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Phosphate binding with a thiophene-based azamacrocycle in water

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Abstract

Structural characterization of the phosphate complex with a thiophene-based macrocycle suggests that two dihydrogen phosphates in a dimeric form are encapsulated in the cavity via several hydrogen bonds from NH···O and CH···O interactions. In the lattice framework, the two dimers are linearly hydrogen-bonded to form a tetramer. ^{1}H NMR titrations suggest that the host forms a 1:1 complex with phosphate, showing an association constant of 120 M^{-1} in D_2O at pH = 5.5. The host guest complexation was further confirmed by ESI-MS in a gas phase.

Keywords

Azamacrocycle; Ditopic complex; Anion binding; Phosphate complex

Phosphate is ubiquitous in Nature and is widely used in the production of fertilizers [1]. It is also used as preservatives in foods and as additives to household detergents. Furthermore, phosphate is a key component of nucleic acids (DNA and RNA) and is known to play an important role in many enzymatic reactions [2]. Furthermore, crystallographic findings can aid in identifying accurate bonding patterns involved in a host guest complex. Therefore, there is an increasing interest in understanding interactions of phosphate anions with synthetic receptors particularly in aqueous solution [3]. However, the high free energy of hydration of phosphate significantly reduces its ability to complex with a synthetic molecule in water [4]. Phosphate binding has been reported by several classes of neutral receptors including amides [5], thioamides [6], ureas [7], thioureas [8], pyrroles [9] and indoles [10]; however, most of these binding studies have been performed in organic solvents. On the other hand, polyamines tend to be soluble in a polar solvent and can effectively be used in binding phosphate anions in water over a wide range of pH [11]. For examples, Martell and coworkers reported a *m*-xylyl-based hexaazamacrocycle forming an inclusion complex with a pyrophosphate, where the anion is hydrogen bonded to four protonated amines through two oxygen atoms [12]. Bianchi, García-España, Paoletti and coworkers studied a smaller macrocycle[18]aneN_6 which in its tetraprotonated form was found to interact with two pyrophosphate anions via NH···O and CH···O bonds [13]. Bowman-James and coworkers isolated crystals of [26]aneN_6C_6 with mixed phosphoric acid/dihydrogen phosphate anion grown at low pH, showing a ditopic complex in which two anions were held above and below the macrocycle [14]. Increasing the dimension from monocycle to bicycle, Lu and coworkers obtained crystals of phosphate complexes with both hexa- and octa-protonated

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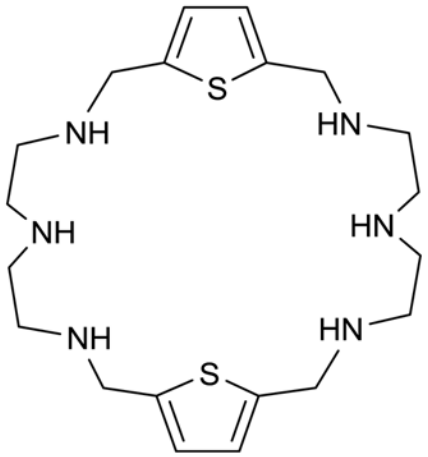
forms of *p*-xylyl-based cryptands [15]. However, only the octaprotonated cryptand provided an inclusion complex with one phosphate anion, suggesting that electrostatic interactions play a key role in the complex formation.



L

In Nature phosphate was structurally identified in the phosphate binding protein (PBP) in which the anion was held with a total of 12-hydrogen bonds [16]. Katayev, Sessler and coworkers synthesized an oligopyrrolic macrocycle which was found to complex a dianioic phosphate showing hydrogen-bond networks similar to those present at the active sites of PBP [17]. During the course of our study, we isolated crystals of phosphate complex of **L**, and characterized a dimeric phosphate species in the form of $(H_2PO_4)_2^{2-}$ within the macrocyclic cavity. Herein, we report the binding aspects of **L** with phosphate in water and structural characterization of the phosphate complex.

The compound **L** was previously reported by Dancey *et al.*[18] and prepared as described before [19]. Phosphate salt was obtained by adding a few drop of phosphoric acid in a solution of the free amine **L** (50 mg) in CH_3OH (2 mL). The white precipitate formed immediately was filtered and washed with diethyl ether. Crystals suitable for X-ray analysis were obtained from slow evaporation of the salt solution in water-methanol system.

