Off-lattice simulation of the solid phase DNA amplification

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Abstract

Recent simulations of the solid phase DNA amplification (SPA) by J.-F. Mercier et al (Biophys. J. 85 (2003) 2075) are generalized to include two kinds of primers and the off-lattice character of the primer distribution on the surface. The sigmoidal character of the primer occupation by DNA, observed experimentally, is reproduced in the simulation. We discuss an influence of two parameters on the efficiency of the amplification process: the initial density of the occupied primers from the interfacial amplification and the ratio of the molecule length to the average distance between primers. The number of cycles till the saturation decreases with roughly as $p_0^{-0.26}$. For $r = 1.5$, the number of occupied primers is reduced by a factor two, when compared to the case of longer molecules. Below $r = 1.4$, the effectivity of SPA is reduced by a factor 100.

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1 Introduction

The standard polymerase chain reaction (PCR) allows to produce multiple copies of DNA in vitro. It is known as a revolutionary technique for molecular biology [1]. The reaction takes place in the whole volume of a vial. If different molecules of DNA are to be amplified, the reaction products must be separated by other methods [2]. This separation is successfully omitted in a recently introduced technique of the solid phase DNA amplification [3][4], which can be classified as an application of the emerging biochip technology [5][6].

The technique, termed as SPA, contains three stages. At first, primers of two kinds are attached by their 5’ ends to a solid surface. They are to be complementary to two ends of the investigated DNA strands, then their proportions should be 1:1. Next, small amount of DNA is attached to some of the primers in one thermal cycle. This stage is termed as the interfacial amplification (IA). At third stage (surface amplification, SA), the solution of DNA is washed out, and it is only the attached strands which are multiplied in subsequent cycles. They attach their free ends to neighbouring primers. Next, they are copied by standard PCR. One thermal cycle can be separated in three distinct steps -
annealing, extension and denaturation, which are repeated in an iterative way. In this way, the concentration of occupied primers (i.e. primers with attached DNA) increases. In this final stage of SPA the area of occupied primers widens by a slow motion of its borders: the border velocity is not larger than the strand length per cycle. A detailed and transparent description of the process can be found in \cite{2}, together with first Monte Carlo simulations.

The aim of this work is to investigate numerically the range of experimental parameters, where the surface amplification is effective. Our contribution can be treated as a computational supplement to Ref. \cite{2}. However, there are two main differences between our approach and Ref. \cite{2}. First, our simulation is performed in the off-lattice scheme, i.e. the positions of the primers on the surface do not form a square lattice, but they are randomly distributed. Second, we take into account the fact that there are primers of two kinds. Both modifications are introduced to make the simulation more realistic. In particular, we intend to capture the limitation of SPA which arise when the length of the strands used is of the order of the mean distance between the primers. The distance distribution between primers is deformed by the assumption of the square lattice, and this deformation is particularly strong for short distances. Here this assumption is omitted. On the other hand, once a molecule of DNA is attached to a given kind of primer by one end, its free end can only be attached to the primer of the other kind. If the distance between primers is of the order of the molecule length, this condition additionally limits the surface amplification process. Again, here this limitation is captured properly.

We also investigate an influence of other parameters: of the efficiency of the interfacial amplification and of the mutual proportion of two kinds of primers. The results can be useful for designing the range of parameters, where SPA is optimal. If the concentration of occupied primers after the interfacial amplification is low, the number of SPA cycles must be large, what raises costs. Note that this concentration is connected directly with the density of the investigated DNA in the sample, and we are obviously interested in the possibility of detecting low amounts of DNA.

In the next section we discuss the values of the input parameters for the calculation. One of them, the density $\rho_0$ of occupied primers at the beginning of the simulated process, is the output of IA and therefore it can be treated as a measure of its efficiency. In this section we describe also the simulation method. In subsequent section our numerical results are reported. The text is closed by discussion.

