Low Concentrations of Sodium Dodecyl Sulfate Induce the Extension of β_2-Microglobulin-Related Amyloid Fibrils at a Neutral pH[†]

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ABSTRACT: In β_2-microglobulin-related (Aβ2M) amyloidosis, partial unfolding of β_2-microglobulin (β2-m) is believed to be prerequisite to its assembly into Aβ2M amyloid fibrils in vivo. Although low pH or 2,2,2-trifluoroethanol at a low concentration has been reported to induce partial unfolding of β2-m and subsequent amyloid fibril formation in vitro, factors that induce them under near physiological conditions have not been determined. Using fluorescence spectroscopy with thioflavin T, circular dichroism spectroscopy, and electron microscopy, we here show that at low concentrations, sodium dodecyl sulfate (SDS) converts natively folded β2-m monomers into partially folded, α-helix-containing conformers. Surprisingly, this results in the extension of Aβ2M amyloid fibrils at neutral pH, which could be explained basically by a first-order kinetic model. At low concentrations, SDS also stabilized the fibrils at neutral pH. These SDS effects were concentration-dependent and maximal at approximately 0.5 mM, around the critical micelle concentration of SDS (0.67 mM). As the concentration of SDS was increased above 1 mM, the α-helix content of β2-m rose to approximately 10%, while the β-sheet content decreased to approximately 20%, a change paralleled by a complete cessation of fibril extension and the destabilization of the fibrils. Detergents of other classes had no significant effect on the extension of fibrils. These findings are consistent with the hypothesis that in vivo, specific factors (e.g., phospholipids) that affect the conformation and stability of β2-m and amyloid fibrils will have significant effects on the kinetics of Aβ2M fibril formation.

In β_2-microglobulin-related (Aβ2M)[1] amyloidosis, a frequent and serious complication in patients on long-term dialysis (*1*), carpal tunnel syndrome and destructive arthropathy with cystic bone lesions ensue on the deposition of Aβ2M amyloid fibrils in the tissue (*2*, *3*). Intact β_2-microglobulin (β2-m) is a major structural component of amyloid fibrils deposited in the synovial membrane of the carpal tunnel (*4*−*7*), but details of the mechanism of the deposition of these amyloid fibrils still remain unknown. Although the retention of β2-m in the plasma appears to be prerequisite (*8*), other factors, such as the age of the patient, the duration of dialysis, and the type of dialysis membrane used, may also be involved (*9*−*11*).

A nucleation-dependent polymerization model has been proposed to explain the general mechanism of amyloid fibril formation in vitro in various types of amyloidosis (*12*−*17*). This model consists of two phases, that is, a nucleation phase and an extension phase. Nucleus formation requires a series of association steps of monomers, which represent the rate-limiting step in amyloid fibril formation. Once the nucleus (*n*-mer) has been formed, further addition of monomers becomes thermodynamically favorable, resulting in the rapid extension of amyloid fibrils according to a first-order kinetic model (*12*, *14*, *16*).

In the mechanism of amyloidogenesis of natively folded proteins such as β2-m and transthyretin, partial unfolding of them is believed to be prerequisite to its assembly into amyloid fibrils both in vitro and in vivo (*17*−*20*). The extension of Aβ2M amyloid fibrils, as well as the formation of the fibrils from β2-m, is greatly dependent on the pH of the reaction mixture, the optimum pH being around 2.0−3.0 (*16*, *17*). On the other hand, they readily depolymerize

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[1] Abbreviations: Aβ, amyloid β-protein; Aβ2M, β_2-microglobulin-related; ANS, 1-anilinonaphthalene-8-sulfonic acid; β2-m, β_2-microglobulin; CD, circular dichroism; CMC, critical micelle concentration; DTAC, dodecyl trimethylammonium chloride; GAG, glycosaminoglycan; GM1, GM1 ganglioside; PG, proteoglycan; r-β2-m, recombinant β2-m; SB12, lauryl sulfobetain; SDS, sodium dodecyl sulfate; TFE, 2,2,2-trifluoroethanol; ThT, thioflavin T; Tx100, Triton X-100.

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into monomeric β2-m at neutral pH (*18*). At pH 2.5, where the extension of Aβ2M amyloid fibrils is optimum, β2-m loses much of the secondary and tertiary structures observed at pH 7.5 (*17*, *18*). Once incorporated into Aβ2M amyloid fibrils at pH 2.5, β2-m becomes highly rich in a β-sheet structure and shows secondary and tertiary structures strikingly different from monomeric β2-m at both pH 7.5 and 2.5 (*21*). Recently, we reported that 2,2,2-trifluoroethanol (TFE) at a low concentration causes the extension of Aβ2M amyloid fibrils at neutral pH, inducing a subtle change in the tertiary structure of β2-m, as well as stabilizing the fibrils (*19*). However, factors that induce partial unfolding of β2-m and subsequent amyloid fibril formation under the near physiological conditions in vitro remain to be determined. Thus, to understand the molecular pathogenesis of Aβ2M amyloidosis, we need to find and characterize the biological molecules that could induce amyloid fibril formation by affecting the conformation and stability of β2-m and amyloid fibrils.