The structure of the phosphate complex of **L** was determined by X-ray diffraction analysis [20]. As shown in Figure 1, all the secondary amines are protonated forming a charged cavity. The macrocyle adopts a rectangular shape and two aromatic rings are almost parallel with an Ar···Ar distance (centroid to centroid) of 9.394 Å. The distance between the central nitrogens (N1···N14 is 6.741 Å which is larger than 6.296 Å observed in the perchlorate complex of **L** [19]. The macrocyle is found to host two dihydrogen phosphates via several hydrogen bonds from NH···O and CH···O interactions (see Table 1). One phosphate (labeled A) is bonded to the macrocycle via two strong NH···O interactions (N1···O2A = 2.69(2) and N14···O1A = 2.723(16) Å) with the central nitrogens (N1 and N14) and one weak NH···O interaction (N4···O2A = 3.21(3) Å) with one protonated secondary amine (N4) connected to an aromatic group. While other phosphate (labeled B) is bonded to the macrocycle via one strong NH···O (N4H···O4B = 2.70(2) Å) and two CH···O (3.162 and 3.276 Å) interactions. Therefore, each anion is connected to the macrocycle with three hydrogen bonds, suggesting that the charged macrocycle effectively interacts with the negatively charge phosphate.

The observed oxygen-nitrogen bond distances for the phosphate binding interactions with an average of 2.83 Å are comparable to those reported for the phosphate complex of *m*-xylyl-

based macrocycle ranging from 2.60 to 2.75 Å [12] and [18]aneN_6 ranging from 2.71(6) to 3.037 (5) Å [13]. The anions remain on the both side of the macrocycle, as observed in the phosphate structure of [26]aneN_6C_6 reported previously [14]. However, in the present case the two phosphate anions are connected via one strong hydrogen bond (OH···O = 2.533 Å) to form a dimer. As shown in Figure 1B, the dimer sits in a perpendicular fashion to the "macrocyclic plane" rather than coplanar with it. Such arrangement was observed for the pyrophosphate structure with *m*-xylyl-based macrocycle reported by Martell [12]. Interestingly, each phosphate group is further connected linearly with another phosphate through two hydrogen bonds to form a tetramer as $(H_2PO_4)_4^{4-}$ (Figure 2A), as viewed in an extended structure along the *c* axis. The linear tetrameric phosphate is encircled by two macrocycles which are anti-parallel to each other (Figure 2B). The structural evidence of dimeric phosphate $(H_2PO_4)_2^{2-}$ formed by synthetic hosts was previously reported [21]. In our case the dimer is further H-bonded to two additional macrocycle-bound $H_2PO_4^-$ to form a linear tetramer.

Solution binding affinity of the host for phosphate was carried out by ^{1}H NMR titrations in D_2O at two different pH (pH = 7.0 and 5.5). The solution pH was adjusted by the concentrated solution of TsOH and NaOD dissolved in D_2O. The addition of NaH_2PO_4 (50 mM in D_2O) to the host solution (5 mM in D_2O) resulted into a downfield shift of ligand's protons. The change in the ^{1}H NMR signals with an increasing amount of the anion solution was analyzed by the non-linear regression method [22], giving the best fit to a 1:1 binding model (Figure 3).The calculated association constant (K_{as}) of **L** for phosphate was 120 M^{-1} at pH 5.5. However, the host was found to interact weakly (K_{as}<20 M^{-1}) under neutral condition (pH = 7.0), suggesting that the binding is primarily influenced by electrostatic interactions.

The formation of the host-guest complex was further supported by ESI-MS experiments in a positive mode. For this purpose, a solution of phosphate complex of **L** was prepared in the mixture of MeOH and H_2O (50:50, v/v). As shown in Figure 4, there is an intense peak at *m/z* 520.8 which is assigned for the singly charged species, $[H_4\mathbf{L}PO_4]^+$. The peak at m/z 260.6 is due to the formation of doubly charged $[H_5\mathbf{L}PO_4]^{2+}$ formed during the experiment. The peak at *m/z* 423.2 and 212.2 correspond to the free ligand $[H\mathbf{L}]^+$and $[H_2\mathbf{L}]^{2+}$, respectively. The results obtained from this experiment confirm the formation of a 1:1 complex between the charged macrocycle and the phosphate anion in a gas phase, supporting the stoichiometry observed in solution.

In summary, we have structurally characterized that a simple macrocycle encapsulates a dimeric form of dihydrogen phosphate by strong hydrogen bonding interactions. The two dimers are further connected in a linear fashion to form a phosphate tetramer. Such assembly of anions is assisted by two macrocycles which are encircled around the tetramer with multiple hydrogen bonds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary material

CCDC 808448 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at doi: ???.

Highlight

- A thiophene-based macrocycle encapsulates two dihydrogen phosphates.
- The two phosphate dimers are linearly hydrogen-bonded to form a tetramer.
- The host binds a phosphate with an association constant of 120 M^{-1} in D_2O at pH = 5.5.
- The host guest complexation is confirmed by ESI-MS in gas phase.

