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Kinetic model for surface-active enzymes based on the Langmuir adsorption isotherm: Phospholipase C (*Bacillus cereus*) activity toward dimyristoyl phosphatidylcholine/detergent micelles

(surface adsorption/detergent-phospholipase binding/surface dilution kinetics)

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ABSTRACT **A simple kinetic model for the enzymatic activity of surface-active proteins against mixed micelles has been developed. This model uses the Langmuir adsorption isotherm, the classic equation for the binding of gas molecules to metal surfaces, to characterize enzyme adsorption to micelles. The number of available enzyme binding sites is equated with the number of substrate and inhibitor molecules attached to micelles; enzyme molecules are attracted to the micelle due to the affinity of the enzyme active site for the molecules in the micelle. Phospholipase C (*Bacillus cereus*) kinetics in a wide variety of dimyristoyl phosphatidylcholine/detergent micelles are readily explained by this model and the assumption of competitive binding of the detergent at the enzyme active site. Binding of phospholipase C to pure detergent micelles is demonstrated by gel filtration chromatography. The experimentally determined enzyme–detergent micelle binding constants are used *directly* in the rate equation. The Langmuir adsorption model predicts a variety of the characteristics observed for phospholipase kinetics, such as differential inhibition by various charged, uncharged, and zwitterionic detergents and surface-dilution inhibition. The essential idea of this model, that proteins can be attracted and bound to bilayers or micelles by possessing a binding site for the molecules composing the surface, may have wider application in the study of water-soluble (extrinsic) protein–membrane interactions.**

The interaction of water-soluble proteins with biomembrane surfaces plays an important role in fat digestion, cell–cell communication, and numerous other cellular functions. Characterization of these phenomena is difficult because of the complexity of biomembranes and the need for sensitive binding assays. The interaction of water-soluble phospholipases with micellar structures offers a useful model system for extrinsic protein–membrane interactions. Many phospholipases have been purified to homogeneity and are available in relatively large quantities (1–3). Micelles form optically clear solutions and can be studied by a variety of conventional physical techniques (4–7). If one of the micellar components is a substrate for the phospholipase, then the observed activity serves as a direct test for theories of enzyme–micelle interactions.

Phospholipase action toward phospholipid molecules in a surface is much greater than that toward monomeric substrates ["interfacial activation" (1)]. Enzyme-specific activity also depends on the matrix used to form the surface—i.e., detergent mixed micelles (8), short-chain lecithin micelles (9, 10), bilayers (11), monolayers (12). A variety of kinetic models have been applied to these phenomena. The simplest model, applied to snake venom phospholipase A_2 action toward short-chain lecithins, proposes normal Michaelis–Menten kinetics and different V_m and K_m values for monomeric and micellar lipid with the monomer as a competitive inhibitor of micellar lecithin (13). Another model, proposed for pancreatic phospholipase A_2, accounts for interfacial activation by proposing a second site on the enzyme that "anchors" or "recognizes" surfaces (14). Different surface-active molecules can interact differentially with the two sites and hence modulate the activity. These models have not been extended in a systematic fashion to binary or more complex surfaces except in cases in which the added surface molecule is a substrate analogue. The only detailed binary component kinetic model is that of Dennis and co-workers (15). This "surface as cofactor" model was developed for phospholipase A_2 and phospholipase C kinetics using Triton X-100/lecithin micelles as substrates. The model is quite complex, requiring estimation of the surface area/head-group ratio and several assumptions (16) to fit observed activities. It is based on surface association of the enzyme followed by substrate binding in the active site to form the Michaelis complex; i.e., two distinct binding steps are involved.

To generalize a kinetic model for surface-active enzymes such as the phospholipases, we have examined the action of phospholipase C (*Bacillus cereus*) toward dimyristoyl phosphatidylcholine ($Myr_2PtdCho$) in mixed micelles with four different detergents: Triton X-100 (nonionic), Zwittergent 3-14 (zwitterionic), deoxycholate (anionic), and trimethylcetylammonium bromide ($Me_3CetNBr$; cationic). The data are interpreted by using a simple model based on the Langmuir adsorption isotherm and competitive inhibition of detergent. Detergent binding is estimated independently by gel filtration. This model, which postulates a single binding site on phospholipase C that has different affinities for amphiphilic molecules, yields unique kinetic constants for processing $Myr_2PtdCho$ and predicts the surface saturation and surface-dilution inhibition kinetics experimentally observed in each detergent system.

