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Kinetic studies of protein L aggregation and disaggregation

Troy Cellmer [a,1], Rutger Douma [a,2], Ansgar Huebner [a,3], John Prausnitz [a,b], Harvey Blanch [a,*]

[a] Department of Chemical Engineering, University of California, Berkeley, Berkeley, CA 94720, United States
[b] Chemical Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, United States

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Abstract

We have investigated the aggregation of protein L in 25% (vol/vol) TFE and 10 mM HCl. Under both conditions, aggregates adopt a fibrillar structure and bind dyes Congo Red and Thioflavin T consistent with the presence of amyloid fibrils. The kinetics of aggregation in 25% TFE suggest a linear-elongation mechanism with critical nucleus size of either two or three monomers. Aggregation kinetics in 10 mM HCl show a prolonged lag phase prior to a rapid increase in aggregation. The lag phase is time-dependent, but the time dependence can be eliminated by the addition of pre-formed seeds. Disaggregation studies show that for aggregates formed in TFE, aggregate stability is a strong function of aggregate age. For example, after 200 min of aggregation, 40% of the aggregation reaction is irreversible, while after 3 days over 60% is irreversible. When the final concentration of the denaturant, TFE, is reduced from 5% to 0, the amount of reversible aggregation doubles. Disaggregation studies of aggregates formed in TFE and 10 mM HCl reveal a complicated effect of pH on aggregate stability.

Keywords: Protein aggregation; Aggregation kinetics; Amyloid; Fibrils

Protein aggregation has been associated with more than twenty human diseases [1]. These diseases can be divided into two categories. In the first, the origins of disease can be traced to the aggregated protein's inability to carry out its biological function; such diseases include cystic fibrosis, Marfan syndrome, and many forms of cancer. In the second category, the protein aggregates exhibit a cytotoxic effect. Such behavior is believed to cause Alzheimer's, Parkinson's, and Huntington's diseases.

Despite the variety of proteins that form pathological aggregates, the aggregates themselves show many similarities. In many cases, they adopt a β-sheet-rich, fibrillar structure that binds dyes in a manner similar to starch (amylose) [2]. These aggregates have thus been called amyloid fibrils. However, much debate remains regarding the characteristics of the toxic aggregates. A variety of studies have suggested that precursors to the fibrillar aggregates are more toxic than the mature fibrillar aggregates [3,4], although evidence for mature-aggregate toxicity has been presented [5].

A number of recent studies has shown that proteins not associated with any known disease can form amyloid fibrils [6–8] as well as toxic aggregates [3,4]. These observations have led to the suggestion that the ability to form fibrils is a generic property of the peptide chain, and that generic mechanisms of aggregation and aggregate toxicity exist. Studies of non-disease-related proteins can be employed to determine the features of such mechanisms. For example, studies of non-disease-related proteins have shown that destabilization of the native state is the crucial factor that directs a normally soluble protein into an amyloidogenic conformation [6,9–11]. Further, the availability of a wide-range of kinetic data for the aggregation of disease and non-disease-related proteins has facilitated an attempt to rationalize protein-aggregation rates from a small number of physiochemical parameters [12]. Such an effort is very important for rational protein design, because it is helpful in narrowing the

* Corresponding author. Tel.: +1 510 642 1387.
E-mail address: blanch@berkeley.edu (H. Blanch).
[1] Laboratory of Chemical Physics, National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health Bethesda, MD 20892-0520 USA.
[2] Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands.
[3] University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge, CB2 1EW UK.

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search for mutations or environmental perturbations that impart an increased resistance to aggregation.

In this study, we investigate the kinetics of aggregation and disaggregation of the small α/β protein, protein L. It is comprised of 63 amino acids, and its folding properties have been addressed in detail [13]. Our motivation for studying the aggregation of protein L is two-fold. First, it is an ideal protein to study aggregation phenomena *in silico*. A minimalist model of protein L has been developed to study protein folding [14]. The computational tractability of the model has been further exploited to study aggregation phenomena [15,16]. Therefore, the experimental data presented here can be used to validate computational findings. Second, our study contributes to the effort to establish the factors responsible for protein aggregation.

Under physiological conditions, protein L forms a stable three-dimensional structure. To induce aggregation, we use 25% aqueous trifluoroethanol solutions buffered near the p*I* of the protein (pH 5.0), and conditions of low pH (10 mM HCl) coupled with high temperature (65 °C). We first present results pertaining to aggregate morphology. Next, kinetic data are reported for aggregation and disaggregation.

1. Materials and methods

1.1. Materials

Thioflavin T and 2-2-2 Trifluoroethanol (TFE) were purchased from Sigma. Sodium acetate was purchased from Fisher.

