Chemical Physics Letters 663 (2016) 51–56

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

Research paper

Kinetics of enzyme-mediated hydrolysis of lipid vesicles

Vladimir P. Zhdanov [a,b,*], Björn Agnarsson [a], Fredrik Höök [a]

[a] Division of Biological Physics, Department of Physics, Chalmers University of Technology, S-41296 Göteborg, Sweden
[b] Boreskov Institute of Catalysis, Russian Academy of Sciences, Novosibirsk 630090, Russia

ARTICLE INFO

Article history:
Received 28 July 2016
In final form 10 September 2016
Available online 15 September 2016

Keywords:
Enzymatic catalysis
Lipid membranes
Mean-field kinetic equations
Monte Carlo simulations
Stochastic kinetics

ABSTRACT

Membrane enzymatic reactions can now be experimentally studied at the level of single sub-100 nm lipid vesicles. To interpret such experiments, we scrutinize theoretically various aspects of the hydrolysis of vesicles by single enzyme molecules and enzyme (e.g., PLA2) supplied with the constant rate. Using the mean-field kinetic model, we illustrate the shape of the corresponding kinetics and the dependence of the time scale of the reaction on the vesicle radius. Stochastic effects are illustrated as well. In addition, we discuss the likely mechanisms of the reaction-induced pore formation and bilayer rupture.

1. Introduction

In cells, volume- and membrane-confined enzymes control highly complex networks of chemical reactions in an efficient, timely, and spatially defined way [1]. In particular, the hydrolysis of lipid membranes representing one of the key reactions determining the lipid metabolism is controlled by PLA2 [2–4]. This reaction is also of interest in the context of drug delivery [5]. Mechanistically, it occurs via the conventional Michaelis-Menten (or Briggs-Haldane) steps [2] (see [6,7] for general retrospective)

$$E + S \rightleftharpoons ES \rightarrow E + P, \quad (1)$$

where E is an enzyme, S is a substrate (lipid), and P is a product (after digestion, a part of a lipid remains in the bilayer while another part leaves it). Despite the apparent simplicity of this mechanism and long history of the corresponding experimental and theoretical studies, the understanding of enzymatic reactions belonging to this class is still limited due to their dependence on the type of lipids and numerous complications inherent to membrane processes. One general factor here is enzyme transport in solution which may limit the reaction rate under certain conditions. Another general factor is membrane heterogeneity which may be present before reaction (especially *in vivo* due to complex membrane composition [8–10]) and/or arise during reaction. Membrane curvature is also important, and it can influence not only enzymatic reactions but also other processes running in membranes [11,12] (e.g., peptide-induced membrane pore formation and lysis [13–16]). In particular, the kinetic parameters may depend on curvature. A more specific factor is transmembrane lipid (flip-flop) transport. At the late stage, the reactions under consideration may be or are accompanied be membrane reconfiguration, rupture, and degradation. The latter processes, occurring also during peptide-induced membrane lysis, are physically complex and their description is not reduced to the conventional mean-field chemical kinetics.

The early academic studies of the PLA2-mediated hydrolysis of lipid membranes were performed with suspended vesicles [2]. The interpretation of the corresponding kinetics was complicated by likely mass-transport limitations and vesicle fusion. Later on, the reaction was explored under well-controlled conditions on tethered or supported vesicles at the ensemble level and on supported lipid bilayers (see e.g. Refs. [17–19] and [3,18,20–23], respectively). The experiments of the latter category showed that under certain conditions the lipid hydrolysis may occur with the formation of various patters [3,20,22,23]. Our group recently studied the PLA2 function on single sub-100 nm lipid vesicles immobilized at the support [24–26]. In particular, the hydrolysis of fluorescently labeled vesicles each processed by a single PLA2 molecule was tracked by using total internal reflection fluorescence microscopy [24,25]. The Michaelis-Menten parameters were found to depend on the membrane curvature. The label-free measurements were executed by employing evanescent light-scattering microscopy with the emphasis on the reaction regime with the PLA2 supply to vesicles [26]. With increasing time, the vesicle digestion was observed to be nearly complete or at least yielded vesicles or aggregates with sizes below the detection limit. Another interest-

* Corresponding author at: Division of Biological Physics, Department of Physics, Chalmers University of Technology, S-41296 Göteborg, Sweden.
E-mail address: zhdanov@catalysis.ru (V.P. Zhdanov).

http://dx.doi.org/10.1016/j.cplett.2016.09.018
0009-2614/

ing observation was that some of the vesicles showed traces of step-like digesting, a feature probably indicating the membrane rupture and reconfiguration.

In parallel with experiments, various factors complicating the enzyme-mediated lipid hydrolysis were clarified by using generic kinetic models. In particular, we illustrated the likely role of the formation of rafts in the membrane [27,28], membrane curvature [27], and product-induced membrane strain (in the absence of membrane rupture) [27]. Monte Carlo (MC) simulations of the hopping and scooting reaction modes were performed in Ref. [29]. Models focused on product-induced enzyme adsorption and a mixed lipid bilayer including inert lipids were proposed in Refs. [30] and [31], respectively. (For some other kinetic models of membrane-active enzymes, see Refs. [32,33].)

