ATP Recognition and sensing with a phenanthroline-containing polyammonium receptor†

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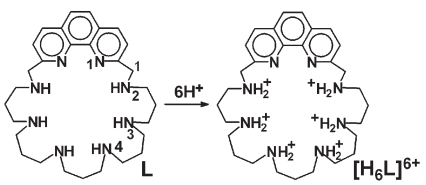
A new polyammonium receptor is able to selectively recognise and sense ATP among triphosphate nucleotides, thanks to ATP-induced quantitative quenching of its fluorescence emission.

There is a growing interest in the design of fluorogenic receptors able to recognise and signal analytes in aqueous solutions,[1] due to their potential use in medicinal and environmental chemistry. ATP is undoubtedly one of the preferred targets, due to its basic role as a center for chemical energy storage and transfer in the bioenergetics of all living organisms. Polyammonium receptors containing fluorogenic units are often chosen as chemosensors for anionic analytes, including ATP,[2–7] since their ability to give charge–charge interactions with anionic species may lead to the formation of stable host–guest complexes in aqueous solutions. Selective coordination, however, requires also the incorporation of sites capable of interactions with the sugar moiety or the nucleic base.[8–13]

Although many fluorogenic synthetic systems able to recognise and signal ATP have been reported,[2–13] in most cases they act as selective chemosensors for ATP with respect to the less charged ADP, AMP or inorganic phosphate anions. Selective recognition and sensing of ATP over the other triphosphate nucleotides is a much more difficult goal, due to the similar charge gathered on the nucleotides; in fact, only one example of a chemosensor able to selectively sense GTP over ATP has been recently reported.[4]

The new phenanthroline-containing macrocycle L (Scheme 1) protonates in aqueous solutions affording polyammonium cations of the type $[H_xL]^{x+}$ (x = 1–6). The protonation constants of the receptor range between 10.14 and 6.9 log units, higher than those reported for 1,10-phenanthroline (log K = 4.96).[14] As already reported for other phenanthroline-containing polyamines,[15] protonation takes place on the aliphatic amine groups, while the phenanthroline nitrogens, less basic than amine groups, are not protonated in our experimental conditions. The hexaprotonated receptor $[H_6L]^{6+}$ is the prevalent species in solution for pH $\leqslant$ 7 (Fig. 1) and displays the typical fluorescence emission band of phenanthroline at 365 nm. Deprotonation of $[H_6L]^{6+}$ to give less protonated forms leads to a complete quenching of the emission (Fig. 1), due to the presence of deprotonated amine groups which can quench the emission through an electron transfer process. In particular, ^{1}H NMR spectra recorded at different pH values show that deprotonation of the $[H_6L]^{6+}$ cation to give the $[H_5L]^{5+}$ and $[H_4L]^{4+}$ ones implies release of acidic protons from the benzylic nitrogens N2, adjacent to phenanthroline. In fact, a marked upfield shift (*ca.* 0.8 ppm) of the signal of the benzylic methylene groups H1, adjacent to N2, accompanies deprotonation of $[H_6L]^{6+}$ to form the $[H_5L]^{5+}$ and $[H_4L]^{4+}$ species (see inset of Fig. 1). Therefore, in the $[H_5L]^{5+}$ and $[H_4L]^{4+}$ cations the fluorescence emission is quenched by the benzylic amine groups N2, through an electron transfer process to the excited phenanthroline. Of course, protonation of these nitrogens in the hexaprotonated receptor $[H_6L]^{6+}$ leads to renewal of the fluorescence emission. In the cations with protonation degree lower than 4, however, the N3 and N4 nitrogens may also contribute to the quenching process.