MATERIALS AND METHODS

Materials. $Myr_2PtdCho$ was obtained from Calbiochem. Phospholipid purity was monitored by TLC in $CHCl_3/CH_3OH/H_2O$ (65:24:4). Triton X-100 (Amersham), Zwittergent 3-14 (Calbiochem), and $Me_3CetNBr$ and sodium deoxycholate (Sigma) were used without further purification.

Enzymatic Assays. Phospholipase C (*B. cereus*) was purified as described (17). Enzymatic hydrolysis of $Myr_2PtdCho$ was measured by pH-stat (pH 8 end point) (8) at 30°C. Assay mixtures contained 0.1–20 mM $Myr_2PtdCho$ and 0.5–100 mM detergent.

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Abbreviations: $Myr_2PtdCho$, dimyristoyl phosphatidylcholine; $Me_3CetNBr$, trimethylcetylammonium bromide; cmc, critical micelle concentration.

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Gel Filtration. Micellar detergent binding constants (K_{Dm}) to phospholipase C were estimated by gel filtration using a column (0.7 × 50 cm) of Sephadex G-100 equilibrated with enzyme at 0.01 mg/ml in 50 mM Tris·HCl/1.0 mM Zn^{2+}, pH 7.5 (column buffer). The column was standardized with blue dextran (Sigma) to mark the void volume and ADP to mark the column volume. Detergent samples (0.5–1.0 ml of 10–100 mM detergent) were applied to the column. Detergent elution was monitored by OD_{278nm} in the case of Triton X-100 and by a colorimetric amine assay (18) for Zwittergent and $Me_3CetNBr$. Enzyme–detergent binding was monitored by the appearance of an activity peak above baseline values coincident with the detergent peak and a subsequent activity trough centered about the elution volume for phospholipase C [M_r, 23,000 (19)]. Binding constants were estimated by averaging the amounts of excess enzyme in the detergent peak and deficient enzyme activity in the trough and using this value for the amount of enzyme–detergent complex formed, knowing the amount of detergent applied to the column and the concentration of free enzyme in the buffer (20). Each K_{Dm} is the mean of two or more columns.

Computational Analysis. Best-fit solutions for the kinetic data were determined on a PDP 1160 computer. Monomeric and micellar concentrations for substrates and detergents were calculated by Raoult's law (21). Critical micellar concentrations (cmc) used were $Myr_2PtdCho$, 0.1 μM; Triton X-100, 0.8 mM; Zwittergent, 0.5 mM; deoxycholate, 3 mM; $Me_3CetNBr$, 0.9 mM. Starting with reasonable guesses for the kinetic parameters based on conventional concepts of K_m and maximal velocity (V_{max}) and using the experimentally determined K_{Dm} values, the total error was calculated. The solution space of this equation is considered a 3-space with axes k_d, k_a, and k_{cm}, (the kinetic parameters desired); the minimum was found by following the path of least error to a true minimum. The K_{Dm} values were then optimized for the concentration range surrounding the experimentally observed K_{Dm} values; true error minima were observed for all four K_{Dm} values. Additional rounds of refinement for kinetic parameters, K_{Dm} values, and then kinetic parameters caused no significant changes in any of the constants.

The least-error path (in the 3-space) from the original guess to the final least-error solution was reasonably linear, suggesting that no nearby minima exist. Other reasonable combinations of the kinetic parameters were tried to sample the solution space; all gave errors more than 10 times the best-fit error. The kinetic parameters were each optimized to one significant figure.

RESULTS AND DISCUSSION

Derivation of Kinetic Model. We propose that the observed initial velocity, V_i, for a surface-active enzyme (Fig. 1) is

$$V_i = k_{cm}[ES_m] + k_c[ES].$$

The different catalytic rate constants for monomeric and micellar substrate are not important in this study; the aqueous solubility of $Myr_2PtdCho$ is very low, and kinetic studies of phospholipase C (9) and phospholipase A_2 (13) with pure short-chain lecithin monomers and micelles suggest that $k_c \ll k_{cm}$. The monomer term is retained in the derivation for generality.

