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MALDI-TOF Mass Spectrometry Study of the Phosphorylated Tau Peptides

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Abstract: Fragmentation of phosphorylated Tau peptides in matrix assisted laser desorption-/ionization-time of flight-mass spectrometry (MALDI-TOF-MS) has been investigated. According to the post-source decay (PSD) in MALDI-TOF-MS, there are two different patterns of cleavage in phosphopeptides, which can be used to determine the phosphorylated site in peptides. In the synthetic tau peptides, the fragmentation at proline residue occurs strongly and this is useful to determine the structure of tau peptides.

Keywords: Mass spectrometry, PSD method, tau peptide, phosphorylation.

An important step in the establishment of Alzheimer's disease is the hyperphosphorylation of the tau protein in nerve cells, which results in an altered interaction of tau with the microtubulin and its association to paired helical filaments and the formation of neurofibillary tangles1,2. However, the molecular details governing these processes are still unclear and subject to an intense debate. To study the phoshorylation in tau protein in the molecular level is very important to understand the role of phosphorylation in the tau protein. In this paper, MALDI-TOF-MS was used to study the peptides and phosphopeptides in the tau protein.

Table 1 Synthetic peptides[a] corresponding to the repeat units of the human Tau-binding C-domain of β-tubulin

Tau peptide	Sequence	Amino acids/repeat number
R1	V K S K I G S T E N L K H Q P G G G	256-273/first repeat
R1p	V K S K I G S[P] T E N L K H Q P G G G	256-273/first repeat phosphorylated
R4	V Q S K I G S L D N I T H V P G G G	350-367/fourth repeat
R4p	V Q S K I G S[P] L D N I T H V P G G G	350-367/ fourth repeat phosphorylated

[a] The superscripted p stands for phosphorylation

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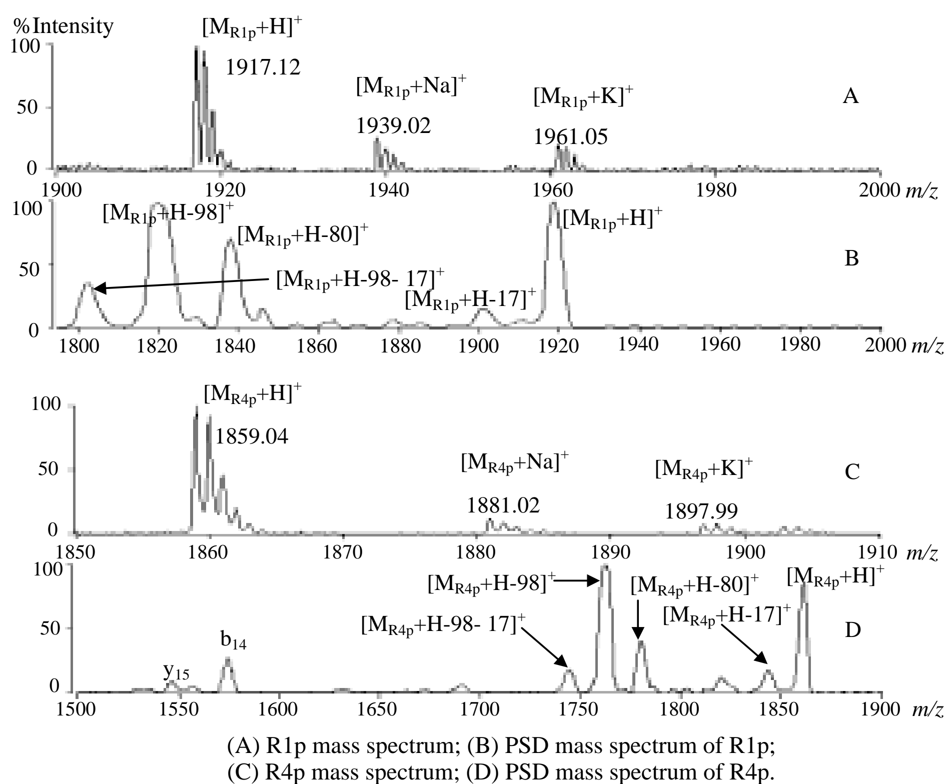
Figure 1 Positive ion reflector MALDI-TOF-MS of phosphopeptide with continuous extraction and CCA as a matrix.



(A) R1p mass spectrum; (B) PSD mass spectrum of R1p;
(C) R4p mass spectrum; (D) PSD mass spectrum of R4p.

R1, R1p, R4 and R4p were synthesized by Fmoc strategy using a simultaneous multiple peptide synthesizer, Model PSSM-8 (Shimadzu Corp, Kyoto Japan). Phosphoserine was incorporated as Fmoc-Ser(PO_3HBzl)-OH. The crude peptides were purified by reverse phase HPLC. The molecular weight of the peptide was determined by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The sequence of the tau peptides and phosphopeptides were shown in **Table 1**.

MALDI-TOF mass spectrometry experiments were performed in positive ion mode on an AXIMA instrument (Shimadzu, Kyoto, Japan). All MALDI-PSD fragment spectra were measured under the following conditions: nitrogen laser, 337 nm; positive-mode detection; reflectron mode. α–Cyano-4-hydroxycinnamic acid (CCA) was used as a matrix without further purification.

Phosphorylation is one of the most common posttranslational covalent modifications[3]. In MALDI-TOF-MS, the modification-specific fragmentation of phosphopeptide was observed as metastable fragmentation as demonstrated in **Figure 1** for the serine phosphopeptide R1p and R4p.

