Nucleated polymerisation in the presence of pre-formed seed filaments

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Abstract

We revisit the classical problem of nucleated polymerisation and derive a range of exact results describing polymerisation in systems intermediate between the well-known limiting cases of a reaction starting from purely soluble material and for a reaction where no new growth nuclei are formed.

I. INTRODUCTION

The classical theory of nucleated polymerisation[1] describes the growth of filamentous structures formed through homogeneous nucleation[2–7]. This framework was initially developed by Oosawa and coworkers in the 1960s[1, 8] to describe the formation of biofilaments, including actin and tubulin. This theory has been generalised to include secondary nucleation processes by Eaton and Ferrone[9] in the context of their pioneering work elucidating the polymerisation of sickle haemoglobin, and by Wegner[10] in order to include fragmentation processes into the growth model for actin filaments.

For irreversible growth in the absence of pre-formed seed material and secondary nucleation pathways, in 1962 Oosawa presented solutions to the kinetic equations which were very successful in describing a variety of characteristics of the polymerisation of actin and tubulin. The other limiting case, namely where seed material is added at the beginning of the reaction and where no new growth nuclei are formed during the reaction, is also well known. In this paper, we present exact results which encompass all cases between these limiting scenarios, extending the results of Oosawa for a system dominated by primary nucleation to the case where an arbitrary concentration of pre-formed seed material is present. We also discuss a range of general closed form results from the Oosawa theory for the behaviour of a system of biofilaments growing through primary nucleation and elongation. We then compare the behaviour of systems dominated by primary nucleation to results derived recently for systems dominated by secondary nucleation.

II. RESULTS AND DISCUSSION

A. Derivation of the rate laws for the polymer number and mass concentrations

The theoretical description of the polymerisation of proteins such as actin and tubulin to yield functional biostructures was considered in the 1960s by Oosawa[8]. For a system that evolves through primary nucleation of new filaments, elongation of existing filaments, and depolymerisation from the filament ends, the change in concentration of filaments of size j,

denoted $f(j,t)$, is given by the master equation[1, 8]:

$$\begin{aligned}\frac{\partial f(t,j)}{\partial t} &= 2m(t)k_+ f(t,j-1) - 2m(t)k_+ f(t,j) \\ &+ 2k_{\text{off}} f(t,j+1) - 2k_{\text{off}} f(t,j) \\ &+ k_n m(t)^{n_c} \delta_{j,n_c}\end{aligned} \tag{1}$$

where k_+, k_{off}, k_n are rate constants describing the elongation, depolymerisation and nucleation steps and $m(t)$ is the concentration of free monomeric protein in solution. The factor of 2 in Eq. (1) originates from the assumption of growth from both ends. For the case of irreversible biofilament growth, the polymerisation rate dominates over the depolymerisation rate; from Eq. (1), the rate of change of the number of filaments, $P(t)$, and the free monomer concentration, $m(t)$, were shown by Oosawa under these conditions [1, 8] to obey:

$$\frac{dP}{dt} = k_n m(t)^{n_c} \tag{2}$$

$$\frac{dm}{dt} = -2k_+ m(t) P(t) \tag{3}$$

Combining Eqs. (2) and (3) yields a differential equation for the free monomer concentration[1]:

$$-\frac{d^2}{dt^2}\log(m(t)) = 2k_+ k_n m(t)^{n_c} \tag{4}$$

.

Here, we integrate these equations in the general case where the initial state of the system can consist of any proportion of monomeric and fibrillar material; this calculation generalises the results presented by Oosawa to include a finite concentration of seed material present at the start of the reaction. Beginning with Eqs. (2) and (3), the substitution $z(t) := \log(m(t))$ followed by multiplication through by dz/dt yields:

$$-\frac{d}{dt}\left[\frac{n_c}{4k_+k_n}\left(\frac{dz}{dt}\right)^2\right] = \frac{d}{dt}e^{n_c z} \tag{5}$$