We propose normal Michaelis–Menten kinetics for monomers; the steady-state approximation ($d[ES]/dt = 0$) yields

$$\frac{ES}{E} = \frac{S}{K_s}, \text{ where } K_s = \frac{k_{-1} + k_c}{k_1}.$$

For micellar substrate, the Langmuir adsorption isotherm is proposed as the appropriate relationship for the steady-state approximation ($d[ES_m]/dt = 0$). In the Langmuir equation, formation and breakdown of the enzyme–micellar substrate complex are as follows:

$$E + S_m \underset{k_d}{\overset{k_a}{\rightleftharpoons}} ES_m \xrightarrow{k_{cm}} E + P_m$$

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow{k_c} E + P$$

$$E + D_m \underset{k_{-I}}{\overset{k_I}{\rightleftharpoons}} ED_m$$

$$E + D \rightleftharpoons ED$$

FIG. 1. Parameters for kinetic model. All concentrations are bulk average solution concentrations. E, enzyme concentration; S, monomeric substrate concentration; D, monomeric detergent concentration; S_m, micellar substrate concentration; D_m, micellar detergent concentration; P and P_m, monomeric and micellar product concentrations, respectively. Complexes are indicated by combining the appropriate symbols.

Association = rate constant × concentration of free enzyme in solution above the surface × *fraction* of binding sites unoccupied

$$= k_a(E)\left(1 - \frac{ES_m}{S_m + ES_m}\right) \cong k_a(E)\left(1 - \frac{ES_m}{S_m}\right)$$

(this approximation is valid because 10 nM enzyme is typically used in an assay, while micellar substrate is in the millimolar concentration range) and

Dissociation = rate constant × *fraction* of sites occupied

$$= k_d\left(\frac{ES_m}{S_m}\right).$$

This treatment circumvents the surface dimensionality problem (16). The full steady-state approximation for micellar substrate is therefore

$$\frac{d[ES_m]}{dt} = 0 = k_a(E)\left(1 - \frac{ES_m}{S_m}\right) - k_d\left(\frac{ES_m}{S_m}\right) - k_{cm}(ES_m).$$

Solving this equation and assuming that

$$\frac{k_d + k_{cm}S_m}{k_a} \gg (E) \qquad \text{(shown below)},$$

we find that

$$\frac{ES_m}{E} = \frac{S_m k_a}{k_d + k_{cm}S_m}.$$

Defining

$$\frac{k_d + k_{cm}S_m}{k_a} = K_{sm} \qquad [= f(S_m)]$$

then gives

$$\frac{ES_m}{E} = \frac{S_m}{K_{sm}}.$$

This term is of the same form as the corresponding term for monomers, but K_{sm} is a function of S_m, the micellar substrate concentration. The approximation shown above, that (k_d +

$k_{cm}S_m)/k_a \gg E$ is now $K_{sm} \gg E$. K_{sm} has a binding term (k_d/k_a) and a kinetic component. Other studies have estimated lecithin or analogue binding constants to phospholipases ranging from 0.1 to 5 mM. This term alone is greater than E (the total enzyme concentration is typically 10 nM); the additional kinetic term will only increase the difference. S_m in these assays is 0.1–20 mM. As shown below with the kinetic constants determined for this system, K_{sm} is 4–8 mM, while total enzyme concentration is typically 10 nM and free enzyme concentration is even less, verifying the approximation.

Langmuir Binding Term for Inhibitors. Steady-state approximation for this complex yields

$$\frac{d[ED_m]}{dt} = 0 = k_I(E)\left(1 - \frac{ED_m}{D_m}\right) - k_{-I}\left(\frac{ED_m}{D_m}\right),$$

giving

$$\frac{E}{ED_m} = \frac{k_{-I}}{k_I}\left(\frac{1}{D_m}\right) + \frac{E}{D_m}.$$

Defining $k_{-I}/k_I = K_{Dm}$ and assuming again that $K_{Dm} \gg E$ gives

$$\frac{E}{ED_m} = \frac{K_{Dm} + E}{D_m} \approx \frac{K_{Dm}}{D_m}$$

or

$$\frac{ED_m}{E} = \frac{D_m}{K_{Dm}}.$$

As long as $K_{Dm} \gg E$, the binding of enzyme to detergent micelles occurs in a form similar to that in bulk (isotropic) solution. If the affinity of the protein for the binding site is stronger—i.e., $K_{Dm} \leq E$, then the appropriate binding term would be $(ED_m/E) = (K_{Dm} + E)/D_m$, which can be solved iteratively.

