Conformation of β2-Microglobulin Amyloid Fibrils Analyzed by Reduction of the Disulfide Bond*

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β2-Microglobulin (β2-m), a major component of dialysis-related amyloid fibrils, has an intrachain disulfide bond buried inside the native structure. We examined the conformation of β2-m amyloid fibrils by analyzing the reactivity of the disulfide bond to a reducing reagent, dithiothreitol. Although the disulfide bond in the native structure was highly protected from reduction, the disulfide bonds in the amyloid fibrils were reduced at pH 2.5 were progressively reduced at pH 8.5 by 50 μM dithiothreitol. Because β2-m amyloid fibrils prepared under acidic conditions have been known to depolymerize at a neutral pH, we examined the relation between depolymerization and reduction of the disulfide bond. The results indicate that the disulfide bonds in the amyloid fibrils were protected from reduction, and the reduction occurred during depolymerization. On the other hand, the disulfide bonds of immature filaments, the thin and flexible filaments prepared under conditions of high salt at pH 2.5, were reduced at pH 8.5 more readily than those of amyloid fibrils, suggesting that the disulfide bonds are exposed to the solvent. Taken together, the disulfide bond once exposed to the solvent upon acid denaturation may be progressively buried in the interior of the amyloid fibrils during its formation.

β2-Microglobulin (β2-m)1 is a major component of amyloid fibrils deposited in dialysis-related amyloidosis with clinical manifestations such as carpal tunnel syndrome and destructive arthropathy associated with cystic bone lesions (1–5). β2-m consists of a polypeptide chain of 99 residues (molecular weight of 11,800) and has a β-sandwich structure stabilized by a single cross-sheet disulfide bond formed by Cys25 and Cys80 (Fig. 1A) (6, 7). However, β2-m, existing on the surfaces of nearly all cells as the constant portion of the light chain of the major histocompatibility complex class 1 antigen (6), is a normal constituent of plasma, where its concentration in adults is 1.1–2.7 mg/liter. The daily production of β2-m in mono- and dimeric forms was solubilized from prepurified [2-m by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by analyzing 2-Microglobulin amyloid fibrils by 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(apoE), a representative amyloid-associated protein, formed a stable complex with β-amyloid fibrils and inhibited the depolymerization of the fibrils, suggesting that apoE could enhance the deposition of amyloid fibrils in vivo.

As described above, the deposition of β-amyloid fibrils is determined by an intricate balance of the stability of the native state and polymerization and depolymerization of amyloid fibrils. To analyze these conformational features, the reactivity of the disulfide bond, Cys25–Cys80, might be a useful probe. In this study, we focused on the reduction of the disulfide bonds in β-amyloid fibrils prepared at pH 2.5. We also used immature fibrils prepared under high salt conditions at pH 2.5. We employed reversed-phase high pressure liquid chromatography (HPLC) and thioflavin T (ThT) fluorescence to monitor the reduction of disulfide bonds and the formation of amyloid fibrils, respectively. Our results indicated that the disulfide bonds of amyloid fibrils are highly protected from reduction as in β-amyloid in the native state, but those of the immature fibrils are not.

EXPERIMENTAL PROCEDURES

Recombinant β-amyloid—Recombinant β-amyloid with three signal peptides, Glu-Ala-Glu-Ala-Tyr-Val, Glu-Ala-Tyr-Val, and Val–, added at the amino-terminal Leu of intact β-amyloid was expressed in methylotrophic yeast Pichia pastoris and then purified as described previously (17, 25). In this study, we used β-amyloid with one additional amino acid residue. The conformational and amyloidogenic properties of this species were indistinguishable from the intact β-amyloid purified from patients.

Polymerization and Depolymerization—β-amyloid fibrils were formed by the fibril extension method described by Yamaguchi et al. (21). Seed fibrils were prepared by the extension reaction of original fibrils with monomeric recombinant β-amyloid. The seeds used here were 6th generation from the original fibrils taken from patients. The seeds were added to yield a final concentration of 5 ng/μl in a reaction mixture containing 25 μM recombinant β-amyloid, 50 mM citrate buffer (pH 2.5), and 0.1 M KCl at 37 °C. Immature fibrils of β-amyloid were formed at 50 μM β-amyloid in a high salt concentration without seeds at pH 2.5 as described (18).

