Simulation of *Dictyostelium Discoideum* Aggregation via Reaction-Diffusion Model

B. N. Vasiev,[1,2] P. Hogeweg,[2] and A. V. Panfilov[2,*]

[1]*Theoretical Physics, Faculty of Physics, Samarkand State University, University Boulevard 15, Samarkand 703004, Uzbekistan*
[2]*Department of Theoretical Biology, University of Utrecht, Padualaan 8, 3584 CH, Utrecht, The Netherlands*
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We propose a reaction-diffusion model for simulation of the process of aggregation of *Dictyostelium discoideum* amoebae. The model is based on FitzHugh-Nagumo–type equations for cyclic adenosine 3′-monophosphate waves and a continuity equation for amoebae motion. We simulate the process of aggregation induced by a periodic point source and by spiral wave. We show that the aggregation pattern is formed as a result of front instabilities due to dependence of wave velocity on density of amoebae. This instability can also result in formation of wave breaks and generation of spiral waves.

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The present Letter deals with the problem of pattern formation in excitable reaction-diffusion systems. This problem is common in the physical, chemical, and biological sciences [1]. The main property of excitable media is conduction of propagating waves [2]. Usually these waves do not have an effect on the parameters of the medium itself. However, in several important situations the wave of excitation can change the properties of excitable media and cause the formation of spatial patterns. Aggregation of *Dictyostelium discoideum* (Dd) amoebae is an example of such a phenomenon. The monolayer of the starving amoebae is an excitable medium which conducts excitation waves of the intracellular mediator [cyclic adenosine 3′-monophosphate (cAMP)] [3]. Since cAMP is a chemotactic attractant for the amoebae, the waves of cAMP cause motion of the amoebae [4]. As a result of this motion amoebae are organized into streams which usually form branching radial multicellular structures. There are two major types of cAMP sources forming aggregates: a point source and a spiral wave. Figure 1 shows streams which were induced by a spiral wave of cAMP.

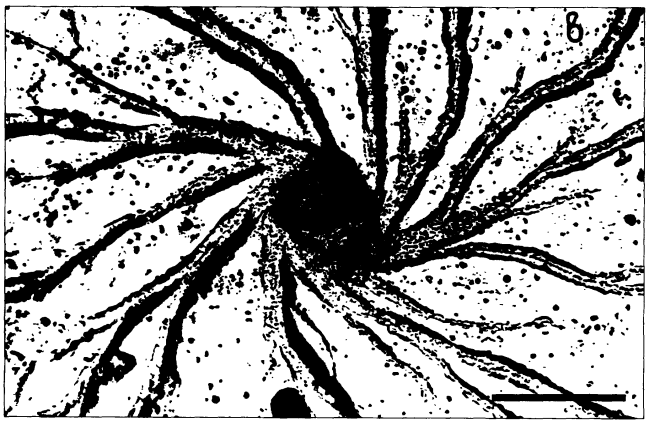

FIG. 1. View of the aggregative structure formed by a starving population of *Dictyostelium discoideum*. Bar is 150 μm. Given from [12].

There are two main approaches for modeling Dd aggregation. The first approach which considers amoebae as discrete cells was developed in papers [5] where occurrence of aggregation structures was shown numerically. The second approach which describes amoebae motion in terms of a continuous density variable was used in [6] to find conditions for density induced instability. Levine and Reynolds [7] have studied a modified Martiel-Goldbetter model [8] coupled to equation for chemotactic motion. They performed a linear stability analysis of the traveling wave solution for these equations and found that the real part of the growth rate exponent of the perturbation can be positive. They suggested that this instability can initiate the formation of transverse spatial structure. This paper gave an important insight into understanding this phenomenon; however, the linear analysis performed in [7] can only predict an occurrence of instability, and cannot give its consequences. In our Letter we were able to study numerically the process of aggregation of Dd in a continuous model. We found that the front instability proposed in [7] initiates the process of aggregation and gives a spatial pattern similar to those from the experiment. We also found that the extreme manifestation of such instability, the lack of wave propagation at low densities of amoebae, can initiate formation of spiral waves.

