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Zn(II) enhances nucleotide binding and dephosphorylation in the presence of a poly(ethylene imine) dendrimer

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ABSTRACT

The interaction of a second-generation poly(ethylene imine) dendrimer (L) with the nucleotides AMP, ADP and ATP was analysed by means of potentiometric titrations (0.1 M Me_4NCl, 298.1 K), in the absence and in the presence of Zn^{2+}, to perform speciation of the systems and determination of complex stability constants. Protonated forms of L interact with anionic forms of the nucleotides giving rise to stable anion complexes. In the presence of Zn^{2+}, ternary complexes (ion-pair complexes) become the main species in solution, the presence of each partner enhancing the binding of the other one (positive cooperativity effect). The stability constants determined by the same method and under the same experimental conditions for the formation of PO_4^{3-}, $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$ complexes with L showed a strict similarity between the binding of AMP, ADP and ATP and their inorganic counterparts, indicating that the main interaction of nucleotides with protonated ligand forms takes places through their inorganic tails.

Dephosphorylation of ATP to form ADP and phosphate was monitored by means of ^{31}P NMR spectra at pH 3 and 9 in the presence of L and of its Zn^{2+} complexes. The rate of ATP dephosphorylation is enhanced by about 2 times at pH 3 and 5 times at pH 9 in the presence of L and by about 4 times at pH 3 and 6 times at pH 9 in the presence of its metal complex, relative to the uncatalysed process, the dephosphorylation reactions being much faster in the acidic media where successive cleavage of ADP to give AMP and phosphate was also observed. Depending on the pH, the observed dephosphorylation enhancements have been interpreted in terms of formation of phosphoramidate intermediates, formation of ATP–Zn^{2+} coordinative bonds and positive allosteric effects due to metal ion coordination.

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1. Introduction

The appreciation that anions play important roles in both abiotic and biological systems has attracted a great deal of interest toward the use of synthetic receptors for the binding and recognition of anionic substrates [1–7]. Among anions of biological interest, the purine mononucleotides ATP, ADP, AMP, and the related inorganic phosphates, were among the first targets of synthetic receptors [8,9]. It is known that ATP, along with ADP and inorganic phosphate, from which they are formed, take part in more chemical reactions than any other compound except water [10]. ATP is mainly associated with the transport of chemical energy within cells for metabolism, but it is also involved in signal transduction and transmission pathways [11], and is incorporated into nucleic acids by polymerases in the process of DNA replication and transcription [12]. ATP is produced by ATP synthase from inorganic phosphate and ADP or AMP and is converted into his precursors by metabolic processes that use it as an energy source.

Since the seminal works of Kimura and Lehn on nucleotide binding by polyammonium macrocycles in the earlier '80s [8,9], the use of synthetic receptors has become a common strategy to mimic biological binding and recognition of nucleotides by proteins [1,13,14]. Proteins employ a variety of forces to bind anions, their binding sites consisting of both polar and apolar groups such as cationic, hydrogen bonding residues, aliphatic chains and aromatic functionalities giving rise to stacking interactions. The polyfunctional character of proteins can be replicated in synthetic receptors by the introduction of appropriate functional groups and the topological control of their molecular arrangement. A snapshot of the nucleotide–synthetic receptor interaction, showing the variety of binding forces, was recently obtained by X-ray determination of the first crystal structure showing a nucleotide (thymidine 5′-triphosphate) adduct with a polyammonium ligand containing aromatic groups [15]. Cationic groups of anion binding sites of proteins include metal ions. A 2007 review, dealing with the binding motifs found in proteins for recognition of phosphate containing substrates, revealed that 547 of the 3003 protein structures identified in the RCSB protein database as phosphate binders contain a metal coordinating to the anionic group in the substrate [16].

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One of the most interesting points of nucleotide receptors chemistry is the capacity that some synthetic receptors have to induce catalytic processes mimicking the behaviour of ATP-ases or kinases [1–7,14]. The macrocyclic polyamines O-BISDIEN [8,17], [21]aneN_7 [18] and the *meta*-cyclophane [19] shown in Fig. 1, for example, rank as the abiotic receptors producing highest enhancements of ATP cleavage. The insertion of metal ions into the receptor structure may affect the receptor ability to enhance nucleotide dephosphorylation. For instance, investigation of the effect of the biologically significant metal ions Ca^{2+}, Mg^{2+}, and Zn^{2+}, added to O-BISDIEN, revealed striking influences on ATP cleavage and on the formation of the phosphoramidate intermediate and pyrophosphate [20]. While addition of both Ca^{2+} and Mg^{2+} increased the observed percentage of phosphoramidate, only Ca^{2+} provided a significant acceleration of ATP dephosphorylation, almost doubling the first-order rate constant found for free O-BISDIEN in the same experimental conditions. Conversely, the presence of Zn^{2+}, as well as of Cd^{2+}, to O-BISDIEN/ATP solutions decreased the rate of nucleotide cleavage.

Most of polyammonium receptors employed for nucleotide binding and activation studies were macrocycles [1–9,13–15,17–20] although also several linear [21] and few branched [22] molecules were also considered.

