CONFORMATIONAL FLEXIBILITY OF β2-MICROGLOBULIN: A COMPROMISE BETWEEN EFFICIENCY AND AGGREGATION RISK

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The conformational flexibility of the loop region containing Trp60 is likely to play a key role in the early steps of fibrillogenesis of β2-microglobulin (β2-m), the protein responsible for dialysis related amyloidosis (DRA).
This conclusion arises from the whole body of experimental evidence obtained by comparing wild type and W60G β2-m. The interest in this single-point mutant was first stimulated by inspection of molecular dynamics trajectories at the early steps of β2-m aggregation: the intermolecular contacts captured by the simulation snapshots suggested that Trp60 should play a most relevant role together with nearby N and C-terminal residues.
The recombinant W60G mutant was expressed and its structure was shown, by NMR spectroscopy, to maintain the general wild type folding. The mutant species exhibited, however, a higher thermodynamic stability compared to wild type, and no fibril formation under mild fibrillogenic conditions, *i.e.* 20% TFE neutral aqueous solution in the presence of seeds.
In order to explain this striking result, ^{15}N relaxation measurements were performed to assess structural mobility. A lower value of the overall correlation time (τ_c) was estimated for the mutant β2-m, consistent with faster rotational dynamics with respect to wild type protein, probably due to a larger association extent of the latter. In addition, but most intriguingly, several residues in the region around Trp60 (DE loop) were observed to undergo a slow time-scale conformational exchange in wild type β2-m that proved substantially absent in W60G β2-m. Given the observed fibrillogenesis attitudes, it was argued that the "risky" conformational flexibility of the natural β2-m sequence may be a necessary compromise to enforce proper affinity with the α-chain of class I MHC, at the price of partial unfolding danger. This unavoidable condition should be eventually responsible for pathological aggregation of β2-m at non-physiological high concentrations.
Binding assay confirmed that mutant W60G has a much lower affinity for the heavy chain of MHC-I than wild type protein. Besides the loss of Trp60 indole specific interactions, also conformational rigidity should conceivably determine the observed affinity decrease.
The consistency of different lines of experimental data suggests that our interpretation of β2-m conformational plasticity may provide new insights for DRA therapies.