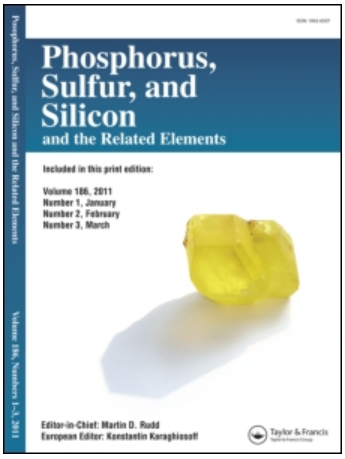

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The Investigation of Interaction Competition Between ATP and DIPP-Ala, Boc-Ala, or Ala by ESI-MS/MS and Theoretical Calculation

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THE INVESTIGATION OF INTERACTION COMPETITION BETWEEN ATP AND DIPP-Ala, Boc-Ala, OR Ala BY ESI-MS/MS AND THEORETICAL CALCULATION

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The interactions between ATP and N-(O,O-diisopropyl) phosphoryl-L-alanine (DIPP-Ala), N-(tert-butoxycarbonyl)-L-alanine (Boc-Ala), or L-alanine (Ala) were investigated by electrospray ionization tandem mass spectrometry (ESI-MS/MS). The non-covalent complexes between ATP and Boc-Ala or DIPP-Ala were observed, while the complex between ATP and Ala was not found in the mass spectra. The affinity of DIPP-Ala for ATP was confirmed to be stronger than that of Boc-Ala by competition experiment. Through molecular modeling calculations, it was found that the non-covalent complexes were stabilized by intermolecular hydrogen bonds, and the affinity sequence for ATP was DIPP-Ala > Boc-Ala > Ala by comparing their binding energy, −35.407 kcal/mol, −15.634 kcal/mol, −6.555 kcal/mol, respectively. The results implied that a phosphoryl group was a very important functional group to provide an interaction site between amino acids and ATP, and that N-phosphoryl amino acids can be used as a good model of protein in the studies of molecular recognition of ATP.

Keywords ATP; ESI-MS/MS; mini-model of protein; molecular dynamic simulation

INTRODUCTION

It is well known that adenosine 5′-triphosphate (ATP; Scheme 1a) plays an essential role in all forms of life. It not only functions as the energy carrier, but also conducts the

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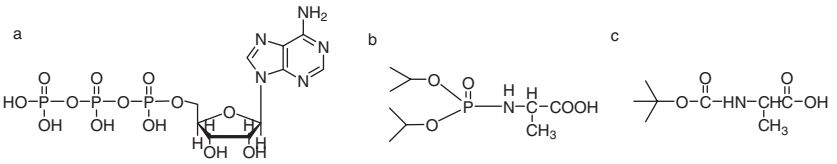

Scheme 1 The structure of ATP, DIPP-Ala, and Boc-Ala.

regulation of complex biochemical processes via phosphorylation of proteins.[1–3] The study of molecular recognition of ATP by proteins, which is essential for many signal transduction and energy transfer processes in the cell, is of great importance for understanding enzymatic mechanism and drug study.[4–6] However, it is difficult to study the interaction between ATP and proteins directly for the characteristic of proteins, such as large molecular mass, structural multiplicity, etc. So it is essential to find a good model to mimic proteins in the investigation of molecular recognition of ATP in detail.

As we know that amino acids are the building block of the proteins and peptides, the compounds to be ideal models of a protein should contain some amino acid moiety. Our group has found that N-phosphoryl amino acids (DIPP-AA) have many interesting biomimic reactivities, such as ester exchange on phosphorus, esterification, N to O migration, peptides and nucleotides formation,[7–12] and have been called "mini-type activating enzymes." So N-(O,O-diisopropyl) phosphoryl-L-alanine (DIPP-Ala; Scheme 1b) was used as a mini-model of protein to investigate its interaction with ATP. Furthermore, N-(tert-butoxycarbonyl)-L-alanine (Boc-Ala, Scheme 1c) contains not only an amino acid moiety, but also an acylamide bond like that of the peptide and protein. Therefore, L-alanine(Ala), Boc-Ala, and DIPP-Ala were selected to be used and compared in the studies of molecular recognition of ATP to study the different interactions between ATP and small model molecules and to find which was the best mini-model of protein.

