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Minireview

Glimpses of the molecular mechanisms of β_2-microglobulin fibril formation in vitro: Aggregation on a complex energy landscape

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ABSTRACT

β_2-microglobulin (β_2m) is a 99-residue protein that aggregates to form amyloid fibrils in dialysis-related amyloidosis. The protein provides a powerful model for exploration of the structural molecular mechanisms of fibril formation from a full-length protein in vitro. Fibrils have been assembled from β_2m under both low pH conditions, where the precursor is disordered, and at neutral pH where the protein is initially natively folded. Here we discuss the roles of sequence and structure in amyloid formation, the current understanding of the structural mechanisms of the early stages of aggregation of β_2m at both low and neutral pH, and the common and distinct features of these assembly pathways.

1. Introduction

The aggregation of proteins into amyloid fibrils has been studied in detail for more than 50 years, yet elucidation of the exact structural mechanisms of this process still provides a considerable challenge [1]. The heterogeneous nature of the protein aggregation landscape contributes to the complexity of this problem. Indeed, even at the earliest stage of this process the monomeric precursors of amyloid formation can be found as a complex mixture that can include natively folded, partially folded and highly unfolded protein species, any one of which could initiate the aggregation process. Furthermore, since the inter- and intra-molecular interactions that are involved in aggregation and protein folding are similar, much work remains to improve our understanding of the competition between these events, especially at the initial stages of amyloid formation (Fig. 1A) [2]. Moreover, multiple practical challenges exist as the formation of fibrils is stochastic and many of the species populated en route to the fibril product are unstable and only transiently formed. Understanding the biophysical nature of amyloid formation, however, is an important goal, not only because a number of diseases involve the deposition of amyloid fibrils [3], but also because the ability to form amyloid is known to be a fundamental property of all polypeptide chains under the appropriate conditions, independent of sequence [4]. However, the manner in which the generic cross-β structure of amyloid is accommodated by the differing sequences of proteins within their fibrillar architecture is not yet understood.

To comprehend the process of amyloid fibril formation in atomistic detail much work has been performed on a range of proteins in vitro. Here, we review some of the techniques employed and the results obtained in the study of the structural and molecular mechanisms of fibril formation of the protein β_2-microglobulin (β_2m). This protein folds natively into a β-sandwich fold consisting of two β-sheets, one containing four strands (ABED) and the other three (CFG) (Fig. 1B) [5]. A disulphide bond between two cysteines (residues 25 and 80) covalently links these sheets [5]. Intact wild-type β_2m is the major component of amyloid fibrils deposited in the joints of patients on long-term haemodialysis, in a condition known as dialysis-related amyloidosis (DRA). In vivo, β_2m is the non-covalently attached light chain of the human major histocompatibility complex 1 (MHC1) [5]. Naturally, β_2m is shed into the serum and degraded by the kidneys, maintaining the serum β_2m concentrations at 0.09–0.17 μM in healthy individuals [6]. However, in patients with renal failure the haemodialysis membrane is unable to remove β_2m, resulting in increases in the serum concentration by up to 60-fold [7]. This rise in concentration alone is deemed not to be sufficient for fibril formation [8]. Thus other, as yet unknown, contributing factors lead to deposition of the full-length β_2m as amyloid fibres in the synovium of joints.

Abbreviations: DRA, dialysis-related amyloidosis; NMR, nuclear magnetic resonance; SEC, size exclusion chromatography

