Inhibition of Alzheimer β-Peptide Fibril Formation by Serum Amyloid P Component*

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A 39–43-amino acid residue-long fragment (β-peptide) from the amyloid precursor protein is the predominant component of amyloid deposits in the brain of individuals with Alzheimer’s disease. Serum amyloid P component (SAP) is present in all types of amyloid, including that of Alzheimer’s disease. We have used the in vitro model to study the effects of purified SAP on the fibril formation of synthetic Alzheimer β-peptide 1–42. SAP was found to inhibit fibril formation and to increase the solubility of the peptide in a dose-dependent manner. At a 5:1 molar ratio of Ap1–42 peptide to SAP, fibril formation was completely inhibited, and approximately 80% of the peptide remained in solution even after 4 days of incubation. At lower SAP concentrations, e.g. at peptide to SAP ratio of 1000:1, short fibrillar like structures, lacking amyloid characteristics, were formed. These structures frequently contained associated SAP molecules, suggesting that SAP binds to the polymerizing peptide in a reaction which prevented further fibril formation.

EXPERIMENTAL PROCEDURES

Materials—Alzheimer’s peptide (98% pure) and α1-antichymotrypsin C-terminal peptide (amino acids 358–394; >90% pure) were purchased from Saveen (Copenhagen, Denmark). DEAE-Sepharose and heparin-Sepharose were from Pharmacia Biotech (Uppsala, Sweden). Purification of SAP—SAP was prepared from human plasma by barium-citrate absorption followed by chromatography on DEAE-Sephacel and heparin-Sepharose, as described previously (26). The purified protein gave a single band of 25 kDa in SDS-polyacrylamide gel electrophoresis. Three different batches of SAP were tested, and they gave similar results. The concentration of SAP was determined by absorbance at 280 nm using an extinction coefficient of 18.2 (27).

Turbidity Assay—The Ap1–42 (20 μl of a 40 μM solution) was mixed with different amounts of SAP in 15 mM Tris·HCl, 150 mM NaCl, pH 7.4 (TBS; final volume 500 μl) and incubated at 37 °C. The final concentration of Ap1–42 was kept constant at 1.6 μM, SAP concentrations being 1.6 μM (1000:1), 16 μM (100:1), 32 μM (50:1), and 320 μM (5:1). Ap1–42:SAP ratios are given in parentheses. At different times, the light scattering at 400 nm was measured and compared with controls not containing the peptide. Presented results represent the mean of two experiments.

Electron Microscopy—Samples were applied to carbon-coated copper grids, negatively stained with 2% (w/v) uranyl acetate and examined in a JEOL 200CX electron microscope. The results shown are representative of samples taken from each test solution.

Radioassay—The Ap1–42 peptide was labeled with 125I using the lactoperoxidase method according to Thorell and Larson (28) and stored in TBS containing 0.02% NaN3. The radiolabeled peptide was mixed...
Inhibition of Aβ-Peptide Fibril Formation by SAP

RESULTS AND DISCUSSION

To examine the effect of SAP on Aβ1–42 fibril formation, an in vitro model was used in which the Alzheimer Aβ1–42 peptide spontaneously adopts β-sheet conformation and forms elongated, approximately 7–8 nm thick, fibrils. The fibril formation of the Aβ1–42 peptide was monitored by light-scattering and electron microscopy (Fig. 1). In the absence of SAP, the light scatter increased to a maximum after 72 h (Fig. 1A), and elongated fibrils, having amyloid characteristics (29), were observed in the electron micrographs (Fig. 1B). The addition of SAP resulted in a dose-dependent inhibition of fibril formation. At a peptide to SAP ratio of 5:1, there was almost no increase in light scattering (Fig. 1A), and the fibril formation was completely inhibited (Fig. 1B). A distinct inhibitory effect was observed also at the highest molar ratios of peptide over SAP. Even at a peptide to SAP ratio of 1000:1, the solubility of the peptide was increased as compared to the control, and short, flexible fibrils were formed as revealed by electron microscopy (Fig. 1D) and as reflected by a slight but significant increase in light scatter (Fig. 1A). These fibrils were 1–2 nm thicker than those formed by Aβ1–42 alone. Moreover, they did not exhibit green birefringence after Congo red staining, which is characteristic of amyloid fibrils.