Aβ2M amyloid deposits in patients contain many kinds of amyloid-associated molecules, for example, glycosaminoglycans (GAGs), proteoglycans (PGs) (*22*, *23*), apolipoprotein E (*24*), serum amyloid P component (*25*), and plasma proteinase inhibitors (*26*). The earliest deposition of Aβ2M amyloid fibrils is observed in the cartilage tissue highly rich in GAGs and PGs (*27*, *28*). Previously, we reported that GAGs and PGs accelerate Aβ2M amyloid fibril formation at an acidic pH (*29*) and that apolipoprotein E, GAGs, and PGs stabilize the fibrils and inhibit their depolymerization at neutral pH (*18*, *29*). Furthermore, some GAGs, especially heparin, enhance the extension of Aβ2M amyloid fibrils at neutral pH in the presence of low concentrations of TFE, suggesting that they bind to the fibrils and enhance their deposition in vivo (*19*).

Recently, many groups have reported that lipid molecules may be involved in the conformational change of various amyloid precursor proteins, as well as in the amyloid fibril formation in vitro (*30*−*32*). Sodium dodecyl sulfate (SDS) is an anionic detergent that mimics some characteristics of biological membranes and is considered to be a good model for anionic phospholipids. SDS binds to various kinds of proteins (*33*) and changes both their secondary and tertiary structures (*34*−*36*). Furthermore, SDS induces some proteins or peptides to form aggregates or amyloid-like fibrils in vitro (*37*, *38*). These results suggest that the interaction of certain lipid molecules with β2-m may induce partial unfolding of β2-m and subsequent amyloid fibril formation under the near physiological conditions.

In this study, we investigated the effects of SDS and other detergents on the conformation of β2-m, the stability of Aβ2M amyloid fibrils, and the extension of amyloid fibrils at neutral pH, using fluorescence spectroscopy with thioflavin T (ThT), circular dichroism (CD) spectroscopy, and electron microscopy.

EXPERIMENTAL PROCEDURES

Materials. SDS and Triton X-100 (Tx100) were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Dodecyl trimethylammonium chloride (DTAC) was obtained from Avocado Research Chemicals (Lancashire, England). Lauryl sulfobetain (SB12) was obtained from Sigma Chemical Co. (St. Louis, MO). 1-Anilinonaphthalene-8-sulfonic acid (ANS) was obtained from ICN Biomedicals Inc. (Aurora, OH).

Preparation of Aβ2M Amyloid Fibrils and Seeds. Unmodified Aβ2M amyloid fibrils composed solely of recombinant β2-m (r-β2-m) were formed by the repeated extension reaction at pH 2.5 with r-β2-m expressed in methyltrophic yeast *Pichia pastoris* (*39*) and the patient-derived S0 seeds as described previously (*40*). By repeating the algorithmic protocol six times, F6 fibrils were obtained from S5 seeds. S6 seeds were prepared by the extensive sonication of F6 fibrils.

Extension Assay of Aβ2M Amyloid Fibrils. The reaction mixture was prepared on ice and contained 0−60 μg/mL S6 seeds, 0−25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and various concentrations of detergents: that is, 0−10 mM SDS or 0.1−5.0 mM DTAC, SB12, or Tx100. After being mixed by brief vortexing of the mixture, 30 μL aliquots were put into oil-free PCR tubes (0.5 mL in size, Takara Shuzo Co. Ltd., Otsu, Japan) on ice. The reaction tubes were then transferred into a DNA thermal cycler (PJ480, Perkin-Elmer Cetus, Emeryville, CA) and incubated at 37 °C without agitation. After incubation for 0 to 120 h, the reaction was stopped by placing the tubes on ice. From each reaction tube, 5 μL aliquots were removed in triplicate and subjected to fluorescence spectroscopy with ThT (*16*). The mean value of each triplicate was determined.

Measurement of Critical Micelle Concentration of Detergents Using Fluorescence Spectroscopic Analysis with ANS. The critical micelle concentrations (CMCs) of SDS, DTAC, SB12, and Tx100 were determined using the fluorescence probe ANS (*41*). Optimum fluorescence measurements of ANS bound to the micelle were obtained at the excitation and emission wavelengths of 385 and 475 nm, respectively, on a Hitachi F-4500 fluorescence spectrophotometer. The fluorescence of the reaction mixture containing 0−1.6 mM detergents, 50 mM phosphate buffer (pH 7.5), and 100 mM NaCl was first measured at 37 °C as a blank. After addition of 10 μM ANS, the fluorescence of the mixture was measured at 37 °C, and the average and standard deviations of three independent experiments were determined. Two straight lines defining the fluorescence in an essentially aqueous environment and in the micellar environment were traced, and the point of intersection of these lines was used to define the CMC.