In recent years, it has been found that the non-covalent complex can be ionized intact by electrospray ionization (ESI) and then be detected by the mass analyzer easily and sensitively. ESI-MS technique has become a useful tool to assess non-covalent interaction of biomolecules.[13–16] Therefore, in this article, to find the best mini-model of protein, the interaction competition between ATP and Ala, Boc-Ala, or DIPP-Ala was studied by ESI-MS, and the binding affinity sequence obtained was confirmed by molecular dynamic simulation using Sybyl 7.1.

EXPERIMENTAL

Chemicals

Adenosine 5′-triphosphate disodium salt (ATP) was purchased from Sigma Chemical Company (St. Louis, MO, USA). L-Alanine was purchased from Baitai Biochemical Company (Shanghai, China). DIPP-Ala and Boc-Ala were prepared according to the methods in the literature.[17,18]

Mass Spectrometry

An Esquire 3000 ESI-MS with an ion trap mass spectrometer (Bruker Daltonik Gmbh, Germany) was used. Sample solutions of ATP and Ala, Boc-Ala, or DIPP-Ala

(10^{-4}M each) were prepared with methanol solution (methanol:water = 1:1, V/V) and continuously infused into the ESI chamber at a flow rate of 4 μL min^{-1} by a Cole-Parmer 74900 syringe pump (Cole-Parmer Instrument Company). The MS/MS spectra were obtained by collision-induced dissociation (CID) with helium after isolation of the appropriate precursor ions. Ionization of analysis was carried out using the following setting of the ESI: nebulizer gas flow 7 psi, dry gas 4.5 L/min, dry temperature 300°C, spray voltage 4000 V. Calibration of *m/z* was performed using a standard ESI-tuning mixture. Scan range was 200–1000 *m/z*, and scan resolution was normal (13000 *m/z/s*).

Calculation Method[19,20]

All the modeling calculations were carried out on a SGI workstation using the Sybyl 7.1 molecule modeling package.[21] The X-ray structures of ATP-2Na, Ala, Boc-Ala, and DIPP-Ala was obtained from the Cambridge Structural Database(CSD).[22–25] Using Tripos force field and molecular mechanics calculation, all the initial structures were optimized with 1000 steps of steepest descent method and 0.05 kJ/mol gradient as the terminate condition. The optimized structures of ATP, Ala, Boc-Ala, and DIPP-Ala were used as docking models. The DOCK method in Sybyl was used to investigate the non-covalent complex of ATP and the studied compounds. The binding energy of the complex was calculated using the Tripos force field in the Sybyl 7.1 by the following equation:

$$\Delta E_{binding} = E_{complex} - E_{ATP} - E \quad (1)$$

Where $E_{complex}$ is the lowest energy of non-covalent complex of ATP and Ala, Boc-Ala, or DIPP-Ala, E_{ATP} is the lowest energy of ATP, and E is the lowest energy of Ala, Boc-Ala, or DIPP-Ala.

