Biophysical Chemistry 160 (2012) 77–78

Contents lists available at SciVerse ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Letter to the Editor

A revisit to the one-form kinetic model of prothrombinase: A comment on the rebuttal

Keywords:
Prothrombinase
Enzyme kinetics
Thrombin
Activation
One-form model
Two-form model
Channeling
Ratcheting

Dear Editor,

The authors of the preceding letter [1] continue the discussion of whether the conversion of prothrombin to thrombin involves one-form or two-form of prothrombinase and whether channeling and ratcheting must be added to kinetic models to accommodate concentration profile data. Central to the prior discussion was work [2] by these authors (Nesheim group = NG) which argues that two-form are necessary, by the Krishnaswamy group (KG) [3], which argues that one-form is sufficient, and by our group (PG) [4] which showed that there were mathematical flaws in the [2] models and which suggested that a minimalist model may reasonably fit the data and also relate to 3D structure. NG now presents new data fits [1] using (apparently) equations corrected as we suggested and imply in conclusion that their original conclusions (prothrombinase has two interconverting forms) still hold. While our prior work [4] considered fits to both the NG and KG data sets, as well as combined (scaled and averaged) data from both groups, in this letter we will focus on the original NG data (Fig. 2 of [2]). We find that

1. while the new one-form model fit without channeling and ratcheting presented in Fig. 1 [1] appears to be correct, we are unable to reproduce the new fit given in [1] for the two-form model, using the derived rate constants in Table 1 of [1]. In particular the details of the two-form model for B-chain and prethrombin-2 from 0 to 180 s differ. Compare Fig. S1 in the Supplementary Information (SI) with Fig. 1 in [1]. We used Matlab [5] to solve the one-form model of Scheme 1 (12 parameters) and the two-form model of Scheme 2 (14 parameters) of [2]. We must assume the NG in [1] made all of the necessary mathematical corrections for the two-form model for [2] as [2] had a number of mathematical errors for both models. We have adopted the definition for thrombin concentration suggested in [1].
2. when, however, we use an iterative Matlab procedure to solve the one-form and two-form differential equations derived from Schemes 1 and 2 [2], we obtain fits (Fig. S2 and Table 1 in the SI) that differ in detail (one-form vs. two-form). The fits, however, do not clearly distinguish between the two models.
3. when we include channeling and ratcheting (22 parameters for the one-form model, 24 parameters for the two-form model), we find improved fits as shown in Fig. S3 and Table 1 in the SI. It appears in this case that the one-form model fits better than the two-form model.
4. we could adopt an alternate set of definitions for the concentration of the intermediates (i.e., M and Pre-2) in which the parent intermediates and its direct derivatives are summed. Also, the B-chain (B) concentration may be alternately defined as in [2]: $[B] = [P]_0 - [P] - [P2] - [P21]$. A test with the alternate definitions is to repeat the process that led to Fig. S3 in the SI. The result is Fig. S4 in the SI. Fig. S3 and Fig. S4 are quite similar and so either definition suffices.

We believe some of the differences seen in [1,2,4] and here are that [1,2] rely apparently on using a curve fitting procedure whereas we solve the differential equations directly and iteratively. And, as we pointed out in [4], it is possible to use incorrect equations with curve fitting and still fit the data, as happened in [2]. In cases similar to this, we would recommend, curve-fitting models to the data, followed by use of the derived parameters (rate constants) as starting parameters for direct (and iterative) solution of the system of differential equations.

We have recently developed a 3D structural model [6] of human prothrombinase with bound full-length human prothrombin. This work is based on a new crystal structure of prethrombin-1 [7], which has the meizothrombin cleavage position at R320 still intact, and an earlier model [8] that we developed of the essential core of prothrombinase (FVa/FXa). The solution-equilibrated system of prothrombin by all—atom molecular dynamics simulation has the two cleavage positions (located on loops) geometrically accessible to the FXa active site. This argues in favor of the minimalist kinetic model [PT → M → T; PT → Pre-2 → T] suggested by us in [4]. When we fit directly the simple four-constants mechanism with analytical solution using Mathematica [9] to the NG data [2], we find a reasonable fit as shown in Fig. 1. Additionally, we find the addition of one rate constant to the minimalist model improves the fit to the NG data as shown in Fig. S5 in the SI. The simplicity of the four or five constants model fits, which are true to the data, and its interpretation (flexible loops) in terms of the proposed structure, is provocative.

Acknowledgements

This work was supported by the National Institute of Health (HL-06350) and the National Science Foundation (FRG DMR 0804549).

Appendix A. Supplementary information

Supplementary information to this article can be found online at doi:10.1016/j.bpc.2011.09.005.

0301-4622/$ – see front matter
doi:10.1016/j.bpc.2011.09.005

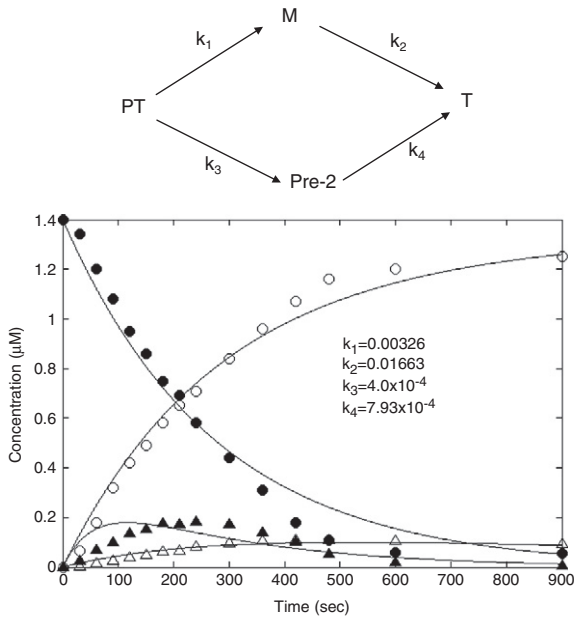

Fig. 1. A fit of minimalist kinetic model for the conversion of prothrombin to thrombin which involves only four rate constants to the NG data: PT (closed circle), B-chain (open circle), M (closed triangle) and Pre-2 (open triangle). The units of four pseudo rate constants (k_1, k_2, k_3, and k_4) are s^{-1}.

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Chang Jun Lee[1]
Sangwook Wu[1]
Lee G. Pedersen*
Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599-3290, USA
*Corresponding author at: University of North Carolina at Chapel Hill, CB 3290, Chapel Hill, NC 27599, USA.
Tel.: +1 919 962 1578; fax: +1 919 962 2388.
E-mail address: lee_pedersen@unc.edu.
[1] These authors contributed equally to this work.

31 August 2011