ISSN 0006-2979, Biochemistry (Moscow), 2011, Vol. 76, No. 3, pp. 366-373. © Pleiades Publishing, Ltd., 2011.
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Modeling Amyloid Fibril Formation

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Received September 22, 2010
Revision received January 13, 2011

Abstract—No detailed step-by-step model of protein rearrangements during amyloid structure formation has been presented in the literature. The aim of this work was to design a kinetic model for description of the amyloid formation process on the basis of the most recent experimental data. A general kinetic model is proposed for description of the amyloid formation process including the nucleation mechanism of polymerization with consecutive monomer attachment to oligomer and autocatalytic growth of amyloid aggregates implying all types of exponential growth such as branching, fragmentation, and growth from the surface. Computer simulations have shown that the model correctly describes experimentally observed growth stages of amyloid fibrils and that the presence of exponential growth stage in the model is critical for modeling amyloid fibril formation. The key feature of the proposed model is the stage of the exponential growth of the aggregate. Such stage can simultaneously describe several versions of aggregate enlargement by branching, fragmentation, or growth from the surface. Data obtained using this model suggest conclusions concerning the significance of each stage in amyloid fibril assembly.

DOI: 10.1134/S0006297911030114

Key words: amyloid fibril, critical concentration, lag period, aggregation, oligomeric particle

The need for investigation of the mechanism of amyloid fibril formation is being recognized all over the world because of outbreaks of prion diseases such as rabies in cattle and Creutzfeldt–Jakob disease in humans. Now over 20 human diseases associated with accumulation of amyloid structures are known. Among them are infectious, sporadic, and hereditary diseases. Diseases associated with senescence and nerve degeneration, including Alzheimer's and Parkinson's diseases and Down's syndrome, should be considered as a special group. Due to the increasing number of aging people [1] and the consequent increase in the number of people suffering from these diseases, investigation of molecular mechanisms of these diseases as well as development on this basis of therapeutic and surgical methods for therapy and prevention of these diseases is crucial at the present time.

Amyloid formation is a kind of polymerization reaction. Nevertheless, there is experimental evidence of some features characteristic just of amyloid formation [2] that make classical schemes elaborated for protein polymerization inappropriate. Two main mechanisms can be distinguished within the large volume of works dealing with description of protein polymerization: mechanisms of protein aggregation by consecutive addition of monomers, and mechanism of prion aggregation. Most of these works stem from the work by Oosawa et al. of the end of 1950s [3]. A kinetic scheme for actin polymerization was proposed in this work. The experimental data showed that the process of polymerization is very similar to a condensation reaction: the reaction takes place only if the concentration of initial reactant (actin) exceeds some critical threshold. The assumption was justified. Cooperation of the polymerization reaction is supported by two facts: (i) increase in actin concentration resulted in increased reaction rate at early stages of the reaction; (ii) addition of a primer with already formed aggregate resulted in immediate aggregation of total free actin.

In 1974 Hofrichter et al. [4] used the mechanism of consecutive joining of monomer to the aggregate for explanation of hemoglobin fibril formation in sickle cell anemia (Scheme (1)). It should be noted that this scheme includes a stage of nucleus formation, i.e. a stage of nucleation. Equilibrium constants of the nucleation reaction were assumed to be below one unit, while after nucleation the reaction equilibrium constants exceeded one unit, i.e. attachment of monomers before nucleus formation is thermodynamically disadvantageous, while it was profitable after nucleus formation, i.e. during polymerization. Thus, these authors defined the nucleus as the least thermodynamically stable aggregate able to initiate further growth of fibrils.

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$$\begin{gathered} M + M \leftrightarrow M_2, \\ \ldots \\ M + M_{n^*-1} \leftrightarrow M_{n^*}, \\ M + M_{n^*} \leftrightarrow M_{n^*+1} \\ \ldots \end{gathered} \quad (1)$$

To explain the observed effect of "extreme autocatalysis" and strong concentration dependence, Ferrone et al. [5] designed the model of "heterogenous nucleation". The following history of events is supposed in this model: the fibril is formed at the beginning, during normal nucleation, and then additional fibrils can be formed on its surface. This model included equations for homogeneous nucleation (first stage) and for heterogeneous nucleation (second stage); in this case the equations were solved numerically. The concept of heterogeneous nucleation was the new and important contribution to development of the protein aggregation theory.

Frieden and Goddette [6] in 1983 used the model of consecutive joining of monomer to the aggregate for description of actin polymerization. In this case it was mentioned that each step of monomer joining has its own reaction rate constant (Scheme (2)), and the whole reaction begins from monomer activation, which in the case of actin corresponded to conformational changes in the protein proper due to ligand binding.