RESULTS AND DISCUSSION

For the samples of ATP mixed with Boc-Ala or DIPP-Ala in the solvent CH_3OH/H_2O, ESI-MS showed that, in addition to the peaks corresponding to ATP, Boc-Ala, and DIPP-Ala, more peaks were observed in the spectra, for example, protonated molecular ions and sodium adduct ions exactly at *m/z* 763 [ATP(3Na) + Boc-Ala + H]$^+$, 785[ATP(4Na) + Boc-Ala +H]$^+$, 807[ATP(4Na) + Boc-Ala + Na]$^+$, 829[ATP(4Na) + Boc-Ala(Na) + Na]$^+$ or *m/z* 805[ATP(2Na) + DIPP-Ala + H]$^+$, 827[ATP(3Na) + DIPP-Ala + H]$^+$, 849 [ATP(4Na) + DIPP-Ala + H]$^+$, 871 [ATP(4Na) + DIPP-Ala + Na]$^+$, 893[ATP(4Na) + DIPP-Ala(Na) + Na]$^+$ as shown in Figure 1. They were related to the adducts between ATP and Boc-Ala or DIPP-Ala (Figure 1)[19] and implied that both Boc-Ala and DIPP-Ala interacted with ATP. These complexes were stable enough to be isolated and fragmented. For example, the MS/MS spectra of protonated or sodium adduct ions of ATP with Boc-Ala or DIPP-Ala are shown in Figure 2. They all lost one small molecule, Boc-Ala or DIPP-Ala, to produce [ATP(4Na) + H]$^+$ ion at *m/z* 596. The results clearly indicated that one molecule Boc-Ala or DIPP-Ala interacted with one molecule ATP to form the non-covalent complex. However, in terms of the solution of ATP mixed with Ala, no adduct ion was observed by ESI-MS.

Both Boc-Ala and DIPP-Ala possessed affinity for ATP, but then which had stronger affinity? The complex ions would disappear by increasing the value of capillary exit of mass spectrometry, so the capillary exit was tuned to compare the affinity of ATP with Boc-Ala

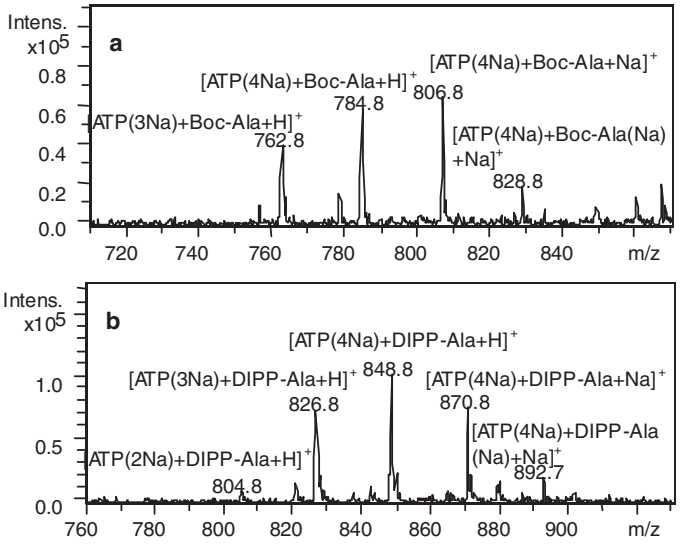

Figure 1 The ESI-MS spectra of the interaction of ATP-2Na with (a) Boc-Ala and (b) DIPP-Ala.

or DIPP-Ala. When the capillary exit was tuned to 236.6 V, the adduct ion between ATP and Boc-Ala disappeared. However, until the capillary exit was tuned to the limit of the mass spectrometry (320 V), the non-covalent complex ion between ATP and DIPP-Ala still existed. Therefore, it was the initial proof that the interaction between ATP and DIPP-Ala was stronger than that between ATP and Boc-Ala. Another proof was obtained when ATP, Boc-Ala, and DIPP-Ala were mixed all together and the mixture was analyzed by ESI-MS.

