Abstract

Identification of the Sequence Cleavage Preference for the Protease BACE2 Using Proteomics, Mass Spectrometry, and Bioinformatics

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Proteases play critical roles in vital biological processes including the regulation, breakdown, and recycling of proteins. Understanding the preferred amino acid sequence for a given protease can elucidate its substrates and therefore its function. The information from this characterization can be used to develop inhibitors of a protease to treat disease or to suggest potential undesired effects resulting from its inhibition.

β-secretase (BACE) membrane associated aspartyl protease family consists of two homologs, BACE1 and BACE2. BACE1 has been studied in depth as the primary member of this family leading to Alzheimer's disease (AD) while its homolog of BACE2 has been largely ignored because BACE2 mRNA is minimally expressed in the brain. However, recent studies suggest that both BACE1 and BACE2 are involved in the generation of beta-amyloid (Aβ) which aggregates to form the plaques that are a hallmark pathology of AD. Thus, characterization of the substrate cleavage sequence preference of BACE2 should help to identify putative substrates and its native and AD-related functions.

Proteomic identification of cleavage sites (PICS) is a combined proteomic and mass spectrometric method for characterizing the cleavage site preferences of proteases. It is less biased than previous methods requiring the synthesis of a small number of peptides designed around a specific amino acid sequence. Briefly, peptide fragments generated by the BACE2 cleavage of semi-random peptide libraries are sequenced using mass spectrometry. The remaining portion of recognition and cleavage sequence is determined using bioinformatics and database searching. With the preferred sequence(s) determined, we will then search the human proteome for novel putative substrates of BACE2. In summary, a first step towards a better understanding of BACE2 is to analyze its substrate specificity and then use that information to identify its native substrates which will enable us to elucidate its native and AD-related functions.

DLPC Liposomes Inhibit Aß Fibrillation and Remodel Preformed Fibrils Through a Detergent-like Mechanism

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The aggregation of amyloid-β (Aβ) on neuronal lipid bilayers is implicated in the neurotoxicity associated with Alzheimer's disease. It is suggested that the aggregation on lipid bilayers is accelerated. This aggregation, in turn, is capable of disrupting bilayer integrity which induces cell death. We have found, however, that lipid bilayers composed of dilauroyl phosphatidylcholine (DLPC) undergo a unique mechanism of disruption. Through a combination of fluorescence measurements, microscopy, CD spectroscopy, and solid state NMR we have found that DLPC liposomes are rapidly disrupted through interactions with monomeric and early oligomeric forms of Ab. This disruption generates free lipid which successfully impedes amyloid formation through a detergent-like mechanism and stabilizes non-toxic, off pathway oligomers. DLPC liposomes are also capable of remodeling preformed fibrils of Ab into on-pathway, toxic protofibrillar species. All previous evidence of membrane disruption by Aβ has

been the result of fibrillation, pointing to a unique interaction occurring at the surface of DLPC membranes which is capable of halting amyloid formation in the early stages.

Supermetallization of Peptides and Protiens Studied by High Resolution Mass Spectrometry

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Transition metal ions are involved in neurodegenerative diseases.

Recently we have reported the supermetallization phenomenon, which is the formation of complex ions of peptide–metal in the gas phase when the peptide accepts an unexpectedly large number of metal atoms. This supermetallization takes place during electrospray ionization when charged droplets are evaporating at relatively high temperature (ca 400°C). The effect has been demonstrated for the divalent $Zn2+$ atom and several peptides and proteins. It is observed that small protein ubiquitin can incorporate up to 20 Zn atoms and the attachment of each Zn requires the removal of two hydrogen atoms. Here we report the first observation, to our knowledge, of gas-phase complexes of peptides and proteins with tetravalentmetals.

Supermetallized complexes were studied by FTICR MS in combination with CID/ECD fragmentation. It was found that during ECD complexes with many metal atoms do not dissociate and only decrease their charge by capturing the electron. Using high resolution FTICR it was observed that with the increase of the number of metal atoms in the supermetallized complex there is a trend toward forming the $z+1$ fragments instead of the z fragments in ECD experiments. The effect of hydrogen rearrangement for c fragments was also observed but was found to be very weak.

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References:

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Key asparagine and glutamine residues promote cross-species prion conversion

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The central event underlying prion disease transmission is the conversion of the cellular prion protein, PrPC, into a misfolded, self-templating conformer called PrPSc. Sequence similarity between PrPSc and PrPC is critical for efficient prion templating, yet the specific interacting residues and the mechanism by which a residue promotes or inhibits conversion remain unclear. Previous studies have shown that single residue differences between PrPSc and PrPC may dramatically impede conversion, as illustrated by the G127V variant of human PrPC, which confers protection against transmission of human prions (1). However, the transmission of prions between species suggests that many PrPC residue differences are not protective, or may even promote prion cross-seeding. Recent studies have found that the PrPC sequence of bank voles, a European rodent, is permissive to conversion by a wide array of prions from many species (2, 3) despite residue differences between the PrPSc and vole PrPC sequences. We recently demonstrated that human PrPC having only two bank vole amino acid substitutions (E168Q