Protonation of the receptor enables L to form stable complexes with anionic forms of triphosphate nucleotides ATP, GTP, CTP or TTP as well as with ADP and AMP. A potentiometric study



Scheme 1

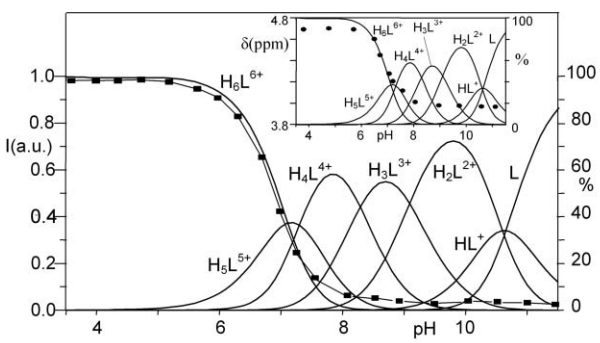


Fig. 1 pH dependence of the fluorescence emission at 365 nm (-■-, left y axis) and distribution diagrams of the protonated species of L (solid lines, right y axis. Inset: pH dependence of chemical shift of the benzylic protons H1 (-●-) superimposed on the species distribution of L (0.1 M NMe_4Cl, [L] = 2.5 × 10^{-5} M.

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† Electronic supplementary information (ESI) available: Experimental details for L synthesis, potentiometric and spectroscopic measurements and MD simulations, protonation constants of L, formation constants of the adducts with ATP, ADP, AMP, GTP, CTP and TTP, distribution diagrams for each system L–nucleotide, CIS values for the receptor–nucleotide complexes, atomic coordinates for the lowest energy conformers of adducts with ATP and CTP. See DOI: 10.1039/b611031b

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shows that all nucleotides forms stable 1 : 1 complexes of the type $[H_xLA]^{(x-4)+}$ (see ESI†).

The comparison between the binding constants of nucleotides to the protonated forms of the receptor, however, is complicated by the remarkably different acid–base characteristics of the substrates, and by the presence of overlapping protonation equilibria involving both receptor and anions in the same pH range. This problem can be conveniently overcome by considering a competitive system containing receptor and nucleotides in equimolar concentrations and calculating the overall percentages of the different complexed anions over a wide pH range.[16] Plots of the percentages *vs.* pH produce species distribution diagrams from which the binding ability of the receptor can be interpreted in terms of selectivity. Fig. 2 displays the plot obtained for a competitive system containing L, ATP, CTP, TTP and GTP in equimolecular ratio; the formation of ATP adducts with L prevails over a wide pH range, *i.e.*, ATP is selectively bound with respect to the other triphosphate nucleotides. For instance at pH 6, 80% of ATP is complexed, while CTP, the most competitive substrate for ATP complexation with L, is complexed at less than 15%.

A similar plot can be also obtained for a competitive system containing ATP, ADP and AMP. In this case, ATP is selectively complexed in the pH range 2–9 in a percentage greater than 90%. (see ESI, Figure S7†).

^{31}P Spectra recorded on solutions containing the nucleotides and the receptor in a 1 : 1 molar ratio show remarkable downfield shifts of the ^{31}P signals of the P_γ and P_β phosphorus atoms of the phosphate chain with respect to the nucleotides in the absence of the receptor, indicating the formation of strong salt bridges between the anionic triphosphate moiety and the ammonium groups of the receptor. Complexation is also accompanied by upfield shifts of the ^{1}H signals of the nucleobases and the phenanthroline of the receptor (see Fig. 3 for ATP), suggesting the presence of π-stacking interactions between the nucleobases and the heteroaromatic unit of the receptor.

ATP shows the most relevant complexation-induced chemical shifts (CIS) in both ^{31}P and ^{1}H NMR spectra (see ESI, Table S6 and S7†). This would confirm the presence of an overall stronger interaction of ATP with the receptor with respect to the other nucleotides. Among the other triphosphate nucleotides, CTP, the most competitive nucleotide toward ATP, displays the highest CIS values for the ^{31}P signals (3.8 and 1.2 ppm for the Pγ and P_β phosphorus atoms) and the lowest one in the ^{1}H signals for its aromatic protons (−0.28 and −0.20 ppm), suggesting the formation of rather strong charge–charge contacts and weak π-stacking interactions with the receptor.

