Title:	Study of oligomerization of human β2 microglobulin
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Abstract:	We found β2 microglobulin (β2m), the causative protein of Dialysis Related Amyloidosis (DRA), forms oligomers when it was loaded with non-reducing sample loading buffer in an SDS-PAGE. Upon treating the sample with reducing sample loading buffer, these oligomers dissociated into monomers, indicating that the intermolecular disulfide bonds might be responsible for its oligomerization. This result was confirmed by further experiments with di-cysteine mutants, which, as anticipated, didn't show any oligomerization. We have hypothesised that due to the burial of cysteines in β2m's native structure, we were not able to observe any higher-order oligomers. Hence, in order to enhance the propensity of formation of the intermolecular disulfide bonds, we decided to carry out the purification under denaturing conditions (with 8M urea), so as to expose the buried cysteines. As expected, we found a profound increment in intensity and the number of higher-order oligomers, which, we propose, could be used as a protein ladder (for SDS PAGE). We next thought to enrich these higher-order oligomers by crosslinking the lower order oligomers with glutaraldehyde, which would lock the formed oligomers and drive the equilibrium towards more populated lower-order oligomers, which in turn increase the probability of molecular collision between them to form more higher-order structures. Surprisingly, we found that glutaraldehyde was not able to crosslink β2 microglobulin. Further, we tracked the formation of these oligomers during the denaturing purification and found that they are forming just after the lysis of the cell. In a different study, our lab has shown that β2 microglobulin forms amorphous aggregates in presence of Ca2+, which, if incubated for 3-4 weeks, gets converted into amyloid aggregates. In order to check if Ca2+ is enhancing these disulfide-linked oligomers, we loaded these Ca2+ induced amorphous aggregates with non-reducing sample loading buffer in an SDS-PAGE. However, the SDS-PAGE revealed a single band corresponding to monomer, indicating a totally different nature of these Ca2+ induced oligomers. In order to further characterize these Ca2+ induced oligomers, we monitored intrinsic tryptophan fluorescence, ANS binding, and intrinsic blue fluorescence, both in presence and absence of Ca2+. We have been able to show the exposure of some hydrophobic patches upon Ca2+ binding, which we propose to be the initial driver of Ca2+ induced β2m self-assembly. Additionally, to check if β2 microglobulin phase separates into liquid condensates on its pathway to amorphous aggregates, we performed confocal imaging just after the addition of Ca2+, which showed mesh-like networks eliminating Liquid-Liquid Phase Separation (LLPS) of β2m.
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