

# Kinetic dependence of phospholipase A<sub>2</sub> activity on the detergent Triton X-100

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**Abstract** A kinetic analysis is presented for the dependence of one form of phospholipase A<sub>2</sub> from cobra (*Naja naja*) venom on the presence of the nonionic detergent Triton X-100 for its activity towards egg phosphatidylcholine and synthetic dipalmitoyl glycerophosphorylcholine as substrates. An automatic recording pH-stat apparatus was employed in order to continuously monitor enzyme activity. The results obtained in this study are interpreted in terms of a change in the physical state of the phospholipid when Triton X-100 micelles convert phospholipid bilayers into mixed Triton X-100-phospholipid micelles; this is consistent with the requirement of this enzyme for substrates which are in micellar form rather than either monomers or bilayers. An apparent inhibition of phospholipase A<sub>2</sub> activity at high concentrations of Triton X-100 is described and discussed in terms of the micellar nature of the substrate.

**Supplementary key words** egg phosphatidylcholine · dipalmitoyl glycerophosphorylcholine · mixed micelles · phospholipid

**D**ETERGENTS are used extensively in the purification and assay of enzymes of phospholipid metabolism, yet their precise function is not clearly understood. In particular, the nonionic detergent Triton X-100 is proving to be increasingly popular for these purposes. For example, in our previous work (1, 2) on the membrane-bound enzyme phosphatidylserine decarboxylase, the assay was carried out in the presence of Triton X-100 and this enzyme has now been solubilized and purified in the presence of this detergent (3). In order to better understand the exact role which the nonionic detergents play when used in studies on enzymes of phospholipid

metabolism, we have studied in detail the kinetic dependence of a well-characterized and highly purified enzyme of phospholipid metabolism, phospholipase A<sub>2</sub> (EC 3.1.1.4; for recent review see Ref. 4), on Triton X-100. Phospholipase A<sub>2</sub> offers a further advantage for these studies in that it is a soluble enzyme. In addition, the main role of the Triton X-100 appears to be in the alteration of the physical state of the substrate phospholipid, rather than in any specific solubilization of the enzyme, but both factors may be involved in the action of membrane-bound enzymes on phospholipid substrates.

The need for some alteration of the physical state of the phospholipid during assay for phospholipase A<sub>2</sub> has long been recognized, and thus the enzyme has traditionally been assayed in ether (5), sodium deoxycholate (6), or Triton X-100 (7). With more highly purified enzymes, workers have attempted to study phospholipase A<sub>2</sub> without the presence of detergent by using "soluble" synthetic substrates containing short-chain fatty acids (8-10), following the example of pancreatic lipase (11). De Haas et al. (9) studied the activity of phospholipase A<sub>2</sub> towards a series of these synthetic substrates with different chain lengths and found that the dioctanoyl glycerophosphorylcholine reacted significantly better than phospholipids containing either longer or shorter fatty acid chains. They concluded from their studies that phospholipase A<sub>2</sub> requires a micellar surface of a specific geometry for activity, although phospholipase A<sub>2</sub> will also hydrolyze monomers, albeit rather poorly. Thus, they found that only the specific phosphatidylcholine containing two octanoic fatty acid groups forms the proper surface for maximal activity without the addition of detergent. Wells (10) has recently reported more extensive mechanistic studies on this enzyme. However, since he used the relatively inactive molecularly dispersed dibutyroyl glycerophosphorylcholine as substrate, the possibility of answering certain questions

Abbreviations: CMC, critical micellar concentration; diacyl glycerophosphorylcholine, 1,2-diacyl-*sn*-glycero-3-phosphorylcholine; Triton, Triton X-100.

relevant to normal substrates in aggregated form in biological systems is questionable. The presence of Triton X-100 during assay of this enzyme offers the potential advantage of allowing the use of a variety of biologically relevant substrates and may provide additional information about the apparent requirement of this enzyme for phospholipid in a particular physical state. Along these lines, Entressangles and Desnuelle (11) found that pancreatic lipase would hydrolyze both micelles and detergent emulsifications of the same short-chain substrate at about the same maximal rate, suggesting that catalysis occurs similarly in both systems.

