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Original Article

Kinetic law leading to a hyperbolic growth rate for protein crystals

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ABSTRACT

In a recent paper, [Barlow D. A., LaVoie-Ingram E. and Bayat J. 2022, J. Cryst. Growth, 578, 126417], it was demonstrated how reported experimental data giving the time dependence for crystal size in the case of protein crystals growing from a batch supersaturated solution, obey an empirical curve in the shape of the hyperbolic tangent. Using this empirical law, population balance models were used to derive a variety of useful kinetic relationships including the relative supersaturation, the homogeneous nucleation rate and the crystal size distribution function. In this report, we present a model which leads to a governing differential equation that directly yields a solution for the crystal radius as a function of time which is of the form of the hyperbolic tangent function. This model gives the linear growth rate as a sum of incorporation and dissociation terms which are described in detail here. The final result has two undetermined parameters, the maximum size at equilibrium and a rate constant. From other reports in the literature, we note how the maximum equilibrium size can be related to the initial supersaturation. We discuss how the rate constant could be determined assuming that it obeys an Arrhenius law with a negative activation energy.

KEYWORDS: biocrystallization, crystallization, lysozyme, proteins, reaction rates.

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1. Introduction

Details of the conditions that control the process of protein crystallization continue to be an area of significant research interest to scientists and engineers. Typically grown from aqueous solution, factors that determine the final size, shape, structure and quality of these crystals are of principle concern. Also of interest, is the kinetic behavior of the nucleation and growth transformations.

The subject of this report will be the kinetic nature of the linear growth rate, and thus also the crystal size, for the batch growth of protein crystals grown from aqueous solution at constant temperature and pressure. In particular, results from the crystallization of lysozyme and beta-lactoglobulin will be considered. It is assumed in all cases that activity occurred in the intermediate stage of growth and therefore, no details concerning the induction period or post equilibrium ripening are considered.

Recent reports give kinetic data for the size of these protein crystals during growth that clearly obey a hyperbolic tangent law [1, 2, 3, 4]. The general form for this expression is

$$r(t) = r_{\mathrm{m}} \tanh(kt) \qquad (1)$$

where r gives the length of a characteristic dimension of the growing crystal at time t, r_{m} is the crystal size at equilibrium, that is, the maximum possible crystal size, and k is a rate constant. In the application of Eq. (1) to the data reported in the above mentioned reports, isothermal and isobaric conditions are assumed.

Recently, Barlow and co-workers [5], fitted Eq. (1) to data given by Heijna [2] and Sauter [1]. They showed how such an expression could be used as a starting point within the framework of an often used phenomenological kinetic model for crystal nucleation and growth [6, 7, 8, 9, 10] to arrive at a time dependent expression for the supersaturation which was also shown to accurately describe reported experimental data for the kinetics of supersaturation decay. Additionally, they were able to derive expressions for the distribution of crystal sizes and the homogeneous nucleation rate. However, no physical justification was given for Eq. (1) in the above mentioned report but was rather suggested as a topic for future work.

Here, we take up the challenge and find a governing differential equation for $r(t)$. Its form suggests that a model for the growth rate be made up of two terms: an attachment/incorporation rate and the other a dissociation rate. This two-term approach mirrors commonly used models for evaporation such as Hertz-Knudsen [11] and the, so-called, 'relationships' due to Schrage [12]. Such models for the growth rate have also been fruitful in simulated studies of protein crystal growth [13, 14] and in the description of inorganic crystal growth in geology [15]. We give explanations here for the incorporation and dissociation terms and show that they can be written with only one completely undetermined parameter, k.

Protein crystal growth from solution is most effectively accomplished by using additives which affect the solubility of the protein. The salt concentration and the pH, for example, are found to have a profound effect upon solubility, the solubility typically being suppressed at high salt concentrations [16]. A comprehensive discussion of the related salting-in effect can be found in the paper by Lee, Kim and Baird [17]. It is assumed in the case of the experimental protein crystal growth experiments cited here that all were carried out in the range of optimal salt concentration, that is, within the so called 'crystallization slot' [18]. Additionally, we expect that they were also carried out in an optimal range of the protein concentration-temperature phase diagram [19] and that the solution pH was adjusted by way of a buffer. Other supplements used to enhance the growth process include various types of gel which were used in two of the cases cited here [3, 4].

2. The governing equation

One can show by direct substitution that Eq. (1) is a solution to the first order, ordinary differential equation,

$$\frac{dr}{dt} = A - Br^2 , \tag{2}$$

where A and B are constants. Eq. (2) gives an expression for the linear growth rate. Our required final conditions are

$$\lim_{t\to\infty} \frac{dr}{dt} = 0 , \tag{3}$$

$$\lim_{t\to\infty} r(t) = r_m , \tag{4}$$

and an initial condition is

$$r(0) = 0, \tag{5}$$

where r_m is the crystal size at equilibrium. It should be noted that this model assumes that the volume of the individual crystal can be described by the product of the cube of a lone dimensional coordinate, that is, r^3, and a shape factor. For example, in the case of a sphere, the shape factor is $4\pi/3$.