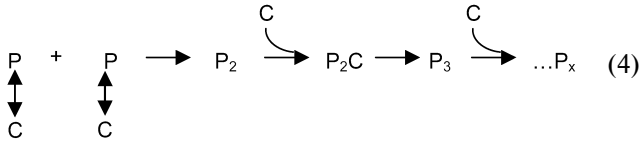
$$\begin{gathered} \overset{K}{M \leftrightarrow M_1}, \\ \overset{K_1}{M + M_1 \leftrightarrow M_2}, \\ \overset{K_2}{M + M_2 \leftrightarrow M_3}, \\ \ldots \\ \overset{K_j}{M + M_j \leftrightarrow M_{j+1}} \\ \ldots \end{gathered} \quad (2)$$

In 1986 Goldstein and Stryer [7] modified the model of consecutive joining of monomer to the aggregate for description of protein polymerization. They defined the nucleus as a "primer" of size *s*, the achievement of which results in changes in kinetic constants (Scheme (3)).

$$\begin{gathered} \overset{k_+}{M + M_n \leftrightarrow M_{n+1}} \quad (n < s), \\ \underset{k_-}{} \\ \overset{g_+}{M + M_m \leftrightarrow M_{m+1}} \quad (m \geqslant s) \\ \underset{g_-}{} \end{gathered} \quad (3)$$

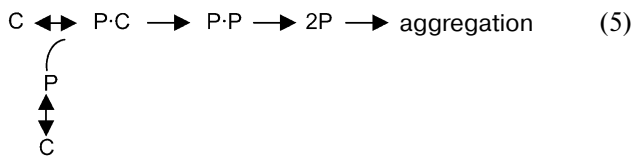
It was shown for the example of amylin that nucleation can proceed in two ways: fibril-independent (or primary) and fibril-dependent (secondary). The contribution of these processes depends on outer interface. In the presence of such interface the primary mechanism is dominant, whereas the second mechanism is dominant in the absence of such surface [8]. Heterogeneous nucleation can be considered in a different aspect, as autocatalytic growth on formed nuclei. It was not shown experimentally whether nuclei are formed on the existing surface or growth continues simply from the surface of one and the same aggregate.

It is also worth considering several mechanisms suggested for description of prion organization. In 1967 Griffith [9] proposed three possible theoretical mechanisms that could explain self-replication of an agent causing scrapie in sheep (Scheme (4)). The schemes are based on the supposition that the infectious agent can be a protein having at least two conformations, normal C conformation and prion P form. In these schemes transition between C and P is thermodynamically disadvantageous. However, if the P state is sufficiently profitable, it can serve as driving force for conversion of two C monomers to the P_2 oligomer. Further transition of C monomers to the aggregate P form is supported by the presence of formed P oligomers from 2 to *n* in size that serve as patterns for further C packing. In this mechanism the $P_x (x \geq 2)$ oligomer is an infectious agent, and fibril growth proceeds via assembly on oligomers and monomer transition from C to P state.



(4)

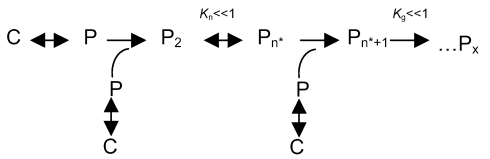
In 1982 Prusiner [10] presented sufficient experimental proof that the infectious agent of scrapie is a protein called a prion. In 1991 Prusiner proposed a scheme for transmission of prion infection [11] (Scheme (5)). In this mechanism the monomer P, able to catalyze the slow reaction of C monomer transition to P monomer (via dimer C • P formation), is considered as the infectious agent. Growth in this situation follows the mechanism of autocatalysis, because P is both reactant and reaction product.



(5)

Based on the work by Prusiner, in 1993 Lansbury published (Scheme (6)) work [12] in which the mecha-

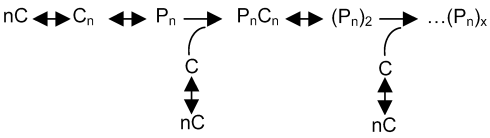
nism of consecutive joining of monomer to the nucleus was used for analysis of kinetic data. The infectious agent in this scheme was P_x oligomer exceeding the nucleus in size, because after reaching an appropriate size, aggregation became advantageous and resulted in further growth.



(6)

In 1996 Eigen [13] kinetically analyzed this model and concluded that catalysis proper is not obligatory for the proposed mechanism. Eigen then concluded that neither of the proposed mechanisms (Lansbury's or Prusiner's) are probable candidates for correct description of the prion aggregate formation and growth kinetics.

In 2000 Susan Lindquist [14] suggested another scheme of prion formation and growth–nucleation–conformational conversion (Scheme (7)). In this scheme the formation of oligomer C precedes slow formation of the P nucleus. In the presence of P, further assembly is rapid. Formation of large aggregates involves participation of a P nucleus with the help of which rearrangement of C oligomer occurs. In this mechanism the P nucleus is considered as the infectious unit.



