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Kinetics of the enzyme–vesicle interaction including the formation of rafts and membrane strain

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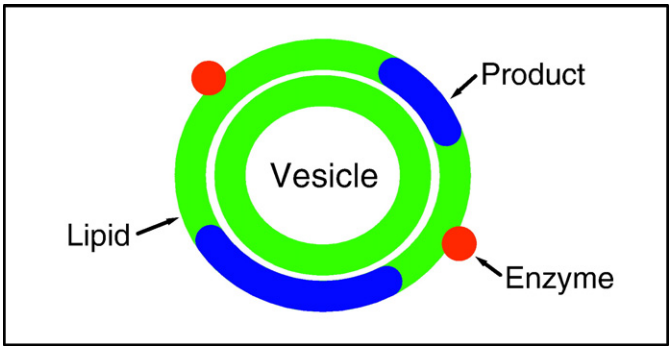
HIGHLIGHTS

- The analysis is focused on enzyme-catalyzed hydrolysis of lipids.
- The formation of rafts composed of product is taken into account.
- The product-induced membrane strain is taken into account as well.
- Various scenarios of lipid conversion are described.
- The conditions of the appearance of the lag phase are scrutinized.

GRAPHICAL ABSTRACT



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ABSTRACT

In cells, an appreciable part of enzymes is associated with lipid membranes. Academic experimental studies of the function of membrane enzymes (e.g., PLA_2 representing a prototype for interfacial enzymology) are often focused on the enzyme–vesicle interaction or, more specifically, on conversion of lipid forming the external leaflet of the vesicle membrane. The corresponding kinetics are complicated by many factors inherent to the interfacial physics and chemistry. The understanding of the relative role of such factors and how they should be quantitatively described is still limited. Here, we present the mean-field kinetic equations, taking the formation of rafts in the membrane and the product-induced membrane strain into account, and analyze various scenarios of lipid conversion. In particular, we scrutinize the conditions when the kinetics may exhibit a transition from a relatively long latency period to a steady-state regime with fast nearly constant reaction rate. Specifically, we discuss the likely role of the pore formation in the external lipid layer in this transition. The latter effect may be caused by the product-induced tensile strain in this layer.

1. Introduction

Enzymatic reactions are usually interpreted and described in the framework of the classical Michealis–Menten (MM) paradigm including reversible binding of substrate (S) to enzyme (E), enzyme-catalyzed conversion of substrate to product (P), and product detachment,

$$S + E \rightleftharpoons SE \rightarrow E + P. \quad (1)$$

If the reaction occurs in solution, the conventional MM steps (1) are often sufficient for an analysis, and the reaction rate per enzyme is given by

$$w = \frac{k_2 k_1 c_S}{k_1 c_S + k_{-1} + k_2}, \quad (2)$$

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where k_1 and k_{-1} are the rate constants for reversible S–E association, k_2 is the reaction rate constant, and c_S is the S concentration. The experimental and theoretical studies scrutinizing and/or extending the MM paradigm are numerous. For general readership, we refer to recent reviews [1–5], original theoretical works focused on stochastic effects [6,7], conformational dynamics [8–10], diffusion-related dynamical corrections [11–14], multistep schemes [15], large-scale networks [16], and references therein.

In cells, roughly one-half of the protein is estimated to be membrane associated, and many enzymes are membrane associated as well [17]. Such enzymes may access substrate either directly from the lipid-water interface or from the aqueous phase. The kinetic models focused on these cases combine customarily the standard MM steps and the steps describing the enzyme and/or substrate transport to or from the interface and employ conventional mass-action-law equations [17]. All these steps can be complicated by numerous factors inherent to the interfacial physics and chemistry. Here, we refer to the formation of rafts and product-induced membrane strain. During the last decade, it has become clear that the rafts (or, in other words, domains) are common in the membranes containing proteins and/or composed of different lipids (see reviews [18–21] and recent theoretical studies [22–26]). The size of rafts, observed in different systems, ranges from a few nm to μm. The membrane strain is known to influence or may influence both cellular and vesicle behaviour, e.g. cell division, endo- and exocytosis [27–29], function of membrane channels [30], rupture of vesicles attached to a surface [31], or processes occurring in or on the vesicle lipid membrane, e.g. peptide-induced pore formation [32] (for general treatment of elastic properties of lipid membranes, see review [33]). The understanding of the role of these two factors in the kinetics of enzymatic reactions occurring on lipid bilayers in general and on vesicles in particular is now limited. The goal of our work is to contribute to the conceptual basis in this area and the corresponding formalism with emphasis on these factors.

