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

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Introduction

Amyloidosis results from abnormal aggregation of native or proteolyzed proteins into amyloid fibrils [1] 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 [2, 3]. In each case, a protein or a proteolyzed fragment aggregates to form unbranched fibrils 10-20 nm wide and hundreds of nm long [4]. 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 [5]. The diversity of the proteins implicated in amyloid diseases [6] 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 [7, 8]. 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 [9-11], β-sandwiches [12], α+β proteins [13, 14], the presence of edge strands makes β-sheet proteins particularly susceptible to aggregation. Human β–2 microglobulin (β_2M), 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 [15-21]. The protein is routinely secreted into the blood stream as part of its catabolic cycle. Most patients undergoing hemodialysis eventually develop dialysis-related amyloidosis due to an accumulation of β_2M in the serum [22]. The monomeric structure of the protein has been determined using both X-ray crystallography [23] and NMR [24]. These crystallographic ($\beta_2M_{X\text{-}Ray}$) and solution (β_2M_{NMR}) structures are highly similar, and both consist of seven β strands (A-G) grouped in two antiparallel β-sheets (Fig. 1). There are also several differences between the two structures. Most notably, in β_2M_{NMR} one of the edge strands (strand D) has a pronounced bulge, whereas the corresponding strand in $\beta_2M_{X\text{-}Ray}$ does not contain the bulge. Similarly, the

crystal structure of β_2M in complex with the HLA heavy chain (β_2M_{HLA}) [25] shows a bulge in strand D involving D53 and L54. Thus strand D of β_2M 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 [26]. As a result the native conformations often must first be destabilized through mutation [27, 28] or exposure to environmental stress [19] in order for β-sheet proteins to become amyloidic. This is consistent with the observation that most proteins require partial denaturation to become amyloidic [19, 29-36]. Biochemical and spectroscopic studies have suggested that the D strand of β_2M may be directly involved in amyloid formation [15, 37-39]. Trinh et al. proposed that the conformation with a straight edge of the D strand may correspond to a rare species trapped by crystallization [23]. 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 β_2M is not known, the protein may be coaxed to form amyloids *in vitro* by reducing the pH of the solution to pH = 3.6 [18, 40] or by adding Cu^{2+} ion to the buffer [41, 42]. The structure of monomeric $\beta_2M_{X\text{-}Ray}$, with its straight edge strand, was determined at pH = 5.7 [23], while that of β_2M_{NMR} was determined at pH = 6.6 [24]. Given the amyloid forming properties at low pH and the straight strand observed in $\beta_2M_{X\text{-}Ray}$, 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 [43-47]. In the present study, molecular dynamics simulation was used to investigate how the conformational properties of β_2M may be modulated by pH. To examine the structural response of β_2M 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 β_2M may suggest a mechanism by which low pH predisposes the protein for amyloid formation.

Figure Captions

Figure 1

Two crystal structures of β_2M. (Left) β_2M_{HLA} corresponds to the structure of β_2M in the HLA complex determined to 1.8 Å resolution (PDB: 1DUZ). (Right) $\beta_2M_{X\text{-}Ray}$ was determined as a monomer, also to 1.8 Å (PDB: 1LDS).

Figure 2

The rms deviation of main chain atoms at the end of 3 ns simulations started from either $\beta_2M_{X\text{-}Ray}$ (—■—) or β_2M_{HLA} (- - ❡ - -). Bars indicate secondary structure in $\beta_2M_{X\text{-}Ray}$ corresponding to the strands A, B, C, C', D (black), E, F and G.

Figure 3

$\beta_2M_{X\text{-}Ray}$ was simulated at medium pH with 100 mM NaCl and snapshots were obtained at different time points: (i) 38 ps, (ii) 638 ps, (iii) 750 ps, (iv) 1.55 ns, (v) 2.48 ns, (vi) 3.0 ns.

Figure 4

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

Figure 5

Two 6 ns simulations were performed with the intermediate conformation $\beta_2M^{\ddagger}$ (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 ns at low pH was superimposed with the frame (i) of Figure 3 (two trajectories). (Right) The structure obtained after 6 ns at medium pH was superimposed with β_2M_{HLA}.