Herein, complementing our previous experimental and theoretical studies [24–28], we present analytical mean-field (MF) treatments of the kinetics of hydrolysis of single vesicles in the two regimes when, respectively, each vesicle is processed by a single PLA2 molecule as in experiments [24,25] (Section 2.1) and the reaction is accompanied by irreversible supply of PLA2 as in experiments [26] (Section 2.2). The corresponding MF equations are similar to the conventional Michaelis-Menten (or Briggs-Haldane) equations. The only difference is that we operate with the lipid population while the conventional equations are written in terms of concentrations. Earlier, the MF equations we use were already presented and employed in Refs. [24–26]. Our present analysis is focused on the dependence of the time scale of the reaction kinetics on the vesicle radius. The corresponding results [Eqs. (10), (20), and (22)] are novel. Complementing the MF analysis, we present MC simulations illustrating the likely stochastic effects in the kinetics under consideration (Section 3). In addition, we scrutinize the likely scenarios of the vesicle rupture (Section 4). The corresponding results (Sections 3 and 4) are novel as well. Finally, in Conclusion (Section 5), the results obtained are briefly discussed in the context of the experiments [24–26].

2. Mean-field kinetics

2.1. Single-enzyme kinetics

In this section, we analyze the simplest MF kinetic model of hydrolysis of a single vesicle by a single enzyme (PLA2) molecule. Our key assumptions are as follows. The enzyme attachment to vesicles takes place after their immobilization at the support. After attachment, an enzyme molecule is located in the external layer of the vesicle lipid bilayer. The lipids and product molecules are distributed in the bilayer at random. Their "flip-flop" exchange between the bilayers is assumed to be rapid (concerning this assumption, see Section 4), and the spherical structure of vesicles is considered to be maintained up to high conversion of lipid. The reaction occurs according to scheme (1). In reality, of course, some of these assumptions are expected to be not fulfilled. With this reservation, the corresponding analysis is, however, instructive, because it helps to form the framework for the understanding of the kinetics of the reaction under consideration.

To describe the single-enzyme reaction regime, we operate with the S and P populations, N_S and N_P, and the probabilities that an enzyme is free or associated with S, p_E and p_{ES}. The equations for these variables are as follows

$$dN_S/dt = -k_1 p_E N_S/N_0 + k_{-1} p_{ES}, \tag{2}$$

$$dN_P/dt = k_{cat} p_{ES}, \tag{3}$$

$$dp_{ES}/dt = k_1 p_E N_S/N_0 - k_{-1} p_{ES} - k_{cat} p_{ES}, \tag{4}$$

$$p_E + p_{ES} = 1, \tag{5}$$

where $N_0 \equiv N_S(0)$ is the initial S population, and k_1, k_{-1} and k_{cat} are the rate constants of steps (1).

Introducing the normalized S population, $\theta = N_S/N_0$, and solving, as usual, Eq. (4) in the steady-state approximation, one can rewrite Eq. (2) as

$$\frac{d\theta}{dt} = -\frac{k_{cat}\theta}{N_0(\theta + K)}, \tag{6}$$

where $K = (k_{-1} + k_{cat})/k_1$ is the Michaelis-Menten constant. The integration of this equation yields (Fig. 1)

$$\theta - 1 + K \ln \theta = -k_{cat} t/N_0. \tag{7}$$

This expression indicates that at any given value of K the characteristic time of the reaction scales as

$$\tau \propto N_0/k_{cat}. \tag{8}$$

The initial number of lipids and the vesicle radius, R, are related as

$$N_0 = 8\pi R^2/a, \tag{9}$$

where a is the lipid bilayer area per lipid. Substituting (9) into (8) results in

$$\tau \propto 8\pi R^2/a k_{cat}. \tag{10}$$

Thus, the time scale of the single-enzyme reaction kinetics is expected to be proportional to R^2 provided the dependence of the Michaelis-Menten parameters, k_{cat} and K, on R is negligible.

2.2. Kinetics with the enzyme supply

Here, we analyze the situation when the vesicle hydrolysis occurs in parallel with irreversible attachment of enzyme molecules. The rate of attachment is assumed to be independent of the extent of lipid conversion. The enzyme population on each vesicle is considered to be small and accordingly the enzymes function independently. The other assumptions are as in Section 2. To describe the reaction, we operate with the S, P, E, and SE populations, N_S, N_P, n_E and n_{ES}. The MF kinetic equations for these variables are read as

$$dN_S/dt = -k_1 n_E N_S/N_0 + k_{-1} n_{ES}, \tag{11}$$

$$dN_P/dt = k_{cat} n_{ES}, \tag{12}$$

$$dn_E/dt = k_a c_E - k_1 n_E N_S/N_0 + k_{-1} n_{ES} + k_{cat} n_{ES}, \tag{13}$$

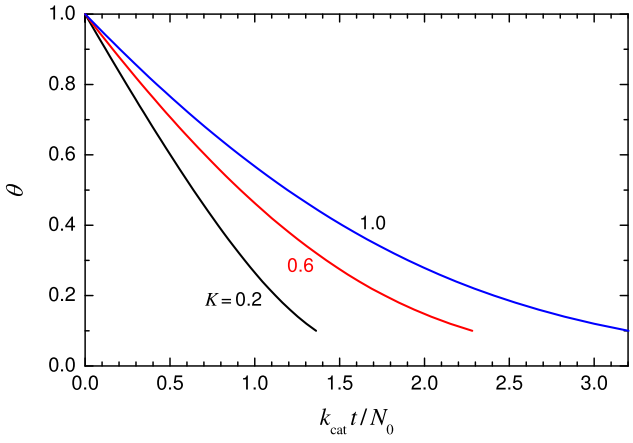

Fig. 1. Kinetics of hydrolysis of a vesicle by a single enzyme according to Eq. (7) with $K = 0.2$, 0.6, and 1.0.