In mass spectrometry, peptides were easily binding with metal ions, such as sodium and potassium. Most peptides can be found as $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$

fragments in the mass spectra. In **Figure 1A**, there are three cluster peaks near 1917, 1939, 1956 for $[M_{R1p}+H]^+$, $[M_{R1p}+Na]^+$ and $[M_{R1p}+K]^+$ fragments, respectively. In **Figure 1C**, there are also three cluster peaks near 1859, 1881, 1897 for $[M_{R4p}+H]^+$, $[M_{R4p}+Na]^+$ and $[M_{R4p}+K]^+$ fragments, respectively. Mostly in phosphopeptides, $[M+H-HPO_3*]^+$ and $[M+H-H_3PO_4*]^+$ fragments are detected as metastable fragments at *m/z* values lower than their correct molecular mass in the PSD mass spectra[4]. In **Figure 1B** and **Figure 1D**, the $[M+H-HPO_3*]^+$ and $[M+H-H_3PO_4*]^+$ fragments both for R1p and R4p phosphopeptide in the PSD mass spectra were very clear and have relatively high intensities. Furthermore, the $[M+H-NH_3]^+$ and $[M+H-H_3PO_4*-NH_3]^+$ fragments also have strong intensities. These fragments in the PSD mass spectra showed that there is a phosphoryl group in the synthesized tau peptides R1p and R4p.

Figure 2 General fragmentation of peptide

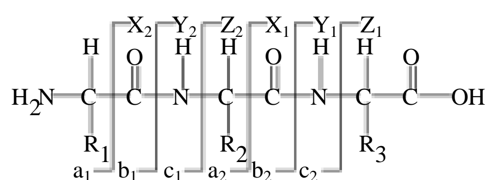
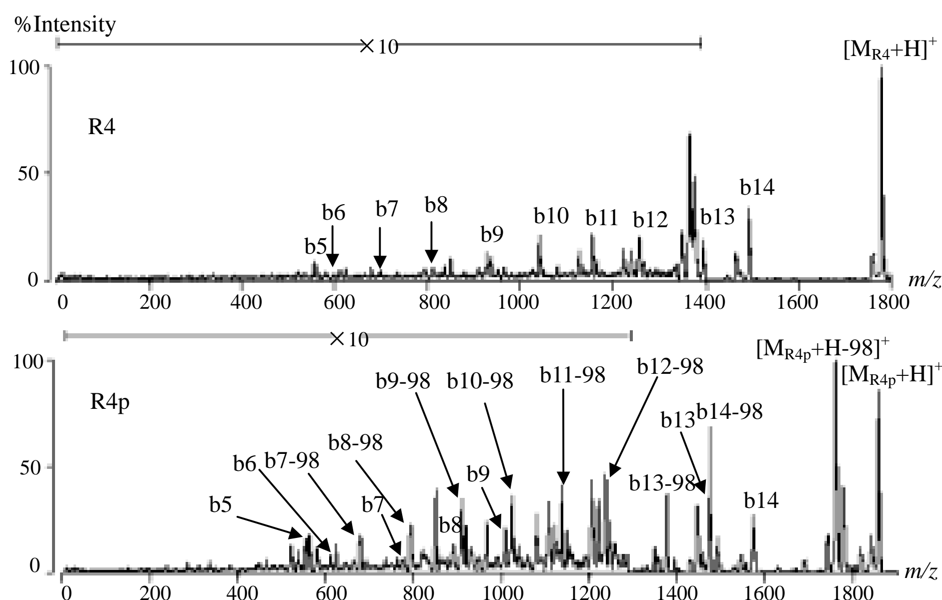


Figure 3 The positive ion reflector PSD mass spectra of R4 and R4p



After a phosphopeptide has been identified, next step is to determine which residue carries the modification. The most common method of identifying phosphrylation sites is analysis of the fragmentations of the MALDI mass spectrometry[5]. General fragmentation of peptide was shown in **Figure 2**. In the experiments, both the b-type ions and ions with masses of bn-98 Da are found for the fragments that contain the phosphoserine. In the PSD mass spectra of both R1p and R4p, there are over 20% phosphopeptide molecules which had normal peptide backbone cleavage but not the phosphoryl group cleavage. Thus in the MALDI-TOF mass spectra of R1p and R4p, there exist two different patterns of fragmentations, one is b_n and another is b_n-98, which is the very important one (**Figure 3**). In the experiments, we found that the N-terminal b_n and b_n-98 cleaveage fragments have higher intensity than the others and that it would be better to be used for determining the phosphorylated site. From the **Figure 3**, we can deduce that serine 7 was a phosphorylated site for there was only cleavage of b-type ions *prior to* the serine 7 in R4p peptide. All data about the b_n cleavage fragmentations of the R4 and R4p were shown in **Figure 3**.

In the tau peptides, there is only one proline residue. This proline residue located in the conservative region, which was thought as the β-tubulin binding domain and can maintain the microtubule[6]. In the experiments the fragmentation of proline residue occurred more strongly than others. B14 fragmentations in R1 peptide and R4 peptide occurred more strongly than other bn cleavages. Both b14 and b14-98 fragmentations showed the same results in R1p peptide and R4p peptide. In tau Protein, there are three or four PGGG motifs in the domain region. Probably there exist several similar conformations at PGGG motifs, which would be easily cleaved at this location. The MALDI-TOF mass spectrometry can be used to study the conformation of human tau peptides and provide many useful conformational information of phosphorylation

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