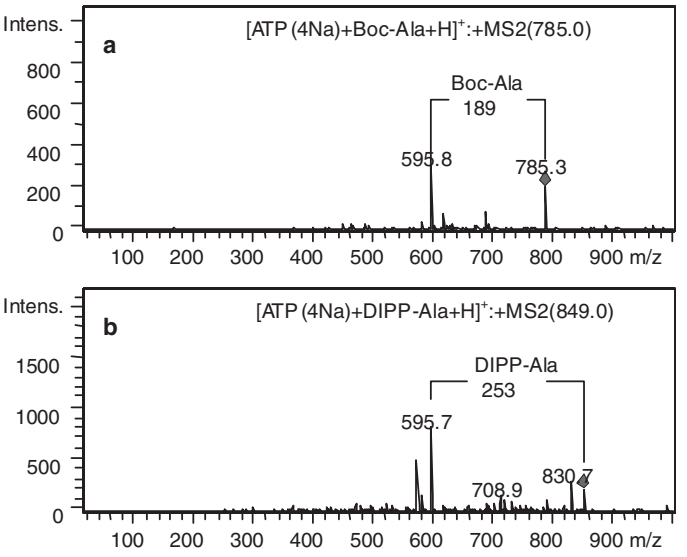

Figure 2 The ESI-MS/MS spectra of the complex between ATP and (a) Boc-Ala or (b) DIPP-Ala ([ATP(4Na) + Boc/DIPP-Ala + H]$^+$ as the example).

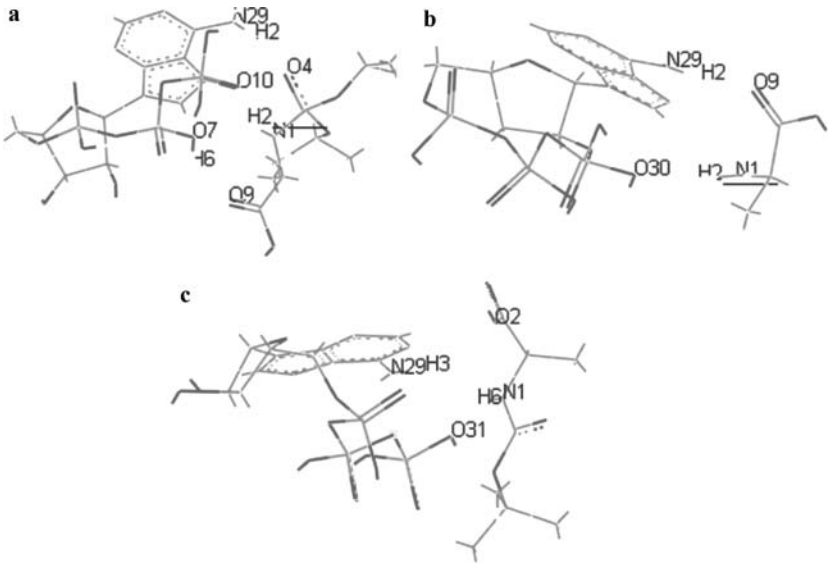

Figure 3 Definition of the lowest energy non-covalent complex and the hydrogen bonds scheme included (a) the complex of ATP and DIPP-Ala; (b) the complex of ATP and Ala; (c) the complex of ATP and Boc-Ala.

ESI-MS spectra showed that only the non-covalent complex ions between ATP and DIPP-Ala could be observed, while the ions related to the adduct of ATP and Boc-Ala could not be observed. The results indicated that DIPP-Ala possessed stronger affinity for ATP than Boc-Ala. Therefore, the affinity sequence for ATP was DIPP-Ala > Boc-Ala > Ala. According to these results, it could be concluded that after it was attached to the phosphoryl group, Ala possessed stronger affinity for ATP, which indicated that the phosphoryl group played an important role in the interaction between Ala and ATP.

In order to further investigate the interaction and explain the non-covalent formation between ATP and the studied compound, the molecular dynamic simulation was used. The structures of non-covalent complexes with lowest binding energy were obtained as shown in Figure 3 using the DOCK method in Sybyl 7.1. The hydrogen bonds in the complex

Table I The geometry data of hydrogen bonds

Compound name	X—H—Y(symm code)* (AA—ATP)		X—Y(nm)	X—H—Y (degree)
DIPP-Ala	N(1)-H(2)	O(10)	2.414	132.42
	N(1)-H(2).	O(7)H	2.671	126.72
	C═O(9)	O(7)H(6)	2.531	132.41
	P═O(4)	N(29)-H(2)	2.683	165.47
Ala	C═O(9)	N(29)-H(2)	2.644	163.04
	N(1)-H(2)	P(γ)-O(30)Na	2.851	160.39
Boc-Ala	C═O(2)H	N(29)-H(3)	3.307	128.47
	N(1)-H(6)	P(γ)-O(31)Na	2.659	154.05
	N(1)-H(6)	O(9)	2.987	120.72

*Atom number shown in Figure 3.