(7)

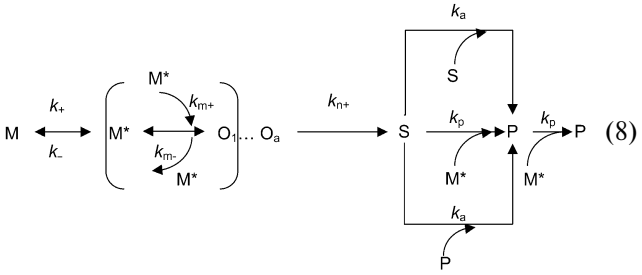
This review shows that protein polymerization can follow very different pathways. There are many works dealing with investigation of amyloid formation by various proteins under different conditions. However, no one has succeeded in designing a detailed step-by-step pattern of rearrangements happening with proteins during amyloid structure formation. At the same time, the available experimental material points to existence in practically all reactions of a nucleation stage that is, however, not enough for explanation of the experimental curves, as was already stated by Ferrone [2]. It is known that experimental kinetic curves for processes of amyloid formation have an important feature – a lag period, i.e. a delay before amyloid growth which often cannot be approximated by a quadratic function as should be the case for a polymerization reaction following a simple nucleation mechanism.

The main reason for a lag period is exponential growth of amyloid fibrils during aggregation. Exponential growth of aggregates can be the result of three scripts: 1) fragmentation; 2) branching, and 3) growth from the surface. Although, generally speaking, the above-mentioned processes are different in kinetic aspect, they are experimentally indistinguishable. To define the mechanism realized in this experiment, additional non-kinetic experimental data are necessary, which directly point to the mechanism. Many mathematical models have been proposed for description of a polymerization reaction on the basis of combination from a nucleation mechanism in the first stage of amyloid formation and exponential aggregation in the second stage, when fragmentation is often a potential candidate for the probable mechanism of exponential growth [15, 16]. Due to technical complications emerging upon solution of the resulting systems of equations, it is not quite understandable how separate stages influence the reaction as a whole, because one has to apply severe simplifications [15, 17] or to model numerically the kinetic scheme with a very large number of parameters. It is incomprehensible to what extent fragmentation is the only mechanism of exponential growth. In this work a general kinetic model is proposed for description of amyloid formation, which includes a nucleation mechanism of polymerization with the consecutive joining of monomer to oligomer and autocatalytic growth of amyloid aggregates, which implies all types of exponential growth.

METHODS OF INVESTIGATION

The model. In this work we have considered a model with different number of described stages. For the complete model, a general scheme of amyloid structure formation was used that was based on a nucleation mechanism with consecutive joining of monomer to oligomer (Scheme (8)). The following designations were used for the model: M, monomer in native conformation; M*, monomer capable of oligomerization; O_s, oligomer of s size; S, seeds; P, polymer; k_+, rate constant of monomer transition to the state for oligomerization (M*); k_-, rate constant for monomer transition to native state; k_{m+}, rate constant of joining (association) of monomer to oligomer; k_{m-}, rate constant of dissociation of monomer from oligomer; k_{n+}, rate constant of seed (the s size oligomer) transition from α state (not associated with β-structural organization like in amyloid fibril) to β state; k_p, rate constant of polymerization; k_a, rate constant of protofibril attachment; s, nucleus size. Now it is known that for transition to the amyloid fibril condition, proteins should not necessarily be denatured or exist in intermediate condition such as molten globule [18]. Thus, in some proteins there are mobile regions of polypeptide chain that are capable of spontaneous transition from structured condition (such as helix) to non-structured condition (loop). In this case, just such regions in non-structured condition can be the following basis for growth of amyloid fibrils, and the protein retains the bulk of native

structure [18]. Thus, transition from native protein with structured region to the native-like protein with non-structured region is the first stage in the proposed model. It was shown in experimental works that proteins in the native-like condition are capable of oligomerization [19]. In this case oligomerization is not a unidirectional process, and oligomers are in dynamic equilibrium with monomers. Thus, the oligomerization stage was included into our model.



(8)

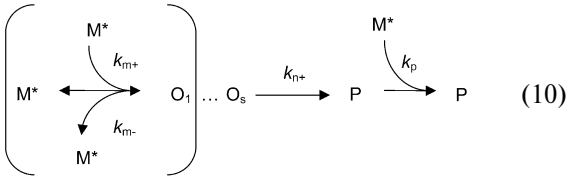
Oligomers that reached critical size are capable of spontaneous and irreversible transition to the amyloid fibril seed state via structural rearrangements. Such seeds are stable and can serve as the basis for amyloid fibril formation. For some proteins an important feature of amyloid formation is a lag period followed by rapid growth of the fibril proper. This event can be due to existence of a nonlinear process increasing the rate of monomer incorporation into the fibril. This was taken into account in the proposed model by addition of a stage of exponential growth of the fibril. For mathematical description the term was chosen in which the number of loci for beginning of growth would be in proportion with the aggregate size (branching script) [M*][P].

