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Theoretical Study of the Sensitivity-Improvement Effect of Phosphoryl Group in Mass Spectrometry of Small Peptides

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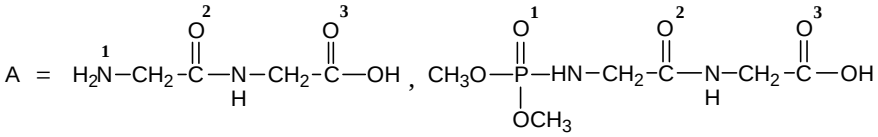
Abstract: It was found that phosphorylation of small peptide could improve the sensitivity in mass spectrometry. Density functional theory calculations showed that the energy for the protonation of N-(O, O′-dimethyl) phosphoryl glycylglycine is lower than that of glycylglycine. These could help to understanding the experimental results.

Keywords: Phosphoryl group, small peptide, mass spectrometry, relative energy.

Mass spectrometry plays an important role in peptide sequencing since the invention of soft ionization techniques, such as fast atom bombardment (FAB), electrospray (ESI) and matrix-assisted laser desorption/ionization (MALDI). In our previous work, it was found that phosphorylation on the N-terminal of small peptides could enormously improve their sensitivity in FAB-MS[1].

To understand the sensitivity-improvement effect of phosphoryl group, the energies for the protonation of glycylglycine (GlyGly) and N-(O, O′-dimethyl)phosphoryl glycylglycine (DMP-GlyGly) were studied by density functional theory. The geometries of all the species were optimized at the B3LYP/6-31G (d, p) level on SGI R12000 working station.

Scheme 1 Ionization process of the two model compounds, glycylglycine and N-(O, O′-dimethyl)glycylglycine, with their proton-binding sites marked.



GlyGly **DMP-GlyGly**

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Results and Discussion

In the computation, a simplified model for the ionization process was proposed (**Scheme 1**). Both GlyGly and DMP-GlyGly have three possible proton-binding sites. Other oxygen or nitrogen atoms also have lone pairs, but it would be much less stable to bind a proton there. The relative energies of protonated GlyGly and DMP-GlyGly were listed in **Table 1**, with the energies of non-protonated forms being zero points. For the dipeptide GlyGly, the best proton-binding site is the amino group. The energy of ion **1**, with the proton binding to the amino group, is much lower than that of ion **2** or **3**, with the proton binding to carbonyl groups. For DMP-GlyGly, the phosphoryl group is the best proton-binding site. Compared with the un-phosphorylated species, the energy of the phosphorylated ion **1** is 13.35 kJ lower than that of the un-phosphorylated one. For the other two proton binding sites **2** and **3**, the energies of phosphorylated species are much lower than corresponding un-phosphorylated ones. In other words, the N-phosphoryl peptide is more easily to ionize. So it could be understood why the sensitivity of N-phosphoryl peptide in mass spectrometry was much higher than the corresponding peptide.

In ESI-MS of N-phosphorylated peptides, characteristic fragmentation patterns different to ordinary peptides were observed[2]. More computations about N-phosphoryl peptides might discover more details about the fragmentation reactions of such peptides in gas phase. These may supply more information in peptide sequencing by mass spectrometry.

Table 1. Relative energies of protonated peptide and protonated phosphoryl peptide, with the energies of GlyGly and DMP-GlyGly set as the zero point. *DE* is the difference between the relative energies of protonated N-phosphorylated and non-phosphorylated peptides.

	Relative energy of protonated peptide/phosphoryl peptide (Unit: kJ/mol)		
	Binding site 1	Binding site 2	Binding site 3
$[GlyGly+H]^+$	-990.25	-902.22	-885.67
$[DMP\text{-}GlyGly+H]^+$	-1003.60	-1002.53	-939.74
DE	-13.35	-100.31	-54.07

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References

1. Y. W. Yin, Y. F. Zhao, Y. Chen, *et al*, *Science in China (Series B)*, **1994**, *24*, 904.
2. Y. Jiang, H. Fu, L. Xu, *et al*., *Rapid Commun. Mass Spectrom.*, **2000**, *14*, 1413.

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