SIMULATION AND ANALYTICAL MODELS FOR THE ANALYSIS OF *IN VITRO* ACTIN POLYMERIZATION

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Abstract

The processes of actin polymerization have significant importance for different biological processes, such as cell cytoskeleton formation and cell motility. However, analytical analysis of the experimental data from these systems gives only rough estimations and has many limitations. In this paper the simulation formalism for the subsequent experimental data processing is proposed and the simulation model developed. The validation of the model has been performed by the comparison of the simulated results with the analytical prediction for the simplest situation.

1 Introduction

This paper is devoted to the simulation and analytical models developed for the data analysis in the study of the actin polymerization. Actin polymerization is a complex cell process (see the review by Pollard [6]), involved in cytoskeleton formation, cell movement and division. The motility of cancer cells and metastasis spread also depends on actin polymerization [2]. Despite a big number of experimental and theoretical works related to this subject, the mechanism of force generation, as well as roles of different proteins have not yet been completely understood. Therefore it is important to develop the computational model, which can be used in a general case to predict the behavior of actin polymerization process.

Another challenging task is to implement methodology for the application of the simulation models directly for data analysis. This approach is used in biophysics, when the studied systems and processes cannot be described analytically with satisfactory precision. In such a case computational simulation models can be used to fit the data as was described early [5]. In the current paper we are aimed at the development of the models for the analysis of the experimental data coming from actin-pyrene experiments [4]: the time-resolved evolution of pyrene fluorescence, which is proportional to the concentration of filamentous actins (F-actins) in a solution.

2 Actin Polymerization Process

Globular monomers of actin protein (G-actins) have the tendency to aggregate under the certain condition and form long filamentous polymers with asymmetrical ends

(barbed and pointed). The energy freed in actin aggregation is used by cells as a source of mechanical forces, which can be transferred into the cell propulsion. The process of actin assembly is influenced by the concentration of G-actins, physical conditions and, what is crucial, different regulative proteins: Arp 2/3, different capping proteins, profilin, formin, ActA, ADC/cofilin, to name just a few [6]. In our study we selected the most relevant proteins, usually used in laboratories for in vitro study of actin polymerization: actin, Arp2/3, ADC/cofilin, capping protein and profilin. Actin is directly involved in filament formation. Arp2/3, ADC/cofilin, capping proteins take part in filament branching, severing, and capping filament ends correspondingly.

3 Models Developed

3.1 Simulation Model for Actin Polymerization

To describe the developed simulation and analytical models, let us use the following notation for types of molecules and reactions (typical values of concentrations for different reagents and rates for the reactions are given in the brackets):
Reagents: actin in G- and F-forms - ACG, ACF ($10\mu M$); filament barbed and pointed ends - FIB, FIP; free and bound Arp2/3 - ARP, ARF ($0.3\mu M$); free and bound capping protein - CAP,CAF ($1\mu M$); ADC/cofilin - ADC ($3\mu M$).
Reactions: filament formation - k_{FORM} ($10^{-6}\mu M^{-2}s^{-1}$); averaged barbed end association - k_{ASSB} ($10\mu M^{-1}s^{-1}$); averaged pointed end dissociation - k_{DISP} ($0.6s^{-1}$); Arp2/3 binding (branching) - k_{ARPB} ($3\mu M^{-1}s^{-1}$); filament capping - k_{CAPB} ($3\mu M^{-1}s^{-1}$); filament severing - k_{SEVR} ($2.3 \cdot 10^{-6}s^{-1}$).

To simulate the reactions in actin system the Gillespie first reaction algorithm, based on the Monte Carlo paradigm, was used [3]. In this algorithm the putative times τ_i for each i-th reaction are generated using the assumption that the flow of reaction-events is Poisson one: $\tau_i = -a_i^{-1} \cdot \ln(\xi)$, where a_i is the concentration-dependent reaction rate, which can be calculated as the product of the reaction rate k_i and quantities of the reacting compounds; ξ is the uniform random value within the range (0,1). The reaction with the smallest τ_i occurs and the system time is increased by τ_i.

3.2 Analytical Model for Actin Polymerization

To write down the system of differential equations we made the following assumption. Short filaments (with length < 3 actins) can appear only as a result of severing or dissociation. When ADF/cofilin severs the filament near ends, a new short filament does not appear. F-actins of the short part directly go to G-actin pool. The full system of differential equations for 8 types of reagents was written down and simplified, taking into account natural dependencies in the system. Sum concentrations of bound and free proteins of one type are constant during experiment; number of barbed ends can be calculated from number filaments (FIP), capped ends (CAF) and branches (ARF). After these transformations the initial system of 8 equations is reduced to the system with 4 linearly independent equations:

$$
\begin{aligned}
\frac{\partial ACF}{\partial t} &= 3 \cdot k_{FORM} \cdot (A - ACF)^3 + k_{ASSB} \cdot (A - ACF) \cdot (FIP + ARF - CAF) - \\
&\quad -k_{DISP} \cdot FIP(1 + 2 \cdot p_{=3}) - k_{SEVR} ACF \left(1 + 3\frac{2 \cdot FIP + ARF}{ACF}\right) \\
\frac{\partial FIP}{\partial t} &= k_{FORM} \cdot (A - ACF)^3 + k_{SEVR} ACF \left(1 - \frac{2 \cdot FIP + ARF}{ACF}\left(3 - 6\frac{ARF}{ACF}\right)\right) \\
&\quad -k_{DISP} FIP \cdot p_{=3} \\
\frac{\partial ARF}{\partial t} &= k_{ARPB}(R - ARF)ACF - 3 \cdot k_{SEVR} ARF \frac{2 \cdot FIP + ARF}{ACF} - \\
&\quad -3k_{DISP} FIP \frac{ARF}{ACF} \cdot p_{=3} \\
\frac{\partial CAF}{\partial t} &= k_{CAPB}(C - CAF)(FIP + ARF - CAF) - 3k_{SEVR} CAF \left(1 - \frac{ARF}{ACF}\right) - \\
&\quad -k_{DISP}\left(1 + 3\frac{ARF}{ACF}\right)\frac{CAF \cdot FIP}{FIP + ARF} \cdot p_{=3}
\end{aligned}
$$

where A, C and R are total concentrations of free and bound actins, capping proteins and Arp2/3 correspondingly; $p_{=3}$ - probability, that the length of a filament at the current moment of time is equal to 3. This probability was the only empirical parameter and its value was approximated by the empirically obtained constant 0.029 during analytical modeling.

4 Results and Models Validation

To test the developed models we compare the results of the simulation and analytical models with different initial concentrations. One of the results is given in the plots below (Fig. 1).

It can be seen, that the behaviors of two models are in a good agreement. Two qualitatively different periods can be seen. In the first period filaments grow fast because there are a lot of free actins and Arp2/3 complexes in the system available, which, in fact, results in an autocatalytic polymerization reaction. In the second period of the evolution, almost all Arp2/3 are bound and most of barbed ends are already capped. Filaments grow only due to appearance of new barbed ends after severing.

5 Conclusions

We present the simulation model for the analysis of actin-polymerization process, taking place in experimental *in vitro* systems. The simulation model was tested by comparison with analytical prediction for the simplest case (minimal number of reagents and minimal number of reactions). Currently the more complex model is developed, which includes about 20 reagents and reactions. This model will be used for the analysis of pyrene-actin fluorescence experimental data in the framework of simulation based fitting.

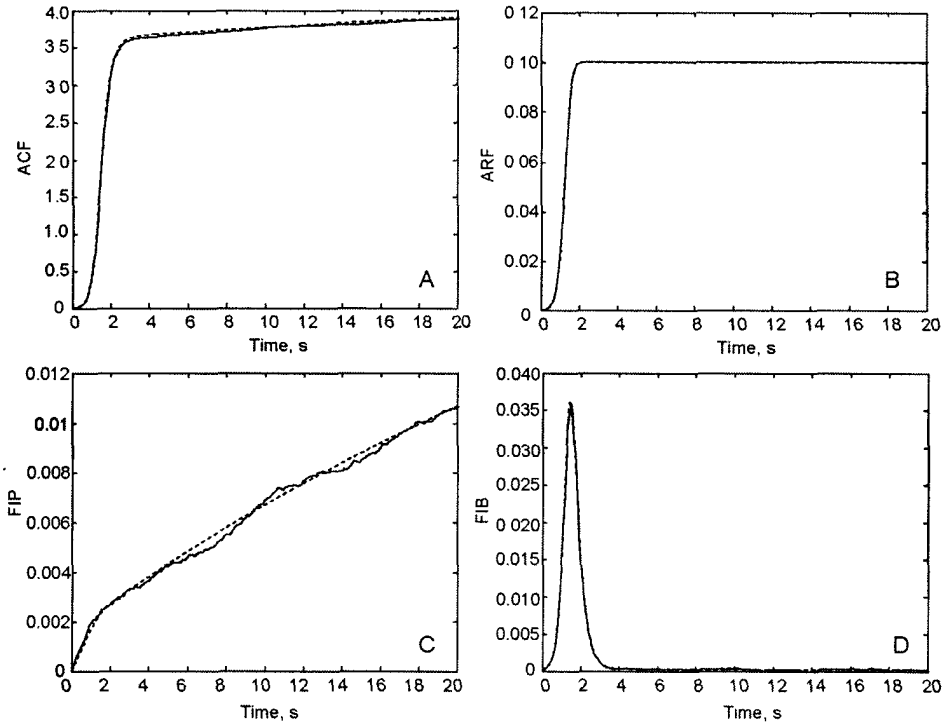

Figure 1: Comparison of the results of simulation (solid line) and analytical (dotted line) models. Concentrations of F-actins, ACF (A), and other reagents: ARF - (B), FIP - (C), FIB - (D) are presented.

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