Figure 6

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

Figure 1

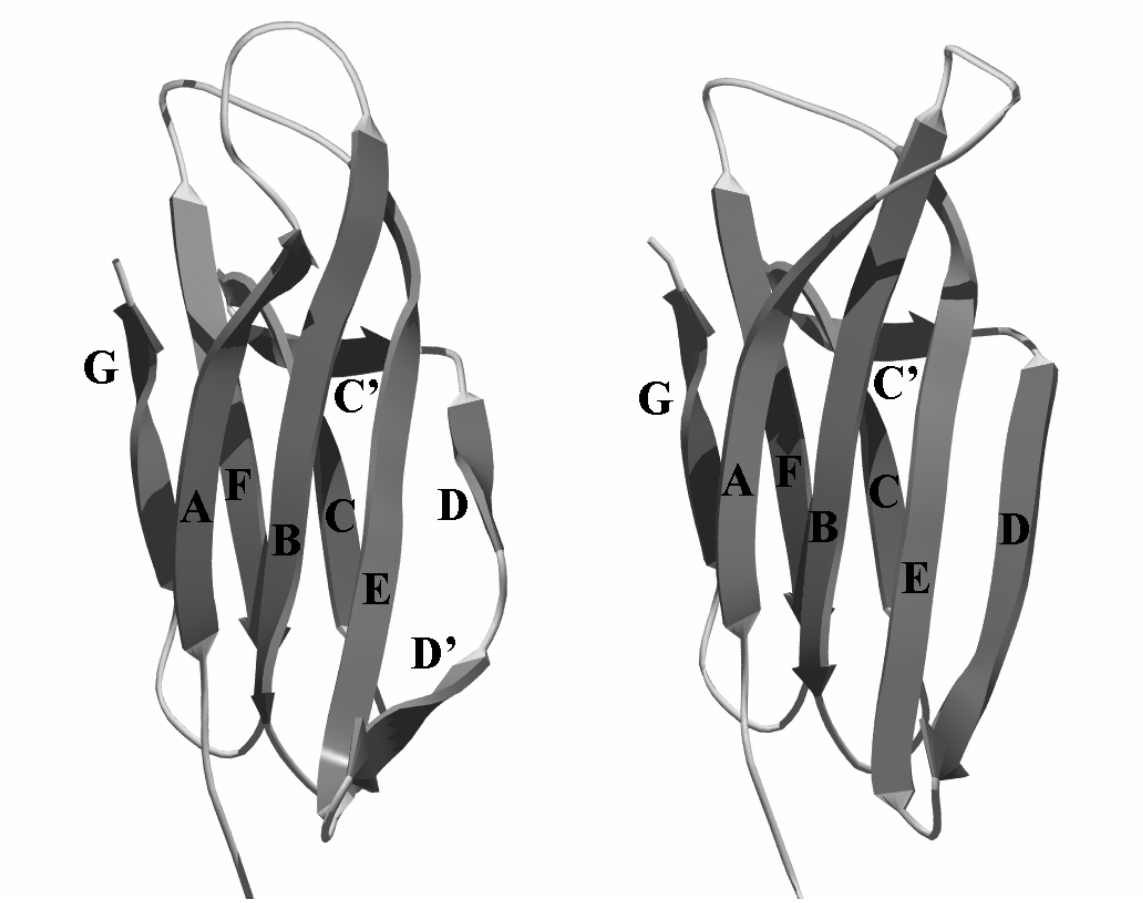

Figure 2

