

The Effect of Phosphoryl Oxygen on the Intermolecular Action of Alanine and Lysozyme *

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Abstract It was found that N-diisopropoxyphosphoryl alanine (DIPP-Ala) could form adduct with hen egg white lysozyme (HEWL) as shown in electrospray ionization mass spectroscopy (ESI-MS), but the non-phosphorylated alanine couldn't. The capability for formation of DIPP-Ala dimer was more stronger than that of alanine. It suggested that a specific non-covalent complex was formed in the solution phase and could be transferred to the gas phase via electrospray ionization (ESI). The results implied that phosphorylated alanine possessed relatively stronger affinities for protein and formed non-covalent complexes with protein more easily than alanine. Using Tripos force field, molecular mechanics calculation on DIPP-Ala dimer showed that such non-covalent adduct formation was due to the intermolecular hydrogen bond.

Keywords: ESI-MS, Intermolecular hydrogen bond, Molecular dynamic simulation

The non-covalent complexes between biomolecules are very important for their functions, such as the assembly of proteins or phosphoryl proteins and antibody-antigen. There is an amazing diversity of reactions and processes that are regulated by the chemically simple event of placing a phosphate group in proteins. Protein phosphorylation might allow direct protein-protein contacts to be established^[1]. In our previous work, we showed that N-phosphoryl amino acids were chemically active species and exhibited biomimetic reactivity^[2-3]. Electrospray ionization (ESI) is sufficiently gentle to allow the ionization and detection of intact non-covalent complexes between proteins and small molecules^[4-5]. It is a rapid, sensitive and accurate method for studying the non-covalent complexes in solution^[6,7]. In this paper, the assembly of DIPP-Ala, which has the simplest structure unit of phosphoryl proteins, was studied by electrospray ionization mass spectroscopy (ESI-MS) and molecular mechanics calculation. It was found that phosphorylation of alanine enhanced the inter-

molecular action.

1 Experimental

Amino acids were obtained from Baitai Company, the phosphoryl alanine was synthesized according published methods^[8], hen egg white lysozyme (HEWL) was obtained from Shanghai Sangon Company, all solvents were chromatography grade. Samples were dissolved in a mixed solvent CH₃OH/H₂O (volume ratio is 1:1). The ESI-MS was performed on a commercial Bruker ESQUIRE-3000 plus ion trap spectrometer equipped with a gas nebulizer probe capable of analyzing ions up to m/z=6000. And the calculations were performed with a software package SYBYL 6.8 of American Tripos Company on the Silicon Graphics O2 computer. Force field parameters were taken from the Tripos force field.

2 Results and discussion

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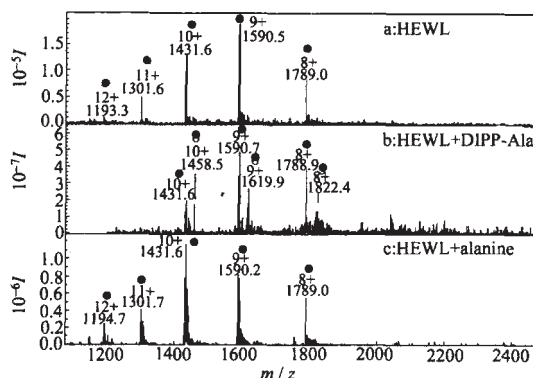


Fig.1 a) ESI-MS of HEWL ($0.026 \text{ mmol} \cdot \text{L}^{-1}$) b) ESI-MS of HEWL ($0.026 \text{ mmol} \cdot \text{L}^{-1}$) and DIPP-Ala ($0.78 \text{ mmol} \cdot \text{L}^{-1}$); c) ESI-MS of HEWL ($0.026 \text{ mmol} \cdot \text{L}^{-1}$) and alanine ($0.78 \text{ mmol} \cdot \text{L}^{-1}$)

identify the multiply charged ion peaks of HEWL-heme-complex ;

