New and Notable

Kinetics and Thermodynamics of the Microgene Polymerization Reaction

Yechiel Shai*
Department of Biological Chemistry,
The Weizmann Institute of Science,
Rehovot, Israel, 76100

In this issue of the Biophysical Journal, Itsko et al. present a sound kinetic model for the microgene polymerization reaction (MPR) (1). The MPR is an empirical method discovered a decade ago by Shiba et al. (2), in which short non-repetitive DNA fragments (homo-duplexes (HDs)) expand into head-to-tail multiple repeated products in heat-cool cycles of polymerase chain reaction. It may reflect primordial molecular evolution of primitive DNA sequences into complex genomes (3,4), and is currently exploited to generate artificial proteins containing repetitive motifs with useful properties (5–7). Rational design in protein engineering requires deep understanding and quantification of the processes underlying the MPR. Itsko et al. have previously analyzed this complex and descriptive phenomenon in strict physical chemistry terms (8,9).

MPR is composed of two major stages: initiation by forming minimal repetitive unit (initial doublet (ID)) from non-repetitive HDs, and propagation of ID into multiple head-to-tail repeats. Initiation stage has been enigmatic until recently because it implies an ambiguous skipping of the gap between two discontinuous templates by DNA polymerase. Itsko et al suggested (8) and thermodynamically substantiated (9) a simple mechanism for MPR initiation: one HD bridges two other HDs into a required proximity for the DNA polymerase to skip over the inter-template gap and generate an ID that is prone to expansion. Based on this mechanism, they continued investigating the propagation stage of the process.

The overall process of MPR propagation, i.e., expansion of the doublets to higher multiple repeats, includes the following recurring set of three steps: a), annealing of reactant complementary single strands of various lengths in a staggered manner; b), association of the DNA polymerase with the staggered homo/hetero-duplex of a); and c), DNA polymerase-mediated filling-in overhangs (created by the expansion of DNA by slippage of the complementary strands), thus generating a longer DNA product. This set is repeated until nucleotides (dNTPs) are exhausted. The first step is characterized by the melting equilibrium constant of the corresponding homo/hetero-duplex whereas the subsequent steps are characterized by common parameters of enzymatic kinetics such as the Michaelis-Menten constant and turnover number of DNA polymerase. Thus, the mathematical formulation of this conceptually straightforward MPR propagation scheme is getting rather complicated as it requires a thorough consideration of multiple steps and parameters involved in the overall process. This was elegantly treated by the authors with necessary simplifications but without losing the essence of the process. Two basic assumptions were made to simplify the formulation of these steps: i), existence of a steady state and ii), equal probability of different alignments between reactant DNA molecules.

The experimental tests of the model lend support to the major conclusions and the quantitative aspects of MPR propagation. The slight discrepancies with the experimental data are reasonably explained. Importantly, treating this phenomenon in terms of polymer chemistry allows predicting the average DNA length in the final distribution of the MPR total product as a function of initial concentrations of the HD and dNTPs. These valuable parameters should contribute to a rational design of MPR reaction mixtures to obtain bioengineered polypeptides with a desirable number of amino acids motifs. This model can be refined by further experimental tests that would alter the reaction conditions. The parameters that explicitly determine the propagation rate, which can easily be experimentally manipulated, are the equilibrium constant of ID and the polymerase chain reaction cycle periods.

In their previous work (9) Itsko et al. discovered an additional stage of MPR, HD-mediated amplification of ID, by deciphering the observed biphasic kinetics of MPR at certain conditions. Their current kinetic model (1) incorporates the amplification stage into the overall propagation by assuming equality between corresponding rate constants, which seem to hold at temperatures that significantly exceed melting temperatures of the corresponding HDs. Model improvement should include the alternative cases, in which these constants differ. Being developed to reconcile apparent inconsistencies with reality, this model will no doubt encourage quantitative investigation in basic and applied fields related to DNA amplification phenomena such as replication and mutagenesis.

REFERENCES

1. Itsko, M., A. Rabinovitch, and A. Zaritsky. 2008. Kinetics of repeat propagation in the Microgene Polymerization Reaction. *Biophys. J.* 1866–1874.
2. Shiba, K., Y. Takahashi, and T. Noda. 1997. Creation of libraries with long ORFs by polymerization of a microgene. *Proc. Natl. Acad. Sci. USA.* 94:3805–3810.
3. Ohno, S. 1984. Birth of unique enzyme from an alternative reading frame of the preexisted, internally repetitious coding sequence. *Proc. Natl. Acad. Sci. USA.* 81:2421–2425.
4. Ohno, S. 1987. Evolution from primordial oligomeric repeats to modern coding sequences. *J. Mol. Evol.* 25:325–329.
5. Shiba, K. 2004. MolCraft: a hierarchical approach to the synthesis of artificial proteins. *J. Mol. Catal., B Enzym.* 28:145–153.
6. Saito, H., T. Minamisawa, and K. Shiba. 2007. Motif programming: a microgene-based method for creating synthetic proteins containing multiple functional motifs. *Nucleic Acids Res.* 35 10.1093/nar/gkm017.

Submitted November 16, 2008, and accepted for publication November 21, 2008.

*Correspondence: Yechiel.Shai@weizmann.ac.il

Editor: Kathleen B. Hall.

0006-3495/09/03/1673/02 $2.00

doi: 10.1016/j.bpj.2008.11.010

7. Shiba, K., and T. Minamisawa. 2007. A synthesis approach to understanding repeated peptides conserved in mineralization proteins. *Biomacromolecules*. 8: 2659–2664.

8. Itsko, M., A. Zaritsky, A. Rabinovitch, and E. Ben-Dov. 2008. Initiation of microgene polymerisation reaction with non-repetitive homo-duplexes. *Biochem. Biophys. Res. Commun.* 368:606–613.

9. Itsko, M., A. Zaritsky, and A. Rabinovitch. 2008. Thermodynamics of unstable DNA structures from kinetics of the microgene PCR. *J. Phys. Chem. B*. 112:13149–13156.