From unfolding intermediates to amyloid protofibrils: the beginning of light chain amyloidosis

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Light chain amyloidosis (AL) is a misfolding disease characterized by the extracellular deposition of immunoglobulin light chains (LCs) as insoluble aggregates [1]. Among the LC families, lambda 6a is frequent in AL patients [1]. Its germline protein (6aJL2) and point mutants (R24G and P7S) are good models to study fibrilllogenesis, because of their stability and fibril formation kinetics are known [2,3]. In addition, acidic pH and the presence of residual secondary structure favor fibril formation in other members of this family [4]. Although many of the clinical aspects of this pathology are known, the molecular mechanism of aggregation remains unclear. The conformational changes resulting in LC intermediates capable to form fibrils have not been characterized at the atomic level. At the very least, these should involve eliminating the anti-aggregation motifs [5] and rotating the β sheets so the β strands become parallel [6]. In order to gain understanding of the effect of mutations and low pH on the dynamics and unfolding of these proteins, we have performed molecular dynamics simulations at neutral and low pH, and at increasing temperatures (298, 398, 448, and 498K). This approach allowed us to sample the conformational landscape, to find intermediates able to form fibrils. We found that mutations and low pH compromise the stability of the anti-aggregation motifs leaving the edges of the β sandwich unprotected, each by a different mechanism, though. Point mutants modify the contact networks and hydrogen bond patterns surrounding the mutation and extend across the protein, affecting the CDR1 and the C'-C'' protective loop. At low pH, the most noticeable effect is the destabilization of the loop connecting strands E-F, close to C-terminus of the protein, allowing water access to the hydrophobic core. From high temperature simulations, we identified unfolding intermediates that are similar to the native conformation while having the sides of the β sandwich denatured. We quenched these at 298K and they held their structured β core during 0.5 μs of simulation time. One of the intermediates of the 6aJL2 protein was stable enough to be extended into a dodecameric protofibril model with two isomeric forms. Both protofibril models were simulated for 1 μs, allowing their structural and dynamic characterization. We found molecular hallmarks that lead to the amyloidogenic pathway in these proteins. Also, this is the first time that a protofibril model that would lead to the correct cross-beta diffraction pattern is proposed for this family of proteins.

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Acknowledgments: CONACyT (PhD scholarship 267623, CB-2009-133294 and INFR-2014-02-231504), and the Laboratorio Nacional de Supercómputo del Sureste de México, CONACYT.