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Acceleration of amyloid fibril formation by carboxyl-terminal truncation of human serum amyloid A

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ABSTRACT

Human serum amyloid A (SAA) is a precursor protein of AA amyloidosis. Although the full-length SAA is 104 amino acids long, the C-terminal-truncated SAA lacking mainly residues 77–104 is predominantly deposited in AA amyloidosis. Nevertheless, the amyloid fibril formation of such truncated forms of human SAA has never been investigated. In the present study, we examined the effect of C-terminal truncation on amyloid fibril formation of human SAA induced by heparan sulfate (HS). Circular dichroism (CD) measurements demonstrated that the C-terminal truncation induces a reduced α-helical structure of the SAA molecule. HS-induced increases in thioflavin T fluorescence for SAA (1–76) peptide and less significant increases for full-length SAA were observed. CD spectral changes of SAA (1–76) peptide but not full-length SAA were observed when incubated with HS, although the spectrum was not typical for a β-structure. Fourier transform infrared experiments clearly revealed that SAA (1–76) peptide forms a β-sheet structure. Transmission electron microscopy revealed that short fibrillar aggregates of SAA (1–76) peptides, which became longer with increasing peptide concentrations, were observed under conditions in which full-length SAA scarcely formed fibrillar aggregates. These results suggested that the C-terminal truncation of human SAA accelerates amyloid fibril formation.

1. Introduction

Amyloid A (AA) amyloidosis, one of the most common forms of life-threatening systemic amyloidoses, is a complication of chronic inflammatory diseases such as rheumatoid arthritis [1]. Human serum amyloid A (SAA), an acute-phase protein whose concentration is markedly increased during inflammation, is a precursor protein detected in the amyloid deposits of patients with AA amyloidosis. The deposition of amyloid fibrils derived from SAA damages tissue structure and function, especially in the kidney [2].

In humans, there are four SAA genes (*SAA1–4*), of which *SAA1* and *SAA2* encode acute-phase proteins, *SAA3* is a pseudogene that is not transcribed, and *SAA4* encodes a constitutively expressed protein [3]. SAA1 is the main protein component found in AA amyloidosis. It is a 104-amino-acid protein in which the C-terminal region (mainly residues 77–104) is cleaved when deposited as amyloid fibrils. Although the underlying mechanism for this enzymatic cleavage remains to be elucidated, the truncation of the C-terminal region may influence the amyloidogenic properties of the SAA molecule. However, since the C-terminal-truncated human SAA (namely, amyloid A protein) has not been produced by recombinant expression systems, we have utilized its shorter fragment peptide and full-length protein for the evaluation of amyloidogenic properties [4–6]. In the present study, we planned to obtain chemically the peptide corresponding to residues 1–76 of the SAA molecule.

The peptide ligation method, which is achieved by the coupling of two or more peptide segments, was originally developed for the chemical synthesis of longer polypeptides possessing more than 50 amino acid residues [7]. In addition, synthetic yields of SAA (1–76) peptide were predicted to be markedly lower as the peptide elongates by conventional solid-phase peptide synthesis since the N-terminal region of the SAA molecule has a high propensity to aggregate. Thus, we decided to synthesize SAA (1–76) peptide by the peptide ligation method.

Heparan sulfate (HS), a glycosaminoglycan (GAG) present in the extracellular matrix, is frequently detected in amyloid deposits, suggestive of its facilitating role in fibril formation [8]. In fact, GAG

Abbreviations: ATR-FTIR, Attenuated total reflection-fourier transform infrared; CD, Circular dichroism; HS, Heparan sulfate; SAA, Serum amyloid A; TEM, Transmission electron microscopy; ThT, Thioflavin T

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promoted the rapid fibrillization of a peptide even with low intrinsic amyloidogenicity *in vitro* [9]. The precise role of GAG in the promotion of fibril formation is unclear, but it probably contributes to the initiation of amyloid formation as a scaffold [10], which is supported by the *in vivo* observation that over-expression of heparanase prevented amyloid deposition in mice [11]. The C-terminal region of the SAA molecule is considered to possess an HS-binding site [12,13]. Thus, the fibril formation of SAA induced by HS is supposed to be affected when the C-terminal region is truncated.

SAA1 has three major isoforms, SAA1.1, SAA1.3, and SAA1.5, which vary in their central region at positions 52 and 57 [14]. Among the three SAA1 isoforms, SAA1.3 is associated with the highest risk of AA amyloidosis in the Japanese population [15]. In contrast, SAA1.1 is associated with the highest risk of AA amyloidosis in Caucasians [16]. Although the reasons for these discrepancies are still unknown, they are probably due to the overwhelming majority of Caucasians having the SAA1.1 isoform, but the frequencies of the three isoforms being approximately equal in Japanese. In the present study, using SAA (1–76) peptide obtained by the ligation method, we examined the effect of C-terminal truncation of human SAA1.3 on the amyloidogenic properties induced by HS.

2. Material and methods

2.1. Materials

Heparan sulfate (HS) and thioflavin T (ThT) were purchased from Iduron Ltd. (Manchester, UK) and Sigma-Aldrich (St. Louis, MO), respectively. Fmoc amino acid derivatives were obtained from the Peptide Institute, Inc. (Minoh, Japan). The recombinant human SAA1.3, hereafter designated as SAA1.3 m because of a single methionine residue at the N-terminus, was produced in *Escherichia coli* as previously described [17]. Unless otherwise noted, 20 mM Tris buffer (pH 7.4) or 10 mM acetate buffer (pH 4.0) was used.