Depolymerization of the fibrils was carried out at pH 8.5 according to the optimal conditions reported by Yamaguchi et al. (21). The fibrils of 25 μM β-amyloid were centrifuged at 15,000 rpm for 30 min at 4 °C. After removing the supernatant, the pellets were resuspended in 0.1 M Tris-HCl buffer (pH 8.5) containing 0.1 M KCl. After mixing by pipetting and vortex, the reaction tube was incubated at 25 °C. Incubation times ranged between 1 min and 24 h. In the case of immature fibrils, the pH was increased to 8.5 by the addition of 2 M HCl to lower the pH and consequently to quench the reaction, and then the remaining fibrils were dissolved completely by adding 90 μl of dimethyl sulfoxide. After a 30-min incubation, the sample was subjected to reversed-phase HPLC.

Electron Microscopy—Reaction mixtures (2.5 μl) were diluted with 25 μl of distilled water. These diluted samples were spread on carbon-coated grids, and the solution allowed to stand for 1–2 min before excess solution was removed with filter paper. After the residual solution had dried up, these grids were negatively stained with 1% phosphotungstic acid (pH 7.0). Again, the solution on the grids was removed with filter paper and dried. These samples were examined under a Hitachi H-7000 electron microscope with an acceleration voltage of 75 kV.

CD Measurements—CD measurements were performed with a Jasco spectropolarimeter, model J-720, using quartz cells with a 1-mm path length. The temperature was controlled with a water-circulating cell holder at 20 °C. The data were expressed as molar residue ellipticity (θ) as described (17).

RESULTS

Conformational States of β-amyloid

We first summarized the different conformational states of β-amyloid (Figs. 1 and 2) as follows.

Native State—Various spectroscopic results including the far-UV CD (Fig. 2) and NMR data (7, 17) are consistent with a typical 7-stranded immunoglobulin fold as shown by x-ray crystallography (Fig. 1A).

Acid-denatured State—The protein is substantially denatured at pH 2.5, as measured by far- and near-UV CD (15–19, Fig. 2) and NMR (17) spectra, although marginal residual
structures were detected as indicated by the ability to bind 1-anilino-8-naphthalenesulfonate (18).

Amyloid Fibrils—Under the same conditions of acid denaturation (at pH 2.5 and 0.1 M KCl), amyloid fibrils were formed by the extension reaction with seed fibrils. With the preparation of fresh amyloid fibrils, extended fibrils, with a diameter of about 12–15 nm and a longitudinal periodicity, were mainly observed (Fig. 1B). The far-UV CD spectrum had a marked negative peak at -220 nm, suggesting an increased amount of the β-sheet content. Recent analysis of amyloid fibrils with H2/H exchange monitored by heteronuclear NMR suggested that the loop regions in the native structure were transformed to a β-sheet structure.2

Aggregated Fibrils—One week after the preparation of the fibrils, we could see by EM large fibrillar clusters, which were not dispersed by dilution or mild sonication (Fig. 1C). We did not see notable changes in the CD spectrum on the aggregation of fibrils (data not shown).

Immature Fibrils—Another type of β2-m fibrillar structure was formed at high ionic strength and low pH (15, 18–19). These filaments, often called immature fibrils, were thin (2–5 nm) and very flexible as observed by EM (Fig. 1D) in comparison with the above amyloid fibrils. The immature fibrils were dispersed, and no aggregation of filaments was observed even after several weeks. The far-UV spectrum of the immature fibrils was different from that of the amyloid fibrils, with the negative peak shifted to a lower wavelength of around -215 nm. However, this is still consistent with a predominantly β-sheet structure. Because CD is an averaging technique, it was difficult to clarify whether the immature fibrils have a conformation intermediate between the acid-denatured state and amyloid fibrils.

Role of Immature Fibrils

We examined the role of the immature fibrils in the formation of typical amyloid fibrils. The immature fibrils were prepared at pH 2.5 under various salt concentrations as reported by McParland et al. (18). The immature fibrils were formed spontaneously without a lag time at 50 μM β2-m, and the amount of immature fibrils measured by ThT fluorescence was sensitively dependent on the salt concentration as reported (18). The kinetics of the immature fibril formation in 0.2 M NaCl was compared with the extension reaction of the amyloid fibrils (Fig. 3A). We found that freshly prepared monomer β2-m, even at concentrations as high as 100 μM, did not bind ThT, whereas both amyloid fibrils and immature fibrils did, even at a concentration as low as 5 μM (data not shown). It is noted that the ThT fluorescence intensity of amyloid fibrils prepared by the extension reaction at 25 μM β2-m and 0.1 M KCl reached a value of 200, more than several times higher than that of immature fibrils at the same concentration. This suggested that the tinctorial property of the immature fibrils is different from that of the amyloid fibrils. Another distinct feature of the immature fibrils was that the rate of formation was slower than that of the extension reaction (Fig. 3A). While the extension reaction with recombinant β2-m was completed in 2 h, the spontaneous formation of immature fibrils took 60 h to complete.