As an example, we analyze one of the simplest mechanisms of the enzyme–vesicle interaction including reversible attachment of enzyme to the external leaflet of a vesicle (V),

$$\mathrm{E} + \mathrm{V} \rightleftharpoons \mathrm{EV}, \tag{3}$$

and enzyme-catalyzed conversion of lipid (L) to product,

$$\mathrm{L} + \mathrm{E} \rightleftharpoons \mathrm{LE} \rightarrow \mathrm{E} + \mathrm{P}. \tag{4}$$

The latter step is considered to represent scission so that one part of L remains in the leaflet while the other part leaves a vesicle. This generic scheme mimics the function of phospholipase A_2 (PLA_2; reviewed in Ref. [34]). This biologically important enzyme (reviewed in Ref. [35]), requiring Ca^{2+}-ions as a cofactor to catalyze the hydrolysis of lipids, has attracted attention already three decades (in addition to reviews [34,35], see e.g. Refs. [36–48] and references therein). Its activity was studied at flat lipid bilayers and also at vesicles.

In the context of steps (3) and (4), the rafts can be formed due to segregation of L and P, i.e., the external leaflet may contain areas enriched by L and areas enriched by P. Our analysis is focused on this case. Specifically, we assume that the areas enriched by L are uniform and the areas enriched by P are uniform as well. Concerning these assumptions, we notice that phase separation may in principle occur even in a bilayer composed of chemically identical lipids. With increasing temperature, such bilayers exhibit a gel-fluid phase transition [49,50]. The coexistence of the corresponding phases can be observed in a narrow region near the phase-transition temperature. It is of interest that in such situations PLA_2 was observed to be located on the boundaries between the two phases, where it appears to exhibit increased conversion rates [47] (see also related simulations [51]). This subject is, however, beyond our treatment.

In the available kinetic models of the PLA_2–vesicle interaction (reviewed in Ref. [38]), the segregation of L and P is usually either ignored (see, e.g., Refs. [37,40]) or described by using the equations operating with the average parameters [38]. Specifically, the effective PLA_2–vesicle binding constant is represented (see Eq. (B9) in Ref. [38]) as a linear combination of two binding constants corresponding to PLA_2–L and PLA_2–P interactions, respectively. During reaction, every bound PLA_2 is assumed to see an average surface L concentration, n, and the average (for the whole area) effective reaction rate (per bound PLA_2) is represented (see Eq. (B10) in Ref. [38]) in analogy with Eq. (2) as

$$r_{\mathrm{eff}} = \frac{\kappa_2 \kappa_1 n}{\kappa_1 n + \kappa_{-1} + \kappa_2}, \tag{5}$$

where κ_1 and κ_{-1} are the rate constants for reversible 2D L-PLA_2 association, and κ_2 is the reaction rate constant. The total reaction rate (per vesicle) is defined as a product of r_{eff} and the number of bound PLA_2.

Strictly speaking, expression (5) corresponds to the case when L and P are located in the external leaflet nearly at random or, in other words, the bilayer is uniform (no rafts). With rafts, the values of the kinetic parameters for the steps occurring on the areas composed primarily of L are expected to be different compared to those for the steps occurring on the areas composed primarily of P. Physically, this difference may be related to the difference in the enzyme–bilayer interaction (this may result in enzyme refolding) and substrate concentration. For these reasons, the average reaction rate is expected to be not reduced to expression (5). In our treatment below, this factor is taken into account.

One of the most interesting kinetic features of the PLA_2–vesicle interaction is a relatively long latency period followed by a steady-state regime with fast nearly constant rate of hydrolysis [36]. Specifically, the steady-state appears to begin after reaching a critical conversion fraction. In principle, an observation of a transition from one kinetic regime to another one is not unusual. What is unusual is a low value (about 5%) of the critical conversion fraction. This feature, observed already about 30 years [36], is believed to be due to the product-assisted binding of enzyme to the substrate bilayer under the conditions close to the attachment–detachment equilibrium [34,38]. Below (in Section 3.2), we show that this explanation does not necessarily hold in the case of L and P segregation. One of our goals was to scrutinize other likely reasons of the lag phase with emphasis on the formation of rafts and product-induced membrane strain. Concerning this phase, one might argue that the complexity of the system is not reduced to these factors and refer, e.g., to slow time-dependent enzyme isomerization and/or conventional (without formation of rafts) enzyme–enzyme or enzyme–product association (see, e.g., the models proposed in Refs. [37,40]), or pre-existing membrane heterogeneity, i.e., defects of the membrane structure [41,43]. Although we would agree that our analysis obviously does not describe all the possible situations, we may notice that slow enzyme isomerization alone appears to be not sufficient in order to explain the lag phase because this phase is known to be sensitive to the reaction products [34,38]. The scenarios including enzyme–product association without formation of rafts can hardly be accepted in order to describe the lag phase, because the substrate-product phase separation (rafts) was explicitly experimentally observed at least in supported bilayers [41,43]. The pre-existing membrane heterogeneity (i.e., defects of the membrane structure) may indeed be important in supported lipid bilayers but can hardly be the main reason of the lag phase in vesicles (where this phase is often observed), because in the latter case the pre-existing defects of the membrane structure are usually negligible.