CD Spectra of r-β2-m in the Presence of SDS. Far-UV CD spectra (198−250 nm) of r-β2-m were recorded on a Jasco 725 spectropolarimeter (Jasco) at 25 °C as described previously (*18*). The reaction mixture contained 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0−10 mM SDS. The results are expressed in terms of mean residue ellipticity. Secondary structure content was calculated using CDPro software package (*42*) available at http://lamar.colostate.edu/~sreeram/CDPro/main.html. The spectral data ranged from 198 to 240 nm at 1-nm intervals were used for the calculation with three programs, that is, CONTINLL, SELCON3, and CDSSTR, included in the CDPro package. Two different reference data sets supplied with the package were used for the analysis. Each of the three programs was run using these reference sets. As a consequence, six independent estimates were obtained for each experimental spectrum. For the measurement of near-UV region (250−350 nm), the CD signals were recorded in a 5 mm path length

quartz cell. Sixteen consecutive readings at a bandwidth of 1 nm, a response of 1 s, and a resolution of 0.2 nm were taken from each sample and averaged, baseline-subtracted, and noise-reduced.

Depolymerization Assay of Aβ2M Amyloid Fibrils in the Presence of SDS. Fresh F6 fibrils extended at pH 2.5 were centrifuged at 18 500 $\times$ *g* for 2 h at 4 °C. The precipitate was washed and resuspended in ice-cold 100 mM NaCl. The reaction mixture was prepared on ice and contained 300 μg/mL F6 fibrils, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0, 0.5, 1.0, or 10 mM SDS. After being mixed by pipetting, 30 μL aliquots were put into PCR tubes. The reaction tubes were then transferred into a DNA thermal cycler and incubated at 37 °C without agitation. After a 0−24-h incubation, the reaction was stopped by placing the tubes on ice. From each reaction tube, three 5 μL aliquots were subjected to fluorescence spectroscopy and the mean of each triplicate was determined.

Detection of the Aggregates of r-β2-m in the Presence of SDS. The reaction mixture containing 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0.5 mM SDS was incubated at 37 °C for 0, 24, and 48 h, then analyzed by size exclusion chromatography. A Superose 12 HR10/30 column (Amersham Biosciences Corp.) was attached to an Äkta Purifier HPLC system (Amersham Biosciences Corp.). One-hundred microliters of the solution was loaded on the column equilibrated at 20 °C with 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0 or 0.5 mM SDS. The column was calibrated in the absence of SDS with molecular weight markers for gel filtration chromatography (Sigma Chemical Co.).

Other Analytical Procedures. Electron microscopy and Congo red staining of Aβ2M amyloid fibrils were performed as described previously (*16*). Protein concentrations of r-β2-m and Aβ2M amyloid fibrils were determined by the method using bicinchoninic acid (*43*) and a commercial protein assay kit (code 23235, Pierce). Statistical analysis was done by one-way analysis of variance with the post-hoc test by Dunnett and linear least-squares fit.

RESULTS

Extension of Aβ2M Amyloid Fibrils in the Presence of SDS at a Neutral pH. As shown in Figure 1A, S6 seeds incubated with 25 μM r-β2-m at pH 7.5 in the absence of SDS, showed a slight decrease in ThT fluorescence, as described previously (*18*). On the other hand, in the presence of 0.5 and 1.0 mM SDS, the fluorescence increased without a lag phase and proceeded to equilibrium after a 120-h incubation. However, in the presence of 10 mM SDS, the fluorescence disappeared immediately after the initiation of the reaction. In the absence of the seeds, no fluorescence increase was observed throughout the reaction, regardless of the presence or absence of SDS (data not shown).

The fluorescence signal increased with SDS concentration, peaking at 0.5 mM before steadily decreasing above this level of SDS (Figure 1B). Above 1.5 mM SDS, the fluorescence signal decreased, indicating the destabilization of the seeds by SDS. An electron microscopic study revealed that the fibrils extended with the helical filament structure in the presence of 0.5 mM SDS (Figure 2A,B), while no fibrils were observed after a 72-h incubation in the presence of 10 mM SDS (data not shown). Moreover, the fibrils in Figure 2B were stained positively with Congo red and showed orange−green birefringence under polarized light (data not shown).