To confirm whether the immature fibrils function as seeds, they were added to the monomeric β2-m solution in 0.2 M NaCl. We observed no acceleration of the fibril formation (Fig. 3A), indicating that the immature fibrils did not act as a seed. We also examined whether the immature fibrils can be converted to mature fibrils by adding the seed amyloid fibrils (Fig. 3B). There was no change in the ThT fluorescence even after incubation for up to 1 month. On the basis of these observations, we may conclude that the immature fibrils are not the intermediate of β2-m amyloid fibril formation but an off-pathway or dead-end product. Even so, the conformation of immature fibrils will be important because it might provide valuable information as to the productive intermediate, as is often addressed for the equilibrium molten globule intermediate of protein folding.

Depolymerization of Amyloid Fibrils

Yamaguchi et al. (24) studied the effect of pH on the depolymerization of amyloid fibrils monitored by ThT fluorescence, and showed that most or all of the initial fluorescence was lost at a pH above 6.0, indicating a pH-induced depolymerization. We examined the depolymerization of amyloid fibrils at pH 8.5 (Fig. 4). We noticed an interesting feature of the depolymerization reaction; the reaction was dependent upon the incubation time at pH 2.5. The fibrils long after their formation were difficult to depolymerize completely at neutral pH. One possible reason for this is the aggregation of the fibrils as shown by the electron micrographs; aggregated or clustered fibrils were predominant for the samples older than 1 week (Fig. 1C).
The disulfide bond of β2-m could not be reduced notably with 10 mM DTT at pH 8.5 and 25 °C in the absence of denaturant as monitored by reversed-phase HPLC, indicating that the bond in the intact β2-m was buried in the interior of the protein molecule, as observed for the constant domain of the immunoglobulin light chain (28, 29). The reduction of a disulfide bond molecule, as observed for the constant domain of the immunoglobulin light chain (28, 29). The reduction of a disulfide bond in several hours.

We assumed first-order kinetics with the apparent rate constant $k_{app}$, $f_{re} = \exp(-k_{app} \times t)$, where $f_{re}$ is the fraction of reduced species, $t$ is the reaction time, and the observed kinetics were analyzed by the least squares curve-fitting procedure to obtain $k_{app}$. The $k_{app}$ value at 2 mM DTT in 4 M GdnHCl, pH 8.5, and 37 °C was 2.7 min$^{-1}$, and the value increased linearly with increase in the concentration of DTT, suggesting that the disulfide bond is completely exposed to the solvent. In contrast, the reaction in the absence of denaturant was still very slow with $k_{app} = 0.010$ min$^{-1}$ in the presence of 200 mM DTT. Taking into account the difference in DTT concentration, the protection factor in 5 μM urea at pH 8.5 and 25 °C was at least 3 x 10$^4$, although the exact calculation could not be done because of the difference in experimental temperatures.

Reduction of a Disulfide Bond in the Native State

The disulfide bond of β2-m was monitored by reversed-phase HPLC, indicating that the bond in the intact β2-m was partly destabilized (Fig. 5A). The HPLC analysis of the reaction product in 5 μM urea revealed a clear separation of the intact and reduced molecules with two sharp peaks appearing at distinct elution times, indicating that HPLC analysis is useful for the quantitative evaluation of the disulfide bond reduction. The fraction of reduced β2-m calculated from its peak area increased exponentially with incubation time, and most of the molecules were reduced after several hours (Fig. 6A). When the reduction by 2 mM DTT was carried out in the presence of 4 M GdnHCl at pH 8.5 and 37 °C, where the protein was completely unfolded, the reduction was completed rapidly in 1 h (Fig. 6B). This result confirmed that the intrinsic reduction rate of the exposed disulfide bond by DTT is very rapid under the conditions used. To estimate the extent of protection in the native state in the absence of denaturant, the reduction of the disulfide bond was carried out in the presence of 200 mM DTT at 37 °C (Fig. 6C). At this high concentration of DTT and elevated temperature, we could observe the complete reduction of the disulfide bond in several hours.

