Abstract

The importance of unstructured termini in the aggregation cascade of beta-2-microglobulin: insights from molecular simulations of D76N mutant

M. Machuqueiro, M. Novo, E. Shakhnovich, P. Faisca

Introduction

The identification of a folding intermediate state of the protein beta-2-microglobulin (β2-m) that is also able to trigger the aggregation cascade [1] [2] showed that folding and aggregation may be competing processes, and that the identification of intermediate states for folding and aggregation is essential both to understand the mechanisms of protein aggregation and to design new therapeutic strategies targeted at conformational disorders.

A well-known example is dialysis related amyloidosis (DRA) [3], a fatal condition that affects ~750000 individuals worldwide with chronic renal failure undergoing long-term hemodialysis, characterized by the deposition of β2-m amyloid fibrils in the osteoarticular tissues.

Recently, a single point mutant of β2-m (D76N) was identified as the causing agent of a hereditary systemic amyloidosis affecting visceral organs [4]. This mutant is considerably more amyloidogenic in vitro at physiological conditions than the wild-type form, thus representing a biologically motivated model for studying β2-m aggregation.

Our goal is to predict the early molecular events of the aggregation mechanism of β2-m using the D76N mutant as a model system. Are there aggregation-prone intermediates populating its folding pathway? What are the main pathways for dimerization? Which residues are critical for the initiation of the aggregation cascade?

Results

Folding thermodynamics and intermediate states

The main structural trait of the conformer is an unstructured and detached C-terminus.

Dimerization mechanism

D76N dimerization hot spots

Residues Arg-3, Tyr-10 and Arg-12 in N-terminus, Phe-30 in BC-loop, Phe-56, Trp-60 and Phe-62, Lys-75 in EF-loop and Trp-66 in C-terminus are hotspots in D76N dimerization.

Comparison with experiment

PhD Thesis: PhD in the DE-loop are designed dimerization spots because they assist the docking of βm onto the MHC-I heavy chain [5].

Several experimental studies have also highlighted the role of the above residues in the DE-loop in β2-m oligomerization [6, 7].

Residue Tyr10 in the strand A [8] have been proposed to be important in β2-m dimerization.

Several reports have provided evidence of the a highly dynamic nature of strand A [9, 10]. An unstructured strand A implicated in βm fibrillogenesis has been reported in [8].

Methods

Monte Carlo Ensemble Docking

We study the initial stage of the de novo aggregation mechanism of D76N by using a three-stage approach based on a plethora of computational tools. We start with I) replica-exchange Monte Carlo simulations of a fully atomistic native-centric Gō model [11] to identify intermediate states in the folding pathway of D76N. We next employ explicit constant-pH molecular dynamics (CpHMD) [12] to investigate up to which pH variations may affect the structures of the identified intermediates. By focusing on the dimerization process, which has been reported to be the first step of βm fibrillogenesis, we ultimately deploy III) Monte Carlo ensemble docking [11] to determine how the dimerization proceeds for the different intermediates at several pH conditions. We significantly improved this method to construct a cost function based simultaneously shape, hydrophobicity, electrostatics and hydrogen bonding complementarily, an evolution to the relation to original cost function exclusively based on shape complementarity.

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References


