Polyfunctional Binding of Thymidine 5'-Triphosphate with a Synthetic Polyammonium Receptor Containing Aromatic Groups. Crystal Structure of the Nucleotide–Receptor Adduct

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Polyfunctional Binding of Thymidine 5′-Triphosphate with a Synthetic Polyammonium Receptor Containing Aromatic Groups. Crystal Structure of the Nucleotide−Receptor Adduct

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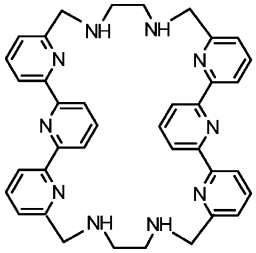
Since the seminal works of Kimura and Lehn,[1] in the earlier 1980s, on ATP, ADP, and AMP binding by polyammonium macrocycles, the use of synthetic receptors has become a common strategy to mimic biological recognition of nucleotides by proteins.[2] Nucleotide binding sites in proteins generally consist of both polar and apolar groups such as cationic, hydrogen binding, aromatic, and aliphatic residues. For instance, as shown by several crystal structures, TTP binding by proteins commonly takes place through the formation of hydrogen bonds and salt bridges between the polyphosphate chain of the nucleotide and protein main chain atoms and/or side chains of Lys or Arg[3] and between carbonyl O atoms of thymine and NH groups from main or side chain residues (i.e., Asp, Asn, Ser, Gln, and Gly).[3a,4] In addition, the thymine group can give rise to π-stacking interactions with aromatic side chains, such as Tyr and Phe in human thymidine kinase hTK1,[5] and/or to CH$\cdots\pi$ and van der Waals interactions of the methyl group with several aromatic and aliphatic protein residues,[6] while the ribose 3′-OH group behaves as both donor or acceptor of hydrogen bonds.[3a,4,6b]

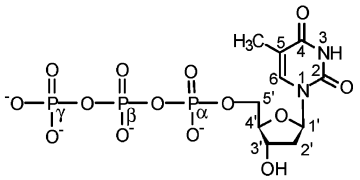
Stacking interactions are of considerable importance since they contribute to stabilize the protein−nucleotide adduct and assist the nucleotide in achieving the correct orientation inside the protein binding site. Due to the polyfunctional nature of these nucleotide-binding sites of proteins, the earlier synthetic receptors based on ammonium groups, acting as Lewis acids in the binding of nucleotide polyphosphate chains via charge−charge and hydrogen bonding interactions, evolved toward more sophisticated receptors containing aromatic moieties displaying enhanced mimicking characteristics and/or binding selectivity.[2e,7]

From the origin of this research field, the nucleotide-binding ability of synthetic receptors, as well as their ability to recognize and activate nucleotides, has been correlated with the mutual substrate-to-receptor arrangement in the adducts and with the nature of the binding forces.[2d,7a] To this purpose, information on the modes of interaction between these partners was obtained by means of spectroscopic methods and computational modeling, which were very helpful in getting a general idea of the adduct architectures,[1,7] but not a single crystal structure of a nucleotide bound to similar synthetic receptors showing detailed structural characteristics of the adduct and the different binding interactions was reported until now.

In the present paper, we report the first crystal structure of this type in which the nucleotide is TTP and the synthetic receptor L is a polyfunctional macrocyclic ligand, composed of two terpyridine moieties linked together by two diamine chains (see Supporting Information for the synthesis), containing both amine/ammonium groups and aromatic residues giving rise to multiple interactions with the nucleotide.