Reduction of a Disulfide Bond in the Amyloid Fibrils

Ohhashi et al. (17) have examined the conformation and stability of the reduced β2-m in comparison with the intact form. They showed by CD and fluorescence measurements that at pH 8.5 the global conformation of the reduced β2-m is similar to that of the intact β2-m, although the stability is decreased significantly. They also showed that reduction of the disulfide bond completely abolishes the ability of β2-m to form mature amyloid fibrils, suggesting a critical role of the disulfide bond in the amyloid fibril formation of β2-m. If so, would the disulfide bonds in amyloid fibrils be protected?

Because the fibrils are prepared and stable under acidic conditions and the fibrils depolymerize at neutral pH, it would be preferable to examine the reduction of the disulfide bond at acidic pH. However, no available disulfide-reducing reagent was reactive at pH 2.5. Therefore, we carried out the reduction of the disulfide bonds with DTT at pH 8.5, although we anticipated that the competition between reduction and depolymerization complicates the interpretation (Fig. 5B). In the absence of the denaturant, the disulfide bond of monomeric native β2-m proteins was only slightly reduced even after the incubation with 50 mM DTT for 30 min at pH 8.5. The fraction of the reduced protein was about 5%. By the same reducing pulse at pH 8.5 the global conformation of the reduced β2-m calcuated rapidly in 1 h (Fig. 6B). This result confirmed that the intrinsic reduction rate of the exposed disulfide bond by DTT is very rapid under the conditions used. To estimate the extent of protection in the native state in the absence of denaturant, the reduction of the disulfide bond was carried out in the presence of 200 mM DTT at 37 °C (Fig. 6C). At this high concentration of DTT and elevated temperature, we could observe the complete reduction of the disulfide bond in several hours.

Depolymerization and Reduction of the Disulfide Bond

The kinetics of the disulfide reduction and depolymerization were carefully compared (Fig. 7A). In this experiment, to pre-
The kinetic with \( k \) followed an exponential curve with \( C \) intact form (Fig. 6). On the other hand, the depolymerization is larger than that of completely unfolded form (Fig. 6B). The fractions of the reduced \( \beta_2 \)-m (\( \bullet \)) were calculated by analyzing HPLC profiles. The solid line indicates the first-order decay kinetics obtained by curve-fitting analysis using the nonlinear least squares procedure. The dotted line in A indicates an inverse curve of the solid line.

**Disulfide Bond Reduction of the Kinetic Intermediates**

Chiti et al. (30) showed that the unfolded \( \beta_2 \)-m refolds to the native state through two major intermediates (I1 and I2). It has been proposed that the highly structured I2 intermediate is similar to the amyloidogenic form of \( \beta_2 \)-m (31). To address the conformational state accessible to DTT during depolymerization, we examined the reactivity of the disulfide bond of \( \beta_2 \)-m during refolding reactions in a monomeric form (Fig. 8). The disulfide bond was reduced with a reducing pulse of 50 mM DTT at pH 8.5 for 5 min at various refolding times initiated from the acid-unfolded state at pH 2.5 or the unfolded state by 5.0 M GdnHCl at pH 8.5. The shortest refolding time of 0 min represents the direct dilution of denatured proteins with the reducing buffer. Irrespective of the initial unfolding conditions, about 30% of the molecules was reduced at time 0, and the fraction of the reduced molecule decreased with increasing refolding time. Independence of the refolding kinetics on the initial conditions is as expected for the normal folding reactions, proposing that the kinetic intermediates observed by Chiti et al. (30) also accumulate during refolding from the acid-denatured state. In fact, the kinetics of protection of the disulfide bond reduction against the refolding time was similar to the kinetics of I2 to native state conversion during refolding (30). With the same reducing pulse, but in the presence of 5.0 M GdnHCl, the disulfide bond was reduced completely (data not shown). This indicated that I1 is less protected from the reduction than the native state and that both I1 to I2 intermediates are more protected than the highly denatured state in 5.0 M GdnHCl.