Our presentation below is divided into Sections 2–6. The general equations are given in Section 2. The kinetics calculated in the absence of membrane strain are described in Section 3. The likely role

of the membrane strain is discussed in Section 4. The single-enzyme case is treated in Section 5. Our main conclusions are summarized in Section 6. In addition, our work contains Appendix A where for comparison we outline the equations corresponding to the situations when the reaction scheme is similar to that in the main text except that L and P are located in the external leaflet nearly at random, i.e., the bilayer is uniform (no rafts).

2. General equations

Our analysis includes steps (3) and (4). The main assumptions of our treatment are as follows.

The E concentration in solution is considered to be maintained constant. The E attachment to V is assumed to be kinetically limited. This situation can experimentally be realized, e.g., in the case of vesicles tethered to the surface under the flow condition in solution (see, e.g., experiments [52]). After attachment, E is considered to be located and operate on the external leaflet of a vesicle. Specifically, E catalyzes conversion [step (4)] of L forming the external leaflet. In reality, E (e.g., PLA_2) may react at the late stage also with L composing the internal leaflet of a vesicle. The latter channel is not taken into account in our treatment.

After conversion, a part of L leaves the surface (for PLA_2, this is the case [45]). The latter step is considered to be irreversible. Using the term "product" ("P") in our presentation below, we refer to the molecules remaining in the external leaflet after conversion of L. The flip-flop jumps of L and P between the external and internal leaflets are neglected. (On the late stage, such jumps may be significant.)

P is assumed to form rafts in the external leaflet of a vesicle (this is the case for PLA_2 [43]), i.e., the external leaflet contains uniform areas enriched by L and uniform areas enriched by P. The chemical compositions of these areas are considered to remain unchanged during reaction. Their size is assumed to be comparable or larger than the E size (the molecular mass of PLA_2 is 12–14 kDa [35] and accordingly the PLA_2 diameter is about 3 nm).

E is attached either to the areas composed primarily of L or to the areas composed primarily of P. In principle, E can be located on the boundaries between these phases. There are kinetic indications that this may happen in the PLA_2 case [43]. The latter scenario is not analyzed here.

The E-catalyzed L conversion to P may also occur on the areas composed primarily of L and on the areas composed primarily of P. In both cases, the effective reaction rate constant can be represented in the MM form [like in expressions (2) or (5)]. As already noticed in the Introduction, the values of the corresponding kinetic parameters for the steps occurring on different areas are different. In particular, the L concentration at the areas composed primarily of P is much lower than that at the areas composed primarily of L. For this reason, the reaction rate (per enzyme) on the former areas may be much lower as well. We focus on this case and consider that the L conversion occurs only on the L areas. This scenario is opposite compared to that treated earlier [38].

The E detachment from and E-catalyzed L conversion on a vesicle are assumed to run independently. This approximation widely adopted earlier [38] seems to be reasonable in the PLA_2 case, because the PLA_2–vesicle interaction includes a multitude of weak PLA_2–L and PLA_2–P interactions, and the formation of an additional PLA_2–L bond during L conversion does not change appreciably the energetics of the PLA_2 detachment.

With the ingredients above, we describe steps (3) and (4), occurring on a vesicle, by using three variables including the numbers of E on the L and P areas, N_l and N_p, and the fraction of conversion of L to P in the external leaflet, θ. The mean-field kinetic equations for these variables are read as

$$dN_l/dt = k_a c s_\circ(1-\theta) - k_d N_l - W_{lp}, \tag{6}$$

$$dN_p/dt = \kappa_a c s_\circ \theta - \kappa_d N_p + W_{lp}, \tag{7}$$

$$d\theta/dt = (k_r s_l/s_\circ) N_l, \tag{8}$$

where c is the E concentration in the solution, k_a, κ_a, k_d, and κ_d are the attachment and detachment rate constants (the attachment rates, $k_a c$ and $\kappa_a c$, are calculated per unit surface area), k_r is the effective rate constant E-catalyzed reaction on the areas covered primarily by L, W_{lp} is the rate of the E transport between the L and P areas, $s_\circ$ is the surface area of a vesicle, and s_l is the surface area per L.

The use of Eqs. (6)–(8) implies that the number of E attached to a vesicle,

$$N \equiv N_l + N_p, \tag{9}$$

is appreciable ($N \gg 1$) but not very large so that the fraction of the vesicle surface covered by E is low (this is usually the case in experiments with PLA_2). In principle, the reaction may occur when a vesicle contains only a few or single E as briefly discussed in Section 5.