L



HTTP

Solution studies (see Supporting Information) showed that interaction of TTP with L occurs in water with the formation of nucleotide complexes deriving from the association of the different ligand and nucleotide species present in solution at the different pH values. Around pH 6.5, the main species is the neutral [(H_4L)-HTTP] adduct, while on lowering the solution pH, the minor species $[(H_4L)H_2TTP]^+$ is first formed followed by the predominant $[(H_5L)H_2TTP]^{2+}$ one (Figure S3, Table S1 in Supporting Information). Attempts to isolate these adducts were successful in affording crystals of $[(H_4L)HTTP]\cdot 12H_2O$ by slow evaporation at room temperature of an aqueous solution, at pH 6.5, containing L and TTP in equimolar amounts. The crystal structure of this compound, solved by means of X-ray analysis, consists of [(H_4L)HTTP] adducts (Figure 1), containing H_4L^{4+} tetraprotonated ligand molecules and $HTTP^{4-}$ anions, and water molecules. A list of selected hydrogen bond distances and interatomic contacts for [(H_4L)HTTP] are reported in the Supporting Information (Table S2) along with the crystal packing of the compound.

As shown in Figure 1 (see also Figure S9), the tetraprotonated macrocycle behaves as a polyfunctional receptor giving rise to tight association with $HTTP^{4-}$ through different binding interactions: (*i*) H-bonds between the polyphosphate chain of the nucleotide and protonated nitrogen atoms of the ligand (N(7)$\cdots$O(2) 2.705(5) Å, N(6)$\cdots$O(2) 2.769(6) Å, N(6)$\cdots$O(8) 2.648(5) Å), (*ii*) one hydrogen bond between a carbonyl oxygen of the nucleotide and one ammonium group of the ligand (N(12)$\cdots$O(13) 2.887(7) Å), (*iii*) CH$\cdots\pi$ interactions involving, respectively, C(1) ($-CH_2-$) and C(8) ($-CH_3$) nucleotide carbon atoms and ligand N(8) and N(4) pyridine units (3.451(8) and 3.734(9) Å), (*iv*) one O$\cdots\pi$ interaction between the nucleotide carbonyl oxygen O(14) and the N(10) pyridine ring (O$\cdots$pyridine centroid 3.566(7) Å) of the ligand.

The protonated macrocycle is S-shaped, with an angle of ca. 17° between each line, defined by the secondary nitrogen atoms belonging to the same aliphatic chain, and the planes defined by the heteroaromatic nitrogen atoms of each terpyridine unit. The three aromatic groups of each terpyridine unit are in *trans* conformation and strongly deviate from planarity, the dihedral angles measured between two adjacent pyridine rings ranging from

10.1021/ja7106977 CCC: $40.75

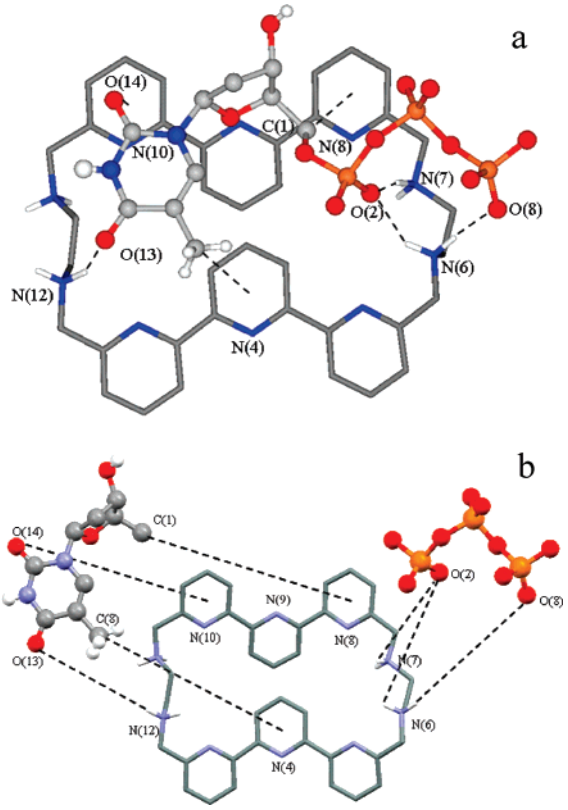


Figure 1. (a) Crystal structure of [(H_4L)HTTP]. (b) Expanded representation of intermolecular contacts.