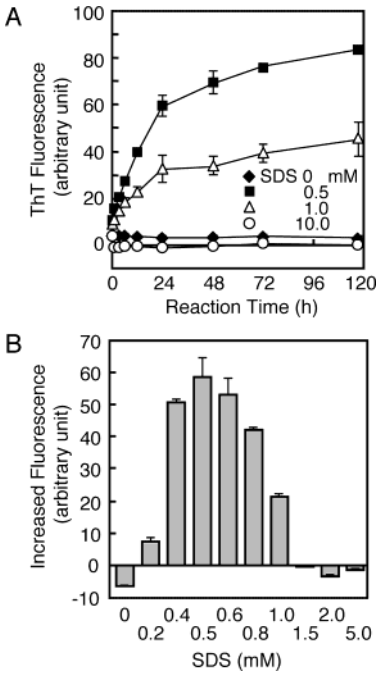

FIGURE 1: Extension of Aβ2M amyloid fibrils in the presence of SDS at neutral pH. In panel A, the reaction mixture contained 30 μg/mL S6 seeds, 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and (◆) 0, (■) 0.5, (△) 1.0, or (○) 10 mM SDS. The reaction mixture was incubated at 37 °C for 0−120 h. At each incubation time, the reaction mixture was analyzed by fluorescence spectroscopy as described in Experimental Procedures. Each point and bar represents the average and standard deviation of three independent experiments, respectively. In panel B, the reaction mixture contained 30 μg/mL S6 seeds, 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0−5.0 mM SDS. The reaction mixture was incubated at 37 °C for 72 h and analyzed by fluorescence spectroscopy as described in Experimental Procedures. Each column and bar represents the average and standard deviation of three independent experiments, respectively.

As shown in Figure 3A, the initial rate of the extension of S6 seeds was linear in relation to the seed concentration (0−60 μg/mL) in the presence of 0.5 mM SDS ($r = 0.998$), while it was not linear in relation to the r-β2-m concentration (0−25 μM) (Figure 3B). These data suggest that, although the extension of the seeds in the presence of 0.5 mM SDS could be explained basically by a first-order kinetic model (*12*, *14*, *16*), SDS may significantly modify the extension kinetics by affecting the conformation and stability of β2-m and extending fibrils, vide infra.

CMC of SDS. The fluorescence probe ANS was used to measure the CMC of SDS under the present experimental condition, that is, 50 mM phosphate buffer (pH 7.5) and 100 mM NaCl at 37 °C. As shown in Figure 4, two straight lines of the fluorescence intensity were defined in the range of 0−0.6 mM and 0.7−1.6 mM SDS, respectively. These two phases indicate that the SDS solution is essentially aqueous and micellar at 0−0.6 and 0.7−1.6 mM, respectively. We estimated the CMC of SDS to be 0.67 mM from the point of intersection of these two lines. This was in good agreement with the CMC of SDS previously reported (*37*, *44*).

Effect of SDS on the Conformation of β2-m. Far-UV CD spectra of r-β2-m were measured at pH 7.5 in the presence

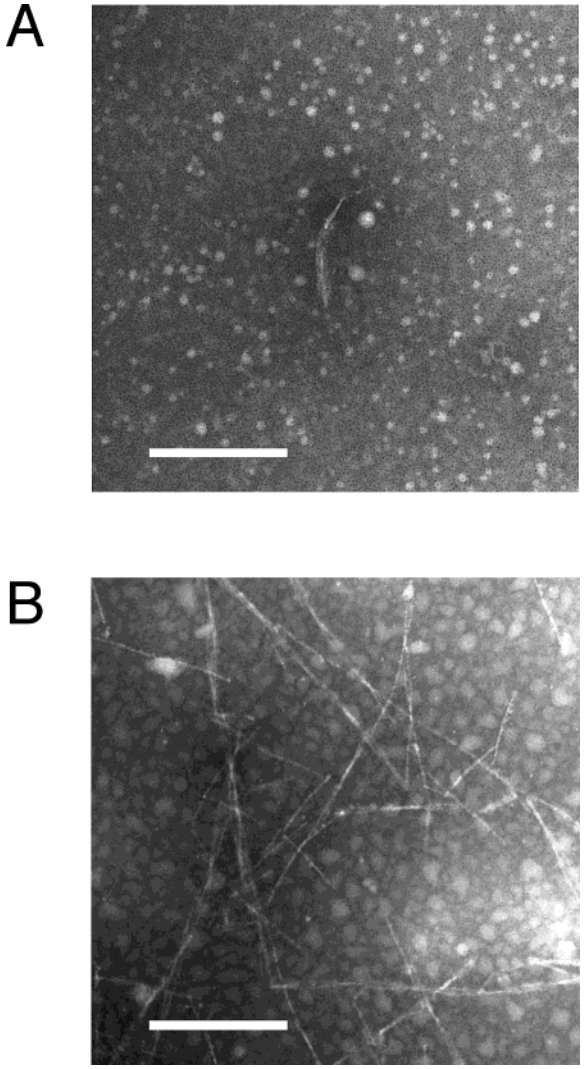

FIGURE 2: Electron micrographs of extended Aβ2M amyloid fibrils in the presence of SDS. The reaction mixture containing 30 μg/mL S6 seeds, 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0.5 mM SDS was incubated at 37 °C for (A) 0 or (B) 72 h. These samples were prepared for electron microscopy as described in Experimental Procedures. Bars are 250 nm long.