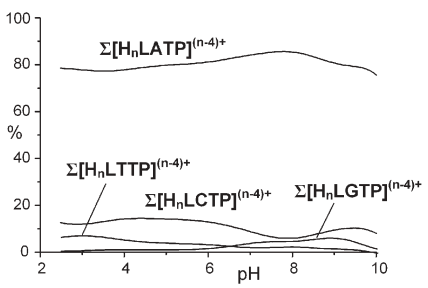

Fig. 2 Overall percentages of L complexed species with ATP, CTP, TTP or GTP as a function of pH in a competitive system containing L, ATP, CTP, TTP and GTP in equimolecular ratio ([L] = [ATP] = [CTP] = [TTP] = [GTP] = 1×10^{-4} M).

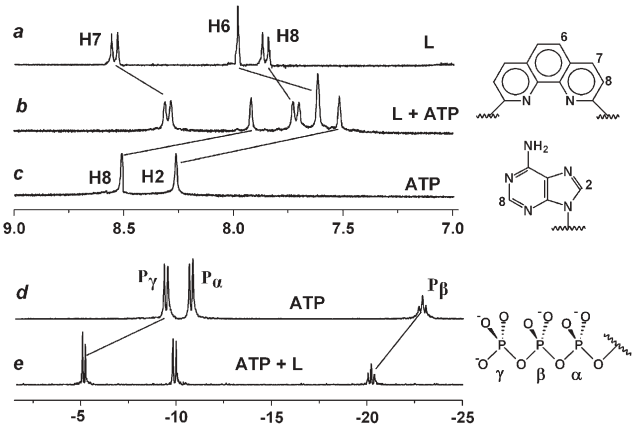

Fig. 3 ^{1}H NMR spectra (aromatic region) of L (a), ATP in the presence of L (1 : 1 molar ratio) (b), ATP (c) and ^{31}P NMR spectra of ATP (d) and ATP in the presence of L (1 : 1 molar ratio) (e).

Spectrofluorimetric measurements show that the receptor can act as effective chemosensor for ATP in aqueous solutions. In fact, addition of increasing amounts of ATP to a solution of L at pH 6 leads to a linear decrease of the fluorescence emission of L. As shown in Fig. 4, the fluorescence of L is completely quenched in the presence of 1 eq. ATP. ADP and AMP give a slight decrease of the fluorescence emission of the receptor (max 10% in the presence of a tenfold excess of ADP). Triphosphate nucleotides also produce a decrease of the fluorescence emission. In this case, however, the quenching effect is only partial even in the presence of a large excess of CTP, GTP or TTP. As shown in the inset of Fig. 4, the larger effect (a fluorescence emission decrease of *ca.* 30%) is observed in the presence of an excess of CTP. Therefore, in the present receptor phenanthroline is not only used for ATP selective binding, thanks to its ability to give strong π-stacking interaction with the nucleobase, but also as signalling unit for this substrate.

To clarify the binding mode of nucleotides, we carried MD calculations on the adducts between $[H_6L]^{6+}$ and ATP or CTP, the most competitive substrate for ATP coordination by L, exploring the potential energy surface by means of simulated annealing. In all the sampled conformers the receptor assumes a folded conformation, with an almost perpendicular disposition between phenanthroline and the aliphatic polyamine chain. Both ATP and CTP display a bent conformation, with the nucleobase–sugar

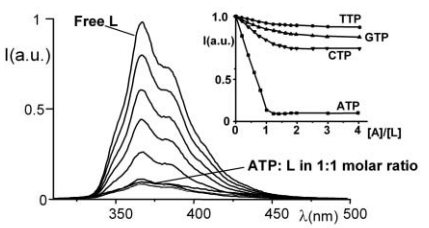

Fig. 4 Fluorescence emission spectra of L in the presence of increasing amounts of ATP at pH 6 (λ_{exc} 270 nm, NMe_4Cl 0.1 M, 298.1 K, [L] = 2.5×10^{-5}). Inset: fluorescence intensity at 365 nm in the presence of increasing amounts of ATP, TTP, GTP or CTP.