In the reduction of the disulfide bond coupled with depolymerization, the kinetics of depolymerization and reduction were similar to each other (Fig. 7A), suggesting the following two conformational states accessible to DTT: the monomeric

![Image](http://www.jbc.org/)

**Fig. 6. Reduction of disulfide bonds of monomeric \( \beta_2 \)-m by DTT at pH 8.5.** The solvent conditions were 10 mM DTT and 5 M urea at 25 °C (A), 2 mM DTT and 4 M GdnHCl at 37 °C (B), and 200 mM DTT in the absence of denaturant at 37 °C (C). The lines show the first-order kinetics with \( k_{app} = 0.0047 \text{ min}^{-1} \) (A), \( k_{app} = 2.7 \text{ min}^{-1} \) (B), and \( k_{app} = 0.01 \text{ min}^{-1} \) (C), obtained by curve-fitting analysis using the non-linear least squares procedure.

**Fig. 7. Comparison between depolymerization and reduction of disulfide bonds.** A, amyloid fibrils incubated 1 day at pH 2.5; B, immature fibrils incubated 3 days at pH 2.5. The concentrations of amyloid fibrils and immature fibrils were 25 and 50 μM, respectively. The reactions were carried out with (△) and without (○) 50 mM DTT (pH 8.5). The fractions of the reduced \( \beta_2 \)-m (●) were calculated by analyzing HPLC profiles. The solid line indicates the first-order decay kinetics obtained by curve-fitting analysis using the nonlinear least squares procedure. The dotted line in A indicates an inverse curve of the solid line.
refolding intermediate and the destabilized fibrillar form. From the low reactivity of the monomeric refolding intermediates, we conclude that the destabilized fibrillar form is likely to be a target of DTT attack.

**DISCUSSION**

**Mature and Immature Amyloid Fibrils—**$\beta$-2-m assumes different conformational states, the relations of which are summarized in Scheme I.

\[
N \equiv U_\alpha \rightarrow AF \rightarrow AF_n \\
\| \\
IF
\]

**SCHEME I**

where IF is immature fibrils, AF is amyloid fibrils, $U_\alpha$ is acid-unfolded state, and N is native state. Typical amyloid fibrils were formed by the extension reaction at pH 2.5 and 0.1 M KCl. The fibrils transformed into fibrillar clusters ($AF_n$) by self-association at pH 2.5. Although the physiological role of this fibrillar-fibrillar interaction is unknown, it is clear that the self-stabilization resulted in the constraint of their depolymerization at neutral pH.

Under high salt conditions at pH 2.5, $\beta$-2-m formed immature fibrils, which exhibited a distinct morphology from the amyloid fibrils. Smith and Radford (19) showed that the reduced $\beta$-2-m in 0.4 M NaCl at acidic pH also forms a filamentous structure similar to these immature fibrils. These results suggest that the immature fibrils were formed more easily than the mature fibrils. These immature fibrils did not transform into amyloid fibrils even in the presence of amyloid fibril seeds, and moreover, the immature fibrils had no seed function (Fig. 3, A and B). These results argue that the immature fibrils might be an off-pathway product as shown in Scheme I. Kad et al. (15) also proposed from their kinetic experiments that the immature fibrils did not represent the intermediate in the assembly of fully mature amyloid. It is likely that the immature fibrils accumulated because unknown factors prevented the cooperative formation of the amyloid fibrils from a certain intermediate, resulting in the formation of a dead-end product. Nevertheless, clarifying the structure and stability of the immature fibrils is important because it might provide valuable information as to the productive intermediate and therefore the mechanism of amyloid fibril formation.

**Reactivity of the Disulfide Bond—**We examined the reactivity of the disulfide bond of different conformational states as shown in Scheme I. Because we had no reducing reagent active at pH 2.5, we had to jump the pH from 2.5 to 8.5 to examine the reactivity of the disulfide bond in different conformational states stable at pH 2.5. Conformations of both monomeric and polymeric states change drastically upon increasing the pH. Although this complicated the interpretation, we carefully obtained several pieces of information addressing the reactivity of the disulfide bond in amyloid fibrils. First, the disulfide bond in the native state was highly protected from reduction as expected from the buried disulfide bond of the immunoglobulin domain (28, 29). Second, the refolding intermediates, either from the acid-unfolded state at pH 2.5 or the unfolded state by 5 M GdnHCl, were somewhat protected from the reducing pulse of 50 mM DTT for 5 min in comparison with the fully unfolded state in 5 M GdnHCl. However, the extent of protection was less than that of the native state, as indicated by the time-dependent increase in the protected molecules (Fig. 8). Third, when coupled with the depolymerization, the reduction of the disulfide bond by 50 mM DTT proceeded almost completely (Fig. 7A). Taken together, whereas disulfide bonds are highly protected from reduction in the amyloid fibrils, they experience a state accessible to DTT attack during depolymerization and refolding to the native state. The low reactivity to DTT of the monomeric refolding intermediate suggests that each $\beta$-2-m exposes the disulfide bond even in the fibrillar form during depolymerization, which is the target of DTT attack.