The conditions imposed by Eqs. (3), (4) and (5) lead to values for the constants A and B and thus the final form for the growth rate equation is

$$\frac{dr}{dt} = k\, r_m - \frac{k}{r_m} r^2 . \tag{6}$$

This result, along with Eq. (1), implies that

$$\frac{dr}{dt} = r_m k\ \operatorname{sech}^2(kt) . \tag{7}$$

Our interpretation of Eq. (6) is that the two terms on the right represent rates of incorporation and dissociation from the crystal face. The term on the far right of Eq. (6) represents the rate of dissociation. The dissociation term is proportional to the square of r and thus grows until $dr/dt = 0$ at equilibrium. Our physical justification for the dissociation term comes from the claim by Pauling that during the sublimation of crystals in air the rate of evaporation is directly proportional to the surface area of the crystal [20]. We hypothesize that an action of this sort occurs for protein crystals in solution and thus $k = k_o g$ where k_o is a detachment

frequency and g an area form factor. For the case of a sphere, $g = 4\pi$, thus leading to the final form of the dissociation rate as $(4\pi k_o / r_m) r^2$.

The first term on the right of Eq. (6) gives the incorporation rate. The incorporation rate is therefore constant, yet directly proportional to the equilibrium size of the crystal. This term can be related to the initial supersaturation Δc, where $\Delta c = c_o - c_s$. Here, c_o is the protein concentration at the start of the growth run and c_s its equilibrium solubility. Several workers have shown that the maximum crystal radius r_m can be related to the cube root of Δc [21, 22]. This would imply that the incorporation rate is proportional to the cube root of the initial supersaturation. This result seems physically appealing as it predicts a very slow attachment process at low initial supersaturations yet the increase of this rate with Δc is only approximately linear for small values of Δc, becoming almost logarithmic as Δc is increased further. In other words, there is a sort of upper limit to the attachment rate as Δc is increased, a result to be expected as it is well known that these crystals cannot be grown to arbitrarily large sizes. Of course the magnitude of Δc is already bounded by the requirement of maintaining a solution within the optimal region of the protein concentration-temperature phase diagram. The full expression for r_m taken from [21] is

$$r_m = \left(\frac{3\Delta c}{4\pi\rho n_o} \right)^{\frac{1}{3}}, \tag{8}$$

where ρ is the crystal density and n_o is the total number of homogeneous nuclei created during the batch growth run.

Albeit n_o would not be trivial to determine experimentally, a reasonable estimate for n_o would be the number of crystals per unit volume in the vessel at equilibrium. With Eq. (8), all of the parameters in Eq. (6) are determined with the exception of k.

3. The rate constant

The rate constant, k, was determined previously from a fit of Eq. (1) to experimental data in Reference [5] for two reported cases of protein crystal growth from batch solution, one for lysozyme [2] and the other for beta-lactoglobulin [1]. We fit Eq. (1) to additional data sets found in the literature here [3, 4]. These data, for lysozyme crystallization, are depicted in Figures 1 and 2. The rate constants from all four cases are given in Table 1 along with their corresponding temperatures. It is interesting to note from the data in Table 1 how the rate constants increase as the temperature decreases. The growth conditions were all different for each of these four runs. However, similar behavior has been

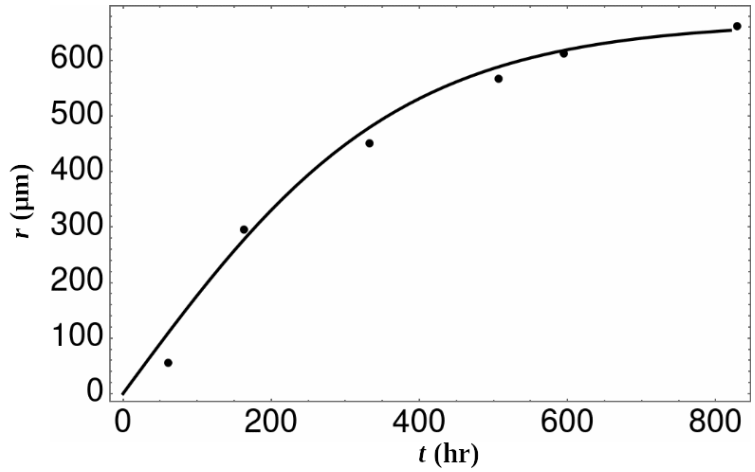

Figure 1. Plot of data for lysozyme crystal size vs. time for a case of batch growth. Data point values are from Reference [4]. The reported linear size was taken to be a diameter and converted to a radius here. The curve is a fit of Eq. (1) to the data. The maximum crystal size was estimated to be 670 μm.

