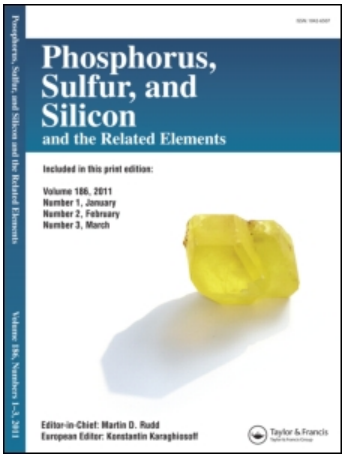

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Studies on the Contribution of Phosphoryl Group to the Non-Covalent Interaction between ATP and α-Aminophosphonic Acids Derivatives by ESI-MS and Molecular Modeling

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To cite this Article Liu, Xiao-xia , Fang, Hua , Liu, Yan and Zhao, Yu-fen(2008) 'Studies on the Contribution of Phosphoryl Group to the Non-Covalent Interaction between ATP and α-Aminophosphonic Acids Derivatives by ESI-MS and Molecular Modeling', Phosphorus, Sulfur, and Silicon and the Related Elements, 183: 2, 791 — 796
To link to this Article: DOI: 10.1080/10426500701808168
URL: http://dx.doi.org/10.1080/10426500701808168

Phosphorus, Sulfur, and Silicon, 183:791–796, 2008

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500701808168

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Studies on the Contribution of Phosphoryl Group to the Non-Covalent Interaction between ATP and α-Aminophosphonic Acids Derivatives by ESI-MS and Molecular Modeling

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In this work, Diisopropyl [phenyl(pyridine-4-carboxamido)methyl]phosphonate was synthesized for investigating the interaction with ATP. The noncovalent complex was observed by the Electrospray ionization Mass Spectrometry (ESI-MS). Through Molecular modeling, it was found that the non-covalent complex was stabilized by two intermolecular hydrogen bonds, which has the binding energy of - 8.999 kcals/mol. In order to investigate the function of phosphoryl group in the interaction, benzoyl phenylglycine isopropyl ester was also tested, but no interaction with ATP was observed by ESI-MS. These results implied that phosphoryl group was a very important functional group to provide effective interaction site between α-Aminophosphonic acids and ATP.

Keywords ATP; ESI; molecular dynamic simulation; noncovalent complex

INTRODUCTION

To investigate molecular recognition of ATP with proteins or structured peptide-based receptor is very important for understanding enzymatic mechanisms and drug design.[1,2] α-Aminophosphonic acids and their derivatives, as phosphorous analogs of amino acid, possess widely biological activities.[3–5] In our previous research, it was

The project is supported by the National Natural Science Foundation of China (No. 20572061) and the Ministry of Education (Key Project 104201).

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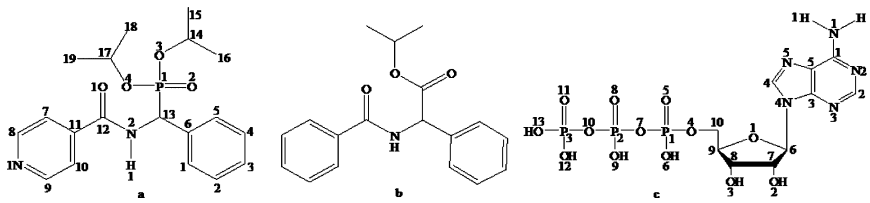

FIGURE 1 The structure of (a) Diisopropyl [phenyl(pyridine-4-carboxamido)methyl] phosphonate and the atom numbering system used in this paper (b) benzoyl phenylglycine isopropyl ester (c) ATP structure and the atom numbering system used in this article.

found that α-Aminophosphonic acid, Diisopropyl [phenyl(pyridine-4-carboxamido)methyl]phosphonate (Figure 1a, compound **1a**) could inhibit KB carcinoma cell.[6] In order to understand the inhibiting function, the interaction of compound **1a** with ATP was investigated first. It is well known that electrospray ionization mass spectrometry (ESI-MS) is a powerful method to observe the intact noncovalent complexes between biomolecules and small molecules.[7,8] Therefore, in this article the noncovalent complex between compound **1a** and ATP were determined by ESI-MS.

MATERIALS AND METHODS

Mass Spectrometry Experimental

ATP was purchased from Baitai Company; compound **1a** and **1b** were synthesized in our lab;[6] all the solvents were chromatography grade. Mass spectra were acquired in positive ion mode using a Bruker Esquire-3000 Plus ion trap spectrometer equipped. The samples dissolved in a mixed solvent CH_3OH/H_2O (volume ratio is 1:1) were ionized by electrospray ionization (ESI) and continuously infused into the ESI chamber at a flow rate of 4 uL/min by a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Co.).