Table II The binding energy of non-covalent complex

Compound name	E(kJ/mol)	E_{ATP} (kJ/mol)	$E_{complex}$ (kJ/mol)	$E_{binding}$ (kJ/mol)*
DIPP-Ala	−19.118	−49.654	−104.179	−35.407
Ala	1.931	−49.654	−54.278	−6.555
Boc-Ala	−6.904	−49.654	−72.192	−15.634

*Calculated from Equation (1).

were determined according to criteria that were used by McDonald and Thornton with a maximum donor to acceptor distance of 3.5 Å, a maximum hydrogen atom to acceptor distance of 2.5 Å, and angles of at least 90° at both the hydrogen atom and the acceptor.[26] The geometry data of the hydrogen bond are shown in Table I. As shown in Figure 3a, N(1)-H(2), P=O(4), C=O(9) of DIPP-Ala formed four hydrogen bonds with the triphosphate moiety and NH_2 on the base moiety of ATP, while N(1)-H(2), C=O(9) of Ala only formed two hydrogen bond and N(1)-H(6), C=O(2)H of Boc-Ala formed three hydrogen bonds with the triphosphate moiety and NH_2 on the base moiety of ATP as shown in Figures 3b–3c. Unlike the NH-C=O of Boc-Ala, the phosphoryl group (P=O (4)) in DIPP-Ala acted as a hydrogen acceptor and formed a hydrogen bond with N(29)-H(2) of ATP, which contributed to the assembling of the complex.

The binding energies were also calculated using the Tripos force field in Sybyl 7.1 followed Equation (1). In the formation of non-covalent complex between ATP and DIPP-Ala, Boc-Ala, or Ala, the energy changes were −35.407 kJ/mol, −15.634 kJ/mol, and −6.555 kJ/mol, respectively (shown in Table II). The energy releasing in the non-covalent complexes formation was all higher than the thermal kinetic energy, which confirmed that all the computed structures of the non-covalent complexes were reasonable. From the number of hydrogen bond and the binding energy, the affinity sequence of the three compounds for ATP was obtained: DIPP-Ala > Boc-Ala > Ala, which was consistent favorably with the conclusion acquired by ESI-MS.

CONCLUSIONS

In this article, the interactions between ATP and the studied compounds (DIPP-Ala, Boc-Ala, and Ala, which were used as mini-model protein) were investigated by ESI-MS/MS. The non-covalent complexes between ATP and Boc-Ala or DIPP-Ala were observed, while no interaction between ATP and Ala was found. By comparing the value of the capillary exit on which the complex disappeared and performing a competition experiment, the complex between ATP and DIPP-Ala was confirmed to be more stable than that of Boc-Ala, so the affinity sequence for ATP was assumed to be: DIPP-Ala > Boc-Ala > Ala. In order to confirm the results obtained by ESI-MS and explain the non-covalent formation mechanism between ATP and the studied compounds, the molecular dynamic simulation was used. Through molecular modeling, it was found that the non-covalent complexes were stabilized by intermolecular hydrogen bonds. The affinity sequence was also DIPP-Ala > Boc-Ala > Ala by comparing their binding energy, −35.407kcal/mol, −15.634 kcal/mol, −6.555kcal/mol, respectively, which was consistent favorably with the conclusion acquired by ESI-MS. These results indicated that phosphoryl group was a very important functional group to provide an interaction site between phosphoryl amino acids

and ATP, and DIPP-AA can be used as a good mini-model of protein in the studies of molecular recognition of ATP.

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