52d An Improved Model of Non-Native Protein Aggregation Kinetics

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Non-native aggregation is a ubiquitous problem during protein and polypeptide product and process development. It has also garnered significant interest from the medical and life science communities due to its possible role in a number of chronic diseases. Although much has been learned at a qualitative level about the mechanism of non-native aggregation over the past three decades, a number of fundamental questions remain unanswered due to difficulties with isolating or identifying key intermediates. As with chemical reaction networks, quantitative, kinetic models based on proposed mechanisms offer an attractive means to gain insights into these intermediates. A general model of aggregation kinetics – one capable of describing a broad range of qualitatively different aggregation behaviors within the same self-consistent framework – has not yet been developed. Such a model would be a valuable tool to enable quantitative, mechanistic comparison of the aggregation behavior of different proteins. It would also aid in designing small-scale, in vitro experiments to better predict large scale as well as in vivo behavior. As a step towards a general model of non-native aggregation, we propose an improved version of extended Lumry-Eyring (ELE) models that expands upon previous work (Roberts, C. J., J. Phys. Chem. B, 107 (2003) 1194.). The improved ELE model accounts not only for "monomer" folding, unfolding, and irreversible association events, but also includes: reversible association between non-native monomers ("nucleus" formation); reversible association steps involving monomers, small oligomers, and larger aggregates; and structural rearrangement events within oligomers and larger aggregates that we hypothesize are required to commit a (non-native) protein chain to the net-irreversible aggregation pathway. It is found that a number of other, previously proposed, models can be shown to be limiting cases or simplified versions of the model proposed here. In addition, the improved ELE model is able to capture recent experimental data for the aggregation of a-Chymotrypsinogen A (aCgn) that cannot be described even qualitatively by other models. In particular, the dramatic effects of changing initial protein concentration (C_0) on qualitative and quantitative aggregation kinetics is found to follow from a C_0-dependent competition between the process of initiating or "nucleating" irreversible oligomers, and the subsequent process of aggregate growth. Finally, data are presented that show aCgn aggregate growth occurs via a sequential process in which reversible association first occurs, followed by one or more irreversible rearrangement steps. To the best or our knowledge this constitutes the first time such a step in the mechanism of non-native aggregation has been shown experimentally.