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Preferential association of serum amyloid P component with fibrillar deposits in familial British and Danish dementias: Similarities with Alzheimer's disease

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Abstract

Two hereditary forms of cerebrovascular amyloidosis, familial British and Danish dementias (FBD and FDD), share striking similarities with Alzheimer's disease (AD) despite structural differences among their amyloid subunits (ABri in FBD, ADan in FDD, and Aβ in AD). Neuropathological lesions in these disorders include neurofibrillary tangles, parenchymal amyloid and pre-amyloid deposits and overwhelming cerebral amyloid angiopathy co-localizing with reactive microglia and multiple amyloid associated proteins including activation products of the complement cascade. Immunohistochemical analysis of FBD and FDD brain lesions unveiled the presence of serum amyloid P-component (SAP) primarily associated with thioflavin positive amyloid deposits in spite of the significant pre-amyloid burden existing in both disorders. Using affinity chromatography and ELISA binding assays we demonstrated specific, calcium-dependent, saturable, high affinity binding interactions between SAP and ABri/ADan peptides, with dissociation constant values in the sub-nanomolar range and within the same order of magnitude as those resulting from the interaction of SAP with Alzheimer's Aβ1-40 and Aβ1-42. The preferential association of SAP with fibrillar amyloid lesions and not with non-fibrillar pre-amyloid deposits is puzzling, suggesting that SAP modulates the assembly and stability of the final fibril rather than participating in the early steps of protein misfolding and oligomerization.

Keywords: Amyloid; FBD; FDD; Alzheimer's disease; SAP; Protein-protein interaction; Binding affinity constants

1. Introduction

Amyloid deposits in systemic and cerebral forms of amyloidosis co-localize with a number of unrelated proteins collectively known as amyloid-associated proteins whose function in the mechanism of amyloidogenesis still remains unclear. Among all these different molecules, serum amyloid P-component (SAP), a protein normally present in body fluids at low concentration, is probably the most extensively studied. SAP is a 204-residue secreted glycoprotein encoded by a single gene on chromosome 1 (reviewed in [1]). It is synthesized as a 223-residues single chain of which the first 19 amino acids correspond to the signal sequence required for transmembrane translocation across the endoplasmic reticulum and are not part of the mature secreted molecule. SAP features an intra-chain disulphide bond between

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residues 55 and 114, a single N-glycosylation site at position 51 and two sites for calcium binding. It belongs to the pentraxin family of proteins which is characterized by the arrangement of their globular subunits in a disc-like configuration with cyclic pentameric symmetry, as originally observed by electron microscopy [2]. The five identical protomers are tightly but non-covalently associated being SDS or strong denaturants such as guanidine or urea required for the disassembly of the pentameric structure. Isolated SAP in solution and in physiological buffers is a stable decamer composed of two pentamers interacting phase to phase [3]. Although it is considered an acute-phase reactant molecule in mice, plasma levels in humans are not markedly modified during acute inflammatory processes (reviewed in [1,4]).

At physiological pH and ionic strength, SAP shows strong calcium-dependent ligand binding properties to a diverse group of substances including glycosaminoglycans, heparan and dermatan sulfate, glycans, DNA and nuclear chromatin [1], as well as to circulating proteins such as fibronectin [5] and C4-binding protein [6], among others. SAP is associated *in vivo* with all types of amyloid deposits in which its presence has been investigated. Although originally thought that the binding to amyloid molecules involved glycosaminoglycans commonly associated with the *in vivo* formed fibrils [7,8], SAP has been demonstrated to bind avidly *in vitro* to fibrils formed exclusively from pure proteins or peptides [1]. It is now believed that the tissue accumulation of SAP in different types of amyloids originates from its particular affinity for the various amyloid molecules. Its abundance in these lesions — in some cases constituting up to about 5–10% of the total amyloid deposits — is remarkable, specially taking into consideration its low concentration in plasma (30–45 mg/l) [1]. Notably, virtually all SAP can be removed from the amyloid deposits after extraction with EDTA-containing buffers, pointing out to the calcium-dependent binding as the only mechanism for SAP association with the deposits [1].

SAP has been found associated *in vivo* with animal and human amyloid deposits irrespective of the biochemical nature of the amyloid subunit (reviewed in [1]) and co-localizing with neurofibrillary pathology in a various neurodegenerative conditions including Creutzfedt–Jacob disease, Parkinson's and diffuse Lewy body disorders, and in the parkinsonism-dementia complex of Guam [4,9–11]. SAP immunoreactivity is also commonly observed in Down's syndrome, AD, and in amyloid disorders with primarily cerebrovascular compromise such as Hereditary Cerebral Hemorrhage with Amyloidosis, Icelandic-type [12–16]. In AD brains, the widespread SAP immunoreactivity is evident in association with plaques and amyloid-laden vessels as well as with neurofibrillary tangles, but its presence in diffuse pre-amyloid deposits is controversial (reviewed in [4]).

Two recently described forms of cerebrovascular amyloidosis, Familial British and Danish dementias (FBB, and FDD, respectively), result from genetic alterations in the *BRI2* gene [17,18] also known as *Itm2b* [19]. In these disorders the deposited amyloid proteins, ABri in FBD and ADan in FDD, are C-terminal proteolytic fragments of a genetically altered BRI2 precursor molecule. The amyloid peptides originate as a result of two different genetic defects at, or immediately before, the BRI2 stop codon with a common final outcome in both diseases: regardless of the nucleotide changes, the ordinarily occurring stop codon is either non-existent (in FBD) or out of frame (in FDD) causing the genesis of an extended precursor featuring a C-terminal piece that does not exist in normal conditions (reviewed in [20]). ABri and ADan, released by a furin-like proteolytic processing, are both 34-residues-long, share 100% homology on the first 22 residues, a completely different 12 amino acid C-terminus and have no sequence identity to any other known amyloid protein. Despite the structural differences among the amyloid subunits these disorders show striking clinical and neuropathological similarities with AD, including the presence of neurofibrillary tangles, parenchymal amyloid and pre-amyloid deposits and cerebral amyloid angiopathy (CAA) co-localizing with inflammatory markers, reactive microglia and activation products of the complement system (reviewed in [20,21]).

The present studies identify the occurrence of SAP predominantly in association with fibrillar deposits in both FBD and FDD and provides a comparative biochemical characterization of the interaction of SAP with ABri/ADan and Aβ species. The predominant association of SAP with fibrillar deposits suggests that SAP participates in the modulation of the final fibril stability rather than influencing the early steps of protein oligomerization and fibrillization.

2. Materials and methods

2.1. Materials

2.1.1. Peptides and proteins

Synthetic peptides DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (Aβ1-42), DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV (Aβ1-40), pEASNCFAIRHFENKFAVETLICSRTVKKNIIEEN (ABri1-34), and pEASNCFAIRHFENKFAVETLICFNLFLNSQEKHY (ADan1-34) were synthesized at the W. M. Keck Facility at Yale University using *N-tert*-butyloxycarbonyl chemistry. The ABri and ADan peptides featured N-terminal pyroglutamate, as found in the FBD and FDD amyloid deposits [22,23]. All peptides were purified by reverse-phase high performance liquid chromatography (HPLC) using a Vydac C18 column (Hesperia, CA) and a (0–80%) linear acetonitrile gradient in 0.05% trifluoroacetic acid, their molecular mass corroborated by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, and their concentrations assessed by amino acid analysis. Aβ-unrelated peptide LGNNIHQWCGSNSNRYEREC (SP19), homologous to amino acids 196–215 of human plasma gelsolin [24] was used as negative