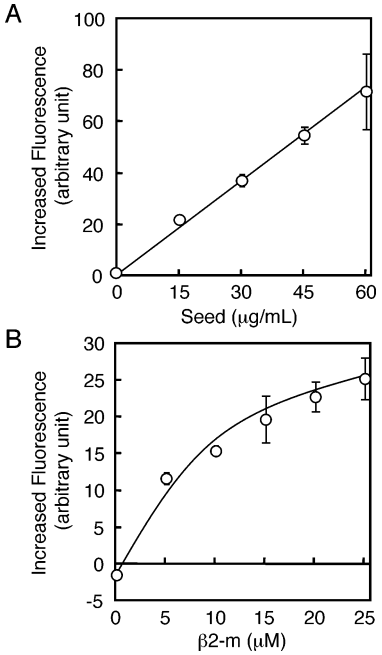

FIGURE 3: Effects of (A) seed concentration and (B) β2-m monomer concentration on the initial rate of Aβ2M amyloid fibril extension. In panel A, the reaction mixture contained 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, 0.5 mM SDS, and indicated concentrations of S6 seeds. The reaction mixture was incubated at 37 °C for 12 h and analyzed by fluorescence spectroscopy as described in Experimental Procedures. Each point and bar represents the average and standard deviation of three independent experiments, respectively. Linear regression and correlation coefficient were calculated (r = 0.998). In panel B, the reaction mixture contained 30 μg/mL S6 seeds, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, 0.5 mM SDS, and indicated concentrations of β2-m monomers. The reaction mixture was incubated at 37 °C for 12 h. Each point and bar represents the average and standard deviation of three independent experiments, respectively.

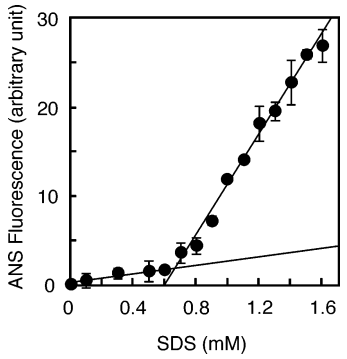

FIGURE 4: CMC of SDS determined by fluorescence spectroscopic analysis with ANS. The reaction mixture containing 10 μM ANS, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0−1.6 mM SDS was analyzed by fluorescence spectroscopy as described in Experimental Procedures. Each point and bar represents the average and standard deviation of three independent experiments, respectively. Two straight lines defining the fluorescence in an essentially aqueous environment and a micellar environment were traced. The point of intersection of these lines was used to define the CMC.

of 0−10 mM SDS (Figure 5A). In the presence of 0−0.1 mM SDS, the spectra of r-β2-m exhibited a positive peak at 202 nm and a negative peak at 221 nm immediately after the addition of SDS at 25 °C. In the presence of 0.5−0.75 mM SDS, which is close to the CMC, the spectra exhibited a transition state. When the concentration of SDS was increased to 1.0−10 mM, a major negative peak at 206 nm and a negative shoulder around 220 nm were observed. In the presence of 0.5−1.0 mM SDS, the spectra changed according to the incubation time (Figure 5B). As shown in Figure 5C,D, r-β2-m in the absence of SDS was estimated to be rich in β-sheet structure, while containing no α-helix structure, consistent with the data of the X-ray crystallography (*45*). With the increase in SDS concentration, the fraction of β-sheet structure decreased significantly, while the fractions of α-helix and random structures increased significantly, reaching a plateau at about 5 mM. In the absence of SDS, the near-UV CD spectrum of r-β2-m exhibited positive peaks at 258, 261, 264, 270, and 294 nm with a shoulder at 288 nm and negative peaks between 272 and 286 nm (Figure 5E). In the presence of 0.1−1.0 mM SDS, which is close to the CMC, the spectra exhibited a transition state. When the concentration of SDS was increased to 5.0−10 mM, the ellipticities of the above-described peaks generally decreased, reaching a plateau at about 5 mM. Thus, the data in Figures 1B, 4, and 5 clearly indicate that a kinetic and structural intermediate of β2-m