In addition, we assume that the E migration along the vesicle surface is rapid (this is the case for PLA_2 [47]) and accordingly there is equilibrium in E distribution on the L and P areas, i.e., N_l and N_p are related according to thermodynamics as

$$\frac{(1-\theta)N_p}{\theta N_l} = \alpha, \tag{10}$$

where $\alpha = exp(\Delta E/k_B T)$, and ΔE is the difference of the E binding energies on the L and P areas. Employing (9) and (10), we obtain

$$N_l = \frac{(1-\theta)N}{1+(\alpha-1)\theta}, \tag{11}$$

$$N_p = \frac{\alpha\theta N}{1+(\alpha-1)\theta}. \tag{12}$$

Relations (11) and (12) between N_l, N_p and N indicate that Eq. (8) in combination with the kinetic equation for N are sufficient in order to describe the system. The latter equation is derived by adding Eq. (7) to Eq. (6),

$$dN/dt = k_a c s_\circ(1-\theta) + \kappa_a c s_\circ \theta - k_d N_l - \kappa_d N_p. \tag{13}$$

The equations presented above have been derived assuming the chemical compositions of the L and P areas to remain unchanged during reaction. In this case, the equations can be used irrespective whether the P-induced membrane strain is taken into account or not. If the strain is negligible (this situation is treated in Section 3), the rate constants employed in these equations are independent of θ. If the strain is significant, one should introduce the corresponding dependence of the rate constants on θ (see Section 4).

3. Kinetics with negligible membrane strain

To illustrate typical kinetics predicted by our equations, we analyze two situations when (i) the E attachment to a vesicle is irreversible (Section 3.1) and (ii) the E attachment and detachment are close to equilibrium (Section 3.2), respectively. In reality, the E (e.g., PLA_2) attachment to the lipid bilayer is usually reversible. The analysis of the kinetics with irreversible E attachment is nevertheless instructive, because even if the E attachment is reversible the E detachment is negligible at least during the initial phase of the kinetics. The understanding of the specifics of this phase is especially interesting in the context of interpretation of the experimentally observed latent period in the beginning of the PLA_2–vesicle interaction (see the Introduction).

3.1. Reaction with irreversible E attachment

If the E detachment is negligible, Eq. (13) is reduced to

$$dN/dt = k_a c s_\circ (1-\theta) + \kappa_a c s_\circ \theta. \quad (14)$$

In addition, we take relation (11) into account and rewrite Eq. (8) as

$$\frac{d\theta}{dt} = \frac{k_r s_l (1-\theta) N}{s_\circ [1 + (\alpha - 1)\theta]}. \quad (15)$$

Eqs. (14) and (15) predict a latency period at $\theta \ll 1$ provided that $\alpha \ll 1$ and $k_a \ll \kappa_a$. To illustrate this prediction explicitly (Fig. 1(a)), we notice that in this limit one can replace $1-\theta$ in Eq. (14) by 1 and neglect $\alpha\theta$ in Eq. (15). This yields

$$dN/dt = k_a c s_\circ + \kappa_a c s_\circ \theta, \quad (16)$$

$$d\theta/dt = (k_r s_l / s_\circ) N. \quad (17)$$

Integrating Eqs. (16) and (17), we obtain

$$\theta = (k_a/\kappa_a)[\cosh(\lambda t) - 1], \quad (18)$$

$$N = (k_a c s_\circ / \lambda) \sinh(\lambda t), \quad (19)$$

where $\lambda = (\kappa_a c k_r s_l)^{1/2}$. According to Eqs. (18) and (19), the slow reaction kinetics with

$$N \simeq k_a c s_\circ t \quad \text{and} \quad \theta \simeq k_a c k_r s_l t^2/2 \quad (20)$$

occurs at $\lambda t < 1$, while the rapid kinetics with

$$N \simeq (k_a c s_\circ / 2\lambda) \exp(\lambda t) \quad \text{and} \quad \theta \simeq (k_a / 2\kappa_a) \exp(\lambda t) \quad (21)$$

takes place at $\lambda t > 1$.

The conditions $\alpha \ll 1$ and $k_a \ll \kappa_a$ used to construct Fig. 1(a) correspond physically to the situation when the E location is thermodynamically favourable at the L areas while the E-attachment rate (per unit area) is faster at the P areas. Initially (at $\lambda t < 1$), the E attachment occurs primarily at the L areas, because θ is low. At $\lambda t > 1$, the kinetics becomes faster, because the E attachment occurs primarily at the P areas with subsequent rapid migration to the L areas and reaction there. The lag phase is, however, not seen very clearly

3.2. Reaction at E attachment–detachment equilibrium

At the attachment–detachment equilibrium in the L and P areas, we have respectively

$$k_a c s_\circ (1-\theta) = k_d N_l, \quad (22)$$

$$\kappa_a c s_\circ \theta = \kappa_d N_p. \quad (23)$$

These equations yield

$$\frac{(1-\theta) N_p}{\theta N_l} = \frac{k_d \kappa_a}{k_a \kappa_d}. \quad (24)$$

According to the detailed balance principle, the latter relation should be the same as relation (10), i.e., its right-hand part should be equal to α.