For our calculations we used the following model:

$$\begin{aligned} \partial r/\partial t &= (g - r)/\tau, \\ \partial g/\partial t &= D_g \Delta g + c^{\alpha}(f(g) - K_r r), \\ \partial c/\partial t &= D_c \Delta c - \nabla(cV(r)\nabla g). \end{aligned} \tag{1}$$

The first two equations are a FitzHugh-Nagumo model which describes the propagation of cAMP waves. Here g represents the extracellular concentration of cAMP, and r the recovery process. Instead of the ordinary cubic function $f(g)$ we used in the second equation the piecewise linear function [9]:

$$\begin{aligned} f(g) &= -Sg && \text{if } g \le 0 \\ f(g) &= K_g(g - a) && \text{if } 0 < g < 1 \\ f(g) &= -S(g - 1) && \text{if } g \ge 0 \end{aligned} \tag{2}$$

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in the limit that S is infinite. Because S in infinite $f(g)$ is defined only on the interval $0 < g < 1$. The first two equations are qualitatively similar to the Martiel-Goldbetter model reduced to two variables which describe the reception and the release of cAMP by amoebae [8]. We suggest that in normal conditions the production and the decay of cAMP are proportional to the cell density $c^{\alpha}(\alpha = 1)$. However, for some computations we will also use other values of the exponent α.

The third equation in (1) describes the chemotactic motion of amoebae. Here c is the local concentration of amoebae, and $V(r)$ is their motility. In our model (as $r \geq 0$) V reaches its maximum at $r = 0$ and decreases to 0, with increasing r. Biologically, this means that cells move if they are not refractory. $V(r)$ has the following shape:

$$V(r) = K_v\left(\frac{K_{\text{thresh}} - r}{K_m + \text{abs}(K_{\text{thresh}} - r)} + 1\right), \qquad (3)$$

where K_v determines the maximal cell velocity, K_{thresh} is a threshold value around which cells lose their ability to move, and K_m determines the slope of $V(r)$ at $r = K_{\text{thresh}}$. The value of $K_v = 0.03$ was chosen such that the average velocity of the cells was about 2% of the velocity of the cAMP waves, which is in agreement with experimental data [4].

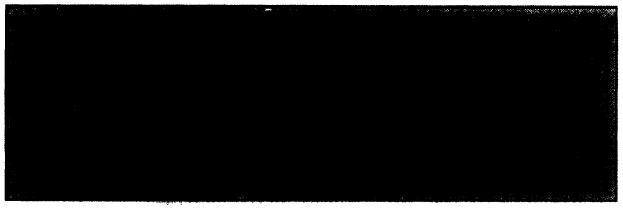
Calculations were performed in a two-dimensional array of 200 × 200 elements by using the explicit Euler method with space step $h_x = 0.4$ and time step $h_t = 0.01$. We used Neumann's "no flux" boundary conditions. The other parameter values were $D_g = 0.1$, $D_c = 0.001$, $\tau = 0.5$, $\alpha = 1$, $K_r = 3$, $K_g = 2.1$, $a = 0.05$, $K_m = 0.01$, and $K_{\text{thresh}} = 0.2$.

To find dimensioned values for space and time units in our model we computed the refractory period for cAMP waves (20 time units) and the velocity of cAMP wave propagation (0.3 space units per time units). In natural populations of *Dictyostelium* cells the refractoriness is about 5 min and the velocity of cAMP waves is 600 μm/min [4,10]. Therefore, our time unit is 15 s and space unit is 500 μm.

Figure 2 shows the formation of an aggregation pattern from an initially random distribution of amoebae. In Fig. 2(a) we have a point wave of the cAMP waves, and in Fig. 2(b) a rotating spiral wave. It can be seen that in both cases the distribution of the amoebae changes over the course of time (compare frames in Fig. 2), and finally they form the pattern of branching streams. Distinct streams become visible after the propagation of 15–20 waves. Afterwards, there is a slow evolution of the stream structure, during which the streams become shorter and the cell concentration at the center increases; i.e., cells collect in the stimulated region.