Gel filtration chromatography of phospholipase C with the appropriate detergent gives this binding constant (K_{Dm}) *directly*. Thus, K_{Dm} is experimentally determined and is not considered a free parameter in the analysis. In all cases, it is considerably greater than E.

Derivation of Kinetic Equation. Returning to the original rate equation, we have

$$V_i = k_{cm}[ES_m] + k_c[ES]$$

$$= (E)\left[\frac{k_{cm}S_m}{K_{Sm}} + \frac{k_cS}{K_s}\right]$$

and

$$E_T = E + ES + ES_m + ED + E_{Dm}$$

$$= E\left(1 + \frac{S}{K_s} + \frac{S_m}{K_{Sm}} + \frac{D}{K_D} + \frac{D_m}{K_{Dm}}\right).$$

In this study where relative inhibition of monomeric versus micellar detergent was not studied carefully, we will assume that

$$K_D = K_{Dm}.$$

Since, under our assay conditions, the concentrations of monomeric detergents are much less (1–10%) than those of the micellar species, K_D would have to be 10 to 100 times K_{Dm} to affect the kinetics. The final rate equation is

$$V_i = \frac{E_T\left[\frac{k_{cm}S_m}{K_{Sm}} + \frac{k_cS}{K_s}\right]}{1 + \frac{S}{K_s} + \frac{S_m}{K_{Sm}} + \frac{D_T}{K_{Dm}}},$$

where D_T is the total detergent concentration.

Results of the Kinetic Model. The kinetic parameters determined for $Myr_2PtdCho$/Triton X-100, Zwittergent 3-14, deoxycholate, and $Me_3CetNBr$ micelles are given in Table 1. The values of the free parameters k_d, k_a, and k_{cm} were derived by minimizing the sum

$$\sum_{i=1}^{49}\left|V_{Calc_i} - V_{Obs_i}\right|$$

for forty-nine assays (done in duplicate) distributed among the four mixed micellar systems. The best-fit values of K_{Dm} for each detergent (for concentrations around the experimentally estimated K_{Dm}) were also determined. Good agreement between best-fit and experimental K_{Dm} values (which have a fairly large error) is observed. Binding of phospholipase C to Triton X-100 is difficult to estimate. In the presence of 1 mM Zn^{2+}, the enzyme shows very weak affinity for Triton. When excess Zn^{2+} is removed from the system, the enzyme shows enhanced Triton binding. Exact evaluation is complicated by a general "stickiness" that phospholipase C develops in solution lacking excess Zn^{2+} and in low ionic strength buffers. Assays are done under conditions in which there is no excess Zn^{2+}. Therefore, the "experimental" K_{Dm} is probably somewhere between these two values.

The K_{Dm} value for $Me_3CetNBr$ is less than the cmc for this detergent. Although direct comparison of these K_{Dm} values with true solution concentrations cannot be proven through this model, the direct correspondence of the gel chromatographic K_D values and the best-fit K_{Dm} values supports this conclusion. This in turn suggests tight binding of monomeric $Me_3CetNBr$ to phospholipase C. Preliminary UV difference spectra of the enzyme (0.7 mg/ml) without and with $Me_3CetNBr$ (<0.6 mM) show that a strong interaction does occur: the enzyme–detergent complex first precipitates and then is resolubilized as larger amounts of detergent are added.

The value of K_s is irrelevant in these assays because the cmc of $Myr_2PtdCho$ is so low (0.1 μM) (21) that monomer hydrolysis does not significantly contribute to the rate. The $Myr_2PtdCho$ cmc would need to be wrong by several orders of magnitude for monomer hydrolysis to be kinetically important. For comparison, Little (9) has found K_m for hydrolysis of monomeric dibutyrylphosphatidylcholine to be 37 mM. The average error per assay for the optimized model is approximately three times

Table 1. Kinetic constants for the Langmuir adsorption model of phospholipase C activity toward $Myr_2PtdCho$/detergent micelles