\section{Calculations}

In our computational model we refer to the data on SPA given in Ref. \cite{4}. There, an optimal value of the density of the primers on a surface has been found to be $15 \text{fmol/mm}^2$. After two cycles of IA and SA, the density $\rho_0$ of occupied primers is $0.0011 \text{fmol/mm}^2$, i.e. a molecule of DNA is attached to one per 15000 primers. This amount comes mainly from IA \cite{4}. Subsequent 28
cycles of SA lead to an increase of $\rho$ to $0.0029 \text{fmol/mm}^2$. If the process of SPA is performed without washing the sample, IA and SA occur simultaneously. In this case, 28 cycles lead to $\rho = 0.019 \text{fmol/mm}^2$.

These data allow to estimate the efficiency of both IA and SA at one cycle of SPA, for the case when $\rho$ is small enough. This condition, true at few initial cycles, allows to neglect the slowing down of the amplification because of the overcrowding by occupied primers. Then, the density $\rho$ increases in subsequent cycles of SA by a constant factor $\alpha$, and $\rho_n = \alpha^n \rho_0$. From the data given above we deduce that $\alpha^{28} = 29/11$, i.e. $\alpha = 1.035$. For such a small rate of amplification, the increase of $\rho$ in first two cycles of IA+SA is due mainly to IA. As we expect that this increase is linear with the number of cycles, we get its value equal to $x = \rho_0/(1 + \alpha) = 0.00055 \text{fmol/mm}^2$ per cycle.

For small $\rho$, at each cycle of IA+SA the density of occupied primers should increase according to the rule:

$$\rho_{n+1} = x + \rho_n \cdot \alpha \quad (1)$$

Applying this rule again 28 times with $\rho_0 = 0.0011 \text{fmol/mm}^2$, we get $\rho_{28} = 0.029 \text{fmol/mm}^2$, which is about 50 percent larger, than the experimental value. We deduce that this value cannot be treated as small, and the effect of a local overcrowding should be taken into account. This means, that Eq.(1) cannot be applied: a simulation is necessary.

The area of the surface used for SPA is about $1 \text{mm}^2$, and the number of primers $pN$ there is equivalent to $15 \text{ fmol}$, i.e. about $9 \times 10^9$ primers. This amount is too large for our computational resources. We work with $K = 15000$ primers, which is equivalent to the area $S$ about $1 \mu\text{m}^2$ covered with the density $15 \text{ fmol/mm}^2$. To improve statistics, we average the results over $k$ runs. The positions of the primers are selected randomly with a constant probability distribution on a squared area with periodic boundary conditions. Then, DNA strands is attached to some primers. The amount of these primers is a measure of the effectiveness of IA. Simultaneously, it is proportional to the starting value of $p$ for the simulation of SA. The amount of occupied primers is

$$\rho S = pK \quad (2)$$

The density value $\rho = 0.0011 \text{fmol/mm}^2$ is equivalent to $p = 1/15000$, i.e. one occupied primer as a starting point for the simulation.

Two kinds of primers fit to two ends of the investigated molecules of DNA. Let us denote the primers by $\pm 1$. For each occupied primer $i$, the algorithm of the simulation of SA selects a primer $j$ which is $i)$ its closest neighbour $ii)$ of opposite sign and $iii)$ it is free. The additional condition is that the distance between $i$ and $j$ cannot exceed the molecule length. During each cycle of SPA, the molecule attached to $i$ with one end is attached with another end to $j$ with probability depending on the distance according to the Gaussian function, multiplied by a phenomenological factor $\gamma$. The choice of the Gaussian function reflects the random-walk character of the free end of the molecule. The factor $\gamma$ is set as to reproduce the experimental value of IA, expressed by $\alpha = 1.035$. This
value means that during one cycle of SPA, only a few percent of DNA strands are amplified. Our algorithm is approximate, because we neglect the possibility that the free molecule end is attached to another primer, which is not the closest one. This simplification is introduced to speed up the calculations. According to the Gaussian distribution, the attaching probability strongly decreases with the distance. That is why this approximation seems to be acceptable.

