

REICHERT LIFE SCIENCES SPR

APPLICATION NOTE 14

Epitope Identification and Affinity Determination of A β specific Antibodies by online SPR- MS

Introduction

The accumulation of extracellular plaques containing the neurotoxic β -amyloid peptide fragment, A β (1-42) of β -amyloid precursor protein (β APP), is one of the characteristics of Alzheimer's disease (AD). Although β APP has been recognised as a key molecule for AD, its molecular (patho)physiological degradation, proteolytic pathways and cellular interactions of A β are still unclear. Studies towards the development of immunotherapeutic methods for AD have yielded initial success in transgenic mouse models of AD and in producing therapeutic antibodies by immunisation with A β (1-42) that disaggregate A β -plaques and fibrils. Using proteolytic excision of the immobilised A β antigen-immune complex in combination with ESI mass spectrometry, the A β -plaque specific epitope was identified as an N-terminal peptide A β (4-10), accessible in A β (1-42) as well as in oligomeric A β -fibrils [1, 2].

Recently, we have identified the epitope recognised by A β -autoantibodies in serum, capable of eliciting a neuroprotective effect to inhibit the formation of A β -plaques, located in the carboxy terminal region of the A β sequence. The differential epitope structures of A β -specific antibodies from healthy individuals and AD patients provides a breakthrough and molecular basis for (i), the development of new immuno-therapeutic approaches by passive immunisation with A β -specific antibodies, and (ii), the development of new diagnostic tools for AD with absolute specificity [2, 3]. The primary structures of polyclonal A β -autoantibodies were elucidated by two-fold A β -epitope specific affinity chromatography from human immunoglobulin G, using a combination of overlapping proteolytic digestion (trypsin, α -chymotrypsin), HPLC isolation, and high resolution MS, which provided sequence data for Fv domains, CDR motifs, and framework regions.

The new online SPR-MS system is capable of detecting and identifying affinity interactions in real time. The reliability of the online interface was established by demonstrating repeatable and comparable K_D determinations and precise mass spectrometric identification of protein. Fast on-line sample processing allows fast throughput of different analytes for biomolecular interaction studies. In this study, the plaque protective A β -epitope of the A β -autoantibody was identified by proteolytic extraction-MS using the online SPR-MS- epitope analyser, and found to reside in the A β (17-28) tryptic peptide sequence.

Experimental

To identify the epitope, the A β - autoantibody was immobilized on a dextran-SPR affinity chip, and a tryptic mixture of A β -peptide fragments was injected through the SPR autosampler followed by ESI-MS. Subsequent online desalting of analyte prior to MS was performed after elution of affinity captured A β -peptide which provided identification of the A β (17-28) epitope peptide (protonated molecular mass 1324,8). Following elution of undigested A β (1-40) through the microfluidic interface, SPR affinity determination revealed high affinity with a K_D of ca. 3.5 nM (Figures 2 and 3). Based on this epitope peptide, interactions of the plaque-protective A β -epitope with two fibril inhibiting peptides, cystatin-C and humanin, were evaluated at the molecular level to gain insight into the mode of action of A β -autoantibodies [4]. A schematic of the setup is shown in Figure 1:

Online SPR-Mass Spectrometry Epitope Analyzer

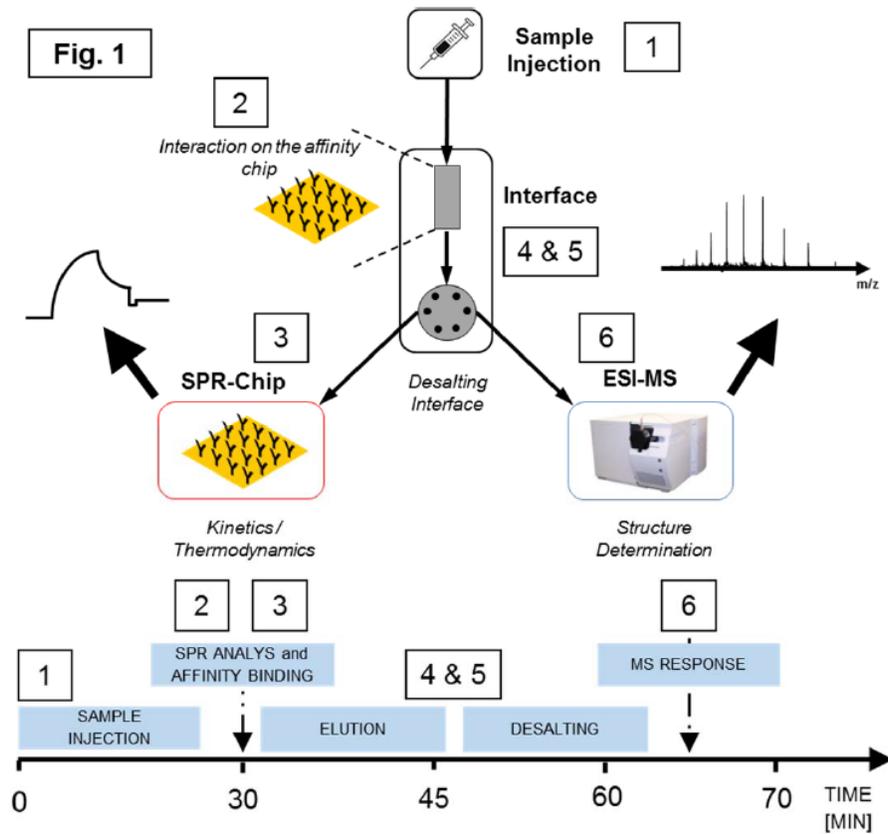


Figure 1

Schematic workflow of epitope & interaction analysis using the online SPR-MS Epitope Analyzer. After sample injection (1), the analyte is captured on the affinity chip (2) followed by the SPR-chip (3) for kinetic analysis of the affinity interaction. After sample processing through the desalting interface (4 and (5), structural analysis is performed by ESI-MS. Time scale is represented on the time axis below.

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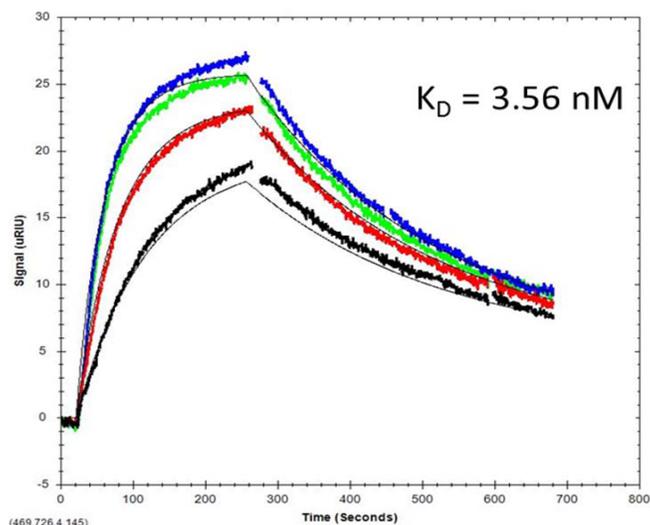


Figure 2 Affinity determination of β -amyloid antibody

SPR determination of a dilutions series of A β (1 - 40) upon processing via the SPR-MS interface. Kinetic evaluation resulted in a calculated KD of 3.56 nM.

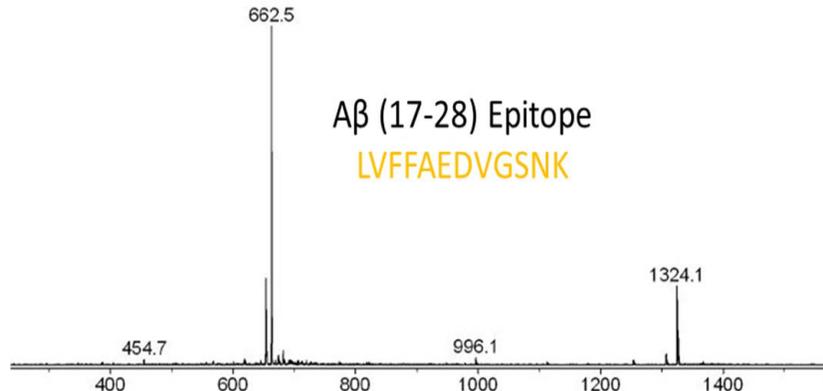


Figure 3 Epitope identification of β -amyloid antibody

ESI-MS identification by online SPR-MS of the epitope A β (17-28) eluted from the A β -antibody upon proteolytic extraction.

Conclusions

In this study we show that the online SPR- ESIMS combination is a powerful tool to enable the simultaneous affinity isolation, structure identification and affinity quantification of an A β - plaque protective epitope from the complex of A β -autoantibodies immobilized on a gold chip. The high application potential of online-SPR-MS has become further evident in recent studies of the identification of an unusual mixed-disulfide antibody epitope of the rheumatic target protein, HLA-B27; and the interaction site identification of chaperone complexes of lysosomal enzymes [5, 6]. Current applications confirm that interaction epitopes as diverse as antigen-antibody and lectin- carbohydrate complexes [7], and binding constants (KD) from milli- to nanomolar ranges are amenable to SPR-MS analysis. These results indicate that applications of the online-SPR-MS epitope analyzer are well feasible to affinity-based biomarker evaluation; identification of protein and peptide epitopes; precise antibody affinity characterization; and direct label-free antigen quantification.

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Contact Information

Corporate Office
Reichert Technologies
3362 Walden Avenue
Buffalo, New York 14043
USA
Tel: +1 716-686-4500
Fax: +1 716-686-4555
Toll Free USA: 1-888-849-8955
reichertspr.lifesciences@ametek.com

www.ReichertSPR.com

European Service Center
Carl-von-Linde Str. 42-85716
Unterschleissheim/Munich
Germany
Tel: +49 89 315 8911 0
Fax: +49 89 315 891

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