Step-by-step recording equations for each stage results in the equation system (9):

$$
\begin{cases}
d[M]/dt = -k_+[M] + k_-[M^*], \\
d[M^*]/dt = k_+[M] - k_-[M^*] - k_{m+}[M^*]^2 - \\
\quad - k_{m+}[M^*]\sum_{n=1}^{n=s-1}[O_s] + k_{m-}\sum_{n=1}^{n=s}[O_s] - k_p[M^*][P] - k_p[M^*][S], \\
\text{from 1 to s}\ \begin{cases} d[O_1]/dt = k_{m+}[M^*]([M^*] - [O_1]) + k_{m-}([O_2] - [O_1]), \\ \ldots \\ d[O_n]/dt = k_{m+}[M^*]([O_{n-1}] - [O_n]) + k_{m-}([O_{n+1}] - [O_n]), \\ \ldots \\ d[O_s]/dt = k_{m+}[M^*][O_{n-1}] - k_{m-}[O_s] - k_{n+}[O_s], \end{cases} \\
d[S]/dt = k_{n+}[O_s] - k_p[M^*][S] - k_a[S]^2 - k_a[S][P], \\
d[P]/dt = 2k_p[M^*][S] + k_p[M^*][P] + k_a[S]^2 + k_a[S][P].
\end{cases}
\qquad (9)
$$

The symbols in the equations correspond to designations used in Scheme (8).

Besides, we have considered a simplified scheme of amyloid aggregate formation on the condition that the number of monomers within critical size oligomer is 2 (Scheme (10)).



(10)

Transition from native to native-like condition is absent from this scheme; in addition, we did not consider transition to protofibril *S* (equation system (11)):

$$
\begin{cases}
d[M^*]/dt = -k_{m+}[M^*]^2 + k_{m-}[O_s] - k_p[M^*][P], \\
d[O_s]/dt = k_{m+}[M^*]^2 - k_{m-}[O_s] - k_{n+}[O_s], \\
d[P]/dt = k_{n+}[O_s] + k_p[M^*][P].
\end{cases}
\qquad (11)
$$

The model under consideration has several assumptions: i) it is supposed that the oligomer size can be changed due to a single monomer joining or dissociation; ii) it is supposed that the monomer attachment to the nucleus is irreversible, i.e. rates of back reactions are zero. Irreversibility of monomer addition to the nucleus was justified by Ferrone [2], while fragmentation into aggregates before and after nucleus formation (oligomers and fibrils) was used in polymerization models with seed formation [7, 8]; iii) it is supposed that all association rate constants are ($k_{m+1} = k_{m+2} = k_{m+3} = ... = k_{m+N} = k_{m+}$), because they are mainly defined by diffusion and remote interactions; iv) it is supposed that all dissociation rate constants are also equal to ($k_{m-1} = k_{m-2} = k_{m-3} = ... = k_{m-N} = k_{m-}$). For fibrils these rate constants sharply decrease because this is due to the higher stability of fibril comparative to oligomers. Because of this, we considered only association constants.

Methods. The standard Runge–Kutta algorithm in the Maple 12 environment (www.maplesoft.com) was used for solution of a complete system of kinetic equations. We used the standard scheme of fourth order of accuracy for our computations. The method is formulated as follows. Let us consider Cauchy problem $dy/dx = f(x,y)$, $y(x_0) = y_0$. The approximate value in subsequent points is computed using the iterative formula $y_{n+1} = y_n + h/6(k_1 + 2k_2 + 2k_3 + k_4)$, where h is the raster unit by x and the new value calculation consists of four steps:

$$k_1 = f(x_n, y_n),$$

$$k_2 = f(x_n + h/2, y_n + h/2k_1),$$

$$k_3 = f(x_n + h/2, y_n + h/2k_2),$$

$$k_4 = f(x_n + h, y_n + hk_3).$$

Total error at the final interval of integration is of $O(h^4)$ order. In our calculations $h = 1 \cdot 10^{-10}$.

Constants. Time in our calculations is in proportion with iteration value in the Runge–Kutta algorithm from which elementary reaction step derives during time interval $h \cdot k_+$. Concentration C in our calculations is expressed through oligomerization constant k_{m+}. Because it is a rate constant of a second order reaction and its dimensionality is $\{t^{-1}C^{-1}\}$ we assumed $C = 0.3$ (C). Generally, in equation solutions the constants were equal to following values: rate constant of monomer transition to the state for oligomerization (M*) $k_+ = 0.1$ $\{t^{-1}\}$; rate constant of monomer transition to native state $k_- = 0.001$ $\{t^{-1}\}$; rate constant of monomer attachment to oligomer $k_{m+} = 0.001$ $\{t^{-1}C^{-1}\}$; rate constant of monomer dissociation from oligomer $k_{m-} = 0.1$ $\{t^{-1}\}$; rate constant of the seed (the s size oligomer) transition from α state to β state $k_{n+} = 0.001$ $\{t^{-1}\}$; polymerization rate constant $k_p = 0.1$ $\{t^{-1}C^{-1}\}$; rate constant of protofibril association (attachment) $k_a = 0.1$ $\{t^{-1}C^{-1}\}$; the nucleus size s varied from 2 to 6 monomers. Deviations from used values are given in legends to appropriate figures.

