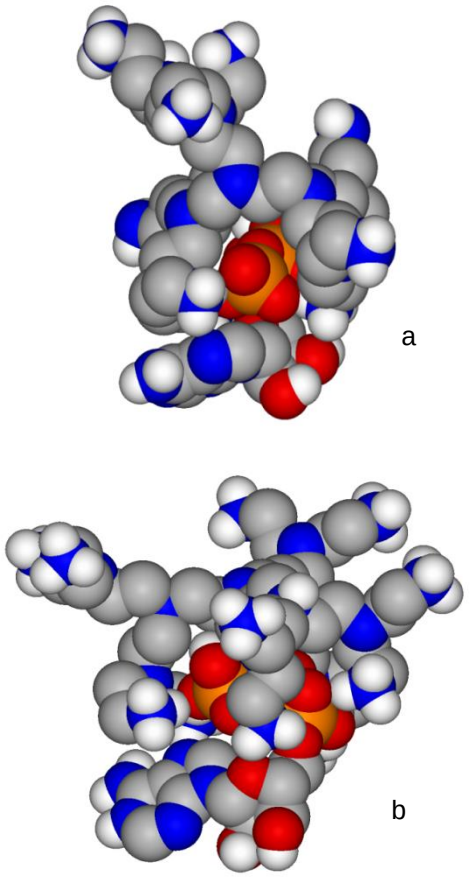

Fig. 4 Space filling views of the minimum energy structures calculated for the adducts $[H_9L(ATP)]^{5+}$ (a) and $[H_{15}L(H_2ATP)]^{13+}$ (b).

According to calculations, ATP is attracted under the dendrimer surface where the phosphate chain of the nucleotide is shielded from solvent interactions (Fig. 4).

The most outstanding feature of these nucleotide/L2 systems is the effect of L2 on ATP dephosphorylation to form ADP and inorganic phosphate. Depending on the solution pH, L2 can enhance or inhibit this process. ATP hydrolysis over time was followed at pH 3 and 9 by ^{31}P NMR at 343.1 K (Fig. S5) with solutions 0.01 M of ATP and 0.001 M of L2. As shown in Table 1, the presence of L2 at pH 9 gives rise to an approximate 6-fold enhancement of the dephosphorylation rate, while at pH 3 the spontaneous ATP cleavage is slowed down by about 30% in the presence of the dendrimer. Such unprecedented behaviour is in agreement with the different ATP adducts formed at the different pH values, with their calculated structures and with the location of ammonium groups in the protonated forms of L2 deduced by ^{1}H NMR spectra (Fig. S6). The latter shows that the first 12 H^+ ions bind the 12 primary N(a) atoms (Fig. 1), which remain protonated in more acidic solutions. The 13th proton binds the central tertiary N(d) nitrogen, while in the two successive protonation stages, until the formation of $H_{15}L2^{15+}$, also N(c) nitrogen atoms get involved. Successive protonation causes a redistribution of protons over all tertiary amine groups.

It is known that when the polyamine receptor contains unprotonated amine groups, nucleotide dephosphorylation proceeds through the formation of a labile phosphoramidate intermediate.[9b,c, 15-18] Indeed, in the principal ATP complex formed by L2 at pH 9, $[H_9L2(ATP)]^{5+}$, there are at least three unprotonated primary amine groups which are available for the formation of the phosphoramidate intermediate.

Table 1. Rate constants (k) determined at 343.1 K for ATP dephosphorylation in the absence and in the presence of L2. [a] Values in parentheses are standard deviations on the last significant figures.

	pH	k ($min^{-1}\times10^3$)
ATP	3	0.68(2)[a]
ATP	9	0.041(4)
L2 + ATP	3	0.48(4)
L2 + ATP	9	0.23(4)

Conversely, in the complexes formed in acidic media, all surface amine groups are protonated while in the inner dendrimer region, where the phosphate ATP chain is attracted, there are only tertiary amine groups that are unable to form the phosphoramidate intermediate. In this environment, ATP, which is present at pH 3 as H_2ATP^{2-}, is protected from solvent interactions and its spontaneous dephosphorylation, that is thought to proceed through the formation of a labile monomeric metaphosphate intermediate,[19] becomes slower.

In conclusion, interaction of ATP with differently protonated forms of the G-3 poly(ethylene imine) dendrimer L2 produces opposite dephosphorylation effects in giving ADP and inorganic phosphate. In alkaline solutions, where ATP finds unprotonated primary amine groups of L2 that can trigger the phosphoramidate-mediated hydrolytic mechanism, dephosphorylation is enhanced with respect to the spontaneous process, while in the acidic region, where unprotonated primary amine groups are no longer available, inclusion of the nucleotide phosphate chain into the inner dendrimer region protects ATP from the spontaneous dephosphorylation reaction, thus inhibiting the process. Previous studies of the effect of synthetic receptors on the process of ATP dephosphorylation have concentrated exclusively on the enhancement aspect and, to the best of our knowledge, a receptor capable of producing both enhancement and inhibition has never been found, neither has one been found that could produce just the inhibition.

The unprecedented behaviour shown by L2 is a further evidence of the peculiar properties characterizing the inclusion compounds of dendrimer molecules.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details of potentiometric, NMR and modelling studies. Table of equilibrium constants for ligand protonation and complex formation with ATP, ADP, AMP, PO_4^{3-}, $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$. Figures with distribution diagrams of protonated L2 species and L2/ATP complexes, pH dependence of the effective stability constants for the complexes of L2 with PO_4^{3-}, $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$, pH dependence of the ^{1}H NMR spectra of L2, ^{31}P NMR spectra of ATP in the absence and in the presence of L2, ^{31}P NMR data for ATP dephosphorylation and related kinetic fitting. See DOI: 10.1039/c000000x/

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