Integrating both sides results in:

$$-\frac{n}{2}\left(\frac{dz}{dt}\right)^2 = 2k_+k_n e^{n_c z} + A = -\frac{d^2 z}{dt^2} + A \tag{6}$$

we obtain a separable equation for dz/dt, which can be solved to yield:

$$\frac{dz}{dt} = \sqrt{\frac{2A}{n_c}}\tanh\left(\frac{\sqrt{2An_c}}{2}(-t+2B)\right) \tag{7}$$

Integration and exponentiation yields the expression for $m(t)$:

$$m(t) = \left[\frac{A}{2k_+k_n} \text{sech} \left(\sqrt{\frac{An_c}{2}}(t - 2B) \right)^2 \right]^{1/n_c} \tag{8}$$

Inserting the appropriate boundary conditions in terms of $m(0)$ and $P(0)$ fixes the values of the constants A and B, resulting in the final exact result for the polymer mass concentration $M(t) = m_{\text{tot}} - m(t)$:

$$M(t) = m_{\text{tot}} - m(0) \left[\mu \, \text{sech} \left(\nu + \lambda_0 \beta^{-\frac{1}{2}} \mu t \right) \right]^{\beta} \tag{9}$$

where the effective rate constant λ is given by $\lambda = \sqrt{2k_nk_+m(0)^{n_c}}$ and $\beta = 2/n_c$, $\mu = \sqrt{1+\gamma^2}$, $\nu = \text{arsinh}\,(\gamma)$ for $\gamma = 2k_+P(0)/(\beta^{\frac{1}{2}}\lambda)$.

We note that this expression only depends on two combinations of the microscopic rate constants, $k_0 = 2k_+P(0)$ and λ. The result reveals that λ controls the aggregation resulting from the newly formed aggregates, whereas k_0 defines growth from the pre-formed seed structures initially present in solution. In the special case of the aggregation reaction starting with purely soluble proteins, $P(0) = 0$, $m(0) = m_{\text{tot}}$, these expressions reduce to $\mu \to 1$ and $\nu \to 0$, and Eq. (9) yields the result presented by Oosawa[1] and the single relevant parameter in the rate equations is λ. Interestingly, generalisations of Eq. (9) which include secondary pathways, maintain the dependence on λ and k_0 but introduce an additional parameter analogous to λ for each active secondary pathway[11–14].

An expression for the evolution of the polymer number concentration, $P(t)$ may be derived using Eq. (9). Direct integration of Eq. (2) gives the result for $P(t)$:

$$P(t) = P(0) + k_n m(0)^{n_c} \mu \frac{\tanh(\nu + \beta^{-\frac{1}{2}}\lambda\mu t) - \tanh(\nu)}{\beta^{-\frac{1}{2}}\lambda} \tag{10}$$

Eqs. (10) and (9) give in closed form the time evolution of the biofilament number and mass concentration growing through primary nucleation and filament elongation.

B. Characteristic features of growth involving pre-formed seed material

Insight into the early time behaviour of the polymer mass concentration can be obtained by expanding Eq. (9) for early times to yield:

$$M(t) \xrightarrow{t \to 0} M(0) + k_0 m(0) t + m(0)[\lambda^2 - k_0^2]t^2/2 + \mathcal{O}(t^3) \tag{11}$$

