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Synthesis and conformational properties of phosphopeptides related to the human tau protein

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Abstract

In the brains of Alzheimer's disease patients, the tau protein dissociates from the axonal microtubule and abnormally aggregates to form a paired helical filament (PHF). One of the priorities in Alzheimer research is to determine the effects of abnormal phosphorylation on the local structure. A series of peptides corresponding to isolated regions of tau protein have been successfully synthesized using Fmoc-based chemistry and their conformations were determined by ^{1}H NMR spectroscopy and circular dichroism (CD) spectroscopy. Immunodominant peptides corresponding to tau-(256–273), tau-(350–367) and two phosphorylated derivatives in which a single Ser was phosphorylated at positions 262 and 356, respectively, were the main focus of the study. A direct alteration of the local structure after phosphorylation constitutes a new strategy through which control of biological activity can be enforced. In our study on Ser^{262} in R1 peptide and Ser^{356} in R4 peptide, phosphorylation modifies both the negative charge and the local conformation nearby the phosphorylation sites. Together, these structural changes indicate that phosphorylation may act as a conformational switch in the binding domain of tau protein to alter specificity and affinity of binding to microtubule, particularly in response to the abnormal phosphorylation events associated with Alzheimer's disease.

Keywords: Alzheimer's disease; Phosphopeptide; ^{1}H NMR; CD; Tau protein

1. Introduction

Alzheimer's disease (AD) is the main form of dementia in today's ageing population [1]. The major histopathological abnormalities that characterize the brains of patients with Alzheimer's disease (AD) include excess neurofibrillary tangles (NFT) and senile plaques (SP) [2]. The tangles are made up of a protein called tau, which is modified by phosphorylation and aggregated into so-called paired helical filaments (PHF) [1]. The tau protein is associated with neuronal microtubules, and it stabilizes them and regulates the transport of vesicles or organelles along them, supports the outgrowth of axons and serves as an anchor for enzymes [3]. Functionally, tau binds to tubulin whereas PHF-tau does not [4–6]. Structural studies of tau have postulated three or four 18-amino-acid repeats that are tubulin binding sites [7]. Indeed, a tau construct that begins within the first repeat of four-repeat tau, and extends 19 amino acids past the last repeat, induced microtubule binding [8].

Ser^{262}, once considered as selectively phosphorylated in PHF-tau [9] but phosphorylation of which was later shown

Abbreviations: AD, Alzheimer's disease; PHF, paired helical filaments; CD, circular dichroism; NOE, nuclear Overhauser enhancement effect; TOCSY, total correlated spectroscopy; NOESY, nuclear Overhauser effect spectroscopy; ppm, parts per million.

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Table 1
Synthetic peptides[a] corresponding to the repeat domain of the human tau441 sequence

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 pS 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 pS L D N I T H V P G G G	350–367/ fourth repeat phosphorylated

[a] The subscripted p stands for phosphorylation.

to be developmentally regulated, is one of the likely abnormal phosphorylation sites [10]. Phosphorylation of Ser^{262} strongly inhibits the binding of tau to microtubules [5]. This site can be phosphorylated by a number of kinases [11], including GSK-3 [12]. In addition to Ser^{262}, which is located in the first microtubule-binding domain, phosphorylation of Ser^{356}, located in the fourth microtubule-binding domain, is also required for maximal inhibition of tau binding to microtubules [13]. Phosphopeptides are useful reagents for the study of protein kinases targets as well as regulatory phosphorylation sites of protein-protein interactions [14,15].

In this study we describe the synthesis of peptides and phosphopeptides relating to the human tau protein (Table 1), containing phosphoserine residue Ser^{262} and Ser^{356} which have been reported as two abnormally phosphorylated. Here, we examine the structural differences between phosphopeptides and nonphosphopeptides using circular dichroism (CD) and high-resolution NMR spectroscopy.

2. Materials and methods

2.1. Peptide synthesis

Peptides were synthesized on Fmoc-Wang resin using the standard Fmoc/tBu chemistry and O-benzotriazol-N,N,N′,N′-tetramethyluronium hexafluorophosphate/1-hydroxybenzotriazol (HBTU/HOBt) protocol [16]. For phosphopeptides, phosphoserine was incorporated as Fmoc-Ser(PO_3HBzl)-OH [17]. The peptides and all protecting groups were cleaved from the resin with TFA containing phenol (5%), thioanisole (5%), ethanedithiol (2.5%), and water (5%) for 120 min [18]. The crude peptides were purified by reverse phase HPLC using an ODS-UG-5 column (Develosil) with a linear gradient of 20% to 50% acetonitrile containing 0.06% trifluoroacetic acid as an ion-pairing reagent. The integrity of the peptides and phosphopeptides was verified by ESI and MALDI-TOF mass spectroscopy. Table 1 listed the synthetic peptides.

2.2. Mass spectrometry

Mass spectra were acquired using a Bruker ESQUIRE-LC ion trap mass spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to *m/z* 6000. Nitrogen was used as the drying gas at a flow-rate 8 l min^{-1}, the nebulizer pressure was 11 psi and the electrospray needle was typically held at 4 kV. The heated capillary temperature was 573K. Samples dissolved in methanol were ionized by ESI and infused continuously into the ESI chamber at a flow-rate of 0.6–0.7 ml h^{-1} by a Cole-Parmer 74 900 syringe pump.

Matrix-assisted laser desorption/ionization time-of-flight (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 matrix without further purification.

2.3. Circular dichroism

CD spectra were recorded with a Jasco 720 spectropolarimeter from 250 to 190 nm in a 0.1 mm path length cell, every 0.2 nm with a 2 s integration-time and a 1 nm bandwidth. The peptides and phosphopeptides concentrations were about 1.0 mg/ml. Four scans were averaged. All spectra were corrected by subtracting the baseline of the solvent buffer solution recorded under the same conditions. The results were expressed as mean residue ellipticity [θ] in units of degrees cm^2 $dmol^{-1}$.

2.4. NMR spectroscopy

Peptide samples for NMR measurements were dissolved in H_2O/D_2O 9:1 (v/v) in 10 mM phosphate or sodium d_4-acetic acid buffer. The pH value was adjusted by adding HCl or NaOH. Sodium 2,2-dimethyl-2-silapentonate-d_4 in a capillary tube was used as the external standard for ^1H NMR chemical shifts. Standard NOESY [19] and TOCSY [20] experiments were collected on a Bruker AMX-500 spectrometer operating at a frequency of 500.13 MHz and Varian Inova-600 spectrometer operating at a frequency of 599.83 MHz for ^1H nucleus. Two-dimensional NMR data were transferred to an Indigo 2 (Silicon Graphics, Inc.) computer workstation and processed using the nmrPipe/nmrDraw program [21] (Biosym/Molecular Simulations, Inc.). Usually, a sine-squared window function shifted by $\pi/4$-$\pi/2$ was applied in both dimensions, with zero filling in f1 to 2K points. Quadrature detection in f1 was achieved using TPPI [22]. H_2O resonance was suppressed either by presaturation of the solvent peak during the relaxation delay (and the mixing time in the NOESY spectra) or by using a pulsed-field gradient technique with a WATER-GATE sequence