Simulation of pH-dependent edge strand rearrangement in human β-2 microglobulin

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Abstract
Amyloid fibrils formed from unrelated proteins often share morphological similarities, suggesting common biophysical mechanisms for amyloidogenesis. Biochemical studies of human β-2 microglobulin (β2M) have shown that its transition from a water-soluble protein to insoluble aggregates can be triggered by low pH. Additionally, biophysical measurements of β2M using NMR have identified residues of the protein that participate in the formation of amyloid fibrils. The crystal structure of monomeric human β2M determined at pH 5.7 shows that one of its edge β-strands (strand D) adopts a conformation that differs from other structures of the same protein obtained at higher pH. This alternate β-strand arrangement lacks a β-bulge, which may facilitate protein aggregation through intermolecular β-sheet association. To explore whether the pH change may yield the observed conformational difference, molecular dynamics simulations of β2M were performed. The effects of pH were modeled by specifying the protonation states of Asp, Glu, and His, as well as the C terminus of the main chain. The bulged conformation of strand D is preferred at medium pH (pH 5–7), whereas at low pH (pH <4) the straight conformation is observed. Therefore, low pH may stabilize the straight conformation of edge strand D and thus increase the amyloidogenicity of β2M.

Keywords: molecular dynamics simulation; amyloidosis; β-2 microglobulin; β-sheet protein; negative design; pH-dependent conformational change

Amyloidosis results from abnormal aggregation of native or proteolyzed proteins into amyloid fibrils (Sipe and Cohen 2000) and is associated with an array of maladies, including Alzheimer’s disease, Parkinson’s disease, spongiform encephalopathies, type II diabetes, and several forms of systemic amyloidosis (Dobson 1999; Selkoe 2003). In each case, a protein or a proteolyzed fragment aggregates to form unbranched fibrils 10–20 nm wide and hundreds of nm long (Serpell et al. 1995). Fiber diffraction and electron microscopy data support a cross-β helical structure in the fibrils, with the main chain of β-strands running perpendicular to the axis of the fiber (Sunde et al. 1997). The diversity of the proteins implicated in amyloid diseases (Huff et al. 2003) and the structural similarity of the resulting amyloid fibers suggest that the formation of amyloid fibrils is a result of general chemical properties of the polypeptide backbone and amino acid side chains rather than the precise amino acid sequence (Bucciantini et al. 2002; Dobson 2003). Thus, it is of interest to explore how changes in environment confer structural changes that predispose proteins to self-associate and ultimately form amyloids.

While the formation of amyloid fibrils is observed for proteins of differing folds, including α-helix proteins (Booth et al. 1997; Bergstrom et al. 2001; Frare et al. 2004), β-sandwiches (Colon and Kelly 1992), and α + β-proteins (Maury and Baumann 1990; Ramirez-Alvarado et al. 2000), the presence of edge strands makes β-sheet proteins particularly susceptible to aggregation. Human β-2 microglobulin (β2M), a 99-residue β-sandwich protein, is an integral part of the MHC I complex, human leukocyte antigen (HLA), and has been studied as a model system for understanding amyloidosis (Esposito et al. 2000;
McParland et al. 2000; Chiti et al. 2001; Hong et al. 2002; Jones et al. 2003; Eakin et al. 2004; Yamaguchi et al. 2004). The protein is routinely secreted into the bloodstream as part of its catabolic cycle. Most patients undergoing hemodialysis eventually develop dialysis-related amyloidosis due to an accumulation of β2M in the serum (Floege and Ketteler 2001). The monomeric structure of the protein has been determined using both X-ray crystallography (Trinh et al. 2002) and NMR (Verdone et al. 2002). These crystallographic (β2M_{X-ray}) and solution (β2M_{NMR}) structures are highly similar, and both consist of seven β-strands (A–G) grouped in two anti-parallel β-sheets (Fig. 1). There are also several differences between the two structures. Most notably, in β2M_{NMR} one of the edge strands (strand D) has a pronounced bulge, whereas the corresponding strand in β2M_{X-ray} does not contain the bulge. Similarly, the crystal structure of β2M in complex with the HLA heavy chain (β2M_{HLA}) (Khan et al. 2000) shows a bulge in strand D involving D53 and L54. Thus, strand D of β2M is capable of adopting more than one stable conformation.