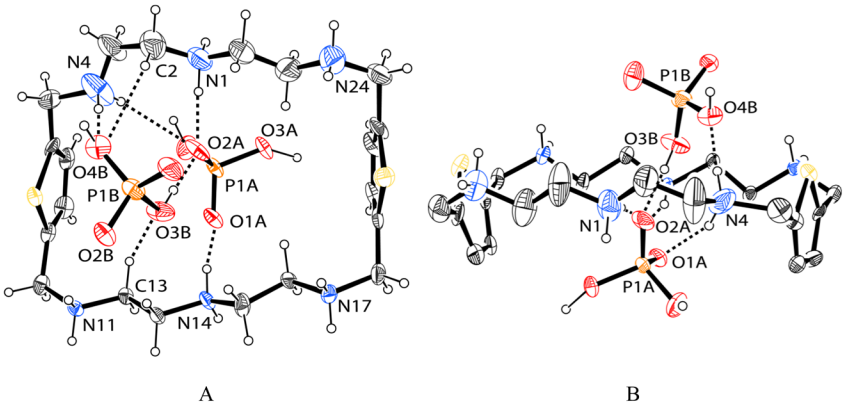

Fig 1.

ORTEP drawing of $[H_6\mathbf{L}(H_2PO_4)_2]^{4+}$ motif, with thermal ellipsoids at the 50% probability level: (A) side view and (B) view along the tertiary N-N axis.

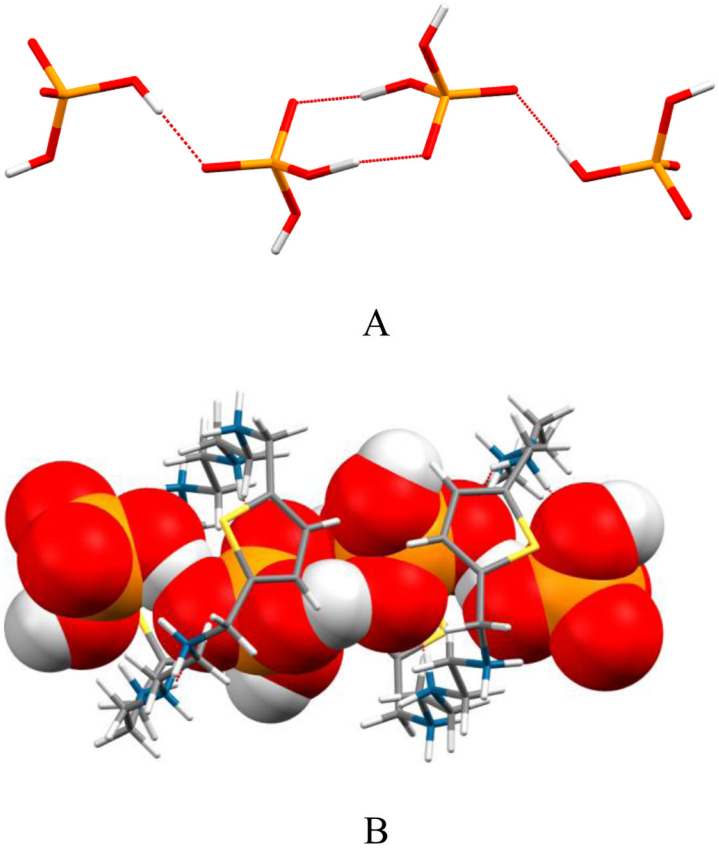

Fig 2.
(A) Tetrameric $(H_2PO_4)_4^{4-}$ and (B) Space filling view of the tetrameric phosphate encircled by two macrocycles.