Constant	Calculated	Experimental
k_d, $mM \cdot s^{-1}$	20,000	—
k_a, s^{-1}	5,000	—
k_{cm}, s^{-1}	1,000	—
K_{Dm}, mM		
Triton X-100	40	≥60; >25 ± 5*
Zwittergent	3	4 ± 2
Deoxycholate	12	10 ± 5
$Me_3CetNBr$	0.2	2.4 ± 0.2; 0.5†
Average specific activity per assay, $\mu mol \cdot min^{-1} \cdot mg^{-1}$	370	—
Average error per assay, $\mu mol \cdot min^{-1} \cdot mg^{-1}$	97	30‡

Unless otherwise noted, K_{Dm} values were measured by gel filtration in the presence of 1 mM Zn^{2+}.
* Estimated by gel filtration in the absence of Zn^{2+} ions.
† Estimated by UV difference spectra suggesting that phospholipase C binds to $Me_3CetNBr$ monomers with a K_D of ≈0.5 mM.
‡ Experimental SD per assay.

the average experimental SD per assay. However, we believe that the SD of two assays done the same day does not accurately reflect the error for a large assay set done over a 4-month period with phospholipase C obtained from three separate purifications. The ability of this model to predict observed specific activities in mixed micellar systems with four structurally dissimilar detergents is a significant improvement over previous models of phospholipase kinetics.

As shown in the derivation of this model,

$$K_{Sm} = \frac{k_d + k_{cm}S_m}{k_a} = \frac{k_d}{k_a} + \frac{k_{cm}S_m}{k_a}.$$

If we compare this with the derivation of the equation for detergent binding and equate k_d/k_a to the binding constant (analogous to K_{Dm}) for $Myr_2PtdCho$, we obtain

$$K_{Sm} = \text{binding constant} + \text{kinetic term}.$$

Substituting the appropriate kinetic constants gives

$$K_{Sm} = 4.0\ \text{mM} + 0.2\ S_m,$$

where S_m is millimolar. The binding constant for $Myr_2PtdCho$ is similar to the value of K_{Dm} for Zwittergent and larger than that for $Me_3CetNBr$. All three molecules contain a quaternary nitrogen and linear aliphatic chains. For the $Me_3CetNBr$ system, the effective inhibition of phospholipase activity is not caused by bromide ion; added NaBr has no effect on other assays (data not shown). Phospholipase C apparently shows little substrate specificity, with binding energy relationships probably dominated by hydrophobic interactions. Structural analyses of this kind can readily be extended to other substrates and detergents to determine the binding specificity of phospholipases.

The maximal specific activity for phospholipase C can be calculated by examining the term S_m/K_{Sm} as S_m goes to infinity:

$$\frac{S_m}{K_{Sm}} = \frac{S_m k_a}{k_d + k_{cm}S_m} \rightarrow \frac{S_m k_a}{k_{cm}S_m} = \frac{k_a}{k_{cm}}.$$

This result and the assumption that monomer kinetic contributions and detergent inhibition are negligible yield

$$\frac{V}{E_T} = \frac{k_a k_{cm}}{k_a + k_{cm}} = 2{,}100\ \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}.$$

This value is similar to the maximum velocity for phospholipase C activity extrapolated for the Triton X-100/egg lecithin system using a surface-as-cofactor model (16).

"Surface Dilution" Kinetics. At a fixed mole fraction of lecithin, phospholipase C activity depends on the total concentration of surfactant (lecithin plus detergent). For the Triton X-100- and deoxycholate-containing micelles, curves somewhat reminiscent of substrate saturation kinetics are observed (Fig. 2 *A* and *C*); for the Zwittergent and $Me_3CetNBr$ micellar systems, the activity is constant and markedly inhibited (Fig. 2 *B* and *D*). In each graph, the line connects the theoretical values; the calculated activities are found at the phospholipid concentrations corresponding to the experimental points. It is not easy to see how direct surface binding can be obtained from these curves, as suggested by Dennis (22).

If, rather than holding the mole fraction of lecithin constant, we maintain a fixed lecithin concentration and vary the detergent concentration, distinct inhibition is observed (Fig. 3). This type of phenomenon, termed surface dilution, has been explained by Dennis and co-workers in terms of a complex kinetic model involving a nonspecific surface binding site and a specific catalytic site on the enzyme. The experimental data points for phospholipase C in the four detergent systems are quite well fit by our Langmuir adsorption model (solid lines), in which only a *single enzyme site* is postulated.