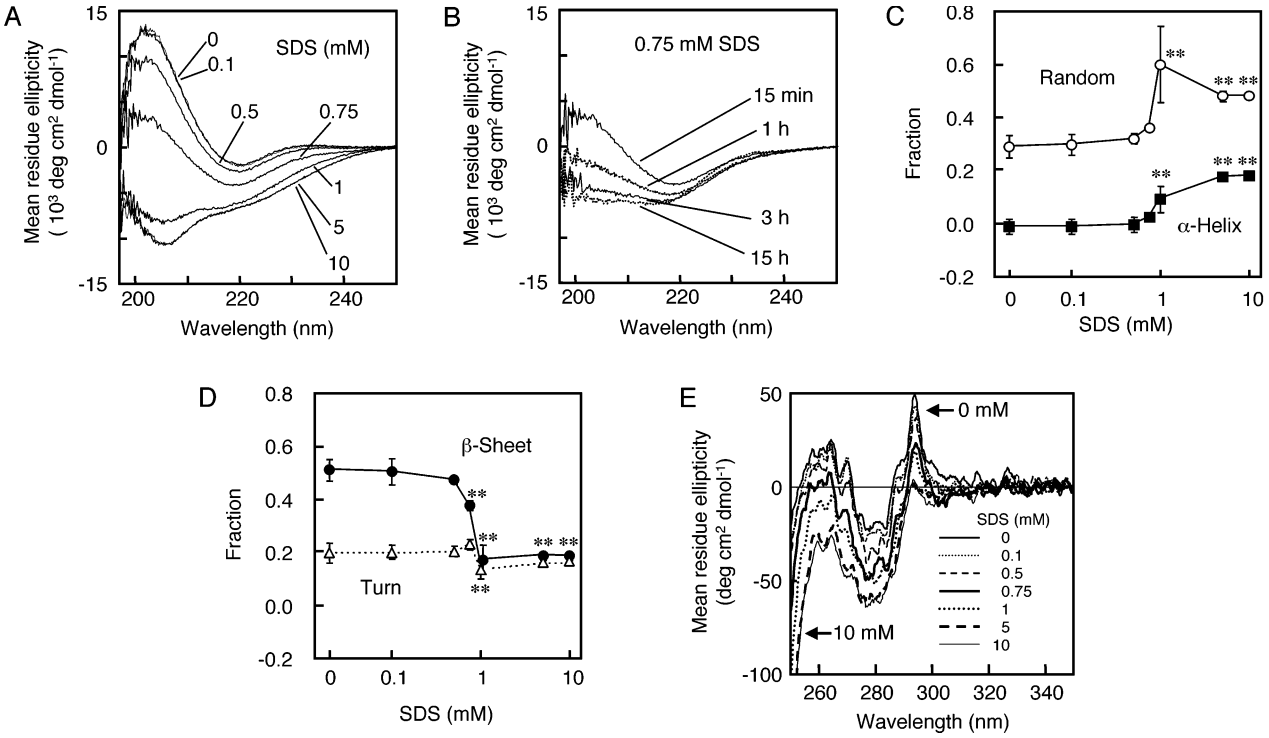

FIGURE 5: Far and near-UV CD spectra of r-β2-m in the presence of SDS. In panel A, far UV CD spectra of the reaction mixture containing 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0−10 mM SDS were recorded at 25 °C immediately after the addition of SDS as described in Experimental Procedures. The results are expressed in terms of the mean residue ellipticity. In panel B, the reaction mixture containing 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0.75 mM SDS was analyzed at 25 °C after the 15-min to 15-h incubation. In panels C and D, the fractions of (■) α-helix, (○) random, (●) β-sheet, and (△) turn structures were calculated as described in Experimental Procedures and plotted against SDS concentration. Each point and bar represents the average and standard deviation of six independent estimates, respectively. Two asterisks (**) denote $p < 0.01$ (one-way analysis of variance, post-hoc test by Dunnett). In panel E, near-UV CD spectra of the mixture containing 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0−10 mM SDS were recorded at 25 °C immediately after the addition of SDS as described in Experimental Procedures.

for the fibril extension reaction may be formed around the CMC of SDS.

Effect of SDS on the Aggregation of β2-m. When 25 μM r-β2-m was incubated at 37 °C for 24 or 48 h in the presence of 0.5 mM SDS, then injected into the column equilibrated with the buffer containing 0.5 mM SDS, a major peak corresponding to monomeric r-β2-m and a minor peak corresponding to the aggregate of r-β2-m (elution volume 12−15 mL) were observed (Figure 6A). About 60% of the peak area corresponding to 0-h incubation was recovered as a monomer peak after 48-h incubation. When the same incubation mixtures were injected into the column equilibrated with the buffer containing no SDS, only a single peak corresponding to monomeric r-β2-m was observed regardless of the incubation time (Figure 6B). These data suggest that at least some r-β2-m may weakly and reversibly aggregate in the presence of 0.5 mM SDS.

Effect of SDS on the Depolymerization of Aβ2M Amyloid Fibrils at a Neutral pH. As shown in Figure 7, when Aβ2M amyloid fibrils extended at pH 2.5 (F6 fibrils) were incubated at pH 7.5 in the absence of SDS, the ThT fluorescence decreased immediately after the initiation of the reaction, as described previously (*18*). On the other hand, in the presence of 0.5 and 1.0 mM SDS, the fluorescence retained more than 90% and 80%, respectively, of the initial fluorescence throughout the reaction. However, in the presence of 10 mM SDS, the fluorescence disappeared completely after the reaction.

Effect of Various Detergents on the Extension of Aβ2M Amyloid Fibrils at a Neutral pH. DTAC, SB12, and Tx100 are the cationic, amphipathic, and nonionic detergents, respectively, and the lengths of the acyl groups of DTAC, SB12, and SDS are equal to each other (C_{12}). The CMCs of DTAC, SB12, and Tx100 under the present experimental condition were determined to be about 1.0, 1.0, and 0.1 mM, respectively. As shown in Figure 8, the fluorescence was not significantly increased by incubation of the S6 seeds with 25 μM r-β2-m at pH 7.5 in the presence of 0.1−5.0 mM DTAC, SB12, or Tx100.