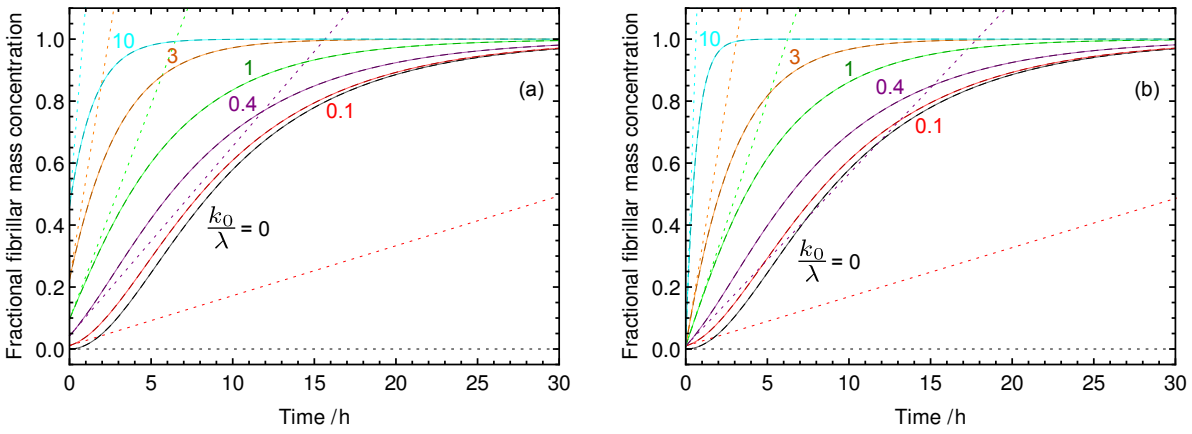

FIG. 1. Nucleated polymerisation in the presence of seed material. The thick dashed lines are the exact solution to the rate equations Eq. (9); the thin solid lines are calculated from numerical simulations of the master equation Eq. (1). The dotted lines are the initial gradients $dM/dt|_{t=0} = M(0) + 2k_+m(0)P(0)t$; a lag-phase exists when the initial gradient is not the maximal gradient. The numbers accompanying each curve are k_0/λ; Eq. (14) predicts that a lag-phase only exists when this ratio is less than unity. (a): Polymerisation in the presence of an increasing quantity of seed material of a fixed average length (5000 monomers per seed) added at the beginning of the reaction. The seed concentrations given as a fraction of the total concentration of monomer present are right to left): 0, 0.01, 0.04, 0.1, 0.2, 0.5. (b): Nucleated polymerisation in the presence of a fixed quantity (1% of total monomer in the system) of seed material of varying average length. The average number of monomer per seed are (right to left): N/A (unseeded), 5000, 1000, 500, 200, 50. The other parameters for both panels are: $m_{\rm tot} = 10\mu M$, $n_c = 3$, $k_n m_{\rm tot}^{n_c-1} = 1 \cdot 10^{-9} s^{-1}$, $k_+ = 1 \cdot 10^5 \mathrm{M}^{-1}\ \mathrm{s}^{-1}$.

This expression recovers the characteristic $\sim t^2$ dependence of the Oosawa theory and has an additional term linear in time relating to the growth of pre-formed aggregates.

In many cases, Eq. (9) describes a sigmoidal function with a lag phase. The time of maximal growth rate, $t_{\rm max}$, can be found from the inflection point of the sigmoid from the condition $d^2M/dt^2 = 0$:

$$t_{\rm max} = \left[\mathrm{artanh}\left(\sqrt{\frac{1}{1+\beta}}\right) - \mathrm{arsinh}\left(\gamma\right)\right](\mu\beta^{-\frac{1}{2}}\lambda_0)^{-1} \tag{12}$$

such that a lag phase exists only for:

$$\operatorname{artanh}\left(\sqrt{\frac{1}{1+\beta}}\right) > \operatorname{arsinh}(\gamma) \tag{13}$$

Using the composition $\sinh(\operatorname{artanh}(x)) = x/\sqrt{1-x^2}$ reduces this to the simple condition:

$$k_0 < \lambda \tag{14}$$

In other words, a point of inflection exists if the growth through elongation from the ends of pre-existing seeds, k_0, is less effective that the effective growth through nucleation and elongation of new material, λ. This result imples that an increased nucleation rate promotes the existence of an inflection point, whereas an increased elongation rate or an increased level of seeding tends to disfavour its existence. In particular, we also note that in the absence of nucleation, an inflection point cannot exist in the polymer mass concentration as a function of time. Interestingly, the result Eq. (14) is analogous to the criterion applicable for fragmentation dominated growth where a lag phase only exists when the parameters controlling fragmentation-related secondary nucleation is larger than ek_0.