identify the multiply charged ion peaks of HEWL-heme-DIPP-Ala complex

2.1 Formation of the adduct of DIPP-Ala with HEWL

The development of ESI technology and the discovery that highly charged ions of proteins are readily formed have led to dramatic growth in the application of mass spectrometry to biomolecules^[10-13]. In this work, the binding affinity of alanine with HEWL and DIPP-Ala with HEWL was investigated by ESI-MS. The results showed that there were complexes formed between HEWL and DIPP-Ala, but there was no adducts formed between alanine and HEWL (Fig.1). Fig.1(a) showed ion peaks of HEWL with varied positive charge from 12+ to 8+. The mass derived from these peaks was 14306 for HEWL. Fig.1(b) showed the ESI-MS obtained from the mixed solution of HEWL and DIPP-Ala. Besides the expected multiply protonated molecular ions at m/z 1431.6, 1590.5, 1789.0, there were three new protonated ions at m/z 1458.5, 1619.9 and 1822.4, which corresponding to $[\text{HEWL} + \text{DIPP-Ala} + 10\text{H}]^{10+}$, $[\text{HEWL} + \text{DIPP-Ala} + 9\text{H}]^{9+}$ and $[\text{HEWL} + \text{DIPP-Ala} + 8\text{H}]^{8+}$, respectively. It clearly indicated that there was one molecule of DIPP-Ala combined with HEWL to form 1:1 adduct. However, for the non-phosphorylated alanine, there were no new peaks in Fig.1(c), which suggested that there were no complexes formed between alanine and HEWL. The results implied that phosphorylated alanine possessed relatively stronger affinities for protein and formed non-covalent complexes with protein more easily than alanine.

2.2 Formation of the dimer of alanine or DIPP-Ala

As the structure unit of proteins and phosphoryl proteins, dimer formation of alanine and DIPP-Ala was studied in our

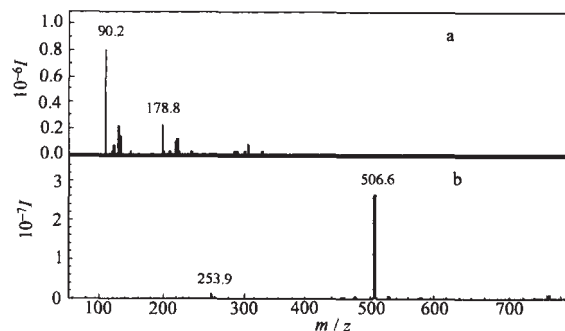


Fig.2 a) ESI-MS of alanine b) ESI-MS of DIPP-Ala

work. Fig.2 showed that DIPP-Ala formed its dimer more easily than alanine. The relative amount of dimer to monomer ($^2R/R$) for alanine was only 0.23. However, when alanine was N-phosphorylated, the $^2R/R$ for DIPP-Ala was 14.29. Therefore, the tendency to form the dimer was enhanced 62 times.

2.3 Theoretical study of the intermolecular hydrogen bonds between two DIPP-Alas

For getting insight into the mechanism of the dimer formation between the DIPP-Ala, a theoretical study of the intermolecular hydrogen bonding was carried out, using Tripos SYBYL 6.8 molecular modeling software system.

2.3.1 Conformation analysis of DIPP-Ala

For lack of experimental data, the molecular structure of Dipp-Ala was built with SYBYL software system^[14-16] and minimized to a local energy minimum conformation as the initial structure for the subsequent analysis. Random search method in SYBYL was used to collect the conformation set with parameter RMSD threshold=0.0200 nm, convergence threshold=0.050, Maximum hits=4, and a conformation set with total number of 295 conformers was attained. The histogram of the distribution

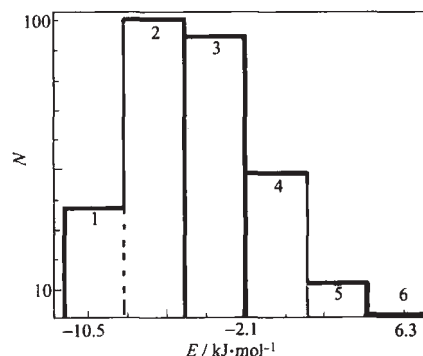


Fig.3 The histograms of the distributions for increment E (energy) and N (total numbers=295) forms based on Tripos force field with random search surface examination

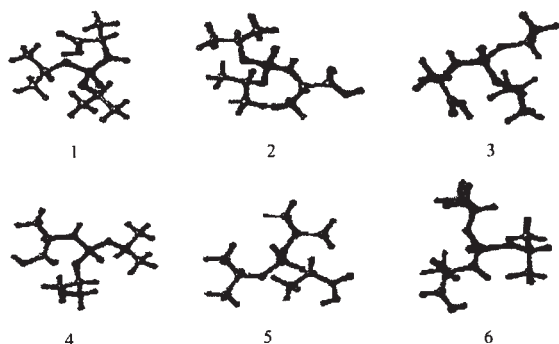


Fig.4 The representative structures of every conformation aggregation

of the numbers of conformers via energy is shown in Fig.3, and the lowest energy conformer from each of the six groups was selected as the representatives of conformation set. The molecular structures of the representatives with numbering scheme were shown in Fig.4, in which structure 1 corresponds to the global lowest energy conformer.