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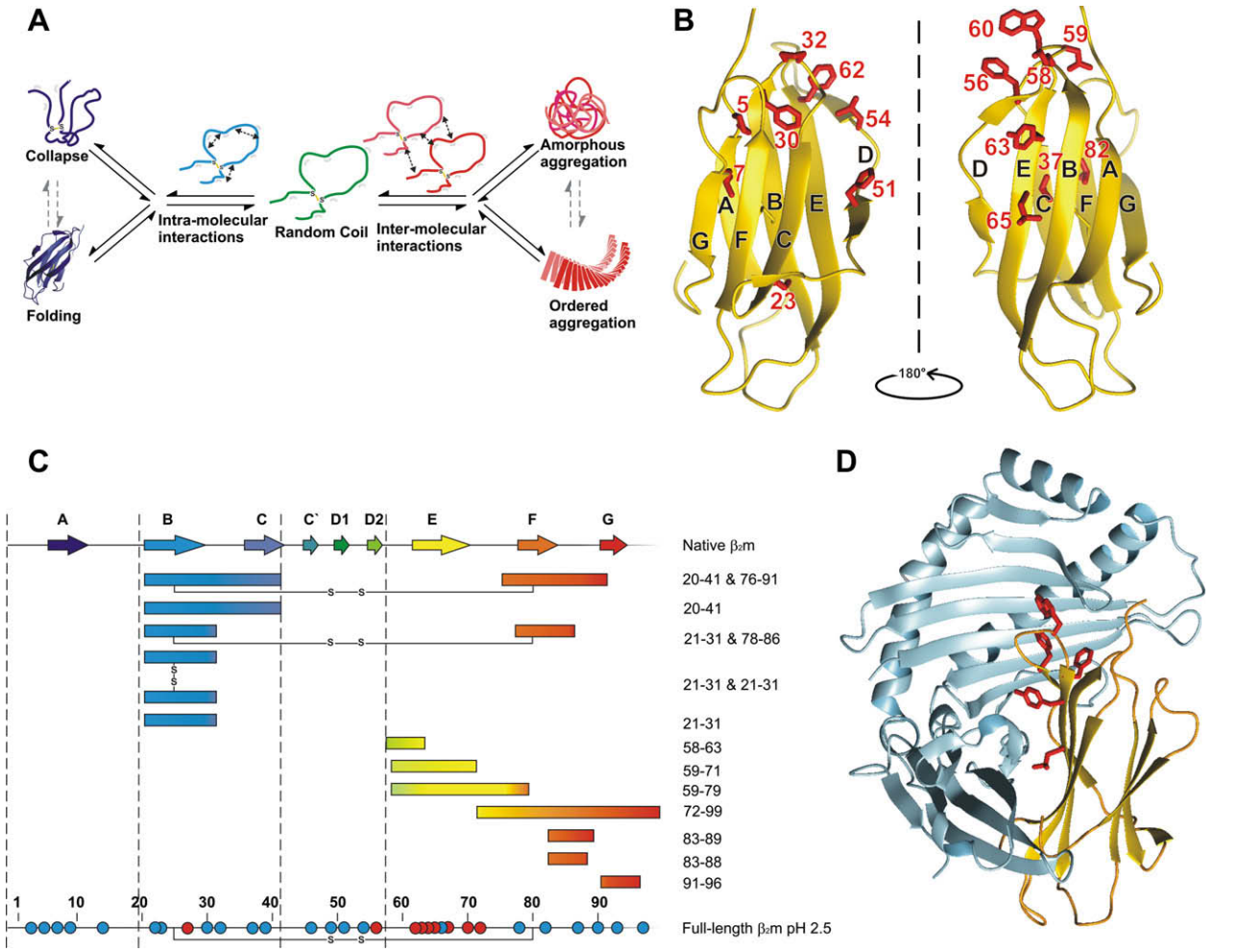

Fig. 1. (A) Schematic illustration of the competition between intra-molecular and inter-molecular interactions in protein folding and assembly. When intra-molecular interactions prevail, proteins fold or form collapsed states (left side). By contrast, when inter-molecular interactions dominate, protein aggregation results (right side). (B) Monomeric β_2m with residues discussed herein highlighted. (C) Amyloid forming properties of the sequence of β_2m when isolated as peptides [10–15], or in the context of the full-length protein (the latter at pH 2.5). Red circles indicate residues that affect fibril formation kinetics, blue circles indicate positions where changes had little or no effect on kinetics [19]. (D) Structure of β_2m (gold) in the MHC-1 complex. Hydrophobic residues present in the β-strand E region of β_2m (Phe56, Trp60, Tyr62, Tyr63 and Leu65) that contact the heavy chain of the MHC-1 are highlighted.

Here we consider the current state of research into the mechanism of β_2m fibril formation in vitro. We discuss the fibril formation pathways under different conditions, how they may be related to one another and to the mechanism occurring in vivo; and how the amyloid forming potential of β_2m is modulated by sequence and structural changes.

2. Sequence versus structural determinants of fibril formation

Studies using prediction algorithms and peptide models have indicated that ~60% of the sequence of β_2m is highly amyloidogenic (Fig. 1C) [9–15]. However, the innate amyloid forming potential of the polypeptide sequence of β_2m is modulated by structure, as the natively folded protein is impervious to aggregation [8,16]. At least a partial unfolding event must therefore take place in vivo to allow exposure of one or more aggregation-prone region(s) of the sequence to initiate amyloid formation. To study fibril formation of β_2m in vitro, many destabilising conditions have been applied, by adding co-solvents, detergents or denaturants at neutral pH or by reducing the pH to drive the aggregation process on a biophysically feasible timescale [17].

At low pH (<pH 3.0), low ionic strength (⩽50 mM) and encouraged by agitation, β_2m spontaneously aggregates in vitro to form fibrils with all of the hallmarks of amyloid [16]. Kinetic analyses of fibril growth coupled with a mutational screen have indicated that a single region approximately 10 residues in length (~60–70) is important for determining the rate of fibril nucleation and elongation of the full-length protein under these conditions (Fig. 1C) [18,19]. This region of the sequence is enriched in aromatic residues and is predicted to be highly aggregation-prone by amyloid algorithms [9]. Other studies of the full-length β_2m sequence at low pH have indicated that its ability to form amyloid is related to the stability of the fibrils and that introduction of the β-sheet breaking amino acid, Pro, in place of certain residues, especially Leu23, His51 or Val82, causes a reduction in fibril elongation kinetics [20]. Comparison of the effects of sequence alteration on the fibril growth kinetics of the intact, oxidised protein at low pH with the results of peptide studies is striking. While fragments corresponding to three regions (residues 20–40, 60–70 and ~80–99) all form amyloid in isolation [10–15,19], in the context of the full-length protein chain mutation of residues in only one region (~60–70) alters the fibril formation kinetics (Fig. 1C) [18,19]. One explanation of this observation is that structure in the initial denatured state may modulate the amyloid potential of the polypeptide chain, even when the initial amyloid precursor is highly unfolded (Fig. 1A) [19]. Indeed, nuclear magnetic resonance (NMR) analysis