The edge strands of a β-sheet often exhibit structural features designed to protect against further β-sheet interactions, such as β-bulges, prolines, charged residues, short edge strands and loop coverage (Richardson and Richardson 2002). As a result, the native conformations often must first be destabilized through mutation (McCutchan et al. 1993; Hammarstrom et al. 2002) or exposure to environmental stress (McParland et al. 2000) in order for β-sheet proteins to become amyloidic. This is consistent with the observation that most proteins require partial denaturation to become amyloidic (Litvinovich et al. 1998; McParland et al. 2000; Khurana et al. 2001; Quintas et al. 2001; Arora et al. 2004a,b; Plakoutsi et al. 2004; Uversky and Fink 2004; Vernaglia et al. 2004). Biochemical and spectroscopic studies have suggested that the D strand of β2M may be directly involved in amyloid formation (Hong et al. 2002; Hoshino et al. 2002; McParland et al. 2002; Monti et al. 2002). Trinh et al. (2002) proposed that the conformation with a straight edge of the D strand may correspond to a rare species trapped by crystallization. Since the loss of the bulge in strand D would likely predispose the protein to aggregation through its exposed edge strand, it is important to understand what factors contribute to its loss and give rise to alternative, potentially aggregation-prone conformations.

Although the in vivo mechanism of amyloid formation from β2M is not known, the protein may be coaxed to form amylloids in vitro by reducing the pH of the solution to pH 3.6 (Kad et al. 2001; Jones et al. 2003) or by adding Ca^{2+} ion to the buffer (Morgan et al. 2001; Villanueva et al. 2004). The structure of monomeric β2M_{X-ray}, with its straight edge strand, was determined at pH 5.7 (Trinh et al. 2002), while that of β2M_{NMR} was determined at pH 6.6 (Verdone et al. 2002). Given the amyloid forming properties at low pH and the straight strand observed in β2M_{X-ray}, it is of interest to probe how the local structure of the β-strand bulge varies with pH. A number of simulation studies have provided molecular insight on amyloid forming proteins (Gsponer et al. 2003; Yang et al. 2003; DeMarco and Daggett 2004; Nowak 2004; Tarus et al. 2005). In the present study, molecular dynamics simulation was used to investigate how the conformational properties of β2M may be modulated by pH. To examine the structural response of β2M to pH, we performed a series of simulations using different ionization states of its side chains His, Asp, and Glu, and the C terminus. Strand D adopts a bulged conformation when only His side chains are protonated (here referred to as “medium pH”), but takes on a straight edge conformation when all three types of residue are protonated (“low pH”). Since a β-strand bulge may be an important deterrent against nonspecific oligomerization, the pH-dependent edge strand rearrangement seen in the simulation of β2M may suggest a mechanism by which low pH predisposes the protein for amyloid formation.

**Simulation of the rearrangement of strand D**

To examine the conformational response of β2M to a change in the ambient pH, we simulated the dynamics of the protein at different pH values, using either β2M_{X-ray} or β2M_{HLA} as the starting structure. While more sophisticated methods exist for constant pH molecular dynamics (Baptista et al. 1997; Onufriev et al. 2001; Baptista et al. 2002; Lee et al. 2004; Morgan et al. 2004), we modeled the effects of pH by specifying the ionization state of side chains and the C terminus. To simulate low pH conditions (pH < 4), we protonated the side chains of Asp, Glu, and

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**Figure 1.** Two crystal structures of β2M. (Left) β2M_{HLA} corresponds to the structure of β2M in the HLA complex determined to 1.8 Å resolution (PDB 1DUZ). (Right) β2M_{X-ray} was determined as a monomer, also to 1.8 Å (PDB 1LDS).
His, and amidated the C terminus. A medium pH was simulated by protonating the imidazole ring of His but leaving the Asp and Glu side chains and the C terminus in their ionized (–COO⁻) forms. Exposed His residues often have pKₐs that are elevated (pKₐ = 6.7) compared to those of imidazole derivatives (pKₐ = 6.0) (Cederholm et al. 1991). Also the pKₐ of His side chains can be elevated (pKₐ = 8) depending on local environment, such as the presence of nearby Asp and Glu residues (Tishmack et al. 1997). Thus, the medium pH simulated in our study may roughly correspond to a range of effective pH 5–7. Na and Cl ions were added to obtain concentrations of either 100 mM or 400 mM to test the effects of the ionic strength. While the most dynamic residues are found in loops at all pH values and ionic concentrations tested, there is a significant movement in residues 50–54 among simulations starting from the intermediate open conformation β₂M† (Fig. 3, iii). The velocities were reinitialized to erase memory of prior dynamics before resuming the simulation. (Repeated independent simulations starting from β₂M† were performed.) At low pH the protein reformed the missing hydrogen bonds in registry with strand E, and the structures obtained after 6 nsec of simulation resemble β₂Mₓ-ray (Fig. 5, left). In contrast, at medium pH the simulated structures developed a bulge and the structures obtained after 6 nsec of simulation resemble β₂M₉LA (Fig. 5, right). Therefore, the preferred conformation of strand D in human β₂M corresponds to a straight strand at low pH and a bulged strand at medium pH.