Although phospholipase A<sub>2</sub> and other enzymes of phospholipid metabolism have been routinely assayed in the presence of a given amount of Triton X-100, little attention has been paid to the detailed effect of this detergent on the kinetics of enzyme action. When Triton X-100 is included during assay, the substrate for the enzyme can be considered to be a mixed micelle of Triton and phospholipid; we are currently attempting to characterize these mixed micelles more precisely using nuclear magnetic resonance techniques.<sup>1</sup> An understanding of the kinetic effects of Triton X-100 on phospholipase A<sub>2</sub> action presented here combined with a knowledge of the precise geometry of the substrate phospholipid in the presence of Triton X-100 may provide further information on the mechanism of phospholipase A<sub>2</sub> action on micelles and a model for the study of more complicated membrane-bound enzyme systems in the presence of Triton.

## MATERIALS AND METHODS

Phospholipase A<sub>2</sub> was provided by Dr. Beatriz M. Braganca, Cancer Research Institute, Tata Memorial Centre, Bombay, India. The enzyme was obtained from the venom of the cobra *Naja naja*, and the major form of phospholipase A<sub>2</sub> (12) was purified 20-fold according to the procedure described elsewhere (13). The purified enzyme is homogeneous on starch gel electrophoresis (13) and is eluted from Sephadex G-100 with an apparent molecular weight of about 11,000 (14). Enzyme purified in this manner was provided by Dr. Braganca as a lyophilized powder and was stored as such at -20°C. Several preparations of enzyme were used; they ranged in specific activity between 0.7 and 2 μmoles min<sup>-1</sup> μg protein<sup>-1</sup> under standard assay conditions. Protein was determined by the method of Lowry et al. (15).

Dipalmitoyl glycerophosphorylcholine refers to syn-

thetic dipalmitoyl-L-α-lecithin (Mann Research Laboratories), which was used without further purification. Egg phosphatidylcholine refers to "highly purified" egg lecithin (Mann Research Laboratories), which was used either as obtained or after further purification according to the method of Singleton et al. (16), followed by lyophilization from benzene. These phospholipids were analyzed by thin-layer chromatography on pre-coated layers of silica gel G on glass plates (Brinkmann Instruments). The developing solvent was chloroform-methanol-water 65:25:4, and the lipids were visualized after the plates were treated with iodine vapors, rhodamine B, or the phosphorus reagent of Dittmer and Lester (17). Each phospholipid produced only a single spot. The molecular weight of the phospholipid was based on a phosphorus determination (18). The molecular weight of Triton X-100 (Rohm and Haas) was assumed to be 628 (19). KOH solutions were prepared from carbonate-free KOH ("Dilut-it analytical concentrate," J. T. Baker Chemicals) by dilution with freshly boiled, glass-distilled water and stored under Ascarite. Potassium acid phthalate (Mallinkrodt Chemical Works) was used as a primary standard, and standard buffers of pH 7 and 10 (Matheson Coleman & Bell) were used for calibration of the electrodes. All other chemicals were of analytical grade. Glass-distilled water was used routinely in these experiments.

The pH-stat apparatus consisted of the following Radiometer components: pH meter PHM-26, titrator TTT-11, Titrigraph recorder SBR-2c, Auto-burette ABU-12 equipped with a 0.25-ml burette assembly B230, and a microtitration assembly TTA-31 equipped with a thermostated jacket V526. The water jacket was maintained at constant temperature within ± 0.1°C with a Lauda-Brinkmann constant temperature circulator K-2/R. The titration assembly was equipped with a Radiometer calomel electrode type K4112 and a Beckman glass electrode 39167. These electrodes were briefly dipped in Clorox bleach followed by a thorough rinsing with water before every experimental point. This was found to be necessary in order to maintain electrode sensitivity and reproducibility. It would not be unreasonable to assume that the Clorox serves to oxidize protein, phospholipid, or detergent remaining on the electrodes and not easily removed with normal washing. After each Clorox treatment and rinsing, electrode response was checked with standard buffers.