Using the relation between N_l and c given by Eq. (22), we rewrite Eq. (8) as

$$d\theta/dt = rc(1-\theta), \quad (25)$$

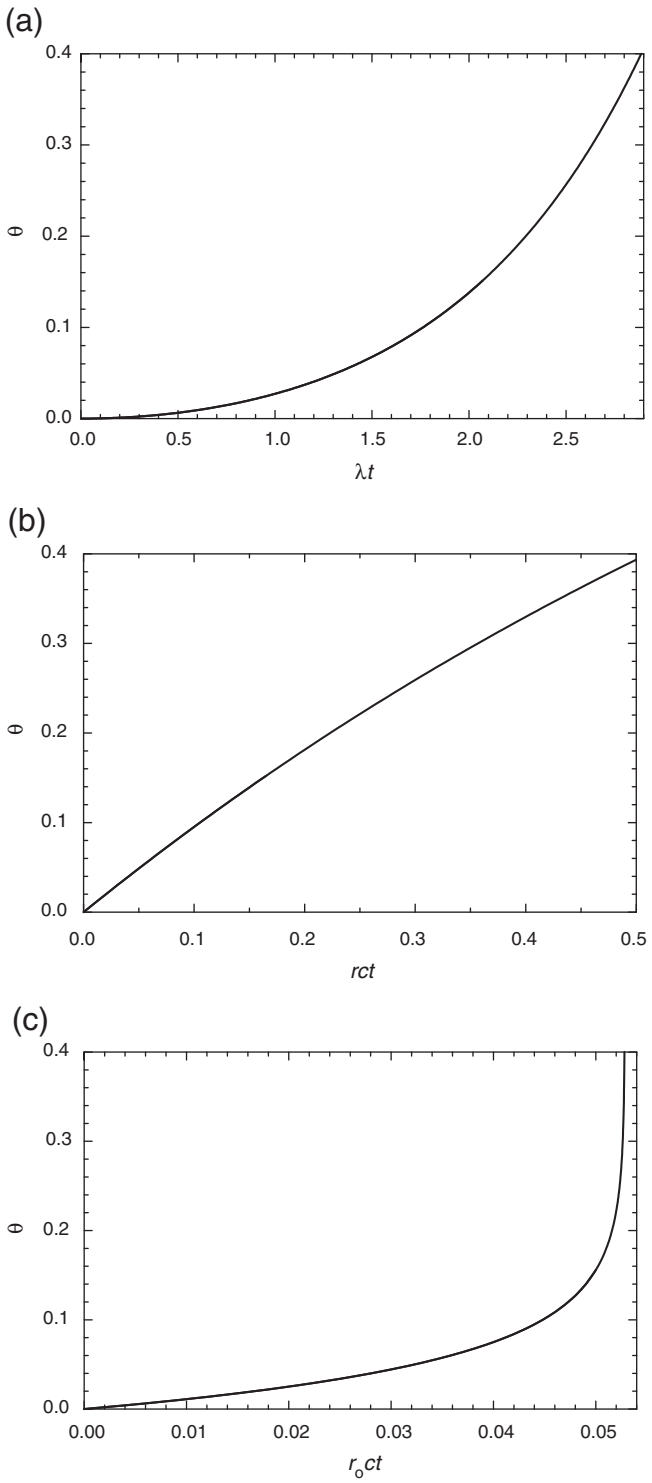

Fig. 1. Conversion of L in the external leaflet of a vesicle as a function of time: (a) irreversible E attachment in the absence of P-induced strain [Eq. (18) with $k_a/\kappa_a = 0.05$]; (b) E attachment–detachment equilibrium in the absence of the P-induced membrane strain [Eq. (26)]; (c) E attachment–detachment equilibrium influenced by the P-induced strain [Eq. (36) with $(A+B)/k_BT = 20$]. Note that the kinetics (a) and (c) are not exponential (the exponential kinetics (b) is nearly linear at these conversions). To focus on the transition from slow to fast kinetics cases (a) and (c), the kinetics are shown only for relatively low conversions (up to $\theta = 0.4$). Mathematically, the duration of the slow (lag) phase cannot here be precisely defined, because the corresponding equations [(18) and (36)] do not predict bifurcations (or, in other words, kinetic phase transitions). In case (a), the transition from slow to fast kinetics takes place visually approximately at $\lambda t \simeq 1.5$. In fact, this feature is not seen very clearly. In case (c), the transition taking place at $r_o ct \simeq 0.044$ is manifested much better. In the experiments, the situation with the definition of the duration of the lag phase is similar. For example, the kinetics (a) and (c) are similar to that measured, respectively, at 17 and 26.5 °C in Ref. [36] (see Fig. 1 there).

where $r \equiv k_r k_a s_l / k_d$. Integrating Eq. (25) and employing relations (22) and (23), we obtain

$$\theta = 1 - exp(-rct), \tag{26}$$

$$N_l = (k_a c s_\circ / k_d)\, exp(-rct), \tag{27}$$

$$N_p = (\kappa_a c s_\circ / \kappa_d)[1 - exp(-rct)]. \tag{28}$$

Eqs. (26)–(28) obviously do not predict a latency period (Fig. 1(b)). In earlier treatments [38], a latency period was predicted at E attachment–detachment equilibrium because the reaction rate (per E) on the areas composed primarily of L was assumed to be equal to that on the areas composed primarily of P, and the E attachment was considered to be thermodynamically preferable on the latter areas.