We have found that a necessary condition for stream formation is nonuniformity in the initial distribution of amoebae density. Figure 3(a) shows a computation

(a)



(b)

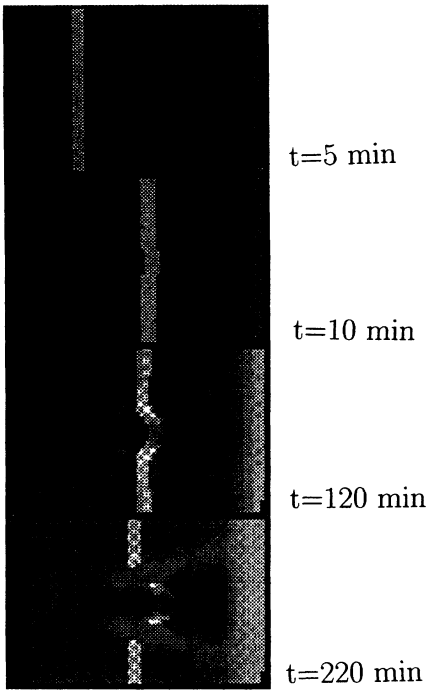
FIG. 2. Formation of the structure in the model (1). Initial distribution of amoebae density is given by random numbers between 0 and 1 [in each grid point on (a) and in each square of 2 × 2 grid points on (b)]; i.e., the average density is 0.5. In (a) the cAMP waves were initiated periodically (with a period of 10 min) by stimulation of the central area of the medium. In (b) the only one wave of cAMP was initiated. Spirals in (b) occur as a result of spontaneous breaking of initiated circular wave. The cAMP wave (white) is superimposed on the pattern of amoebae density (various shades of grey).

similar to that in Fig. 2(a), but with an initially uniform distribution of amoebae. We see that streams were not formed here; amoebae collect in the stimulated area and form a circular spot with high density.

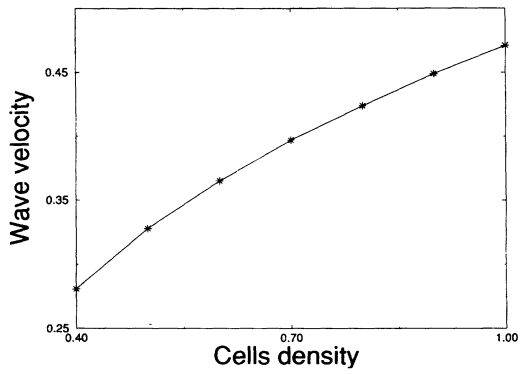
The mechanism of stream formation is associated with the fact that the velocity of the cAMP waves depends on the local density of the amoebae. To demonstrate

(a) (b)

FIG. 3. (a) Aggregation pattern in the model field with initially uniform amoebae density. All settings are as in Fig. 2. (b) Aggregation pattern at $\alpha = 0$ in the model (1). In both cases amoebae collect in the stimulated region without stream formation.



(a)



(b)

FIG. 4. Mechanism of stream formation. (a) Evolution of local inhomogeneity in the amoebae density due to amoebae motion. Computation on the grid of 40 × 60 elements. All other settings are as in Fig. 2. (b) Dependence of the velocity of the cAMP waves on amoebae density ($\alpha = 1$).

this, we studied the wave propagation in the medium with density $d = 0.5$ to which we added a small spot of high density ($d = 1$). Figure 4(a) shows the propagation of plane periodic waves initiated at the left boundary of this field. It can be seen that the velocity of waves in the spot is higher, and this causes a change in the shape of the wave; the wave front becomes curved in such a way that cell motion (along the gradient of cAMP, i.e., in the direction of normal to the wave front) increases the inhomogeneity. This increased inhomogeneity in turn increases the curvature of the front of the next wave, etc. Finally we see the formation of a long black region with a high density of amoebae or, in other words, there is an increase in the initial inhomogeneity. In the case of Fig. 2 the same process causes stream formation in our system. Note that at the same time the spot in Fig. 4 also moves in the direction of the left boundary.

