MODEL OF PCR KINETICS

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Resume

Motivation:
Despite the fact that PCR is a powerful tool in modern biology and medicine, its quantitative model describing product accumulation at later stages of amplification is not developed yet.

Results:
Kinetics of standard amplification is treated as recurrent scheme with a variable amplification factor $H(n)$ introduced. At each cycle number $n$, the $H(n)$ is obtained from solving a system of equations of chemical kinetics, which takes into account the reassociation and destruction of PCR product. The amplification limit is calculated as a fixed stable point $H=1$ of the transformation. A good agreement has been found between the results obtained under the proposed model and the known experimental data. The model can be straightforwardly generalized for use in more realistic PCR computer simulation.

Introduction

PCR is a widely used technique for specific amplification of the target DNA to reasonable quantities. For successful PCR performance an optimal pair of primers is required. The procedure of selection of an appropriate set of primers is implemented in a wide range of computer packages (e.g., [1, 2]) for molecular biology. However, even good-fitted primers require optimal reaction conditions. When approaching high product concentrations, the PCR efficacy is limited by cooperative action of a number of factors (renaturation of the product, polymerase and primers deficiency, etc. [3]), providing a permanent reduction of the amplification factor $H(n)$. Its departure from the maximal value of 2 can be significant. When the value of the amplification factor is close to 1, further amplification does not provide any substantial increase in the product yield ("plateau" effect).

The Theory

Key factors of the plateau effect. Product accumulation is limited by many factors. Among them, renaturation and destruction are most significant ones, as none of the modern PCR techniques can get rid of them. The renaturation of a product inhibits primer annealing and strand synthesis. The destruction of a product is responsible for the plateau effect per se, otherwise the product content would have been growing infinitely, though slowly. While the product concentration, $C$, is high, a strand can be annealed in the process of amplification, and then this annealed strand can be cleft by Taq polymerase possessing 5'-3' exonuclease activity. The higher is the concentration of the product, the higher the rate of the process. The destruction process equilibrates the synthesis when the amplification is at its limit.

A formal description of amplification kinetics. PCR efficacy is usually characterized by some constant amplification factor [4], which in fact is the geometrical mean of the partial amplification factors (each of them describes the product accumulation at a single cycle). Instead, we introduce a variable amplification factor, $H$, at a cycle $n$ in the recurrent form:

$$C_{n+1} = H(C_n) C_n$$

(1)

Obviously, the value of $H$ depends on the PCR parameters and can be calculated within the frame of the scheme of chemical reactions proceeding at each cycle. Under the model being described, the $H$ is obtained in the analytical form. A universal amplification factor allows me to describe the PCR kinetics within the entire range of product concentrations. The transformation (1) has been found to have two fixed points, the unstable ($C = 0$) and the stable ($H = 1$), the latter corresponding to the amplification limit.

The kinetic scheme of a cycle. As limiting factors of PCR, here I consider only renaturation of complementary product strands (with a second order rate constant, $k_a$) and destruction of renatured product strands (with a first order rate constant, $k_d$). The renaturation is assumed to block successful synthesis on either of reassociated strands. For a pair of complementary strands, I calculate the probabilities $P_i$ of the following processes:

a) each strand reproduces a complementary strand;
b) the pair of strands is annealed and neither of them is cleft;
c) the strands are annealed, then either of them is cleft;
d) the strands are annealed, then both are cleft.

The amplification factor. It can be readily obtained by summing over a-d) contributions:

$$H = \sum_i H_i P_i$$  \hspace{1cm} (2)

where $H_1 = 2$, $H_2 = 1$, $H_3 = 0.5$, $H_4 = 0$. It is convenient to go to dimensionless variables $u = k_D C \tau_0$, $T = \tau_0 / \tau$, $D = k_D \tau$. ($C$ is the initial concentration of complementary pairs at the beginning of each cycle, $\tau$ is the duration of the phase of synthesis, $\tau_0$ is the period of annealing-free synthesis. One can simply estimate $\tau_0$ through the product length $L$ and the rate of nucleotide incorporation $k_i$: $\tau_0 = \frac{L}{k_i}$).

Renumbering cycles by dimensionless concentration $u$ (1), one can obtain a general expression:

$$H = 2 \left[ \int_0^u \frac{1}{(1 + ux)^2} \, dx \right]^{1/2}.$$  \hspace{1cm} (3)

As can be seen from Eq. 3, in case the product concentration is small (the "dilute limit"), $H = 2$ and it grows exponentially. This increase entails the monotonous decrease of the amplification factor that finally approaches the value $H=1$.

It is instructive to consider the low $T$ limit ("rapid" synthesis), where the amplification factor is expressed algebraically:

$$H = \frac{2}{u + 1} + \frac{ue^{-D}}{u + 1}.$$  \hspace{1cm} (4)

Comparing with experimental data.

As it is difficult to obtain a reliable value of the amplification factor in the dilute limit (small product concentrations can hardly be detected), I set it equal to 2 throughout. It could be estimated from the dependence of the cycle number required for a given product yield on the initial concentration. However, such a "delay" experiment would produce a very rough estimate of the amplification factor. In particular, a non-specific amplification increases the delay. In [4], the decrease of the initial copy number by 3 orders of magnitude has resulted in a delay of 10 cycles, which corresponds to $H=1.995$. In this process, the non-specific product was not detected, what was the case while further decreasing of the initial product content. Meanwhile, the regression of dependence [4] within the whole range of initial product concentration corresponds to the apparent amplification factor 2.15.

To test the model, I have used the data on amplification kinetics published in [4, 5]. First, since synthesis temperature has a dramatic impact on the rate of nucleotide incorporation $k_i$ [6] and hence on $T$, I have...
estimated the T values by interpolating the known temperature dependence of $k_i$ [6]. The values have been found to be low ($T \approx 0.08$ for kinetics given in [4] and $T \approx 0.03$ - in [5]). Secondly, using the approximation (Eq. (4)), I have reconstructed the amplification kinetics. A good agreement has been found (Fig.1) between calculations performed by my model and the experimental amplification kinetics [4] with excess of polymerase.

Using Eqs. (1, 4) and processing the data [4] and [5], I have estimated the destruction rate as $k_d \approx 10^{-2}$ and $k_d \approx 4 \cdot 10^{-3}$ (sec molecule)$^{-1}$, correspondingly. The difference of the estimates is likely due to the non-excess polymerase concentration (2.5U) of [5].

**Discussion**

The main limitation of my model is the assumption on the ultimate specificity of the primer binding. In other words, it is assumed that some computer tools [1, 2] already optimize the primers. In addition, this simple theory considers neither primers nor polymerase deficiency nor its aging. (The latter decreases the amplification limit and results in apparent reduction of the product yield after plateau.) However, these factors, together with a given distribution of non-specific primer binding sites and other PCR parameters (K and Mg ions concentrations, etc.), can be incorporated into the model by direct numerical solving of phenomenological equations of chemical kinetics at each cycle. While losing speed of analytical solutions (3, 4), this brutal approach would make it possible to simulate more realistically not only standard PCR dynamics but, e.g., asymmetric amplification. In doing so, one can optimize the PCR by varying the initial parameter set. Such computer system for PCR dynamics simulation may be a useful addition to any computer tool searching for optimal primers.

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**References**