The maximal growth rate, $r_{\max}$, is given by:

$$r_{\max} = \frac{2m(0)}{\sqrt{n_c(2+n_c)}}\left(\frac{2\mu^2}{2+n_c}\right)^{\frac{1}{n_c}}\mu\lambda \tag{15}$$

which occurs at a polymer mass concentration $M_{\max}$ given from Eq.:

$$M(t_{\max}) = m_{\text{tot}} - m(0)\mu^{\frac{2}{n_c}}\left(1 - \frac{n_c^2}{(2+n_c)^2}\right)^{\frac{1}{n_c}} \tag{16}$$

The lag time, $\tau_{\text{lag}} := t_{\max} - M(t_{\max})/r_{\max}$, is then given by:

$$\tau_{\text{lag}} = \left[\operatorname{artanh}\left(\sqrt{\frac{1}{1+\beta}}\right) - \operatorname{arsinh}(\gamma) - \frac{m_{\text{tot}} - m(0)\mu^{\frac{2}{n_c}}\left(1-\frac{n_c^2}{(2+n_c)^2}\right)^{\frac{1}{n_c}}}{\frac{2m(0)}{\sqrt{n_c(2+n_c)}}\left(\frac{2\mu^2}{2+n_c}\right)^{\frac{1}{n_c}}}\right](\mu\beta^{-\frac{1}{2}}\lambda)^{-1} \tag{17}$$

Interestingly, from Eq. (17), we note that a point of inflection can never exist for $P(t)$ for simple nucleated polymerisation. By contrast, when secondary pathways are active, an inflection point can frequently be present[12].

	Primary nucleation	**Fragmentation**	**Monomer-dependent secondary nucleation**
Kinetic parameters	λ, k_+	λ, κ_-, k_+	λ, κ_2, k_+
Early time growth	Polynomial	Exponential	Exponential
Scaling behaviour (lag time, max growth rate)	Yes with λ	Yes with κ_-	Yes with κ_2
Positive feedback	No	Yes	Yes

TABLE I. Comparison of biofilament growth dominated by primary and secondary nucleation pathways

C. Comparison between nucleated polymerisation in the presence and absence of secondary pathways

Many systems that evolve through nucleated polymerisation display characteristic scaling behaviour[11–15]. This behaviour can be seen to be a consequence of the fact that under many conditions, the rate equations are dominated by a single parameters that corresponds to the dominant form of nucleation: λ for classical nucleated polymerisation and $\kappa_{2,-}$ for polymerisation in the presence of secondary pathways. These parameters have the general form $\sqrt{2k_+m(0)k_Nm(0)^n}$ where $k_N = k_n, k_-, k_2$ corresponds to the nucleation process and n is related to the monomer dependence of this process: $n = n_c - 1$, where n_c is the critical nucleus size for primary nucleation, $n = 0$ for fragmentation driven growth and $n = n_2$, the secondary nucleus size in cases where monomer-dependent secondary nucleation is dominant. The dominance of a single combination of the rate constants implies that many of the macroscopic system observables will be correlated since they are dependent on the same parameter. A striking examples of this behaviour is provided by the very general correlation between the lag-time and the maximal growth rate[14, 15], which is manifested in the present case in Eqs. (15) and (17) as $r_{\max} \sim \lambda$ and $\tau_{\rm lag} \sim \lambda^{-1}$.

Interestingly the rate equations describe sigmoidal curves both in the presence and in the absence of secondary nucleation processes. For more complex primary nucleation pathways[16, 17] the polynomial form for the early time solution is maintained, but higher-order exponents are obtained. In the absence of secondary processes, however, the lag-phase

is less marked since the early time rise is a slower polynomial relationship rather than the exponential onset characteristic of secondary pathways[17]. This observation implies that the difference between a high-order polynomial and an exponential may not be apparent in experimental data in the presence of noise, and therefore a global analysis of the system under different conditions is required in order to obtain robust mechanistic information[14].

III. CONCLUSION

In this paper, we have provided results for the time course of nucleated polymerisation for systems that are initially in a mixed state and contain both monomeric and fibrillar material. These results generalise the classical Oosawa theory that describes the formation of biofilaments to cases where an arbitrary amount of pre-formed seed material is present in the system. Furthermore, these results represent a reference to which polymerisation driven by secondary pathways can be compared.

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