RESULTS AND DISCUSSION

We set up a system of kinetic equations corresponding to Scheme (8). The lag period with following rapid (compared to the lag period) aggregate accumulation can be successfully simulated in the resulting model. No such effect is possible in schemes with linear growth (with attachment only to the growing fibril termini); therefore, usually only the term is added to equations that is responsible for nonlinearity associated, for example, with the growing fibril fragmentation or with the surface growth. In our system of equations, the term [M*][P] in the equation of amyloid fibril growth describes the latter as autocatalytic because incorporation of new monomers into an aggregate is in proportion with the number of already incorporated monomers. Moreover, the resulting equations simultaneously correspond to several possible scripts: i) fibril fragmentation; ii) new fibril growth from the surface of already grown ones, and iii) fibril branching. The main event in these processes is accelerated accumulation of vacant sites suitable for attachment of monomers incorporated into growing amyloid fibrils. Since the main kinetic curve parameters under consideration will be two intervals (widths of lag period and of the time required for all monomers to transition to aggregate), we shall introduce corresponding definitions: the lag period will mean the time during which no fibril growth is observed, and accordingly, the width of all monomer transitions to aggregate will mean the time during which all monomers will be transformed to aggregate. The scheme of interval determination is given in Fig. 1.

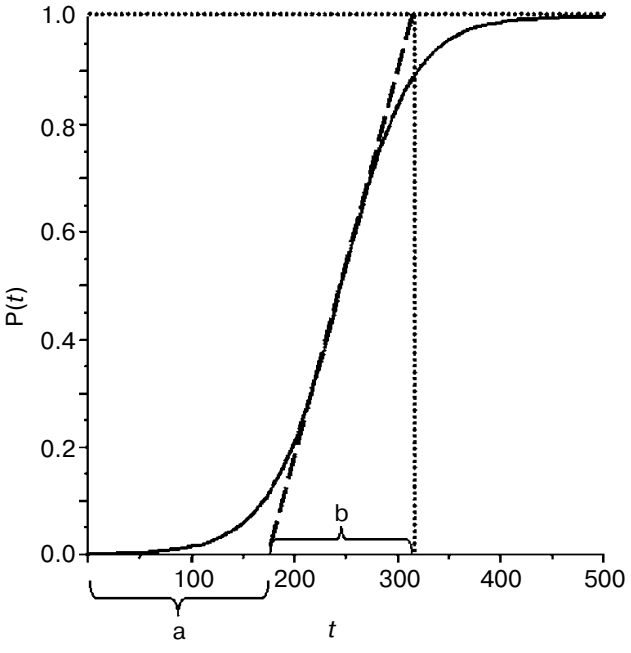


Fig. 1. Definition of terms "lag period" and "the time of transition of all monomers to the aggregate": lag period implies the "a" region cut off by tangent at the curve inflection point; the time of transition of all monomers to the aggregate implies the "b" region also cut off by tangent at the curve inflection point.

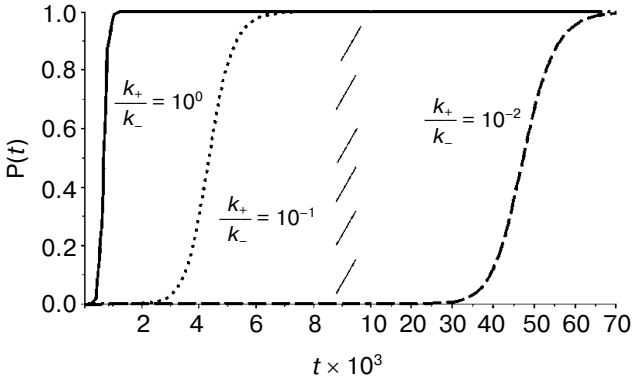


Fig. 2. Kinetic curves of amyloid structure formation: the effect of the ratio of kinetic rate constants k_+ and k_- upon transition from M to M*. P(t), the fraction of monomers incorporated into amyloid. The critical oligomer size is 2.

Besides, we apply an additional condition: the time during which no fibril growth is observed should be much longer than that required for aggregation of all monomers.

It should be noted that although the length of the lag period is connected with nucleation and the width of time of transition of all monomers to aggregate depends on the aggregate exponential growth, just due to the latter the relationship between these two intervals on a kinetic curve may change several-fold. We carried out several tests to estimate the contribution of different stages to the kinetic model (Scheme (8)).