Although the precise mechanism of the molecular interaction between SAP and Aβ1–42 has not been elucidated, the changes in fibril morphology suggest that SAP affects the packing mechanism of Aβ1–42 and disturbs the typical uniformity of the fibrils. SAP is unique in its ability to inhibit fibril formation at all concentrations reported in this study. The amyloid-associated proteins apoE and ACT have been reported either to inhibit or to stimulate the β-peptide amyloid fibril formation. At a low ratio between the Aβ1–42 peptide and apoE (1000:1), a significant delay in the onset of amyloid fibril formation was observed, whereas under other conditions, apoE stimulated fibril formation (20, 22, 30). Similar results have been reported for ACT (19, 21).

To study the effect of SAP on the overall solubility of Aβ1–42 peptide, the amount of radiolabeled peptide remaining in solution after centrifugation and filtration was measured at various times of incubation (Fig. 2). After a 96-h incubation, the solubility of Aβ1–42 peptide reached its minimum with 20% of radioactivity remaining in solution. Addition of increasing amounts of SAP resulted in increased solubility demonstrating that SAP may be able to prevent Aβ1–42 aggregation. To ensure that the experimental conditions (10-min centrifugation at 13,000 × g and filtration through 0.2-μm filters) were sufficient to remove small aggregates of Aβ1–42 peptide, a second experiment was performed in which the peptide:SAP mixtures after a 72-h incubation were centrifuged at 120,000 × g for 1 h. The results confirmed the ability of SAP, at an Aβ1–42:SAP ratio of 5:1, to keep essentially all the peptide in solution (89%) for up to 72 h. At Aβ1–42:SAP ratios of 100:1 and 1000:1, the amounts of peptide remaining in solution were 81 and 60%, respectively. In this experiment, 54% of the peptide remained.
soluble in the absence of SAP.

Direct binding between SAP and the Aβ1-42 peptide was demonstrated by non-denaturing agarose gel electrophoresis, but, in contrast to the complexes between ACT and the Aβ1-42 peptide (21), complexes between SAP and the β-peptide were not stable in SDS (results not shown). Recently, it has been shown that SAP is able to bind to Aβ1-40 immobilized in microtiter plates (24). Together, these data suggest that SAP is able to bind Aβ1-42 under non-denaturing conditions.

The inhibitory effect of SAP on Alzheimer Aβ1-42 peptide fibril formation appears to reflect a general ability of SAP to inhibit amyloid fibril formation. In support for this concept, SAP was found to inhibit the formation of fibrils from a β-sheet containing peptide derived from α1-antitrypsin (31). Complete inhibition of fibril formation was observed at a peptide to SAP ratio of 5:1. Even at a peptide to SAP ratio of 1000:1, a clear attenuation of fibril formation was observed (Fig. 3). It was noteworthy that at this latter experimental condition, the morphology of the aggregated peptide was different from that observed in the experiment using Aβ1-42 (compare Figs. 1D and 3C). Instead of short flexible fibers, dense aggregates of fibers were observed.

Our present data suggest SAP both to impede the seeding process, which initiates fibril formation (32), and to inhibit fibril growth by binding to the peptide and thus preventing peptide-peptide polymerization. Both processes presumably involve binding of SAP to β-sheet structures formed by the polymerizing peptide. If the physiological function of SAP is to inhibit amyloid fibril formation, this process is probably imbalanced in AD, either due to changes in the metabolism of the β-peptide or to the presence of other SAP ligands in the plaques, such as glycosaminoglycans which would inhibit SAP function. Moreover, in such pathological situations, the attachment of SAP to amyloid fibrils may lead to increased resistance to proteolysis (1, 25). SAP has been extremely well conserved through evolution (33) and no deficiency of SAP has been described, suggesting SAP to have important functions. The ability of SAP to inhibit pathological deposition of amyloid-forming peptides may be such a function.

FIG. 3. Inhibition of fibril formation from α1-antitrypsin C-terminal peptide by SAP. Synthetic α1-antitrypsin C-terminal peptide was incubated at 37°C (final concentration 12 μM) in the presence of increasing concentrations of SAP as described for Aβ1-42 under “Experimental Procedures.” In the absence of SAP, the peptide polymerized into extended fibrils as observed by electron microscopy (A). No fibrils were observed at a peptide to SAP ratio of 5:1 (B). At a peptide to SAP ratio of 1000:1, a few number of dense clusters of fibrils were observed (C). It was noteworthy that SAP molecules often appeared associated with the fibrils, as observed in Fig. 1 (arrow). In the upper and lower panels, bars represent 200 and 100 nm, respectively.

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