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Evidence for an extended interacting surface between β-amyloid and serum amyloid P component

István Likó [a], Marianna Mák [b], Éva Klement [c], Éva Hunyadi-Gulyás [c], Tamás Pázmány [a], Katalin F. Medzihradszky [c,d], Zoltán Urbányi [a,*]

[a] *Pharmacological & Drug Safety Research, Gedeon Richter Ltd., 1475 Budapest 10, P.O. Box 27, Hungary*
[b] *Spectroscopic Research Laboratory, Gedeon Richter Ltd., 1475 Budapest 10, P.O. Box 27, Hungary*
[c] *Proteomics Research Group, Biological Research Center, 6726 Szeged, Temesvári krt. 62, Hungary*
[d] *Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143, United States*

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Abstract

Studying the interaction between serum amyloid P component (SAP) and β-amyloid (Aβ) a new Aβ binding site was identified on the SAP near the known binding site at the two bound calcium ions. SAP stabilizes deposits in neurodegenerative diseases, which is manifested via Aβ-binding. Because the inhibition of this interaction is a potential therapeutic target in neurodegeneration, the structural basis of SAP-Aβ binding was studied. The chymotryptic digestion of SAP resulted in a 18,223 Da product identified by mass spectrometry. This cleavage was inhibited by Aβ revealing that this cleaving site between Tyr-140 and Gly-141 is involved in the interaction between SAP and Aβ· These results suggest that the Aβ-binding site on SAP is larger than it was recently assumed.

Keywords: Serum amyloid P component; β-Amyloid; Alzheimer's disease; Limited proteolysis; Structural study; Mass spectrometry

Serum amyloid P (SAP) is a member of the pentraxin serum protein family, consisting of five identical subunits non-covalently associated as pentameric discs. Human SAP is produced mainly in the liver and secreted into the blood circulation. Average plasma concentration of SAP is about 40 mg/l [160 nM]. The serum concentration and even more the synthesis rate of SAP depend on age, sex and status of health. However, total plasma concentration of SAP proved to be nearly constant in chronic diseases. Secretion of SAP in the blood is increased in different illnesses such as malignancy, rheumatoid arthritis, pregnancy and also in central nervous system (CNS) diseases [15,16,21]. These data can be interpreted as the accelerated exchange of SAP between plasma and extracellular space including amyloid deposits [10]. Casein-induced peripheral amyloid deposition is delayed and reduced in SAP-knockout mice compared to wild type control. These data suggest the importance of SAP in the mechanism of amyloid deposit formation as well [2].

Alzheimer's disease (AD) is one of the major types of senile dementia associated with memory loss, impairment of cognition and changes in behavior. Important pathological characteristics of AD include amyloid plaques and intraneuronal neurofibrillary tangles [9,14].

SAP was localized to three main histopathologic characteristics – neurofibrillary tangles (NFT), senile plaques and amyloid angiopathy – of AD. SAP deposits are also seen in other neurodegenerative diseases including Creutzfeldt–Jakob disease, Pick's disease, Parkinson's disease and Lewy body disease [11,12,19]. An unambiguous relation was also established between the plasma concentration of SAP and the degree of cognitive impairment in centenarians [15]. In fact, the concentration of SAP in the cerebrospinal fluid (CSF) is also significantly higher in AD than in healthy controls [8].

Hamazaki suggested that SAP can promote plaque formation by binding to Aβ peptides in a Ca^{2+}-dependent manner [7]. This interaction possibly occurs at the carbohydrate binding site of SAP near the two bound Ca^{2+} ions [3,17]. Moreover, since SAP is highly protease-resistant [13], it could prevent the degradation of peripheral and cerebral amyloid aggregates by phagocytic cells and proteolytic enzymes [4,22]. Furthermore,

* Corresponding author. Tel.: +36 1 431 5590; fax: +36 1 260 2643.
E-mail address: z.urbanyi@richter.hu (Z. Urbányi).

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intranasal administration of SAP in APP over-expressing and wild type control mice revealed that the interaction between SAP and Aβ also prevents the decomposition and clearance of SAP [20]. Considering these facts the assumption is well established that amyloid deposits may regress when the supply of fibril precursor proteins is sufficiently reduced. While serum amyloid P component is a universal stabilizing constituent of amyloid deposits, a competitive inhibitor of the SAP-amyloid fibril interaction has recently been described. It removes SAP from human amyloid deposits in tissues and may provide a new therapeutic approach to amyloidosis, and possibly AD [18].

Glycosaminoglycans can bind to a specific, well characterized binding site of SAP. Our previous studies suggested that they may inhibit SAP-Aβ binding in a different manner [24]. This recognition initiated further investigations aimed at the structural basis of SAP-Aβ interaction at the monomeric state of constituents. In the present study, we demonstrate a potential binding site for Aβ on SAP by limited proteolysis and by the analysis of the distribution of the tryptic and chymotryptic cleavage sites on the SAP surface.

Structural analyses were based on the crystal structure of SAP determined at 2 Å resolution and refined to an R-factor of 0.18 [4]. The program Swiss-PdbViewer Deep View version 3.6 [5] [http://www.expasy.ch/spdbv/] was used to analyze the surface of the protein and to make the graphical representation. To enhance the graphical representation the program MegaPovRay Version 1.0 [http://www.povray.org/] was used.