4. Likely role of the membrane strain

The membrane strain may be induced during reaction (Section 4.1). In vesicles, the membrane is also strained due to its curvature (Section 4.2).

4.1. Product-induced membrane strain

After the E-catalyzed L conversion (step (4)), as already noted in the Introduction, one part of L remains in the leaflet while the other part leaves a vesicle. This means that the surface area per P is smaller (by a factor of 2) than the surface area per L. If the external leaflet of a vesicle were independent of the internal leaflet, this difference could result in its area reduction approximately by a factor of $\theta/2$ compared to that in the initial state before reaction (this and other estimates presented in this section imply that the elastic constants of the L and P areas are comparable). In such a state, the surface area per L could be the same as in the initial state. In reality, the reduction of the surface area of the external leaflet is accompanied by the compression of the internal leaflet. At mechanical equilibrium, the external leaflet becomes stretched, while the internal leaflet becomes compressed (provided that the flip-flop jumps of L and P between the external and internal leaflets are negligible as assumed in our analysis). In particular, the area of the external leaflet is reduced only by a factor of $\theta/4$ compared to that of the initial state (at $t = 0$), i.e., this leaflet becomes stretched by a factor of $\theta/4$, while the area of the internal leaflet is reduced by a factor of $\theta/4$ compared to that in the initial state.

Thus, the P-induced strain of the external leaflet is tensile. Such strain is known to reduce the energy and chemical potential of atoms or molecules absorbed in or adsorbed on the surface of solids (their binding energy accordingly increases). For example, the lattice strain contribution to the chemical potential of H atoms absorbed by metals is represented as $\Delta\mu = -v_\circ \sigma_{ii}/3$, where $v_\circ$ is the increment of the lattice volume per interstitial atom, and σ_{ii} is the trace of the stress tensor [53]. The latter tensor is proportional to the strain tensor or, in our case, to θ. For these reasons, we can describe the effect of the P-induced strain on the energy of E attached to the L areas as

$$E_l = E_l^\circ - A\theta, \tag{29}$$

where E_l° is the energy corresponding to the strain-free state, and A is the constant proportional to the E-L contact area. (For the P areas, the expression is similar.)

The lattice strain results also in the change of the activation energies of adsorption of molecules on and desorption from solid surfaces and diffusion of absorbed atoms (see, e.g., DFT-based studies [54–58]). The corresponding contribution is also proportional to σ_{ii}. In our case, this means that we can represent the activation energies of E attachment and detachment on the L areas as

$$E_a = E_a^\circ - \alpha A\theta, \tag{30}$$

$$E_d = E_d^\circ + \beta A\theta, \tag{31}$$

where α and β are dimensionless coefficients. According to the detailed balance principle, the difference of these energies should depend on θ as predicted by expression (29). For this reason, we should have $\alpha + \beta = 1$.

Employing expressions (30) and (31), we represent the attachment and detachment rate constants as

$$k_a = k_a^\circ exp(\alpha A\theta / k_B T), \tag{32}$$

$$k_d = k_d^\circ exp(-\beta A\theta / k_B T), \tag{33}$$

where k_a° are k_d° are the values corresponding to $\theta = 0$.

For the reaction step, the corresponding equations are as follows

$$E_r = E_r^\circ - B\theta, \tag{34}$$

$$k_r = k_r^\circ exp(B\theta / k_B T), \tag{35}$$

where B is the constant similar to A.

In analogy with the rate processes occurring with participation of atoms and simple molecules [54–58], the constants A and B are expected to be comparable with the activation energies for E detachment and L conversion, respectively. This means that the effect of the P-induced membrane strain on the kinetics under consideration may be appreciable. Due to this effect, the kinetics may exhibit an initial slow phase not only in the case of irreversible E attachment (as discussed in Section 3.1) but also with the E attachment–detachment equilibrium. To illustrate this feature, we use expressions (32), (33) and (35) and rewrite Eq. (25) as

$$d\theta/dt = r_\circ c(1 - \theta) exp[(A + B)\theta / k_B T], \tag{36}$$

where $r_\circ \equiv k_r^\circ k_a^\circ s_l / k_d^\circ$. The kinetics predicted by this equation are shown in Fig. 1(c).

