

Study on the Enhancement of Proton Affinity by N-Diisopropoxy Phosphorylation of Amino Acid in Mass Spectrometry

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Abstract: With introduction of a diisopropoxy phosphoryl group into the N terminal of amino acids, it was found that proton affinity (PA) of amino acid was enhanced in mass spectrometry. Density functional theory calculations showed that the energy for protonation of DIPP-amino acid is lower than that of amino acid, which means PA of DIPP-AA is higher than that of corresponding amino acid. These results, coincident with our empirical results, offer a useful interpretation of experimental observations.

Keywords: Proton affinity, diisopropoxy phosphoryl group, amino acid, mass spectrometry.

In our previous work, it was found that N-phosphorylation of small peptides could improve their sensitivity in FAB-MS. Theoretical study showed that N-dimethoxy phosphoryl dipeptide is more easily to be ionized¹. Further research work was done in this direction. Using the kinetic method developed by Cooks², we obtained proton affinities (PA) of some phosphoryl amino acids in ESI-MS. It was found that introduction of a diisopropoxy phosphoryl group (DIPP) into the N-terminal of amino acids resulted in an enhancement of the PA of amino acid. To demonstrate the result the energies for protonation of DIPP-AA, and corresponding amino acid 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 workstation.

Results and Discussion

In the kinetic method, the proton-bound dimers of DIPP-Ala or DIPP-Leu with different reference base, such as Ile, Pro, Trp, have been formed respectively and dissociated in ESI-MS/MS. Compared with the gas-phase proton affinities of amino acids³, it could be concluded that the PA of DIPP-Ala and DIPP-Leu are between 934 kJ/mol to 948 kJ/mol, about 36-50kJ/mol higher than Ala 898kJ/mol and 20-34kJ/mol higher than Leu 914kJ/mol.

For computational work, we proposed a simplified model for protonation process, two possible proton-binding sites were marked (**Scheme 1**). Using density functional theory, we obtained the relative energies of protonated amino acid and DIPP-AA listed in **Table 1**, the energies of non-protonated forms were zero points. For amino acid, the