Since the protonation state of D53 differs at the low and the medium simulated pH values, its interaction with other neighboring residues may be affected by pH. One factor that contributes to the observed pH dependence appears to be the differing degrees of solvation of the D53 carboxyl side chain at different pH. For example, the number of water molecules Nwat that are within 2.7 Å of the carboxyl side-chain oxygens of unprotonated D53 is 6.5 ± 0.7 at medium pH, while Nwat = 2.7 ± 1.1 at low pH, where D53 is protonated. The reduced solvation at low pH facilitates van der Waals interactions with the aliphatic side chain of L65 on strand E, whereas the corresponding interactions at medium pH are disfavored due to the required desolvation of the charged D53 side chain. The side-chain interactions with L65 maintain D53 in a position that protects main-chain hydrogen bonds between strands D and E. Additionally, the solvation shell around unprotonated D53 side chain may weaken its hydrogen-bonding capacity to H51 at medium pH. Since the straight D strand requires the two side chains to reside on the same side of the β-sheet where they can interact with each other, a loss of the hydrogen bonding interactions with the side chain at position 53 would free the H51 imidazole ring to hydrogen bond with the main-chain carbonyl oxygen of S52, thus stabilizing the bulged conformation (Fig. 6). To evaluate the significance of the side-chain hydrogen bond between H51 and D53, we introduced a mutation D53K in β₂M†, which would preclude a hydrogen bonding with H51 while retaining a hydrophilic side chain. After 3 nsec of simulation, strand D in the D53K mutant adopted a bulged conformation that closely resembles the corresponding strand of β₂M₉LA (data not shown). Therefore, at low pH side-chain interactions involving H51, D53, and L65 may be at least partially responsible for the absence of a bulge in

Figure 2. The RMS deviation of main-chain atoms at the end of 3 nsec simulations started from either β₂Mₓ-ray (■) or β₂M₉LA (○). Bars indicate secondary structure in β₂Mₓ-ray corresponding to the strands A, B, C, C’, D (black), E, F, and G.
\(\beta_2M_{\text{X-ray}}\) and increased population of the straight D strand in solution.

**Coupling between pH and strand conformation**

Protein–protein interactions mediated by \(\beta\)-strands can lead to the formation of large protein aggregates (Sinha et al. 2001; Ding et al. 2002; Liu and Eisenberg 2002; Wang and Hecht 2002). Although the conformation of \(\beta_2M\) in amyloid fibrils is not well understood, a limited proteolysis study showed that residues of strand D are protected in the fibrillar form (Monti et al. 2002). Additionally, H/D exchange combined with NMR analysis suggests that only residues from the middle region of the protein (residues 35–75) form the core of the fibrils (Hoshino et al. 2002), while equilibrium denaturation measured by heteronuclear NMR demonstrated that \(\beta_2M\) partially unfolds noncooperatively at low pH while retaining a stable core structure comprising residues from the C, D, E, and F strands (McParland et al. 2002). Both human and bovine isoforms of \(\beta_2M\) have been shown to contain a bulge in strand D, which may be important as deterrent against aggregation during the assembly of the HLA complex as well as after the secretion of \(\beta_2M\) into the bloodstream. As such, the assembly of monomeric \(\beta_2M\) molecules into amyloid fibrils may involve removal of the bulge in strand D to promote intermolecular association.

Of the two alternative conformations of strand D, the straight arrangement seen in the most recent crystal structure may have been facilitated by the lower ambient pH, which differed by as much as two pH units from the pH used to determine other \(\beta_2M\) structures. Whereas \(\beta_2M_{\text{X-ray}}\) was determined at pH 5.7 (Trinh et al. 2002), the HLA structure was obtained at pH 6.5 (Khan et al. 2000), the NMR structure was obtained at pH 6.6 (Verdone et al. 2002), and the bovine \(\beta_2M\) crystal structure was solved at pH 7.8 (Becker and Reeke 1985). It has been shown that the amyloidogenicity of \(\beta_2M\) as well as the morphology of the resulting amyloid fibrils vary with both pH and salt concentration (McParland et al. 2000). Although the natural state of the protein is likely partially unfolded at pH < 4 (McParland et al. 2000; Borysik et al. 2004), the simulations performed here at low pH were not of sufficient duration to observe such unfolding. Interestingly, simulations at 400–450 K resulted in a pH-dependent conformational change.