The results for immature fibrils contrasted with those for mature fibrils. The disulfide bonds of the immature fibrils were reduced very rapidly before their depolymerization as monitored by ThT fluorescence. It is clear that the disulfide bonds of the immature fibrils are accessible to DTT. Moreover, the depolymerization measured by ThT assay was independent of DTT, suggesting that the stability of immature fibrils is independent of the presence or absence of the disulfide bond. This is consistent with the fact that the reduced $\beta$-2-m formed immature fibrils (17, 19).

Although we know that a cross-$\beta$-sheet is the major structural component of $\beta$-2-m amyloid fibrils, details as to the conformation, in particular the topology of how each molecule is folded in the amyloid fibrils, are unknown. Our results propose that the disulfide bonds are buried in the interior of the amyloid fibrils. This does not mean they are buried in each $\beta$-2-m molecule intramolecularly in the amyloid fibrils. The disulfide bond may be buried by the intramolecular interactions between adjacent molecules. On the other hand, in the case of immature fibrils, the disulfide bond is either located on the surface or is accessible to DTT even if it exists inside the fibrils. Although we do not consider the immature fibrils to be a direct intermediate of amyloid fibril formation or depolymerization, one possibility raised from the high reactivity of the immature fibril disulfide bond is that a conformation similar to the immature fibril exists during the formation and depolymerization of the mature fibrils.

**Intrinsic Potential of Fibril Aggregation—**Our results showed that the amyloid fibrils have an intrinsic potential to aggregate (Fig. 4). The formation of such aggregated fibrils evidently retarded the depolymerization at neutral pH. Walsh et al. (32, 33) suggested that the protofibrils are in equilibrium with low molecular weight $\alpha$-fibrils and mature amyloid fibrils. They also showed that the amyloid fibrils are stabilized by fibrillar aggregation by themselves.

**In vivo,** however, the aggregation of amyloid fibrils has been considered to be promoted by other components including several proteins and hydrophobic molecules. In fact, the amyloid deposits in patient tissue contain various other proteins (34–37). ApoE, which is a cholesterol transport protein that serves as a ligand for low density lipoprotein receptors (38), is colocalized to amyloid deposits in all types of systemic and localized amyloidosis, i.e. Alzheimer’s $\beta$-amyloidosis, prion amyloid disease, amyloid A protein amyloidosis, immunoglobulin light
chain-related amyloidosis, transthyretin-related amyloidosis, and β2-m amyloidosis (33–36). Yamaguchi et al. (24, 39) showed that ex vivo β2-m amyloid fibrils are associated with serum amyloid P component and apoE, resulting in the inhibition of depolymerization of the fibrils at neutral pH. Although it is not clear whether these proteins are “promoters,” also called “pathological molecular chaperones,” of amyloid fibril formation from various precursor proteins, it is clear that they play a role in stabilizing the fibrils.

In addition to these factors, we assume that intrinsic potential of fibril aggregation is important to stabilize amyloid fibrils at neutral pH. Extensively aggregated fibrils prepared at pH 2.5 are also stable at neutral pH. Considering this pH independence, the intermolecular hydrophobic interaction between the exposed hydrophobic residues may be the driving force of fibril aggregation.

Conclusion—We examined the reactivity of the disulfide bonds of various conformational states of β2-m. In the native state, the disulfide bond, buried in the core of the molecule, was highly protected from reduction by DTT. In the acid-unfolded state, the disulfide bond, buried in the core of the molecule, was completely exposed to the solvent judging from the conformation measured by CD or NMR. In the immature fibrils, the disulfide bonds were reactive. Taken together, the disulfide bond completely buried in the native structure of β2-m experiences exposure and subsequent burial during the transformation to rigid amyloid fibrils.

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