3 Results

In Fig. 1 we show the ratio $p$ of the number of occupied primers to their total number against time, the latter expressed in the number $N$ of thermal cycles. The starting value of $p$ is taken as $p_0 = 1/15000$, and it is due to the amount of primers occupied in the interfacial amplification. The increase of $p$ shown in the picture is a consequence of the surface amplification. The curve is obtained by averaging over 14 simulations. The error bars are due to the statistics. The sigmoidal curve allows to evaluate the overall dynamics of the process of SA.

In Fig. 2, the number $N$ of cycles needed to obtain the saturation $p = 1$ is shown as dependent on the number of primers $p_0$ occupied by means of the interfacial amplification. As we see, $N$ decreases as $p_0^{-\phi}$, at least for $p_0$ far from the target value $p = 1$. We get $\phi = 0.264$. Note that the data in Fig. 2 are not equivalent to those in Fig. 1. To obtain each point of the plot, a separate simulation is performed. During the process of SA, spatial correlations between occupied primers develop from an initially random configuration.

In Fig. 3 we show the number $N$ of cycles needed for the saturation, against the ratio $r = L/d$, where $L$ is the molecule length and $d = (S/K)^{1/2}$ is the mean distance between primers. The plot shows a plateau for $r > 1.8$; below this value, $N$ abruptly increases. In Fig. 4 the limit value of $p$ is shown, which stabilizes after many steps of SA, as dependent on the ratio $r$. As we see, below $r = 1.4$, the process of SA does not work.

In Fig. 5 we show how SPA is sensitive to a variation of the ratio $c$ of the numbers of two kinds of the primers. Obviously, an optimum of the technique is for $c = 0.5$, when we have in the average the same numbers of the primers appropriate for both ends of the investigated molecules of DNA. However, the method works also for other values of $c$. We see that for $r = 2$, the saturated state $p = 1$ can be reached for the range of $c$ as wide as $(0.2, 0.8)$. This means, that for shorter molecules or for a smaller density of primers, the proportion of two kinds of primers should be controlled more precisely.

4 Discussion

The exponential character of the results presented in Fig. 2 allow to evaluate the time of measurement by SA as dependent on $p_0$. The value of $p_0$ is directly proportional to the efficiency of the interface amplification, which depends both on the time of IA and on the concentration of DNA in investigated samples.
Further, the results shown in Fig. 3 prove, that the efficiency of the surface amplification depends on the molecule length only if the latter is of order of the distance $d$ between primers. The same conclusion can be drawn from Fig. 4. We note that in this case, $d$ can be evaluated as $\rho^{1/2}$. These results provide a direct information on the range of parameters where the technique of the surface amplification can be used. Also, the obtained limitations of the range of the parameter $c$ can be of interest for applications. Actually, this result show that the technique works quite well even if the proportion of two kinds of primers is between 1:4 and 4:1.

A drawback of our simulation is that interactions between the molecules of DNA is not taken into account. This interaction is known to prevent the saturation; the obtained values of $p$ stabilize near $1/300$ instead of $1.0\,[4]$. This is a severe limitation of the efficiency of the whole technique. However, we expect that with an appropriate rescaling of the value of the parameter of $p$ at saturation, our results remain qualitatively correct.

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Figure 1: Fraction of occupied primers $p$ against the cycle number $N$.

Figure 2: The number of cycles $N$ needed to obtain the saturation $p = 1$ as dependent on the initial density $p_0$. 
Figure 3: The number of cycles $N$ needed to obtain the saturation $p = 1$ as dependent on the ratio $r = L/d$.

Figure 4: The final occupation $p$ as dependent on the ratio $r = L/d$. 

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Figure 5: The final occupation $p$ as dependent on the ratio $c$. 