

Application of Affinity Proteomics and High Resolution Mass Spectrometry in Elucidation of New Amyloid-derived Vaccine Lead Structures against Alzheimer's Disease

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Introduction

Mass spectrometry has become a most important tool in biopolymer structure analysis, e.g. for identifying protein sequences and post-translational modifications, detecting protein biomarkers and novel peptides with affinity to specific targets, characterising combinatorial peptide libraries, and in proteomics [1,2]. The recent development of Fourier transform ion cyclotron resonance (FTICR) mass spectrometry enabled a breakthrough for the ultra-high resolution mass spectrometric analysis of biopolymers using both electrospray (ESI) and MALDI [3]. Using selective proteolytic digestion of the antigen:antibody complex (epitope excision) in combination with high resolution FTICR-M), we have identified the molecular recognition structures of target antigens related to Alzheimer's disease (AD), which opens new lead structures for effective AD vaccine development. Both high resolution and high selectivity have been found critically important in mass spectrometric epitope analysis and evaluation of vaccine peptides, in which FTICR-MS provides unprecedented identification selectivity from complex biological mixtures [4].

Results and Discussion

Immunisation of transgenic mouse models of AD with A β (1-42) has been recently effective to inhibit and disaggregate A β -fibrils, and to reduce both AD-related neuropathology and memory impairments [5]. The mechanism underlying these therapeutic effects has been as yet unclear. Using epitope excision in combination with FTICR-MS, we have identified the epitope recognised by the therapeutically active antibody as the N-terminal A β (4-10) sequence [6]. Essential mass spectrometric data for the identification of the epitope are summarised in Figure 1.

While trypsin digestion of free A β ₄₂ yielded all expected A β peptides (i.e., 1-16, 6-16, 17-28, 29-42; Fig. 1a), trypsin or Lys-C protease digestion of A β ₄₂ within the anti-A β -antibody complex yielded only a single specifically bound peptide - A β ₁₋₁₆ (Fig. 1b). Glu-C-protease and β -chymotrypsin generated only A β ₁₋₁₁ and A β ₁₋₁₀, respectively (Fig. 1b). Further exopeptidase digestion of the antibody-bound A β ₁₋₁₀ chymotryptic fragment using aminopeptidase M identified A β ₄₋₁₀ (FRHDSGY) as the peptide that still bound to the antibody with high affinity. However, further C-terminal digestion from Y10 (using carboxypeptidase A) only yielded peptides with drastically diminished affinity (Fig. 1d) indicating that the minimal core epitope recognized by the therapeutically active IgG antibodies is A β ₄₋₁₀. The essential residues of the epitope, F4, R5, H6, and S8 were found shielded from proteolytic degradation in the immune complex, and were further characterised by alanine mutation scans and ELISA of synthetic peptides. No affinity was found for rat A β (1-42) which lacks the R5 residue, and with antibody isolated from non-immunised control mice [6].

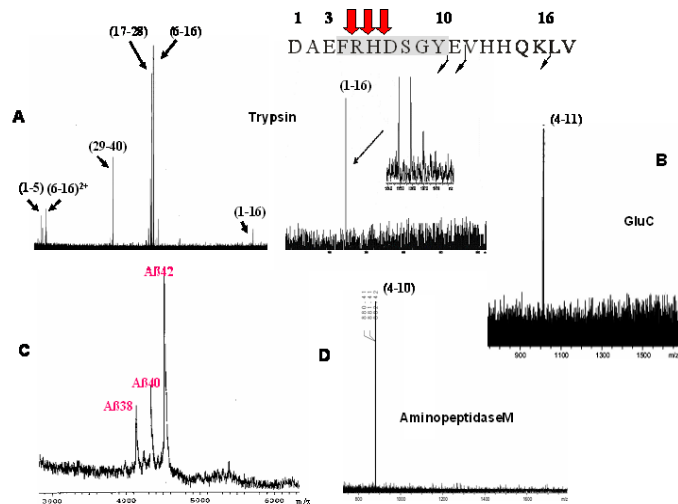


Figure 1. MALDI-TOF- and FTICR-MS identification of A β (4-10) epitope. *A*, MALDI-MS of A β 40 tryptic digest mixture; *B*, epitope excision and FTICR-MS using LysC and GluC protease; *D*, epitope-excision-MALDI-MS from soluble plaques upon chymotrypsin and ApsaseM digestion; *C*, MALDI-MS of A β peptides in mouse plaques. Thin and bold arrows denote accessible and shielded proteolytic sites, respectively.

The N-terminal A β -epitope sequence raised the question of a possible active binding conformation and aggregation state recognised by the antibody. In contrast to the C-terminal transmembrane part of A β (1-42) which may form α -helical and β -sheet conformation (A β -aggregates), molecular dynamics studies suggested an unstructured sequence for the A β (1-10) epitope peptide. A flexible structure would be compatible with a model in which the active antibody could effectively target the epitope in soluble, protofibrillar, and oligomeric A β -species which have been suggested as critical, highly neurotoxic intermediates in the formation of AD plaques [7]. According to this model of epitope accessibility, the therapeutic antibodies might also possibly target A β -precursor molecules, processed by β - or γ -secretase. A number of peptides comprising the N-terminal A β -epitope and C-terminal peptide sequences of the β APP ectodomain were synthesised, including peptides with either the wild-type, or the FAD-mutation β -secretase cleavage residues, K670M671 and N670L671. ELISA studies with these peptides revealed substantially higher affinities to the active antibody compared to A β (1-40). Of particular interest is also the significantly higher affinity (ca. 6-fold) of the FAD-mutation peptide, NL(661-687) compared to the wt-KM(661-687), suggesting a possible effect of the NL-mutation site of β -secretase cleavage on the antibody recognition and conformation of the epitope. This astounding observation and the reactivity of the antibody towards A β -precursor sequences encompassing the β -secretase cleavage residues are presently subject of detailed studies in our laboratory.

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