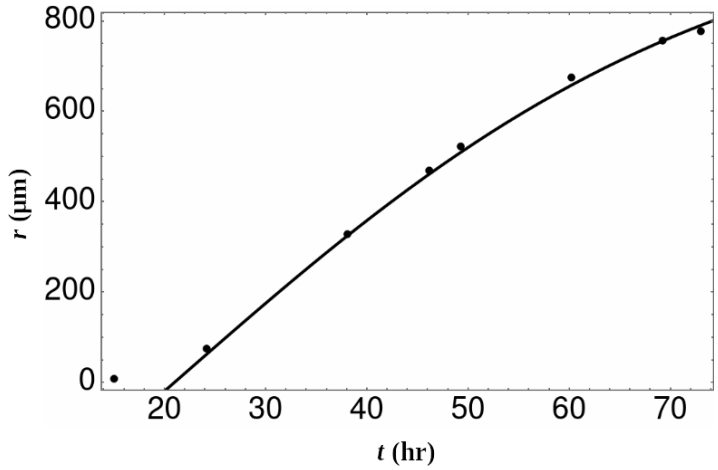

Figure 2. Plot of data for lysozyme crystal size vs. time for a case of batch growth. Data point values are from Reference [3]. The reported linear size was taken to be a diameter and converted to a radius here. The curve is a fit of Eq. (1) to the data. The maximum crystal size was estimated to be 1060 μm. The early time region, not described well by Eq. (1), is likely caused by the diffusion limiting nature of a two-step mechanism also observed in the growth cases reported in References [1, 2] and discussed and described further in Reference [5].

Table 1. Rate constants and temperatures from four different batch crystal growth cases. Rate constants come from a fit of Eq. (1) to reported experimental data.

Protein	***k*** **(hr $^{-1}$)**	***T*** **(K)**
Lys. [2]	0.528 [5]	281
β-Lac. [1]	0.246 [5]	293
Lys. [3]	0.0185	295
Lys. [4]	0.0027	298

reported for protein crystals grown at different temperatures from identical solutions. In protein crystal growth dilatometry experiments, which were sensitive to the supersaturation, Caraballo, Baird and Ng [23] determined the rate constant for the time rate of decay of the supersaturation. Using mass balance considerations, they showed that this rate constant involved a ratio, the numerator of which was a rate constant for the linear advance of a facet, and the denominator of which was the protein solubility. If the temperature dependence of both the linear growth rate constant and the solubility were assumed to be proportional to Arrhenius/Boltzmann factors, their ratio took the form

$$\exp[-\frac{(E_A - \Delta H)}{RT}] \ , \qquad (9)$$

where E_A is the activation energy governing the attachment of a protein molecule to a crystal facet and ΔH is the enthalpy of solution of the protein. They interpreted (E_A - ΔH) as the effective activation energy, E_{eff} , for the decay of the supersaturation. It could be positive or negative depending upon the signs and magnitudes of E_A and ΔH. Whereas the dilatometry experiments were incapable of separating the effects of E_A from those of ΔH, the data in Table 1 indicate that the linear growth rate constant, k, increases with decreasing temperature, consistent with a negative activation energy.

This implies that the protein absorbs heat to denature somewhat upon dissolving. According to Munk [24], 'as temperature increases the ensemble of protein molecules takes on more random configurations.' It is well known that protein crystallization is only favored when the solute molecule is in a non-denatured state. Lowering the temperature then causes the ensemble of molecules to consist more of one identical compact tertiary structure--the so called, native state. Current research seems to indicate that the folding pathway to the native state can be complex and consist of several local minima [25, 26]. This leads us to suggest that, as more protein molecules achieve their native state, these molecules are able to take their place in the growing crystal with greater ease thus increasing the rate constant for growth.

This observation suggests the study of protein batch growth experiments under identical conditions of solution composition but at a variety of temperatures within a range of interest. If an Arrhenius model holds, then the values of k determined by fitting Eq. (1) to crystal size data should yield values for the slope, E_{eff}/R, and the intercept, when plotted in Arrhenius form with $\ln k$ versus $1/T$.

4. Conclusion

In this paper we show how a recently reported empirical relation can be derived from phenomenological considerations. The expression under consideration gives the crystal size over time during batch growth in terms of a hyperbolic tangent function. It is shown here how this result can be arrived at from a differential equation involving the growth rate which is given by two terms: one which gives the rate of dissolution from the crystal face, the other, the rate of incorporation.

The rate of dissolution is taken to be directly proportional to the surface area of the growing crystal. The incorporation term is constant yet proportional to the maximum crystal size. We show how the maximum size at equilibrium can be related to quantities that could be measured or estimated for a particular batch growth run. The result involves an undetermined rate constant which we suggest can be written in Arrhenius form with a negative activation energy. Then, isothermal protein crystal growth runs, with the same solution conditions at different temperatures, should yield values for $\ln k$ versus $1/T$ that fall on a straight line and thus yield a value for the activation energy and the pre-exponential factor. Such experiments are left as future work.

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CONFLICTS OF INTEREST STATEMENT

The authors have no conflicts of interest to report.

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