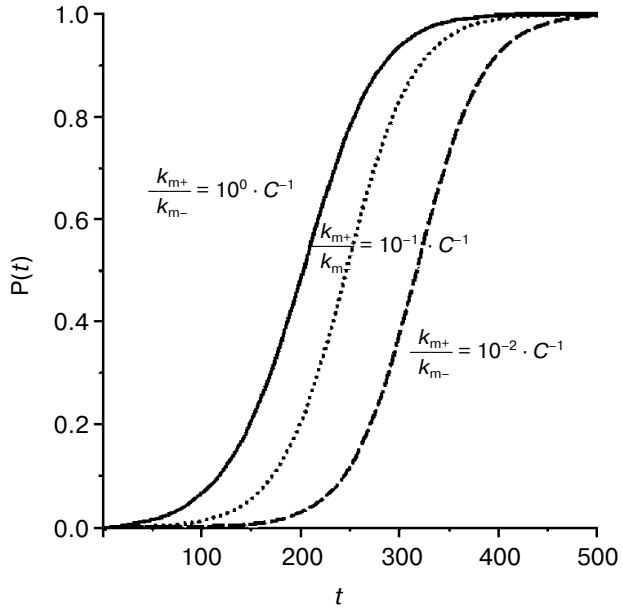


Fig. 3. Kinetic curves of amyloid structure formation: effect of intermediate state stability. P(t), fraction of monomers incorporated into amyloid. The critical oligomer size is two.

The first reaction, the effect of which on the amyloid formation process was analyzed, was protein transition from native to native-like state (Fig. 2). Although the impression can evolve that with the reaction shift towards prevalence of protein with native condition there should appear a pronounced lag period, this does not happen due to linearity of the transition. Thus, estimation of the ratio of the time for aggregation of all monomers to the length of lag period for each of three situations ($k_+/k_- = 1$; $k_+/k_- = 0.1$; $k_+/k_- = 0.01$) shows that it is identical in all three cases. So it was concluded that the change in the k_+/k_- constant ratio can only extend/shorten kinetic curves in time but not in shape.

A quite different situation was observed when we changed the constant ratio at the stage of oligomer formation (Fig. 3). It is seen in the figure that unlike the previous stage, the kinetic curve shape really changes. Moreover, if the ratio of the aggregation time of all monomers to the time of lag period is considered, then it is seen that this ratio decreases. These data indicated that, according to our definition, the change in the k_{m+}/k_{m-} constant ratio at the stage of oligomerization is really able to result in emergence of a lag period. This is due to existence of nucleation.

Besides, we considered the effect of the height of energy barrier upon transition from oligomer to the new phase seed, i.e. the effect of the critical size oligomer transition to the k_s seed. Figure 4 shows that when the barrier height increase (constant k_s decreases) the time of the lag period only increases.

Since the time of the critical size oligomer formation is a function of the number of incorporated monomers, it is not surprising that the most pronounced lag period is observed in the case of enlargement of the critical size oligomer. Figure 5 shows the dependence of the lag period on the number of monomers incorporated in the critical size oligomer.

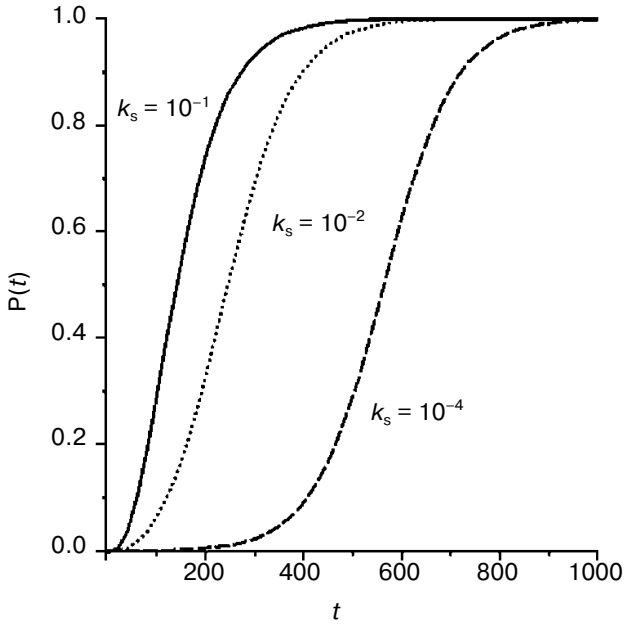


Fig. 4. Kinetic curves of amyloid structure formation: effect of rate constant k_s of the critical size oligomer transition into seed. P(t), fraction of monomers incorporated into amyloid. The critical oligomer size is 2.

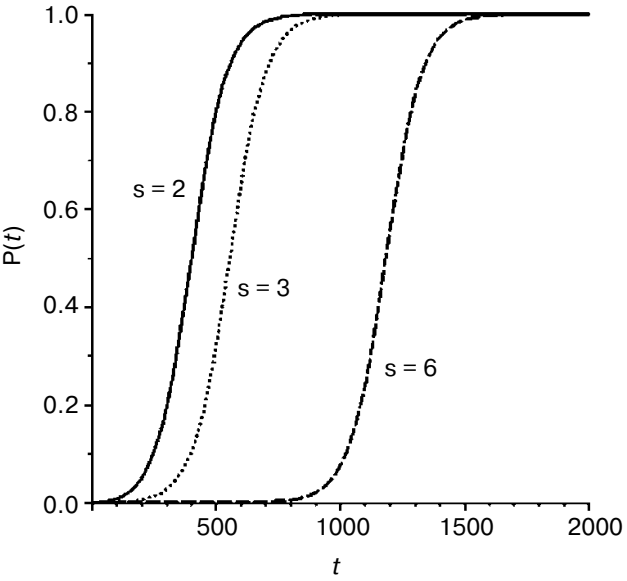


Fig. 5. Kinetic curves of amyloid structure formation: dependence of lag period on number of monomers incorporated in the critical size oligomer.