DISCUSSION

Extension of Aβ2M Amyloid Fibrils at a Neutral pH in the Presence of SDS. β2-m adopts a compact, β-sheet rich conformation at neutral pH, where the de novo formation or extension of Aβ2M amyloid fibrils does not occur (*16*−*18*). Moreover, Aβ2M amyloid fibrils readily depolymerize into monomeric β2-m at neutral pH (*18*). Thus, to observe the extension reaction of Aβ2M amyloid fibrils at neutral pH, we needed to unfold the compact structure of β2-m to an amyloidogenic conformer and stabilize the extended fibrils by modifying the solution, adding other factors or both. At low concentrations, TFE caused the extension of Aβ2M amyloid fibrils, inducing a subtle change in the tertiary structure of β2-m, and stabilized the fibrils at neutral pH (*19*). TFE is known as a cosolvent to weaken the hydrophobic interactions within a polypeptide chain and to strengthen the

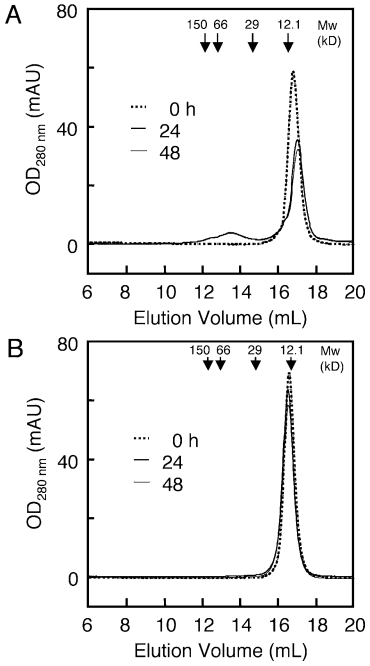

FIGURE 6: HPLC analysis of the effect of SDS on the aggregation of β2-m. In panel A, 25 μM r-β2-m was incubated at 37 °C for 0, 24, or 48 h in the presence of 0.5 mM SDS, then injected into the column equilibrated with the buffer containing 0.5 mM SDS as described in Experimental Procedures. In panel B, the same incubation mixtures as in panel A were injected into the column equilibrated with the buffer containing no SDS.

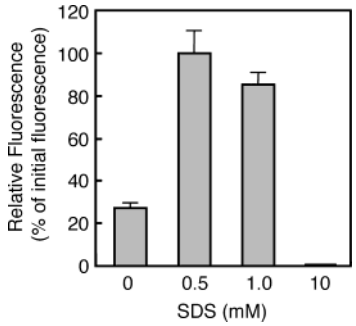

FIGURE 7: Effect of SDS on the depolymerization of Aβ2M amyloid fibrils. The reaction mixture contained 300 μg/mL fresh F6 fibrils, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0−10 mM SDS. The reaction mixture was incubated at 37 °C for 24 h and analyzed by fluorescence spectroscopy as described in Experimental Procedures. Each column and bar represents the average and standard deviation of three independent experiments, respectively.

intramolecular hydrogen bonds between residues close to each other (*46*). As a result of these effects, TFE changes the conformation of various proteins, often producing the α-helical structure stabilized by intramolecular hydrogen bonds (*47*, *48*). However, it is also known that the formation of intermolecular hydrogen bonds in the presence of TFE results in the aggregates (*47*) or amyloid fibrils (*49*). These dual properties of TFE may well explain the effect of TFE to accelerate Aβ2M amyloid fibril extension at neutral pH. TFE would weaken the hydrophobic interactions within β2-m and make β2-m an amyloidogenic conformer by unfolding the tertiary structure (*19*). At the same time, TFE could strengthen the intermolecular hydrogen bonds between β2-m molecules in the fibrils and stabilize the extended fibrils (*19*). We also reported that in the presence of low concentrations

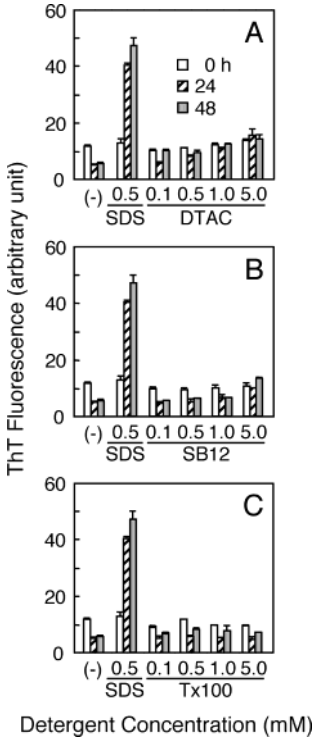

FIGURE 8: Effect of various detergents on the extension of Aβ2M amyloid fibrils. The reaction mixture contained 30 μg/mL S6 seeds, 25 μM r-β2-m, 50 mM phosphate buffer (pH 7.5), 100 mM NaCl, and 0.5 mM SDS or 0−5.0 mM (A) DTAC, (B) SB12, or (C) Tx100. The reaction mixture was incubated at 37 °C for (open) 0, (hatched) 24, and (closed) 48 h and analyzed by fluorescence spectroscopy as described in Experimental Procedures. Each column and bar represents the average and standard deviation of three independent experiments, respectively.