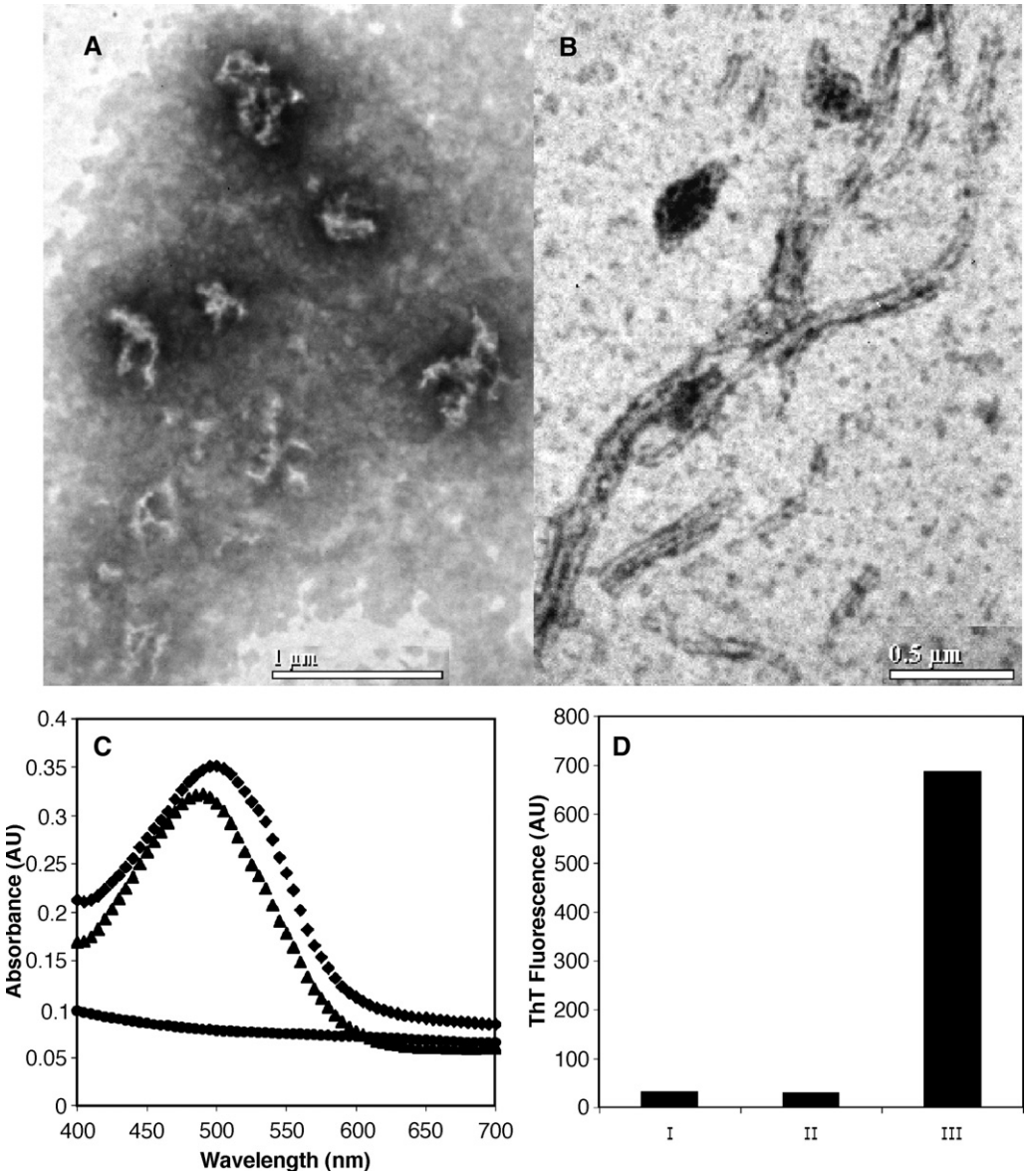

Fig. 1. (Panel A) electron micrographs of aggregates formed in 25% TFE after 5 min (left) and 24 h (right). (Panel B) absorbance spectra of buffer (circles), Congo Red in buffer (triangles), and Congo Red with protein aggregates in buffer (diamonds). Note the red shift in Congo Red absorbance. The difference in absorbance for Congo Red and Congo Red with fibrils was a maximum at 540 nm, consistent with amyloid. (Panel C) ThT fluorescence in the presence of I) protein only II) 25% TFE with 50 mM NaAc, and III) aggregates formed after 24 h in 25% TFE with 50 mM NaAc.