Metal binding sheds light on mechanisms of amyloid assembly

Matthew F. Calabrese and Andrew D. Miranker*

Department of Molecular Biophysics and Biochemistry; Yale University; New Haven, CT USA

Abstract: 

β-2 microglobulin (β2m) is the protein responsible for amyloid deposition in Dialysis-Related Amyloidosis (DRA). Aggregation can be induced by various solution conditions including exposure to divalent metal, incubation at acidic pH, and limited proteolysis. Using Cu2+ as a trigger, we have trapped, isolated, and crystallized a stable oligomer of β2m that is populated under amyloidogenic solution conditions (Calabrese et al. Nat Struct Mol Biol 2008; 15:965–71). This structure reveals that Cu2+-binding is associated with dramatic conformational rearrangements. This has allowed us to postulate a set of structural changes common to all β2m aggregation pathways. Cu2+ serves as a potential trigger in other aggregation systems such as αβ2m, α-synuclein, and mammalian Prion (PrP). A comparison of Cu2+ binding to β2m and PrP reveals common features. Therefore, in addition to providing insight into DRA, induction of structure by Cu2+ binding appears to be a recurring structural motif for pathological changes in conformation.

Key words: amyloid, protein folding, dialysis-related amyloidosis, copper, β-2 microglobulin, prion

β2m is the 99 residue, 12 kDa non-covalent light chain of the Class I Major Histocompatibility Complex (MHC). Its function is to ensure proper folding and cell-surface expression of the MHC.1 In the course of normal turnover, β2m is released to the serum and catabolized by the kidneys. In patients suffering from end-stage renal disease who are treated with hemodialysis therapy, β2m levels rise and amyloid plaques develop throughout the joints and connective tissue.2 Although an elevated serum level of β2m may be necessary for aggregation, it is not sufficient as β2m remains stably folded at > 1 mM concentration at 37 °C.3,4 Furthermore, it can be reversibly folded in vitro5-7 and amyloid is not reported in other diseases with elevated levels in serum.8,9 Therefore, aggregation must be triggered by features unique to hemodialysis therapy.

As β2m exists as a stable monomer under near physiological conditions,3,10 much effort has been focused on understanding the molecular mechanism by which self association is triggered. Many approaches have been used to induce β2m aggregation. These include limited proteolysis,11 incubation at acidic pH,12 and exposure to detergents or organic solvents.13,14 In 2001, we discovered that β2m is a Cu2+-binding protein.4 Whereas β2mapo is not amyloidogenic, β2mholo is aggregation-prone.

Cu2+ Mediated Oligomerization

We are interested in Cu2+-dependent aggregation of β2m for several reasons. First, hemodialysis patients may be exposed to elevated levels of divalent cation as a consequence of therapy. This is apparent in the historical use of Cu2+ in the preparation of dialysis membranes, and in water quality standards which permit as much as 1.6 μM Cu2+ in hemodialysate.15 This is comparable to the ~3 μM affinity of β2mholo.4 Second, early experiments revealed that although Cu2+ is necessary to initiate aggregation, mature fibers remain stable in the presence of a metal chelate.10 This allowed the possibility that Cu2+ action is transient and that periodic exposure to Cu2+ during dialysis therapy may be sufficient to induce aggregation. Finally, the use of an amyloidogenic trigger that operates through a defined binding event provides an opportunity for particularly clear insight into the structural rearrangements which may permit self-association.

β2m binds Cu2+ selectively over other divalents including Ca2+, Zn2+ and Ni2+ also bind, but with much weaker affinities.4,16 Importantly, the binding of Cu2+ but not Ni2+ leads to self-association.10 We have previously asserted that at least three modes of Cu2+-binding may exist for β2m. One is based on coordination to non-native states and is linked to protein destabilization.10 A second involves Cu2+-bound to the monomer in an initial capture complex, and a third represents Cu2+ bound to oligomeric states. Previous studies have implicated His13, His31, and His51 as well as the N-terminus in native-state metal binding.16-19 Of the three histidines above, our own studies have shown that only mutation of His31 affects native-state Cu2+ affinity.16 Continued incubation
Metal binding sheds light on mechanisms of amyloid assembly

We therefore employed mutagenesis to generate a more uniform population. Mutations were targeted based on predicted interfaces and a single point mutant, H13F, was sufficient to increase both the extent and homogeneity of the oligomerization reaction, resulting in efficient formation of a hexamer. Nevertheless, the global stability, Cu\(^{2+}\) affinity, and rate of oligomerization remain similar for H13F and WT \(\beta_2m\).\(^{16,25}\)

The hexamer is organized as a closed ring surrounding a solvent-filled central channel. The ring is three-fold symmetric demonstrating the existence of two distinct classes of interface (Figure 1). Within each hexamer, six Cu\(^{2+}\) atoms are bound consistent with the binding stoichiometry observed in solution.\(^{23,25}\) However, contrary to our initial expectation, metal does not localize to interfaces and does not bridge adjacent subunits. Rather, Cu\(^{2+}\) binding is entirely intramolecular where all coordinating ligands are derived from a single polypeptide chain. Investigation of the Cu\(^{2+}\)-binding site reveals a roughly square-planar geometry comprised of an imidazole ring from His31 together with two amides and a carbonyl derived from the peptide backbone (Figure 2A). Interestingly, this site bears a striking resemblance to a previously observed Cu\(^{2+}\) coordination in the octapeptide region of PrP (Figure 2B).\(^{26}\)