2.3.2 Modeling and computation of Dipp-Ala super molecular dimer

From inspection of the representative conformers, the lowest energy conformer 1 was not preferable for assembling a dimer with maximum number of intermolecular hydrogen bondings, because of its carboxyl group and phosphoryl group pointing towards opposite direction (nearly parallel with the —NH). Meanwhile, conformer 2 having its hydrogen bonding acceptor and donor groups pointing nearly the same direction and without any steric hindrance in their vicinity, and its energy was only $5.23 \text{ kJ}\cdot\text{mol}^{-1}$ higher energetically than structure 1. Therefore, this conformer was a suitable candidate for the formation of intermolecular dimer via hydrogen bondings.

The DOCK method^[17-19] in SYBYL system was used to investigate the stability and structure of the dimer of structure 2. The results showed that the energy of the dimer was lower than the sum of the two monomers by a value of $35.82 \text{ kJ}\cdot\text{mol}^{-1}$. To further investigate on the stability of the dimer, ten cycles

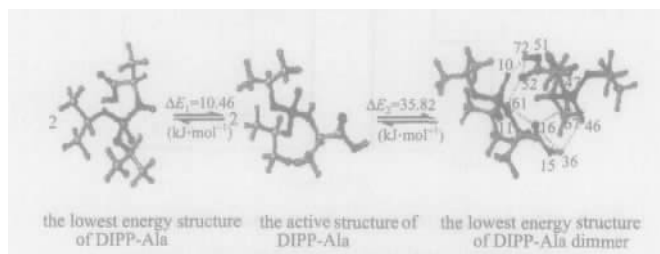


Fig.5 Definition of DIPP-Ala and its 1:1 hydrogen-bonding interaction conformers

Table 1 Hydrogen bonds geometry

X	H	Y(symm code)	X	Y(nm)	H	X/Y(nm)	X	H	Y(degree)
		(M1 M2)							
(M1)O10	H72-	O51(M2)	0.2856	0.2081					137.63
(M1)N11-	H61	O52(M2)	0.2859	0.2058					132.60
(M1)O16	H67-	N47(M2)	0.2730	0.2439					95.06
(M1)O15-	H36	O46(M2)	0.2832	0.2707					87.65

M1 identifies the left molecule of dimer ,M2 identifies the right molecule of dimer.

of molecular dynamic simulation annealing of the dimer from 800 K to 300 K were calculated using MD program in SYBYL. The result of the calculation showed that the dimer was stable at room temperature, which consistent with the mass spectrum experiment. The calculated energies and hydrogen bonds scheme in the dimer were showed in Fig.5, and the hydrogen bonds geometry of the dimer were shown in Table 1. It was clear that the existence of P=O group in molecule made considerable contribution to the dimer formation. The P=O group was essential both to keep the proper conformation of monomer and to form more hydrogen bonds between monomers.

It was found that the ability of the dimer formation could be enhanced remarkably by introducing a phosphoryl group to the N-terminal of alanine (Fig.5). Four intermolecular hydrogen bonds between the two monomers contributed to the assembling of the dimer. The most critical structural groups involved in the dimer formation are those of carboxyl, phosphor-amide group.

3 Conclusion

In the ESI-MS spectra, it was observed that DIPP-Ala could form non-covalent complexes with biological molecule (HEWL), but could not form complexes with the alanine. It should be pointed out that the presence of phosphoryl group in DIPP-Ala could enhance the intermolecular interaction, which was confirmed by the molecular modeling. It was found that there are four pairs of hydrogen bonds in the dimer. And the calculation showed that the dimer was stabilized than the monomer by a value of $35.82 \text{ kJ}\cdot\text{mol}^{-1}$. In a word, the phosphoryl oxygen group in the phosphoryl amino acid and phosphoryl protein would favor the intermolecular hydrogen bond. This specificity might explain the contribution to the molecular

recognition between small molecule and biological molecule in the course of developing new medicine. These results might prove the structure information for the phosphorylated flavonoids possess relatively stronger affinities and form non-covalent complexes with the proteins more easily than the non-phosphorylated compounds^[20].

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磷酸化对丙氨酸与溶菌酶相互作用的影响*

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摘要 在电喷雾离子阱质谱图中发现丙氨酸不能和溶菌酶形成二聚体, 而磷酸化丙氨酸(DIPP-Ala)能和溶菌酶形成二聚体. 进一步研究发现丙氨酸及其他氨基酸磷酸化后, 自身形成二聚能力大大增强. 在 Silicon Graphics 图形工作站上采用 SYBYL 6.8 软件, 利用 Tripos 力场和分子力学方法研究了 DIPP-Ala 最低能量构象, 并用分子对接(DOCK)研究了二聚体的形成. 结果说明磷氧双键的存在增强了分子间的相互作用.

关键词: ESI-MS, 分子间氢键, 分子动力学模拟

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