2.2. Peptide synthesis

Peptide synthesis by the ligation method was performed essentially as described previously [18]. Two peptide segments, SAA1.3 (1–44)-Cys-Pro-OCH_2COTle-NH_2 and Cys-SAA1.3 (46–76), were prepared by standard Fmoc chemistry using an automated peptide synthesizer, ACT440Ω (AAPPTec, Louisville, KY) or Liberty Blue (CEM Corporation, Matthews, NC). After cleavage of peptides from the resin, they were purified by reversed-phase high-performance liquid chromatography (YMC-Pack ProC18 or Cosmosil 5C18-AR-II, 10 × 250 mm). Ligation reactions were performed under the conditions of 50 mM 4-mercaptophenylacetic acid, 20 mM Tris (2-carboxyethyl)phosphine, and 6.0 M guanidine hydrochloride in sodium phosphate buffer (pH 8.0) at 37 °C overnight. Desulfurization reactions were performed under the conditions of 10 mM 2,2′-azobis [2-(2-imidazolin-2-yl)propane] dihydrochloride, 0.10 M sodium 2-mercaptoethanesulfonate, 0.15 M Tris (2-carboxyethyl)phosphine, and 6.0 M guanidine hydrochloride in sodium phosphate buffer (pH 7.0) at 37 °C overnight. After the final purification, peptide structures were confirmed by electrospray ionization mass spectrometry (Thermo Finnigan LCQ Deca XP spectrometer) or matrix-assisted laser desorption ionization-time of flight mass spectrometry (Bruker AutoFLEX spectrometer) and amino acid analysis (Hitachi L-2000 amino acid analyzer).

2.3. Sample preparation

Lyophilized protein or peptides were dissolved in 4 M urea and freshly dialyzed into buffer extensively, and were centrifuged to remove insoluble or aggregated matter before use. Samples were kept at 4 °C throughout the preparation procedure. Protein or peptide concentrations were determined by measuring the absorption at 280 nm (A280). For fibril formation experiments, HS was added to protein or peptide samples, followed by incubation at 37 °C without agitation.

2.4. Fluorescence spectroscopy

All fluorescence measurements were performed on a Hitachi F-7000 spectrophotometer (Tokyo, Japan). ThT fluorescence spectra were recorded in a 4 × 4 mm cuvette from 450 to 600 nm at an excitation wavelength of 440 nm. The ThT and protein or peptide concentrations were 10 μM and 50 μg/mL, respectively.

2.5. Circular dichroism (CD) spectroscopy

Far-ultraviolet CD measurements were performed with a Jasco J-820 spectropolarimeter (Hachioji, Japan). The results were corrected by subtracting the baseline of an appropriate blank sample. The mean residual ellipticity ([θ]) was calculated using the equation [θ] = (MRW) $\theta/10lc$, where θ is the measured ellipticity in degrees, l is the cuvette path length (0.2 cm), c is the peptide or protein concentration in g/mL, and the mean residue weight (MRW) is obtained from the molecular mass and the number of amino acids.

2.6. Fourier transform infrared (FTIR) spectroscopy

Attenuated total reflection (ATR)-FTIR spectra were recorded at ambient temperature (~25 °C) on a Jasco FT/IR-4200 Fourier transform infrared spectrometer (Jasco Co., Tokyo, Japan) purged with N_2 and equipped with a Mercury-Cadmium-Telluride detector. Samples immediately after the CD measurements were dried on the surface of an ATR plate for the FTIR measurements. For each sample, 256 interferograms were averaged at a spectral resolution of 4 cm^{-1} and processed using one-point zero-filling and Hamming apodization.

2.7. Transmission electron microscopy (TEM)

Samples containing SAA peptides were spread on Formvar film-coated copper grids (400 mesh) and were negatively stained with 2% sodium phosphotungstate (pH 7.0). The grids were observed by a JEM-1400Plus transmission electron microscope (JEOL, Akishima, Japan) with an acceleration voltage of 80 kV. Digital images were acquired with a CCD camera.

3. Results

3.1. Peptide design and analytical validation

The amino acid sequence of the SAA1.3 (1–76) peptide is shown in Fig. 1. Owing to the length and the propensity for aggregation, especially in the N-terminal side of the sequence, we decided to employ the ligation method. Although a Cys residue will always be inserted by native chemical ligation, it can be converted into an Ala residue by a desulfurization reaction [19]. Since there are no Cys residues in the SAA sequence, ligation at the Ala residue at position 45 was chosen to consider reducing the deleterious side reactions. Details of the reaction

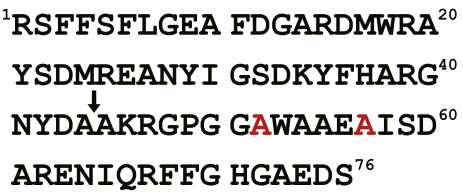

Fig. 1. Amino acid sequence of SAA1.3 (1–76) peptide. Ligation site is indicated by an arrow. Amino acid positions which differ in other two human SAA isoforms are shown in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)