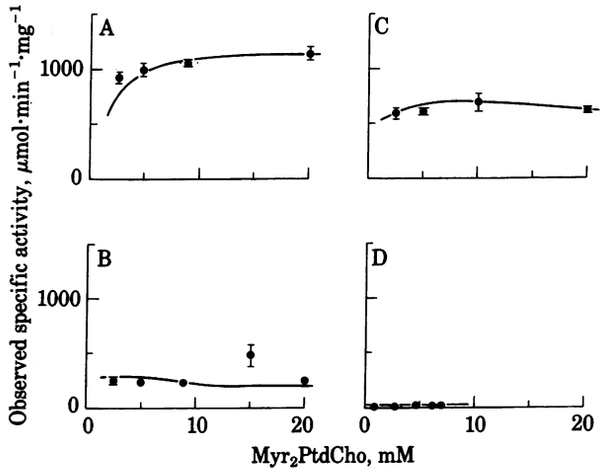

FIG. 2. Experimental and theoretical enzymatic activities at a fixed $Myr_2PtdCho$ mole fraction (f_L). Results are experimental values ± SD (absence of error bars reflect SD values smaller than the point size). ——, Activities calculated by the kinetic model; the calculated activity is found at the same total $Myr_2PtdCho$ concentration as the corresponding experimental point. (*A*) $Myr_2PtdCho$/Triton X-100 micelles; $f_L = 0.19 \pm 0.01$. (*B*) $Myr_2PtdCho$/Zwittergent micelles; $f_L = 0.19 \pm 0.01$. (*C*) $Myr_2PtdCho$/deoxycholate micelles; $f_L = 0.20 \pm 0.02$. (*D*) $Myr_2PtdCho/Me_3CetNBr$ micelles; $f_L = 0.16 \pm 0.03$.

A powerful technique for understanding the kinetics of surface-active enzymes is a three-dimensional plot in which total substrate, total detergent, and observed activity form the *x*, *y*, and *z* axes. Two-dimensional slices of such plots are shown in

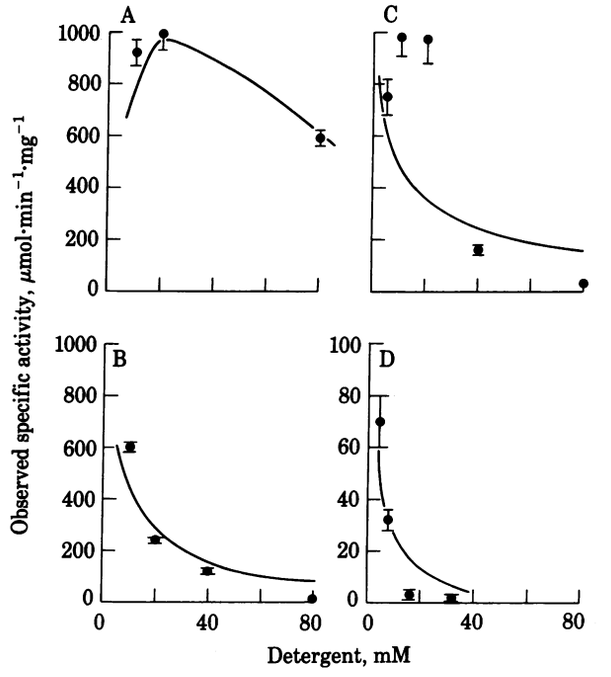

FIG. 3. Surface-dilution experiments for $Myr_2PtdCho$/detergent micelles. Observed phospholipase C specific activity at roughly constant $Myr_2PtdCho$ concentrations is plotted as a function of total detergent concentration. Results are expressed as in Fig. 2. (*A*) $Myr_2PtdCho$/Triton X-100 micelles; average $[Myr_2PtdCho] = 4 \pm 1$ mM. (*B*) $Myr_2PtdCho$/Zwittergent micelles; average $[Myr_2PtdCho] = 4.9 \pm 0.1$ mM. (*C*) $Myr_2PtdCho$/deoxycholate micelles; average $[Myr_2PtdCho] = 2.1 \pm 0.1$ mM. (*D*) $Myr_2PtdCho/Me_3CetNBr$ micelles; average $[Myr_2PtdCho] = 2.4 \pm 0.3$ mM. The apparent peak in the theoretical curve in *A* is caused by variations in $[Myr_2PtdCho]$.