Calculation Methods

All the modeling calculation was carried out on a SGI workstation using the SYBYL 7.1 molecule modeling package.[9] The X-ray structure of ATP was obtained from the Cambridge Structural Database (CSD),[10] and the X-ray structure of compound **1a** was the unpublished data from our lab. Using Tripos force field and molecular mechanics calculation, these two initial structures were optimized with 400 steps of steepest

descent method, then 400 steps of conjugate gradient method. The optimized structures of compound **1a** and ATP were used as docking models. The DOCK method in SYBYL system was used to investigate the noncovalent complex of ATP and compound **1a**. The binding energy of the noncovalent complex was calculated using the Tripos force field in the SYBYL 7.1 by the following equation:

$$\Delta E_{Binding} = E_{1a-ATP} - E_{1a} - E_{ATP} \quad (1)$$

where E_{1a-ATP} is the lowest energy of non-covalent complex between compound **1a** and ATP, E_{1a} is the lowest energy of compound **1a**, and E_{ATP} is the lowest energy of molecular ATP.

RESULTS AND DISCUSSION

The binding affinity of ATP with ligand was investigated by ESI-MS. When pure ATP in the mixed solvent CH_3OH/H_2O was tested by ESI-MS, only the positive ion peaks of ATP with different number of sodium ions were observed, including protonated molecular ions $[ATP+H]^+$at *m/z* 508, $[ATP(Na)+H]^+$ at *m/z* 530, $[ATP(2Na)+H]^+$at *m/z* 552, $[ATP(3Na)+H]^+$at *m/z* 574, $[ATP(4Na)+H]^+$at *m/z* 596, and sodium adduct ion at *m/z* 618$[ATP(4Na)+Na]^+$ (Figure 2a). However, when ATP was mixed with compound **1a** in the solvent CH_3OH/H_2O, ESI-MS showed that, in addition to the peaks corresponding to the pure ATP, there were more peaks showed up, including protonated molecular ions $[ATP+\mathbf{1a}+H]^+$at *m/z* 884, $[ATP(Na)+\mathbf{1a}+H]^+$ at *m/z* 906, $[ATP(2Na)+\mathbf{1a}+H]^+$ at *m/z* 928, $[ATP(3Na)+\mathbf{1a}+H]^+$ at *m/z* 950, $[ATP(4Na)+\mathbf{1a}+H]^+$ at *m/z* 972, and sodium adduct ion $[ATP(4Na)+\mathbf{1a}+Na]^+$ at *m/z* 994, which were related to the adduct between ATP and compound **1a** (Figure 2b). It clearly indicated that one molecule compound **1a** interact with one molecule ATP to form noncovalent complex, which implied that α-aminophosphonic acids derivative compound **1a** possessed affinity for ATP. But for the compound **1b**, which is a carboxyl analogy for compound **1a**, no interaction with ATP was observed by the ESI-MS (Figure 2c). These results implied that phosphoryl group made a great role in the interaction between α-Aminophosphonic acids and ATP.

To investigate the function of phosphoryl group in the interaction and explain the noncovalent complex formation between ATP and compound **1a**, the Molecular dynamic simulation was used. The distribution of hydrogen bonding acceptor and donor on the two top sites of the U type in ATP molecular matched with the distribution of P (1) = O (2), and N (2)-H (1) in the molecule **1a**.