These data indicate that the first stage of the proposed model (monomer transition from native to native-like condition) has no significant effect on the lag period. To find out how the nucleation process is reflected on the kinetic curve behavior in our scheme, we designed a simplified scheme where there was no stage of protein transition from native to native-like condition (Scheme (9)). We plotted kinetic curves clearly showing what really happens at the stage of nucleation (Fig. 6).

At the very beginning of the process, the dynamic equilibrium between all oligomer fractions (O_1, O_2, etc.) and monomer fraction (M) is established, and this is followed by seed accumulation in critical concentration. At a certain moment there begins an avalanche-like growth of aggregates stimulated by the increase in the surface available for association of monomer with aggregate. Thus, it is possible to draw corresponding analogies with a chain reaction in which there is also a time necessary for reaching critical condition and subsequent exponential reaction. In the simplified model, like in the general model, there is an additional parameter that can influence the course of kinetic curves. Figure 7 shows changes in kinetic curves depending on this parameter. Despite reduction in the time of the lag period, 10-fold increase in k_p, the ratio of the time of transition of all monomers to aggregate to the length of the lag period remains constant.

It is known from the literature that the process of amyloid formation is strongly concentration dependent.

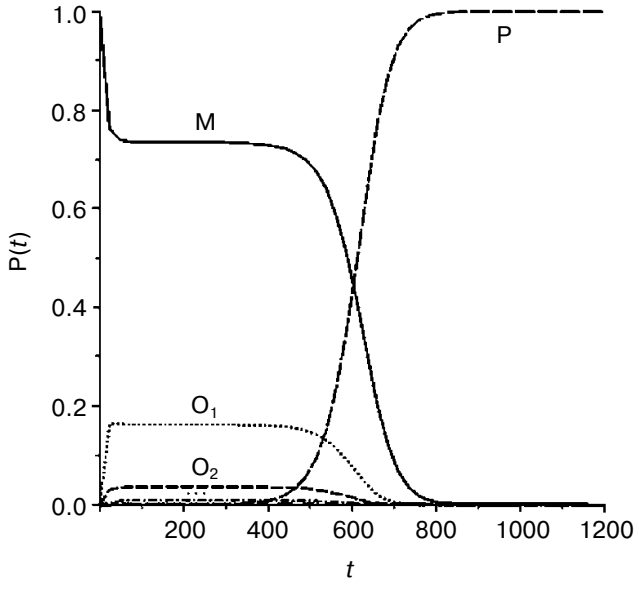


Fig. 6. Kinetics of distribution of different fractions for the simplified model. The simplified model with following constant values was used for plotting: rate constant of monomer association with oligomer $k_{m+} = 0.001$, rate constant of monomer dissociation from oligomer $k_{m-} = 0.1$, rate constant of seed transition (oligomer size $s = 6$) from condition "α" to condition "β" $k_{n+} = 0.001$, rate constant of polymerization $k_p = 0.1$.

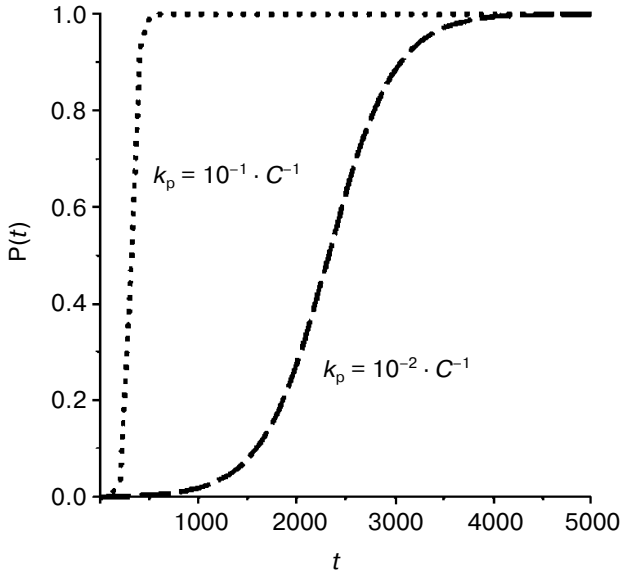


Fig. 7. Effect of rate constant k_p on total course of amyloid formation kinetic curves ($k_p = 0.1$ and $k_p = 0.01$). P(t), fraction of monomers incorporated into amyloid. Other designations as in Fig. 5.

We checked the effect of this parameter in our complete system of equations. Figure 8 (a and b) shows that the change in concentration in itself slightly influences the ratio of the aggregation time of all monomers to the length of lag period, i.e. the curve shape does not change, but the time of aggregate formation proper changes quite strongly.