of TFE, some GAGs, especially heparin, dose-dependently enhance the fibril extension by uniformly binding to the surface of extended Aβ2M amyloid fibrils (*19*). Since GAGs constitute an extended and rigid structure, one GAG molecule would bind to the surface of the fibrils over many β2-m molecules by the electrostatic interaction between the negative charges of GAGs and the positive charges of the specific residues of β2-m molecules.

The present study revealed that SDS at concentrations slightly below or around the CMC accelerates the extension of Aβ2M amyloid fibrils at neutral pH not only by unfolding the compact structure of β2-m to an α-helix-containing aggregation-prone amyloidogenic conformer but also by stabilizing the extended fibrils (see Figures 5−7). SDS is known to interact with various types of proteins between the alkyl chain of SDS and the hydrophobic side chains of the proteins, as well as between the negatively charged sulfate group of SDS and the positively charged side-chains of the proteins (*36*). The submicellar concentration of SDS optimal for the extension reaction (see Figure 1) may indicate that the interaction occurs between SDS and β2-m/amyloid fibrils mainly in an aqueous environment, rather than in a micellar environment. Moreover, both the alkyl chain (C_{12}) and the negatively charged sulfate group of SDS may be essential for the interaction between SDS and β2-m/amyloid fibrils because DTAC (C_{12}), SB12 (C_{12}), and Tx100, cationic, amphipathic and nonionic detergents, respectively, have no effects on the extension reaction (Figure 8). On the other hand, at a high concentration, SDS is known to form micelles

and attack various kinds of native proteins causing them to unfold (*37*). As shown in Figures 5 and 7, SDS at concentrations higher than CMC causes the β2-m molecules to unfold to the α-helix-rich, nonamyloidogenic conformer, as well as the destabilization of the fibrils.

Biological Relevance of Lipids to Aβ2M Amyloid Deposition in Vivo. Various lipid molecules and a detergent, namely, SDS, have been reported to induce the conformational change of various amyloid precursor proteins, as well as to initiate the amyloid fibril formation in vitro (*30*−*32*, *38*, *50*−*52*). For example, GM1 ganglioside-bound amyloid β-protein (GM1/Aβ), found in brains exhibiting early pathological changes of Alzheimer's disease including diffuse plaques, has been suggested to be involved in the initiation of amyloid fibril formation in vivo by acting as a seed (*51*). Kakio et al. reported that (1) Aβ recognizes a GM1 "cluster" in membranes, the formation of which is facilitated by cholesterol and (2) an increase in membrane-bound Aβ concentration triggers its conformational transition from α-helix-rich to β-sheet-rich structures (*52*). Pertinhez et al. reported that SDS significantly affects fibril formation by a peptide from human complement receptor 1 (*38*). In an aqueous solution, the peptide is unfolded but slowly aggregates to form fibrils. In submicellar concentrations of SDS, the peptide is initially α-helical but converts rapidly to a β-sheet structure and large quantities of fibrils form. In SDS above the CMC, the peptide adopts a stable α-helical structure and no fibrils are observed. Although Aβ2M amyloid deposition takes place predominantly in the cartilaginous and tendinous tissues (*27*, *28*), the SDS effects described in this paper support the hypothesis that lipid molecules (e.g., phospholipids) that affect the conformation and stability of β2-m and amyloid fibrils may have significant effects on the kinetics of Aβ2M fibril formation in vivo. This hypothesis is consistent with the observation that hemodialysis patients have a notably higher molar ratio of the plasma lysophosphatidylcholine/phosphatidylcholine and significantly higher concentrations of plasma phosphatidylethanolamine and lysophosphatidic acid as compared to healthy controls (*53*). Further studies on this abnormal phospholipid metabolism seen in hemodialysis patients may significantly contribute to the understanding of the pathogenesis of Aβ2M amyloidosis.

In conclusion, low concentrations of SDS around the CMC not only converted natively folded β2-m monomers into partially folded, α-helix-containing conformers but also stabilized the fibrils, resulting in the extension of Aβ2M amyloid fibrils at neutral pH. These findings demonstrate the sensitivity of Aβ2M fibril formation to solution conditions and suggest a possible role for lipid molecules in the development of Aβ2M amyloidosis. A challenging subject for future study is to elucidate the culprit lipid molecules that induce partial unfolding of β2-m and subsequent amyloid fibril formation both in vitro and in vivo.

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