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Competitive inhibition reaction mechanisms for the two-step model of protein aggregation

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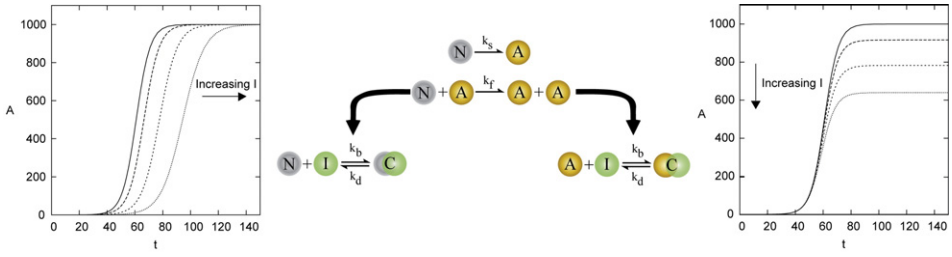
HIGHLIGHTS

- Three mechanisms for competitive inhibition of protein aggregation are introduced.
- Rate equations are derived to estimate aggregation inhibition constants.
- Rate equations are used to distinguish inhibition mechanisms of insulin aggregation.
- Longer insulin peptide inhibitors delay insulin aggregation onset.
- Shorter insulin peptide inhibitors reduce total concentration of aggregated insulin.

GRAPHICAL ABSTRACT



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ABSTRACT

We propose three new reaction mechanisms for competitive inhibition of protein aggregation for the two-step model of protein aggregation. The first mechanism is characterized by the inhibition of native protein, the second is characterized by the inhibition of aggregation-prone protein and the third mechanism is characterized by the mixed inhibition of native and aggregation-prone proteins. Rate equations are derived for these mechanisms, and a method is described for plotting kinetic results to distinguish these three types of inhibitors. The derived rate equations provide a simple way of estimating the inhibition constant of native or aggregation-prone protein inhibitors in protein aggregation. The new approach is used to estimate the inhibition constants of different peptide inhibitors of insulin aggregation.

1. Introduction

Surveillant processes that control protein quality (proteostasis) are critical for functionality and longevity of the cell [1]. Under optimal physiological conditions, protein homeostasis may be viewed as a dynamic network of interconnected processes which are monitored and regulated by quality control mechanisms, ameliorating any instances of inappropriate folding or oligomerization [2]. When the cellular surveillance of protein quality is compromised, proteins start to form misfolded species that are associated with a variety of diseases, many of which are terminal. Therefore, understanding the mechanisms by which proteins aggregate is of paramount importance for the development of effective methods to ameliorate protein folding diseases.

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Misfolding and aggregation can also occur in proteins synthesized industrially. Biosynthetic human insulin is manufactured for widespread clinical treatment of diabetes, but its aggregation in vitro and in the bloodstream makes it pharmacologically ineffective [3–5]. Observations of oligomerization, aggregation and amyloid fibrillogenesis from misfolded proteins have prompted explorations into inhibitory compounds that either prevent unfolding of the native protein or sequester partially folded aggregation-prone intermediates [3].

Misfolded or aggregation-prone proteins have been described as intrinsically disordered proteins, lacking a defined, stable, structured state [6]. In vitro studies have found a correlation between protein stability and aggregation propensity [7–9]. In general, partial unfolding and destabilization of the protein due to mutations or environmental changes increase the aggregation propensity of the protein.

Oosawa and Asakura [10] presented one of the first monographs of protein polymerization and aggregation in 1975. Classical studies of protein aggregation combined kinetics and thermodynamics when attempting to understand the mechanism of protein aggregation [11,12]. Eaton and Hofrichter [13] explicitly employ reaction kinetics to investigate the mechanisms of hemoglobin S gelation both in vitro and in vivo. They also accounted for temperature and pressure to infer the mechanisms of hemoglobin S aggregation [14].

In this work, we focus our attention on the reaction kinetics of the time course of aggregation-prone species to infer minimalistic reaction mechanisms of protein aggregation. In 1997, Watzky and Finke [15] proposed a minimalistic two-step model of protein aggregation which was inspired by classical mechanistic literature from the 1950s on the formation of colloids in homogeneous, initially supersaturated solutions by LaMer [16,17]. The model is also known as the Finke–Watzky two-step model (F–W model) or "Ockham's razor"/minimalistic F–W model [18,19]. In the first step, native protein N is converted with a slow first-order rate into aggregation-prone protein A. The second step is a fast second-order autocatalytic, irreversible conversion of N into A by using an existing A as a template. The reaction scheme for the two-step model is:

$$\begin{aligned} N &\xrightarrow{k_s} A \\ N + A &\xrightarrow{k_f} 2A \end{aligned} \tag{1}$$

where k_s and k_f are rate constants. This reaction scheme has been successfully used to describe the time course of protein aggregation for numerous proteins, including amyloid β, α-synuclein, polyglutamine, prions, and human calcitonin aggregation [15,19,20]. It has also been applied to investigate aggregation inhibition [3]. A limitation of using reaction scheme Eq. (1) to characterize aggregation inhibitors is that it does not explicitly account for the presence of inhibitors and hence neglects the mechanism of inhibition, which is essential to the design of highly specific pharmacological agents [21,22].