In recent papers [23,24], we described the coordination properties of the second-generation poly(ethylene imine) dendrimer (L), showing that this iperbranched ligand is able to form stable complexes with both metal ions (Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}) [23] and anions (NO_3^-, SO_4^{2-}, SeO_4^{2-}, HPO_4^{2-} and $H_2PO_4^-$) [24]. Furthermore, due to the many amine groups present in the molecules, L forms highly protonated metal complexes that, in turn, bind anions giving rise to ion-pair complexes of considerable stability. Molecular modelling calculations showed that both anion–ligand and anion–metal ion interactions can participate in stabilising these ion-pair complexes.

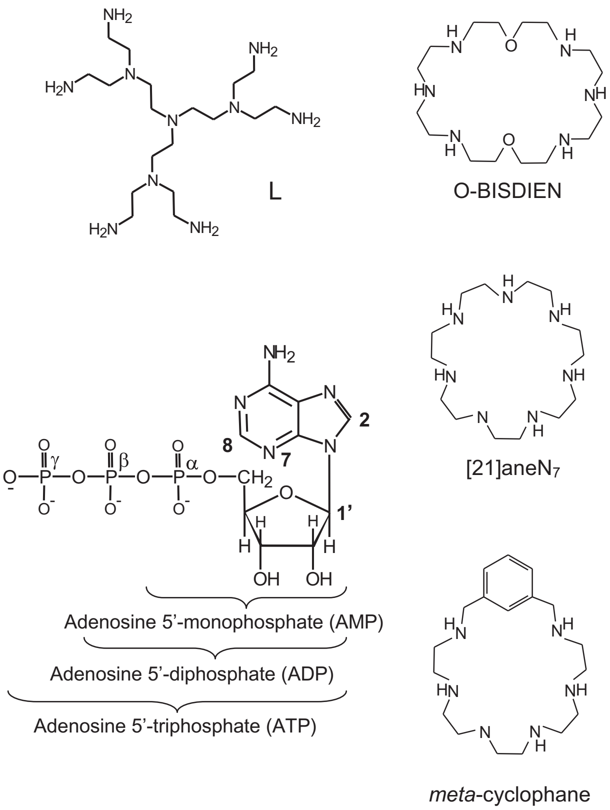

Fig. 1. Ligand (L) and nucleotide drawings. Abiotic receptors producing highest enhancements of ATP dephosphorylation.

Considering the peculiar binding properties of L, we found it interesting to analyse the effect of the dendritic structure of this ligand on the binding of AMP, ADP and ATP, as well as on the possible catalytic activity that it might have in ATP dephosphorylation in the absence and in the presence of the biologically significant metal ion Zn^{2+}. For the sake of comparison, the analysis was extended to the binding of PO_4^{3-}, $P_2O_7^{4-}$ and $P_3O_{10}^{5-}$ which are the inorganic counterparts of these nucleotides. We describe here the results of this study.

2. Experimental

2.1. Materials

Ligand L was synthesised as previously described [23].

2.2. Potentiometric measurements

All pH-metric measurements ($pH = -\log[H^+]$) employed for the determination of equilibrium constants were carried out in 0.1 M NMe_4Cl solutions at 298.1 ± 0.1 K, by using the equipment and the methodology that has been already described [23]. The combined Hamilton glass electrode (LIQ-GLASS 238000/08) was calibrated as a hydrogen concentration probe by titrating known amounts of HCl with CO_2-free NMe_4OH solutions and determining the equivalent point by Gran's method [25] which allows one to determine the standard potential E^o and the ionic product of water ($pK_w = 13.83(1)$ at 298.1 ± 0.1 K in 0.1 M NMe_4Cl). At least three measurements were performed for each system in the pH range 2.5–10.5. The computer program HYPERQUAD [26] was used to calculate the equilibrium constants from e.m.f. data. The concentration of the ligand was 1×10^{-3} M in all measurements. The concentration of anions [A] in anion coordination experiments was in the range $= [L] \leqslant [A] \leqslant 5[L]$. Three measurements were performed for each anion and the titration curves were treated both separately or as a unique set without significant variations in the calculated stability constants. The final stability constants were obtained by processing the whole set of curves. The measurement for the simultaneous binding of metal ions and anions were performed with a metal ion concentration $[M] = 0.8[L]$, to ensure that only mononuclear complexes were formed, while the concentration of the anion was again $[L] \leqslant [A] \leqslant 5[L]$. Three measurements were performed for each system by using the same procedure. Different equilibrium models for the complex systems were generated by eliminating and introducing different species, including species with 1:2 and 1:3 receptor:anion stoichiometries. Only those models for which the HYPERQUAD program furnished a variance of the residuals $\sigma^2 \leqslant 9$ were considered acceptable. Such condition was unambiguously met by a single model for each system. The equilibrium constants for ligand protonation, for the formation of Zn^{2+} complexes with L, for anion protonation and for the formation of Zn^{2+} complexes with anions used in calculation were determined by us in previous works or taken from the literature [23,27,28].

2.3. ^{31}P NMR measurements

^{31}P (161 MHz) NMR measurements were performed in D_2O solutions at 298 K on a Bruker-Advance III 400 MHz spectrometer. Chemical shifts were recorded relative to 85% H_3PO_4 as an external reference. Small amounts of 0.01 M NaOD and DCl were used to adjust the solutions pD. The pH was calculated from the measured pD value by using the relationship pH = pD − 0.40 [29]. The kinetics of

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