Although the kinetics presented in Fig. 1[(a) and (c)] (and discussed in the Appendix A) exhibit an initial slow phase, their shape is somewhat different compared to those observed for PLA_2. In particular, the rapid phase exhibited by these kinetics is exponential, while the experimentally observed rapid PLA_2 phase is rather nearly linear (see, e.g., Ref. [36]). One of our conjectures why the latter phase is nearly linear is related to the possibility of pore formation in the external leaflet due to the influence of the tensile P-induced membrane strain. This may happen if the strain is above the critical one. After the pore formation, the strain may partly relax or its value may be stabilized, and the kinetics may become more linear.

Physically, it is clear that the value of θ corresponding to the critical strain in the external leaflet should be related to the critical value of the relative area expansion, $\Delta S/S$, for transmembrane pore formation and membrane rupture in experiments focused on measurements of the area stretch modulus of lipid membranes (see, e.g., Ref. [59]). As we have already noticed above, the relative area expansion of the remaining part of the external leaflet is about $\theta/4$, and the first guess could be that under the critical conditions $\theta/4$ should be approximately equal to $\Delta S/S$. One should, however, bear in mind that the pore formation in a leaflet is facilitated compared to that in the whole membrane, because the line tension or, in other words, the free energy cost per unit length for creating the edge of the pore in the leaflet is about two times lower than in the membrane. This factor compensates the factor of 1/4 in $\theta/4$. Thus, under the critical conditions, θ should be approximately equal to $\Delta S/S$. As we have already mentioned in the Introduction, the transition from a relatively long latency period to a fast nearly constant steady-state rate of hydrolysis is observed at $\theta \simeq 0.05$ [36]. The formation of transmembrane pores is observed at $\Delta S/S \simeq 0.05$ as well (see, e.g., Fig. 2 in Ref. [59]). The coincidence of these values supports our conjecture that the pore formation in the external leaflet may influence the kinetics under consideration.

4.2. Curvature-related membrane strain

In vesicles, the external part of each lipid layer is slightly stretched while the internal part is slightly compressed [60]. In particular, the linear tensile strain of the external part is $\simeq L/(2R)$, where L is the layer thickness, and R is the vesicle radius. The contribution of this strain to the E binding energies and the activation energies of the processes under consideration can be described by replacing θ by L/R in expressions (29)–(31), (34) and (35). Due to this factor, the reaction is expected to be faster on smaller vesicles.

5. Single-enzyme kinetics

If a vesicle contains only a few E, the kinetics should in general be described by using, e.g., the Monte Carlo technique. Some situations can, however, be treated analytically. For example, we analyze the case when the reaction starts at $t=0$ after attachment of a single E and occurs up to its detachment without attachment of additional E. Experimentally, this regime can be observed provided that c is sufficiently low.

Mathematically, the situation when a vesicle contains a single E is described in analogy with Eq. (8) as

$$d\theta/dt = (k_r S_l/s_\circ)P_l, \tag{37}$$

where P_l is the probability that an E is located on the L areas. This probability is given by the right-hand part of Eq. (11) with $n=1$, i.e. $P_l=(1-\theta)/[1+(\alpha-1)\theta]$. Substituting this expression into Eq. (37) yields

$$\frac{d\theta}{dt} = \frac{k_r s_l(1-\theta)}{s_\circ[1+(\alpha-1)\theta]}. \tag{38}$$

(Note that this equation can also be obtained by employing Eq. (15) with $n=1$.)

To illustrate the kinetics predicted by Eq. (38), we consider as in Section 3.1 that $\alpha \ll 1$ and $\theta \ll 1$. In this case, one can neglect $\alpha\theta$ in the denominator of Eq. (38) and rewrite it as

$$d\theta/dt = k_r s_l/s_\circ. \tag{39}$$

Using then expression (35) for k_r, we have

$$d\theta/dt = (k_r^\circ s_l/s_\circ)\, exp(B\theta/k_B T). \tag{40}$$

According to this equation, the kinetics is linear, $\theta=(k_r s_l/s_\circ)t$, provided that the P-induced strain is negligible ($B=0$). If the strain is appreciable ($B/k_BT \gg 1$) and $\theta \ll 1$, Eq. (40) predicts the kinetics similar to those (Fig. 1(c)) given by Eq. (36).

6. Conclusion

The function of enzymes attached to vesicles and converting the lipid forming the external leaflet of the vesicle membrane can be complicated by the formation of rafts in the membrane and the product-induced membrane strain. We have presented the mean-field kinetic equations, taking these factors into account, and analyzed various scenarios of lipid conversion.

To some extent, our presentation has been focused on the initial phase of the kinetics. Specifically, we have scrutinized the conditions when the kinetics may exhibit a transition from a relatively long latency period to a steady-state regime with fast nearly constant rate of hydrolysis. Our related conclusions are as follows.