We introduce three competitive inhibition reaction mechanisms to the F–W model of protein aggregation Eq. (1). These inhibition mechanisms differ in the assumption that a given inhibitor binds with high specificity to native protein, aggregation-prone protein, or both proteins. We derive rate expressions for the time course and initial rates of the aggregation-prone species formation, which allow the estimation of inhibition constants. We also derive conditions for the validity of our rate equations. We test and validate our rate expressions by studying time courses of the inhibition of aggregation using insulin as an example. This demonstrates how the competitive inhibition mechanisms can be used to model experimental data.

2. The Finke–Watzky two-step model of protein aggregation

Watzky and Finke introduced a two-step model described by the reaction scheme Eq. (1) in 1997 [15,19]. They used this model to study the transition-metal nanocluster formation through two pseudoelementary steps [15,18,19,23]. Thereafter, Finke and colleagues fit the F–W model to 41 kinetic data sets from the literature [18,23]. Fourteen of these kinetic data sets were amyloid protein aggregation [23]. The excellent fit of these 14 data sets with R^2 values larger than or equal to 0.98 showed the wide applicability of the F–W model. Next, Watzky et al. [18] fit the F–W model to 27 prion aggregation kinetic data sets. All 27 fits were good to excellent with a range of R^2 values from 0.764 to 0.999 [18]. The application of the F–W model to 41 kinetic data sets demonstrates the power of minimalistic modeling of aggregation systems with a fully quantifiable model [18,23].

The F–W model was also independently proposed by Saitô and colleagues in 2000 for the fibrillation mechanism of calcitonin as a three-step model that is mathematically identical to the F–W model [20,23]. Thereafter, more researchers used Saitô's version of the F–W model to fit β-amyloid aggregation [24–27] and HET-s, a fungal prion protein [28]. In 2006, Gibson and Murphy [3] used the F–W model to describe the time course of insulin aggregation in the presence of inhibitors.

Applying the law of mass action to reaction scheme Eq. (1), the governing differential equations for the F–W model are:

$$\begin{aligned} \frac{dn}{dt} &= -k_s n - k_f n a \\ \frac{da}{dt} &= k_s n + k_f n a. \end{aligned} \tag{2}$$

where n is the concentration of native protein species N and a is the concentration of aggregation-prone species A. Using the law of mass conservation for the reaction scheme,

$$\frac{dn}{dt} + \frac{da}{dt} = 0, \tag{3}$$

the system Eq. (2) can be analytically solved to obtain a closed-form expression for a as a function of time:

$$a(t) = \frac{n_0\left(e^{k_s t(1+kn_0)} - 1\right)}{kn_0 + e^{k_s t(1+kn_0)}}, \tag{4}$$

where n_0 is the initial concentration of native protein, and $k = k_f/k_s$. In the above expression, it is assumed that aggregates are initially absent from the reaction.

3. Competitive inhibitors of protein aggregation

Given the broad range of pathological conditions and industrial problems associated with aggregation of proteins, there is a great interest in exploring strategies that prevent or delay the onset of protein aggregation. This can occur by either intrinsically modifying the amino acid sequence of the protein or by pharmacologically altering the extrinsic reaction environment of proteins [29]. Structural modifications can be performed by site-specific mutagenesis [30] or by chemical reactions [31]. However, these intrinsic modifications can affect the functional activity of the protein – a problem that may, in many cases, be intractable.

On the other hand, there has been considerable attention paid to enhancing the structural integrity of proteins by changing the local milieu of the protein. This is commonly accomplished by the introduction of excipients or additives to stabilize proteins by preferential interactions, so that aggregation is inhibited [32,33]. For instance, molecules that alter insulin aggregation include lecithins, cyclodextrins, and polymeric surfactants [34–37], carbohydrates and glycerols [38–40], low molecular weight compounds such as betaine, trehalose, and citrulline [41], and small hybrid peptides [3].

The kinetic characterization of inhibitors is a valuable tool for investigating the mechanisms of aggregation, and it is also of practical importance in the search, design and characterization of protein aggregation