The dependence of the velocity of the cAMP waves on the local density of amoebae can cause the formation of spiral cAMP waves. Spiral waves in Fig. 2(b) have occurred due to the breaking of a circular wave in the region with low amoebae density ($d < 0.4$). Frequencies of rotation of the spiral waves depend on the distribution of the cells in the region of their cores. One of the two spirals in Fig. 2(b) ($t = 7$ min) initially rotates faster than the other. As a result of spiral interaction, the slow spiral is destroyed and only the fast spiral wave remains in the medium and causes the formation of aggregation structure [see Fig. 2(b) $t = 125$ min, $t = 225$ min]. To form spirals the wave break has to occur in an area of relatively large size [compare Figs. 2(a) and 2(b)].

In our model the dependence of the velocity of the cAMP waves on amoebae density is relatively strong [see Fig. 4(b)]. The speed of the cAMP waves decreases nearly twofold while the density of amoebae decreases from 1 to 0.4 (waves cannot propagate when the density is less than 0.4). By decreasing exponent α in (1) it is possible to make this dependence less strong. This results in an increase of the time required for stream formation. In the limit case when the wave velocity did not depend on cell density ($\alpha = 0$), the streams were not formed at all [Fig. 3(b)]. In this case, moving amoebae formed a spot of high density around the stimulated area, without stream formation, similar to the case in Fig. 3(a).

The reaction-diffusion model proposed in this Letter describes fairly well the aggregation process in a natural population of Dd. The aggregation pattern obtained by numerical simulation (Fig. 2) looks similar to the pattern in amoebae populations (Fig. 1). Our model also gives the correct number of waves necessary for structure formation. In both cases the pattern appears after the propagation of about 15–20 waves. In simulations presented in Figs. 2, 3, and 4(a) the period of wave stimulation was 10 min which is twice more than the refractory period of the medium. We have also made computations at periods 7.5 and 15 min. In all cases we obtained similar aggregation patterns appearing after the propagation of about the same number of waves.

The aggregation pattern in Fig. 2 was obtained for the average amoebae concentration $d = 0.5$, which corresponds to a cell density of 5×10^5 cells/cm^2 (provided that amoebae sizes are 10×20 μm^2). This is in the range of usual experimental conditions [10]. We have also observed the process of stream formation for d: $0.4 < d \leq 1$, i.e., over the whole range of existence of cAMP waves [Fig. 4(b)]. In all this range, the stream patterns were similar to those in Fig. 2.

From a general point of view the mechanism of stream formation proposed in this Letter can be viewed as wave instability due to the chemotactic motion of amoebae. This phenomenon was predicted analytically in [7]. In [11] it was shown experimentally that chemotactic motion of E-coli can result in wave instability in natural systems.

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*The author to whom all correspondence should be addressed.

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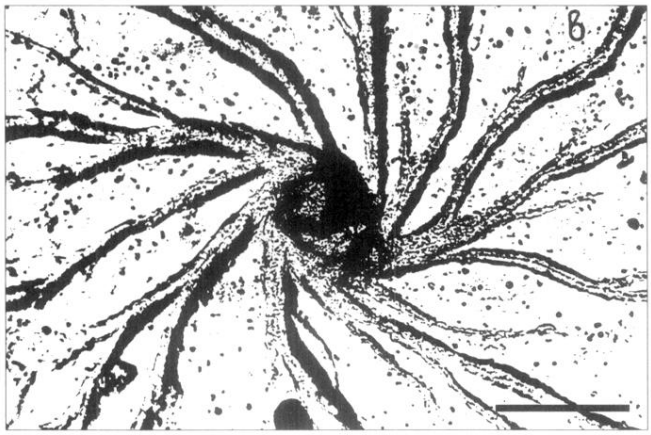