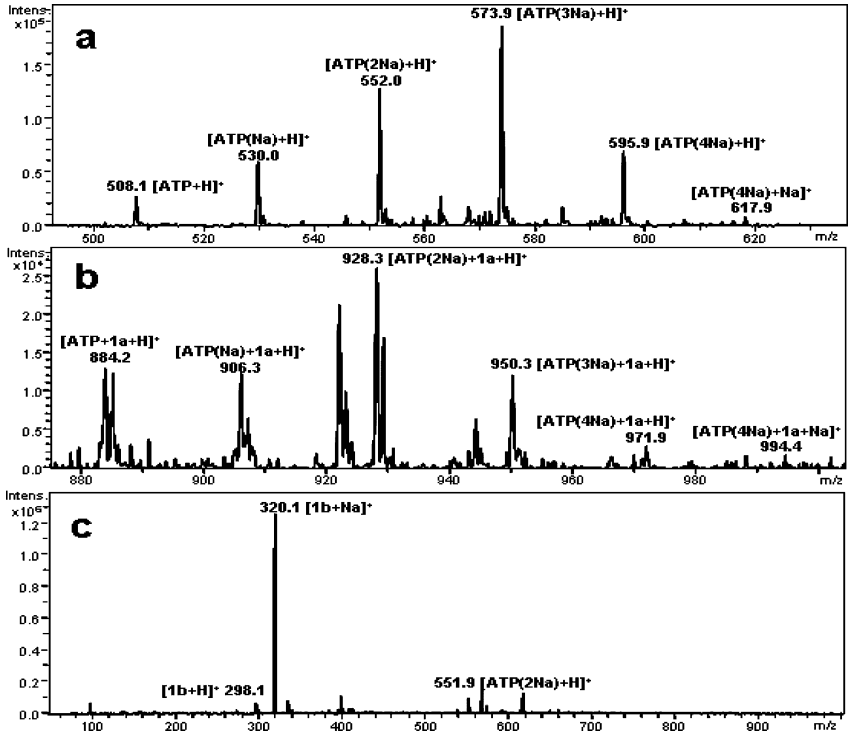

FIGURE 2 ESI-MS of (a) pure ATP in mixed solvent CH_3OH/H_2O (volume ratio is 1:1) (b) ATP (0.5 mmol/L) and compound **1a** (0.5 mmol/L) in mixed solvent CH_3OH/H_2O (volume ratio is 1:1). (c) ATP (0.5 mmol/L) and compound **1b** (0.5 mmol/L) in mixed solvent CH_3OH/H_2O (volume ratio is 1:1).

The DOCK method in SYBYL system was used to investigate the non-covalent complex between ATP and compound **1a** and the conformation with the lowest binding energy was obtained. The definition of the lowest energy non-covalent complex and the hydrogen bonds scheme were shown in Figure 3. N(2)-H(1) and O(2) of the molecule **1a**, formed two hydrogen bonds with O(12) and H(1)-N(1) of the ATP respectively. The hydrogen bonds geometry of the non-covalent complex was shown in Table I. All the hydrogen bonds data were in the normal range. The two intermolecular hydrogen bonds between ATP and molecule **1a** contributed to the assembling of the dimmer. The binding energy of the noncovalent complex was calculated using the Tripos force field in the SYBYL 7.1 as followed:

$$\Delta E_{Binding} = E_{1a-ATP} - E_{1a} - E_{ATP}$$
$$= -57.166 - (-7.266) - (-40.901) = -8.999\,\text{kcal/mol}$$

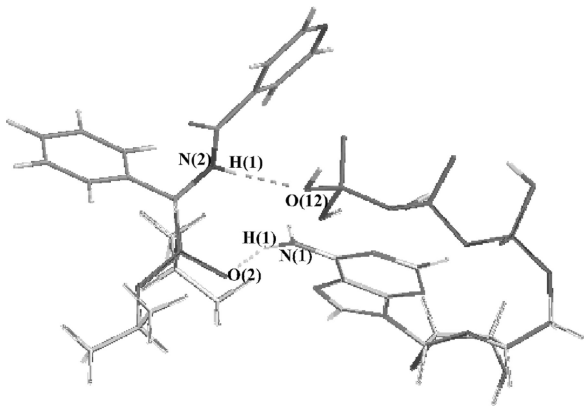

FIGURE 3 Definition of the lowest energy noncovalent complex and the hydrogen bonds scheme included.

The release of the binding energy was much higher than the thermal kinetic energy, which confirmed that the computed structure of the noncovalent complex was reasonable.

CONCLUSION

It was observed by ESI-MS, ATP could form the noncovalent complex with the bioactive α-Aminophosphonic acids derivative compound **1a**, but not with the compound **1b**, the carboxyl analogy to the compound **1a**. Using the Molecular modeling, it was observed that there were two intermolecular hydrogen bonds formed in the noncovalent complex between compound **1a** and ATP. The binding energy was –8.999 kcals/mol, which was reasonable. These results implied that phosphoryl group provided effective interaction site in the noncovalent complex, which was a very important functional group for α-Aminophosphonic acids to interact with ATP. All these results carried the investigation a step forward in the molecular recognition of ATP with proteins or structured

TABLE I Hydrogen Bonds Geometry

X—H—Y(symm code) (compound **1a** —ATP)	X—Y(nm)	X—H—Y(degree)
N(2)-H(1)—O(12)	2.906	134.56
[P(1)]O(2)—H(1)-N(1)	2.877	160.68

peptide-based receptor, which was meaningful for understanding the enzymatic mechanisms and the drug design.

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