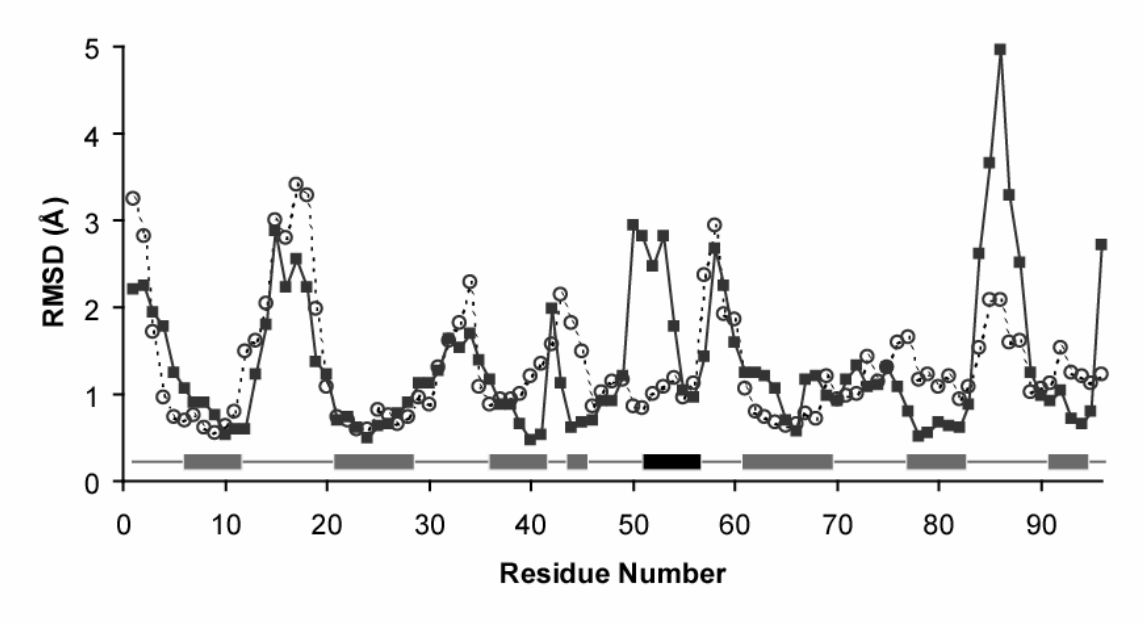

Figure 3

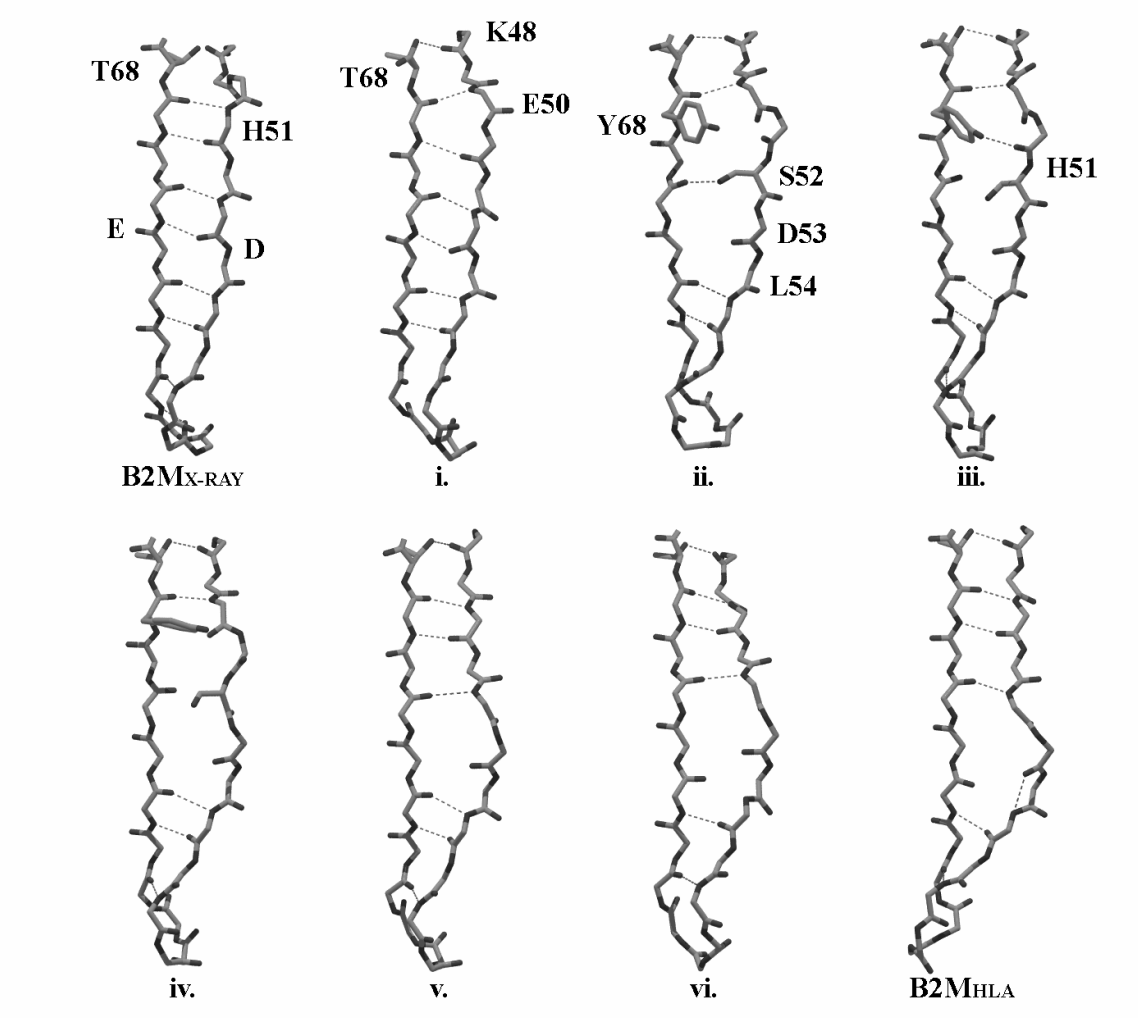

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