Our work suggests the presence of several structurally related pre-amyloid structures.
Metal binding sheds light on mechanisms of amyloid assembly

binding sites. Initially, Cu$^{2+}$ is captured by monomeric $\beta$2m with minimal perturbation to the apo structure. This is followed by binding to reversible oligomeric species. Ultimately, Cu$^{2+}$ is released from a binding site weakened by conformational changes associated with irreversible aggregation. The structure we have recently reported describes the Cu$^{2+}$ binding site within the context of an oligomer. Formation of this site requires disruption of the first $\beta$-strand and local rearrangements proximal to His31. This includes rotation of Phe30 from the hydrophobic core to face solvent, and the cis-trans isomerization of Pro32. These rearrangements cannot be accommodated in isolation, and require a set of compensatory rearrangements that propagate across the molecule (Figure 3). In all, within the context of the hexamer, 26 of the 99 residues of $\beta$2m show large deviation (> 2 Å rmsd) from the position in the apo protein. Cu$^{2+}$ binding may direct some but not all of these rearrangements. The energy of oligomerization may additionally stabilize alterations.

Summary

The conversion of soluble protein to amyloid plaques requires structural perturbations to a protein’s native state. For $\beta$2m, these perturbations can be triggered by covalent modification or altered solution conditions including exposure to Cu$^{2+}$. Using Cu$^{2+}$ as a tool, we have gained unique insight into the detailed changes that initiate self association (Figure 3). Most striking, the trans conformation at Pro32 and the dramatic rotation of Phe30 from the hydrophobic core toward solvent appear to be a critical switch enabling aggregation. This rearrangement may occur in $\beta$2m refolding, proteolytic processing, and acid-induced restructuring. This suggests that all $\beta$2m aggregation pathways converge at an activated state similar to M*. Indeed, an M*-like state can be mimicked by mutation of Pro32.5,6,20 The atomic structure of one such mutant, P32A, reveals a set of rearrangements that are highly similar to those observed in the Cu$^{2+}$-bound hexamer.20,25

In addition to elucidating common themes in $\beta$2m aggregation, several gross similarities to PrP are apparent. PrP binds Cu$^{2+}$ in vivo and this binding has been suggested to play a role in functions including metal homeostasis and response to oxidative stress. Although no such assertion has been made for $\beta$2m, it is intriguing to note that Cu$^{2+}$ binding is specific, and the coordinating histidine and many of the residues that rearrange upon metal binding are conserved.16 For both $\beta$2m and PrP, metal binding can occur in several possible geometries leading to protein destabilization, structural rearrangements, and self-association.4,10,16,30-32 For PrP, multiple modes of metal binding have been determined using electron paramagnetic resonance spectroscopy.30 For $\beta$2m the presence of unique binding modes has been inferred based on the ability of $\beta$2m to bind Cu$^{2+}$ under both native and destabilizing conditions as well as changes in metal affinity and chelate sensitivity during the course of aggregation.16,20,25

At least one binding mode for the octapeptide region of PrP bears a strong resemblance to the Cu$^{2+}$ site in the $\beta$2m hexamer (Figure 2).25,26 Although PrP binds Cu$^{2+}$ in vivo and undergoes alterations in structure, the role of metal binding in both function

Figure 3. Unified model of $\beta$2m amyloid formation: Under physiological conditions, $\beta$2m exists as a stable, well folded monomer (upper panel, PDB 2CLR) characterized in part by a conserved cis proline at residue 32. Upon exposure to a variety of amyloigenic triggers the native structure is perturbed and amyloid formation commences. We have shown (Cu$^{2+}$ or P32X mutation) or conjectured (limited proteolysis or acidic pH) that this transition involves rotation of Phe30 from the hydrophobic core toward solvent and the cis-trans isomerization of Pro32.20,25 We postulate that all pathways converge on a state resembling the activated monomer (M*) in which broad rearrangements occur to compensate for the cavity left by movement of Phe30. These rearrangements precede oligomerization which terminates in a hexamer.10,20,25 The path from oligomer to mature aggregate is not known, however, as the hexamer represents a closed state, aggregation likely requires a ring-breaking event.
and aggregation remains unclear. For example, one report suggests that Cu^{2+}-binding promotes protease-resistance as seen in disease strains33 while others indicate that exposure to Cu^{2+} inhibits aggregation.34 These reports, although apparently contradictory in nature, may result from the structural diversity that can result from different modes of metal-interactions.35 In addition, PrP, like β2m, may have a set of closely related mechanisms for the induction of pathological aggregation with metal cations acting to bias the sampling of alternative states. The ability of metal to perturb structure appears to be a general theme in protein aggregation. Recent work suggests that self-association in diverse systems including Aβ, aggregate.36-38 α-synuclein,39,40 immunoglobulin light chain41, and superoxide dismutase42 may all be modulated by metal. A more thorough understanding of the structural consequences of Cu^{2+} binding in systems such as β2m may therefore provide broader insight into both function and disease associated with aggregation.

Acknowledgements

We thank D.V. Blaho for helpful comments. This work is supported by NIH DK54899.

References