Fig. 4. The assay series for total surface concentration at a fixed mole fraction of lecithin is represented on this plot by a straight line that intersects the origin. For example, the data from Fig. 2*A* for $Myr_2PtdCho$/Triton X-100 micelles are shown in Fig. 4*B* as points connected by the line constant $Myr_2PtdCho$/detergent = 4. At low substrate concentrations, this surface concentration activity line cuts across some of the specific activity contour lines, showing some change in activity. At higher concentrations (i.e., the upper two-thirds of the line), this line runs parallel to the activity isobars, so no change in activity with increasing concentration is indicated. The surface-dilution series is seen on this graph as a line parallel to the abscissa. It can intersect a large number of activity isobars indicating inhibition. An optimized set of experiments is given by the crossline in Fig. 4*A*, which represents fixed total surfactant (phospholipid and detergent) but various mole fractions of phospholipid.

Inspection of the $Myr_2PtdCho$ concentration axes in Fig. 4 *A* and *B* indicates different activities in the absence of detergent. The failure of this model to converge to a common activity in the absence of detergent cannot be examined experimentally for this system because mixed micelles that have high proportions of $Myr_2PtdCho$ are not stable soluble micelles. However, mixed micellar systems with short-chain lecithins and detergents are soluble in all proportions, making the entire line shown in Fig. 4*A* accessible. The cmc of several of the short-chain lecithins are in experimentally convenient concentration ranges, so a full kinetic analysis for monomeric and micellar substrate and monomeric and micellar detergent is possible.

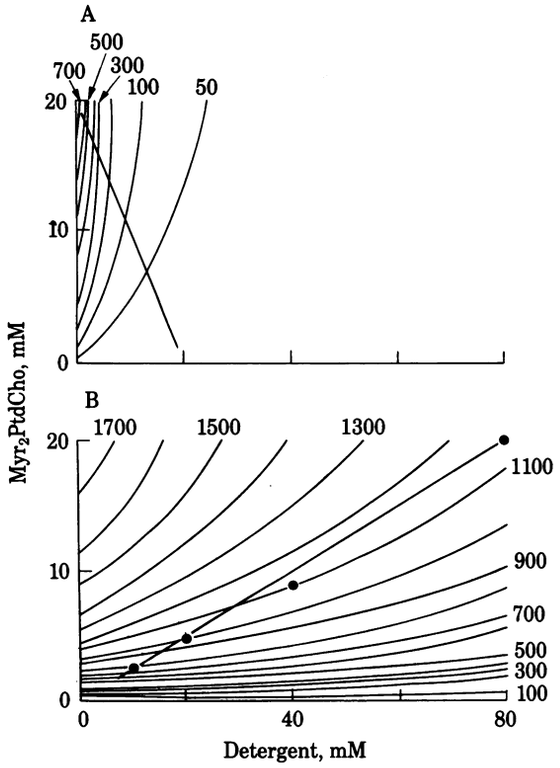

FIG. 4. Contour plots of specific activity versus total $Myr_2PtdCho$ and detergent concentrations. Plots are derived from best-fit values of the model. (*A*) $Myr_2PtdCho/Me_3CetNBr$ micelles: strong inhibition by $Me_3CetNBr$ is clearly demonstrated; the line crossing the activity isobars represents a maximum information assay series. (*B*) $Myr_2PtdCho$/Triton X-100 micelles: points and the line crossing the activity isobars represent the surface concentration experiment shown in Fig. 2*A*.

Extrapolation to Other Substrate Aggregates. The basic idea of the Langmuir adsorption kinetic model is that an enzyme is attracted to a surface via a binding site for individual molecules composing the surface. This allows us to reinterpret the activity of phospholipases in other mixed systems and to predict enzyme affinities for different surface components. For example, phospholipase C (*B. cereus*) activity is sensitive to the presence of cholesterol (23) but not to that of triglyceride (24). Rather than strictly relating "surface" concentrations of these components, this means that phospholipase C must have a strong affinity for cholesterol but little for triglyceride compared with lecithin. The report of Sundler *et al.* (25) that a phosphatidylinositol-specific phospholipase C displayed surface dilution inhibition in Triton X-100/phosphatidylinositol micelles but not with lecithin/phosphatidylinositol sonicated vesicles can be explained by a stronger affinity of that enzyme for Triton than for lecithin. This suggests that the detergent hydroxyl group or oxygen-rich oxyethylene units mimic inositol binding to that enzyme.

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