Figure 4

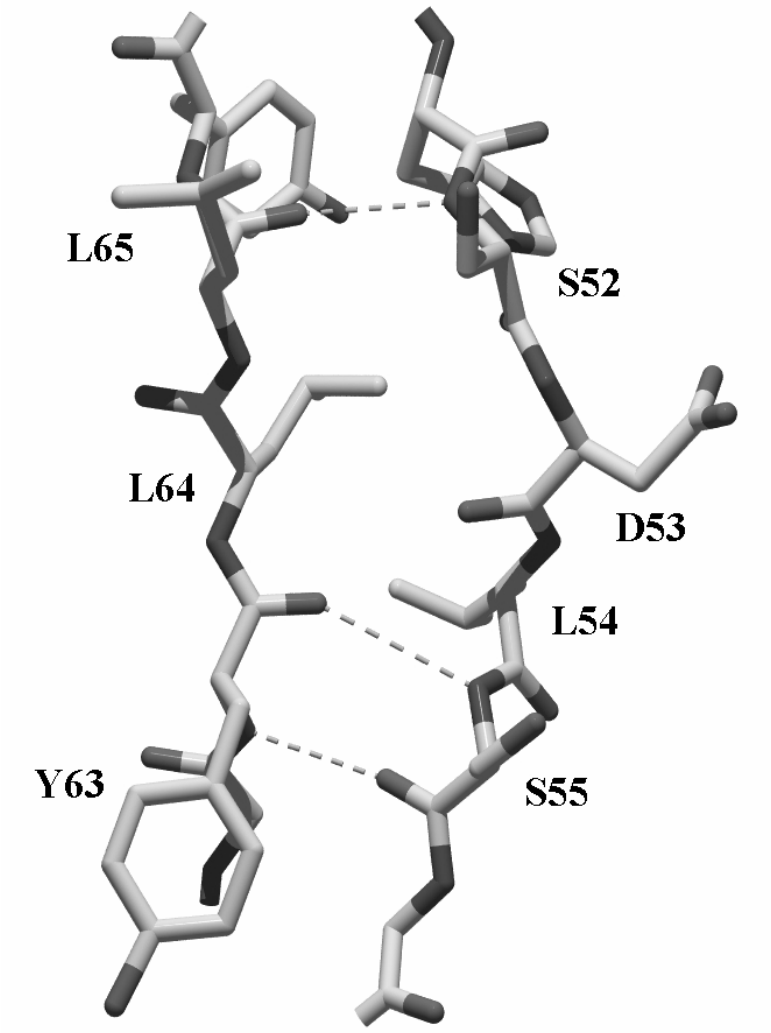

L65
S52
L64
D53
L54
Y63
S55

Figure 5

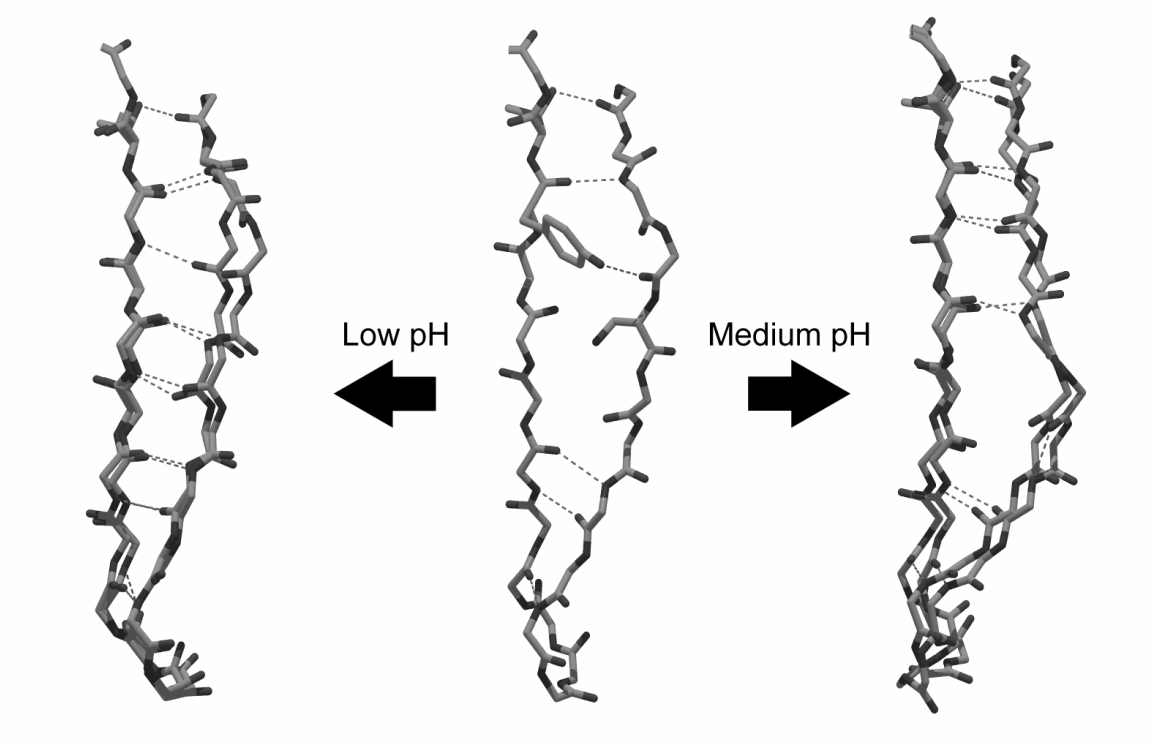
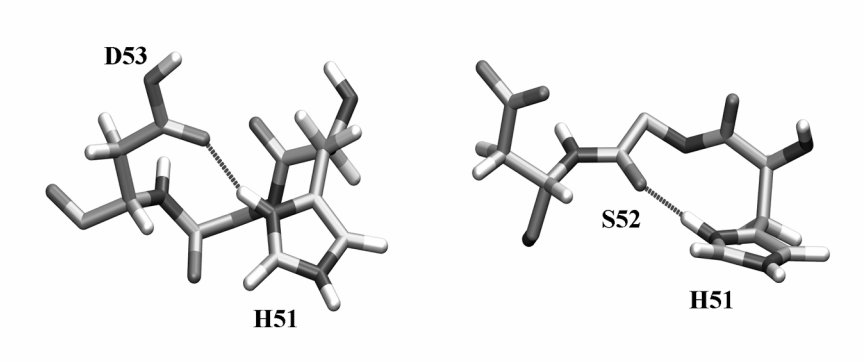


Figure 6



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