Eighteen micrograms human SAP (Calbiochem, La Jolla, CA, USA) was digested with 3 μg porcine trypsin (Promega, Madison, WI, USA) or chymotrypsin (Sigma, St. Louis, MO, USA) in the presence or absence of 31.5 μg freshly prepared Aβ(1–42) (Bachem, Bubendorf, Switzerland) in 100 μl PBS at 37 °C. For these experiments, a 1 mM stock solution was prepared from synthetic Aβ(1–42) in dimethylsulfoxide (DMSO), that was stored at −20 °C. It was freshly diluted in PBS just before application. Controls contained DMSO in the appropriate concentration. The extent of digestion was analyzed by reversed phase HPLC on a BioBasic-18 2.1 mm × 250 mm column (Thermo Hypesil-Keystone, Bellefonte, PA, USA) at 70 °C. Solvent A was: 0.1% trifluoroacetic acid (TFA) 5% acetonitrile in water, solvent B was: 0.1% TFA in acetonitrile. A linear gradient was developed at 0.2 ml/min, peptide elution was monitored at 210 nm. The inhibition of chymotryptic digestion was also monitored by electrophoresis on an Agilent Bioanalyzer 2100 capillary electrophoresis system using Protein 50 chip (Agilent, Palo Alto, CA, USA) according to the instructions of the manufacturer. SDS polyacrylamide gel electrophoresis was carried out in a Tris–glycine–SDS buffer system (Bio-Rad, Hercules, CA, USA) on a 12% polyacrylamide gel (Cambrex, Rockland, ME, USA).

The proteolytic products were analyzed on a Finnigan MAT 95 SQ hybrid tandem mass spectrometer. The eluent was 0.1% AcOH in methanol–water (1:1), at a flow rate of 20 μl/min. The sheath gas applied was N_2. Typical values for capillary and cone voltages were 3.0 kV and 80 V, respectively, with a source temperature of 180 °C.

The silver-stained polyacrylamide gel band was cut and destained with the destainer solutions from the Invitrogen SilverQuest Silver Staining Kit (for MS-compatible silver staining) (Invitrogen, Carlsbad, CA, USA). Disulfide bonds were reduced with dithiothreitol, then the free sulfhydryls were alkylated with iodoacetamide. Digestion with side-chain protected porcine trypsin (Promega) proceeded at 37 °C for 4 h.

The unfractionated tryptic digest was analyzed on a Bruker Reflex III MALDI-TOF mass spectrometer using 2,5-dihydroxy-benzoic acid as the matrix.

An aliquot of the digest was analyzed on an Agilent 1100 nanoLC system coupled to an XCT Plus ion trap mass spectrometer in information-dependent acquisition mode, MS acquisitions were followed by two collision-induced dissociation (CID) analyses on computer-selected multiply charged ions. HPLC conditions: C18, 75 μm × 150 mm column; flow rate 300 nl/min: gradient: 5–45% B in 20 min, then 45–65% B in 10 min; solvent A was 0.1% formic acid in water, solvent B: 0.1% formic acid in acetonitrile.

MS and MS/MS data were searched against the NCBI non-redundant protein database (2005.11.21. 3044223 sequences) using the Mascot search engine (www.matrixscience.com). Monoisotopic masses with peptide mass tolerance of ±2 Da and fragment mass tolerance of ±0.8 Da were considered for the ion trap data and 200 ppm mass tolerance for MALDI-TOF data. No species restriction was used. Only tryptic cleavages were considered, and two missed cleavages were permitted. Cys carbamidomethylation was considered as fixed modification, while acetylation of protein N-termini, pyroglutamic acid formation from N-terminal Gln-residues, and methionine oxidation were permitted as variable modifications. Search results were manually inspected.

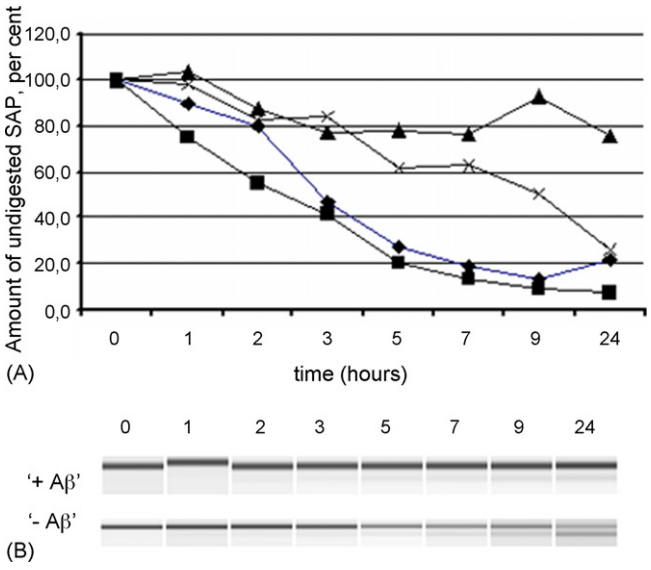

Fig. 1. The influence of Aβ on tryptic and chymotrytic cleavage of SAP was quantified by RP-HPLC. Aβ did not inhibit tryptic digestion (diamond) and inhibited chymotryptic digestion (triangle) of SAP comparing to appropriate controls in the absence of Aβ (square and x, respectively). (A). The inhibition of chymotryptic digestion was also verified by chip gel electrophoresis (B). Results were reproduced in five independent experiments.