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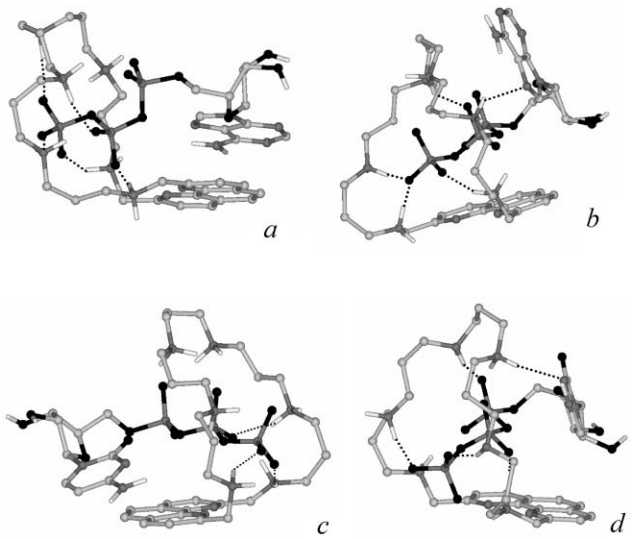

Fig. 5 Low energy conformers of the adducts between $[H_6L]^{6+}$ and ATP in the A (a) and B (b) families and between $[H_6L]^{6+}$ and CTP in the A (c) and B (d) families. Only hydrogen bonds with N–H···O distances lower than 2 Å are reported.

torsion in a *syn* form, which allows the simultaneous interaction of the nucleobases and of the triphosphate chain with the receptor, the terminal γ-phosphate group being encapsulated within the macrocyclic cavity. In both ATP and CTP complexes, the different conformers can be grouped in two different families (herein called A and B), which differ in the interaction mode of the nucleobase with the receptor. The low energy conformers of each family for the ATP and CTP complexes are shown in Fig. 5. In the A family, the most populated for the ATP complex (70%) and the less populated one for CTP (35%), adenine and cytosine interact with the phenanthroline unit *via* π-stacking. The adenosine of ATP, however, gives a stronger π-stacking interaction than cytidine of CTP; while adenine lies above phenanthroline (interplanar distance 3.6 Å) and assumes an almost parallel disposition with respect to the phenanthroline plane (Fig. 5a), cytidine displays a smaller "overlap" with phenanthroline and the plane of cytidine is bent *ca.* 26° with respect to the phenanthroline one (Fig. 5c). The bent conformation of the nucleotides allows the formation of hydrogen bonding interactions of the γ-phosphate groups of both ATP and CTP with the benzylic nitrogens.

The low energy conformer in the B family, the most populated for the CTP complex (65%) and the less populated one for ATP (30%), shows hydrogen bonding interactions between the N7 donor of adenine (Fig. 5b) or the N3 nitrogen of cytosine (Fig. 5d) with an ammonium group of the aliphatic chain of $[H_6L]^{6+}$. Thanks to its smaller dimensions, cytidine can assume a spatial position closer to the macrocyclic cavity than adenine. This determines a different disposition of the triphosphate moiety with respect to the aliphatic chain of the receptor. In fact, the γ-phosphate of CTP is pushed away from the benzylic ammonium groups and cannot give hydrogen bonding interactions with them, while the γ-phosphate of ATP can give a couple of strong hydrogen bonds with these nitrogens.

Therefore, while ATP gives hydrogen bonds with the benzylic ammonium groups in both the A and B families, CTP forms hydrogen bonds with these nitrogens only in the less populated A family.

These results are in accord with the observed high stability of the ATP complexes as well as with the observed complete quenching of the fluorescent emission of phenanthroline in the ATP complex.

In the case of ATP, the nucleotide can assume a conformation suitable to give simultaneously strong π-stacking, charge–charge and hydrogen bonding contacts, which reinforce the overall substrate–receptor interaction. At the same time, in the case of ATP the hydrogen bonding $P–O^-\cdots H_2N^+$ interactions strongly involve the protonated benzylic nitrogens; this can lead to partial transfer of an acidic proton from the benzylic amines N2 to the phosphate chain. Deprotonation of the N2 nitrogens in the $[H_6L]^{6+}$ cation leads to the observed fluorescence quenching.

In conclusion, the present polyammonium receptor represents a unique system simultaneously able not only to selectively recognise ATP over GTP, TTP and CTP, but also to signal ATP through a quantitative quenching of its fluorescence emission.

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