**Figure 3.** \(\beta_2M_{\text{X-ray}}\) was simulated at medium pH with 100 mM NaCl and snapshots were obtained at different time points: (i) 38 psec, (ii) 638 psec, (iii) 750 psec, (iv) 1.55 nsec, (v) 2.48 nsec, (vi) 3.0 nsec.
in significant unfolding of strand A (data not shown), in accordance with chemical denaturation data (McParland et al. 2000), suggesting that longer simulations may ultimately reproduce a large-scale unfolding of the protein. While limited in their sampling, the simulations at the two different pH conditions are suggestive of the following scenario: As pH is lowered, the straight conformation of the D strand becomes increasingly populated, potentially leading to \( \beta \)-strand-mediated protein aggregation and ultimately to amyloid formation by an undetermined mechanism. Furthermore, they suggest that altered interactions of D53 with H51 and L65 may play a central role in inducing the observed conformational change of the strand. Although previous biophysical studies have shown the amide protons of strand D are protected in the fibrils (Hoshino et al. 2002), it remains to be determined whether the amyloid fibrils formed at low pH are truly edge-aligned aggregates of \( \beta_2 \)M or the protein undergoes further conformational changes before forming fibrils.

Engineered \( \beta \)-sheet proteins involving edge strands without interactions that destabilize intermolecular \( \beta \)-strand hydrogen bonding often aggregate to form amyloid-like structures (West et al. 1999; Lopez de la Paz et al. 2002; Wang and Hecht 2002). Hence, a pH-dependent edge-strand rearrangement presents a model for pH-dependent amyloid formation for \( \beta_2 \)M (McParland et al. 2000). We simulated the spontaneous rearrangement of strand D from a straight to a bulged conformation using molecular dynamics and showed that this rearrangement is pH-dependent. A change in pH may shift the underlying distribution between the straight and the bulged conformations by affecting the relative stability of the two conformations. We compared the energetics of the two conformations, straight or bulged, by computing the intramolecular potential energy \( E_{\text{intra}} = E_{\text{conf}} + E_{\text{vdw}} + E_{\text{elec}} \). \( E_{\text{conf}} \) is the sum of bond, angle, and dihedral energies; \( E_{\text{vdw}} \) and \( E_{\text{elec}} \) are van der Waals and electrostatic energies, respectively. The energies involving the solvent were not included. At medium pH, the bulged conformation has an average \( E_{\text{intra}} = -8224.1 \pm 91.5 \text{ kcal/mol} \), much less than that of the straight conformation, \( E_{\text{intra}} = -8056.8 \pm 86.3 \text{ kcal/mol} \). In contrast, at low pH both conformations have similar energies (\( E_{\text{intra}} = -6637.5 \pm 61.6 \text{ kcal/mol} \) for the bulged and \( E_{\text{intra}} = -6603 \pm 70.5 \text{ kcal/mol} \) for the straight conformation). Although a crude gauge of structural preference, these energetics are consistent with an increased population of the straight conformation at the lower pH.

Although conformational changes that depend on pH have been reported in the literature (Mayans et al. 1997; Su et al. 1998; Blanch et al. 1999), in \( \beta_2 \)M the residues that undergo a conformational change are part of a stable secondary structural element (a \( \beta \)-strand) rather than loop sequences. As such, the protein represents an example in which the backbone of a \( \beta \)-strand can pack differently at different pH values. Interestingly, when \( \beta_2 \)M was studied in solution under the same conditions as used to crystallize \( \beta_2 \)MX-ray, the region including residues 50–57 appeared more dynamic than suggested by the crystal structure, and 20% of the protein formed dimers and higher oligomers despite strong NOE cross-peaks indicative of a bulge (Trinh et al. 2002).

![Figure 4](image1.png)

**Figure 4.** A detailed view of frame vi from Figure 3, illustrating the bulge in strand D.

![Figure 5](image2.png)

**Figure 5.** Two 6-nsec simulations were performed with the intermediate conformation \( \beta_2 \)M‡ (middle) corresponding to frame iii of Figure 3. The simulated pH values were set to either low by protonating Asp, Glu, and His, or medium by protonating His only. (Left) The structure obtained after 6 nsec at low pH was superimposed with the frame i of Figure 3 (two trajectories); (right) the structure obtained after 6 nsec at medium pH was superimposed with \( \beta_2 \)MHLA.
The side chain of H51 can form a H-bond with the side chain of D53, forcing the two residues on the same side of a β-strand and constraining the geometry of the backbone (left), or with the main-chain carbonyl of S52, thus allowing D53 to rotate toward the solvent (right).

Figure 6.

period after each increment. Once the temperature reached 310 K, the simulations were continued for additional 3–6 nsec in 1.5-fsec time steps under constant pressure (1 atm) and temperature (NPT) using periodic boundary conditions. All bonds connecting hydrogens and heavy atoms were held rigid. The electrostatic interactions were implemented using the particle mesh Ewald method with 1 Å or less grid spacing. Nonbonded forces were gradually switched off between 10 and 12 Å and the pair list within 13.5 Å separation was updated every 10 steps (i.e., every 15 fsec). Structures were saved every 0.75 psec for analysis.

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References