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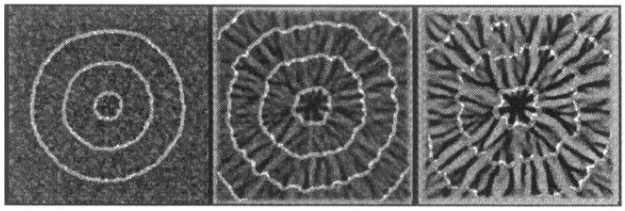
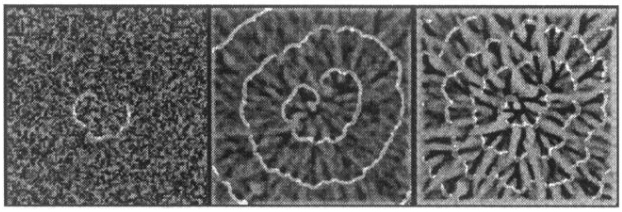


t=25 min t=125 min t=225 min

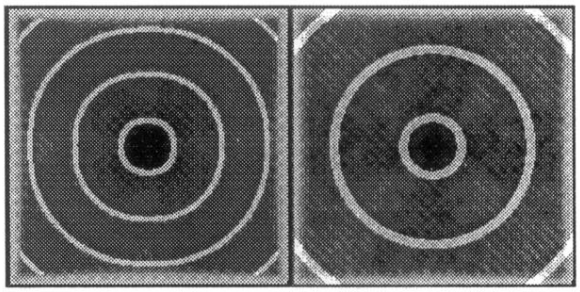
(a)



t=7 min t=125 min t=225 min

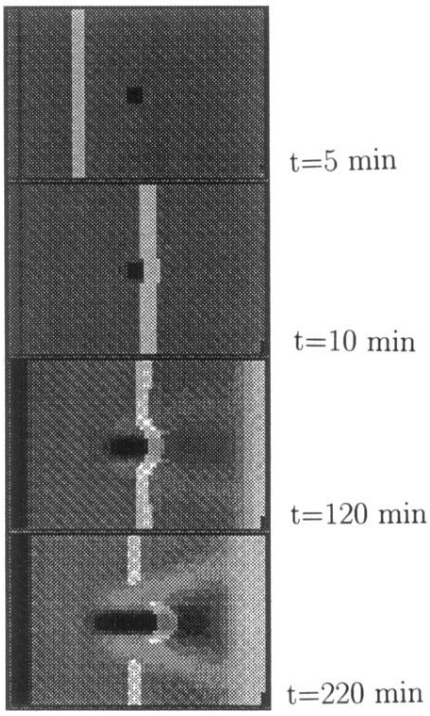
(b)

FIG. 2. Formation of the structure in the model (1). Initial distribution of amoebae density is given by random numbers between 0 and 1 [in each grid point on (a) and in each square of 2×2 grid points on (b)]; i.e., the average density is 0.5. In (a) the cAMP waves were initiated periodically (with a period of 10 min) by stimulation of the central area of the medium. In (b) the only one wave of cAMP was initiated. Spirals in (b) occur as a result of spontaneous breaking of initiated circular wave. The cAMP wave (white) is superimposed on the pattern of amoebae density (various shades of grey).

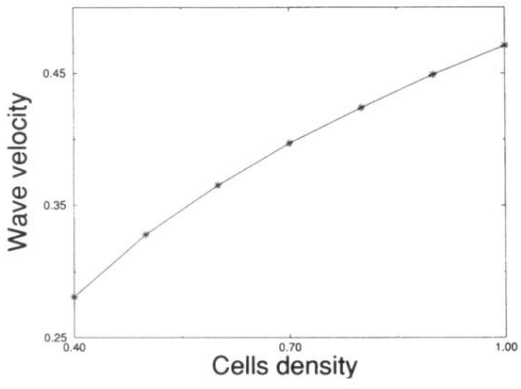


t=225 min (a) t=225 min (b)

FIG. 3. (a) Aggregation pattern in the model field with initially uniform amoebae density. All settings are as in Fig. 2. (b) Aggregation pattern at $\alpha = 0$ in the model (1). In both cases amoebae collect in the stimulated region without stream formation.



(a)



(b)

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