(i) In the literature, the latency period was often explained by the product-assisted binding of enzyme to the substrate bilayer under the conditions close to the attachment–detachment equilibrium. We have shown (Fig. 1(b)) that in the presence of rafts this explanation does not necessarily hold.

(ii) Our analysis indicates that the latency period can be observed (Fig. 1(a)) in the absence of the product-induced membrane strain provided that during this period the E attachment is irreversible. In this case, the transition between the slow and rapid phases is, however, not well manifested.

(iii) With the product-induced membrane strain, the latent phase may be much more distinct (Fig. 1(c)) even at the E attachment–detachment equilibrium.

(iv) The likely role of the strain-induced pore formation in the external leaflet in this transition has been discussed. In particular, our analysis based on the data obtained in the independent measurements of the elastic properties of lipid membrane indicates that under reactive conditions in vesicles the pore formation is expected exactly where the transition between the slow and rapid phases is experimentally observed.

(v) Concerning the latency period, we may notice that its length, t_{lat}, seems to be determined by the condition that the conversion reaches the critical value ($\theta \approx 0.05$). Combining this condition with our analytical results, we conclude that t_{lat} is expected to be inversely proportional to $c^{1/2}$ (see Eq. (20)) provided that during the latency period the E attachment is irreversible. If during the latency period the reaction occurs at E attachment–detachment equilibrium, t_{lat} is expected to be inversely proportional to c (see, e.g., Eq. (36)). The latter dependence follows also from the earlier treatments [37,38]. The physical factors behind the predictions there are, however, different compared to those assumed in our treatment. The accurate measurements of the dependence of the duration of the latency period on c are still lacking to our knowledge.

Our analysis and conclusions above clarify what may happen in the system under consideration and simultaneously show the complexity of this system. Articulating the latter point, we may repeat (cf. the Introduction) that our analysis obviously does not describe all the possible situations.

Finally, we note that although our presentation was focused on the conversion of lipid, the main counterparts of our models and analysis can be used for interpretation of the other types of enzymatic reactions occurring at vesicles.

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Appendix A

The main part of our presentation has been focused on the case when P forms rafts during the E-catalyzed L conversion. For comparison, we briefly analyze here the situation when L and P are located nearly at random in the external leaflet of a vesicle, i.e., there are no rafts. Specifically, we consider that the E–vesicle interaction includes a multitude of weak E–L and E–P interactions (as in the PLA_2 case). The binding of this type is common in many areas of chemical physics and surface science (see, e.g., Ref. [61]). The standard physically reasonable mean-field prescription in this case is that as a first-order approximation one should represent the effective binding energy (or activation energies) as a combination of the corresponding binding energies (or activation energies), i.e., the binding energies (or activation energies) corresponding to the E–L and E–P interactions. Following this line, we describe the E–vesicle interaction in the absence of rafts as

$$dN/dt = k_a c s_\circ - k_d N, \tag{41}$$

$$d\theta/dt = (k_r s_l/s_\circ)N, \tag{42}$$

where k_a and k_d are the attachment and detachment rate constants dependent on θ due to the E–L and E–P interactions, and k_r is the effective reaction rate constant.

To describe the effect of the E–L and E–P interactions on the dependence of k_a and k_d on θ, we employ the mean-field expression for the corresponding binding and activation energies,

$$E = E_\circ - C\theta, \tag{43}$$

$$E_a = E_a^\circ - \alpha C\theta \quad \text{and} \quad E_d = E_d^\circ + \beta C\theta, \tag{44}$$

where C is the constant determined by the interactions, and α and β are the dimensionless coefficients satisfying the condition $\alpha+\beta=1$ (cf. Section 4).

The effective reaction rate constant, k_r, can be represented in the MM form [as in expression (5)]. In general, its dependence on θ may be complex because the corresponding expression contains n (n is related to θ) and three rate constants (each of them may depend on θ). For example, we consider the initial stage of the kinetics where $\kappa_1 n$ is expected to be large compared to κ_{-1} and κ_2. In this case, the reaction itself limits conversion, and we have $k_r \simeq \kappa_2$. The activation energy of the latter rate constant can be neglected, because the L conversion occurs locally inside E, the E–L and E–P interactions are weak, and locally their role is expected to be minor. Under such conditions, k_r is independent of θ.

For comparison with the kinetics described in the main part of the article, let us consider that the reaction occurs near the E attachment–detachment equilibrium, i.e., $k_a c s_\circ = k_d N$. Using this equation and expressions (44), we rewrite Eq. (42) as

$$d\theta/dt = r_\circ c \, exp(C\theta/k_B T), \tag{45}$$

where $r_\circ \equiv k_r k_a^\circ s_l / k_d^\circ$, and k_a° are k_d° are the rate constants corresponding to $\theta = 0$. If the conversion is low ($\theta \ll 1$), Eq. (44) is mathematically similar to Eq. (36), and accordingly the kinetics predicted by Eq. (44) may be similar to those shown in Fig. 1(c).

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