However, decrease in monomer concentration increases the time for oligomer formation as well as the time for following monomer association into fibril. The same effect can also be seen on experimental curves (Fig. 1a [15]). It is seen that as concentration decreases, the time of monomer transition into fibril increases.

It is shown in this work that all parameters influence the behavior of kinetic curves in the proposed model. However, the curvature of the kinetic curve is determined by only three parameters: ratio of oligomer association/dissociation rate constants, rate constant of definite size oligomer rearrangement to amyloid seed, and number of monomers incorporated in the critical size oligomer. All three parameters directly influence the lag period by its reduction or prolongation. However, these parameters have no effect on the rate of monomer association with amyloid aggregate. The other parameters (monomer concentration, rate constant of protein transition from native to native-like form) influence the whole process of amyloid formation in the following way: decrease in monomer concentration retards both oligomer and fibril formation. In the case of transition from native to native-like form, the rate of oligomer formation changes due to existence of dynamic equilibrium between two forms of protein molecules. Thus, the concentration of monomers able to form

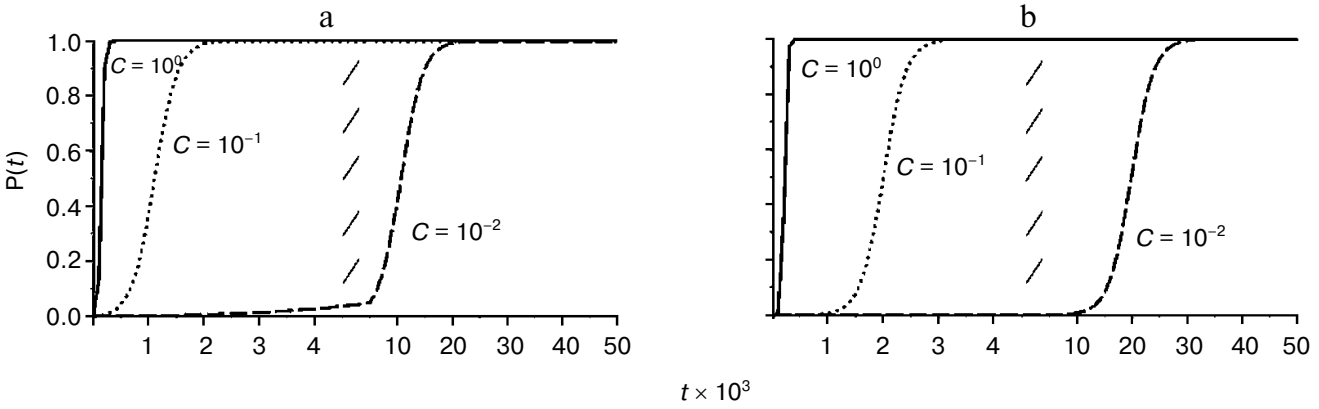


Fig. 8. Kinetic curves of amyloid structure formation for general model: effect of monomer concentration M. P(t), fraction of monomers incorporated into amyloid. a) Conditions without lag period ($k_{m+}/k_{m-} = 1$). b) Conditions with lag period ($k_{m+}/k_{m-} = 0.001$). The critical oligomer size is 2.

oligomers decreases, which results in a decrease at a definite moment of concentration of monomers capable of oligomerization. These results indicate that the model demonstrates a community in description of different scripts of amyloid formation. However, it seems impossible to predict on the basis of the proposed model and experimental data (shape of kinetic curve and initial experimental conditions, some detailed characteristics of aggregation process like the number of monomeric components in the critical size oligomer) the cause of structural rearrangements or the mechanism responsible for the exponential growth of the aggregate; additional non-kinetic experimental data are needed. However, rate constants calculated upon approximation on the basis of a fixed parameter known from experiment, such as concentrations, can point to reasons for a particular form of the kinetic curve.

A question arises to what extent this model is applicable to description of real amyloid formation processes and whether there are some experimental proofs of exponential growth following mechanisms of branching or growth from the surface. Formation of some amyloid fibrils is known to follow the script of heterogeneous nucleation, i.e. growth of amyloid aggregates proceeds from the surface of already formed amyloid fibrils, like in the case of insulin [20], amylin (IAPP) [8], Aβ peptide, and prions [21]. Evidently, "natural" amyloid film present in some organisms (including bacteria) are formed following just this script. Besides, it is known for α-synuclein that the protein half-folded intermediate contains extended regions of hydrophobic surface potentially able to stimulate aggregation and thus stimulate fibril growth [21].

The authors are grateful to A. V. Finkelstein for productive discussion.

This work was supported by the Russian Foundation for Basic Research (grants No. 08-04-00561 and 11-04-00763), the Russian Academy of Sciences (Programs "Molecular and Cell Biology" (01200959110) and "Fundamental Sciences to Medicine"), the Foundation for Promotion of Domestic Science, and the Dmitry Fomin Foundation "The Dynasty" of the Federal Agency on Science and Innovations (02.740.11.0295).

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