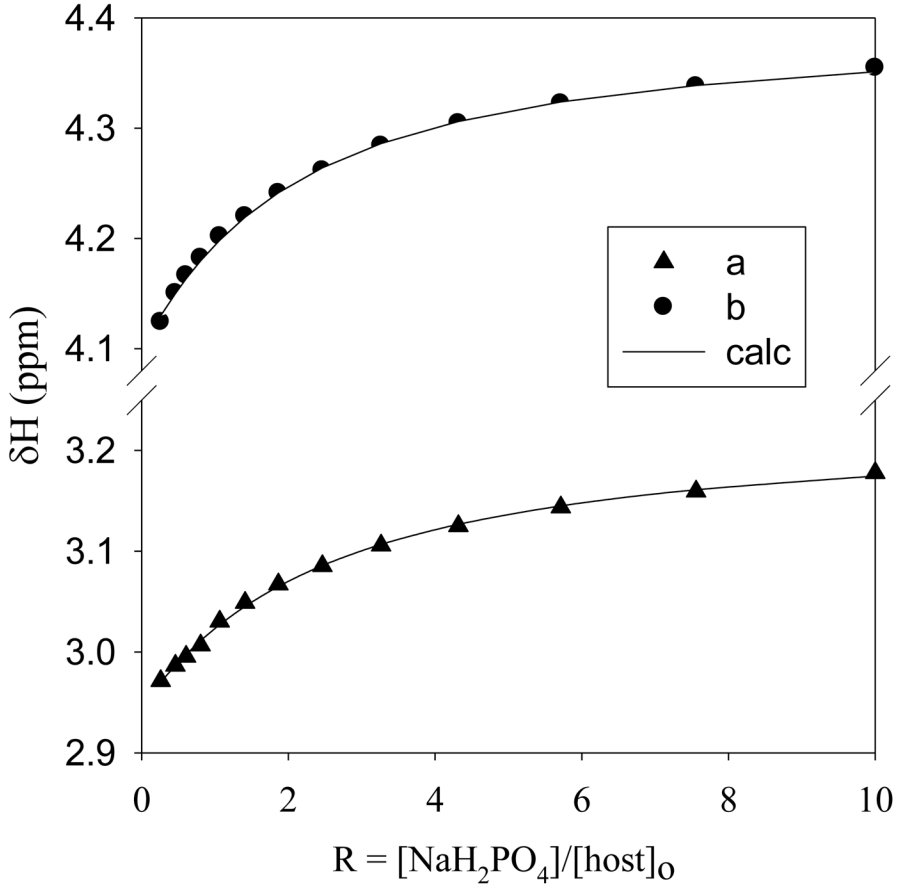

Fig 3.

^{1}H NMR titration curves for phosphate binding with the host (5 mM) in D_2O at pH = 5.5. Changes in the chemical shifts of different protons (a = $NHC\mathit{H}_2CH_2$ and b = $NHCH_2C\mathit{H}_2$ are shown against an increasing amount of NaH_2PO_4 (50 mM) at room temperature.

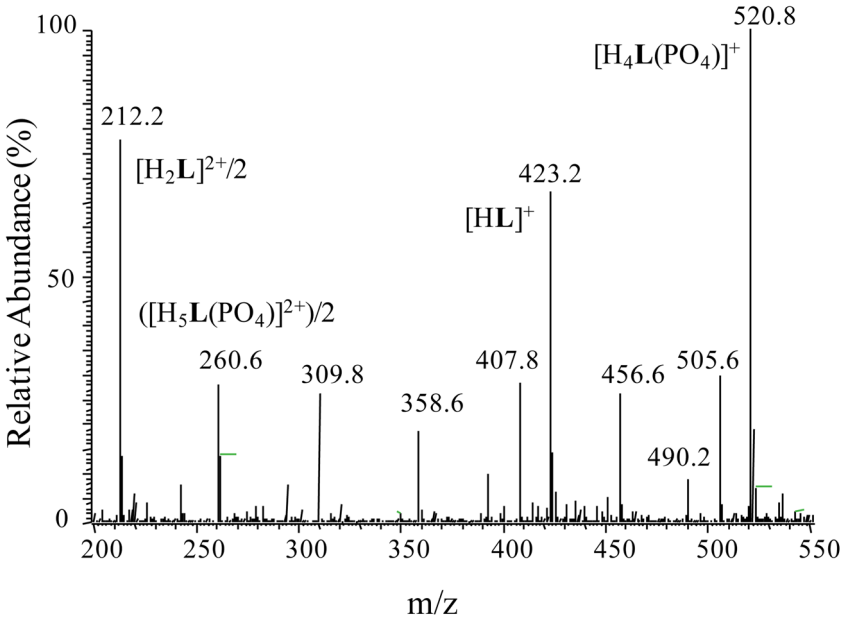

Figure 4.

ESI-MS (positive ion mode) spectrum of the phosphate complex. The solution was prepared from the phosphate salt of **L** (1.0×10^{-5} M) in MeOH/H_2O (50:50, v/v).

Table 1

Hydrogen bonding parameters (Å, °) for $H_2PO_4^-$ binding in **L**

D–H···A	D···A	< D–H–A
N1–H1B···O2A	2.69(2)	172.0
N4–H4B···O2A	3.21(3)	130.6
N4–H4B···O4B	2.70(2)	178.0
N14–H14B···O1A	2.723(16)	157.0
C2–H2A···O4B	3.276	133.69
C13–H13A···O3B	3.162	117.78
O3B–H3BO···O2A	2.531(16)	179.4