4.96(3) to 27.7(2)°. The two central pyridine groups give rise to an intramolecular π-stacking interaction, which can be described as intermediate between face-to-face and edge-to-face, characterized by a distance of 3.864(9) Å between the centroid of the N9 pyridine ring and the carbon atom in *para* position to the nitrogen of the N4 pyridine ring. Details of the crystal packing are reported in the Supporting Information.

The multifunctional character of L in nucleotide binding is also manifested in solution where interactions between the aromatic ligand moieties and the thymidine group of the nucleotide contribute to stabilize the adducts in addition to salt bridges and hydrogen bonds. For instance, the stability of the [(H_4L)HTTP] species in solution (log K = 4.57 for H_4L^{4+} + $HTTP^{4-}$ = [(H_4L)HTTP], Table S1) is significantly higher than the stability observed for similar nucleotide complexes with polyazamacrocycles bearing the same positive charge and having comparable size, with respect to H_4L^{4+}, but which do not contain aromatic groups in their structures.[8] ^{1}H NMR spectra (see Supporting Information) recorded on solutions containing L and TTP in 1:1 molar ratio show that complexation is accompanied by significant upfield shifts of all ^{1}H signals of the nucleobase, suggesting that an extended interaction with the heteroaromatic moieties of the ligand, similar to that observed in the crystal structure of [(H_4L)HTTP]·12H_2O, also exists in solution. The largest complexation-induced chemical shifts (CIS) are found for H6 (0.84 ppm) and CH_3 (0.81 ppm), the nucleobase protons showing CH···π interactions with aromatic rings of L in the crystal structure. Conversely, CIS values in ^{31}P NMR spectra are small (maximum CIS = 0.67 ppm for P_β, pH 4.2), revealing a rather weak interaction between the triphosphate chain of TTP and the ammonium groups of L. Altogether, a tight association between the two polyfunctional partners is accomplished, thanks to the many associative forces, among which, interactions between the nucleobase and the large heteroaromatic regions of the ligand are of primary importance.

Molecular modeling studies (see Supporting Information) were performed to get further information regarding bonding interactions and mutual arrangement of L and TTP in the adduct. Annealing simulations for [(H_4L)HTTP] showed three different families of conformations differing, at most, by 5 kcal/mol. In the three adducts, the main interactions are again hydrogen bonds and salt bridges involving the ammonium groups of L and TTP phosphate oxygen atoms and π-stacking between the nucleobase and ligand heteroaromatic groups. In particular, in the second most populated family, conformations of TTP and L are remarkably similar to that assumed in the crystal structure (Figure S11c, Supporting Information).

In conclusion, we report the first crystal structure of a nucleotide bound to a synthetic receptor. The structure shows a multiple linkage between the two partners mimicking the different binding modes of nucleotide-binding proteins observed, for instance, in human thymidine kinase hTK1,[5] in ribonucleotide reductases of *Salmonella typhimurium*[3] and *Saccharomyces cerevisiae*,[4] in vaccinia virus thymidine kinase,[6a] and deoxyribonucleoside kinase mutant N64D of *Drosophila melanogaster*.[6b] Solution studies reveal that similar interaction modes are active also in solution and lead to thermodynamically stable nucleotide−receptor adducts.

Supporting Information Available: Experimental procedures and data, table of stability constants, species distribution diagrams, fluorescence emission and ^{1}H NMR spectra, list of selected interatomic distances for [(H_4L)HTTP]·12H_2O, ORTEP drawings of the crystal structure, details of the crystal packing of [(H_4L)HTTP]·12H_2O, lowest energy calculated structures for [(H_4L)HTTP], and X-ray crystallographic data of [(H_4L)HTTP]·12H_2O as CIF file. This material is available free of charge via the Internet at http://pubs.acs.org.

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