The standard assay system contained 6 mM phosphatidylcholine, 12 mM Triton X-100, 10 mM CaCl<sub>2</sub>, and 0.4 μg of protein in a volume of 2.00 ml, added as described below and maintained at 40°C and pH 7.75 by the addition of 0.01 N KOH. Phosphatidylcholine was dispersed in water with the Teflon pestle of a Potter-Elvehjem homogenizer (Arthur H. Thomas tissue

<sup>1</sup> For preliminary results, see: Dennis, E. A., and J. M. Owens. 1973. Studies on mixed micelles of Triton X-100 and phosphatidylcholine using nuclear magnetic resonance techniques. *J. Supramolecular Structure*. 1(3). In press.

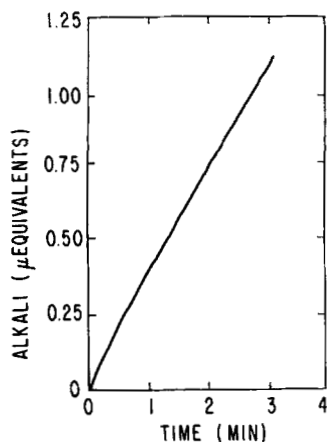


FIG. 1. Time course of action of phospholipase  $A_2$  on dipalmitoyl glycerophosphorylcholine. Standard assay conditions were employed; the recorder output is shown.

grinder) operated by an Eberbach Con-Torque power unit no. 7265 until a uniform dispersion was obtained. The dispersion was then brought to pH 7.5 with 0.01 N KOH. Aliquots of aqueous solutions of Triton X-100 and  $CaCl_2$  at pH 7.5 and the phospholipid dispersion

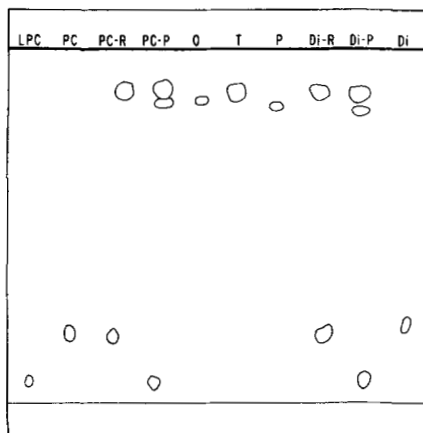


FIG. 2. Demonstration of the products of phospholipase  $A_2$  action, using thin-layer chromatography. An aliquot of solution resulting from an experiment conducted under standard conditions with egg phosphatidylcholine as substrate but allowed to continue to completion was applied to a thin-layer plate as described in Materials and Methods; this is designated *PC-P* for "PC-products." An aliquot of a similar solution, without added enzyme, was treated similarly; this is designated *PC-R* for "PC-reactants." Aliquots from similar procedures carried out on dipalmitoyl glycerophosphorylcholine are designated *Di-P* and *Di-R*. Lysophosphatidylcholine (*LPC*), phosphatidylcholine (*PC*), oleic acid (*O*), Triton X-100 (*T*), palmitic acid (*P*), and dipalmitoyl glycerophosphorylcholine (*Di*) were included as standards. All standards were prepared as 6 mM aqueous solutions except for Triton X-100, which was prepared as a 12 mM solution corresponding to the concentrations of phospholipid and Triton in the reactant vessels. 10  $\mu$ l of each standard as well as of the "reactants" and "products" was applied to the thin-layer plate. Thus, all "reactants" and "products" are at comparable concentrations with the standards.

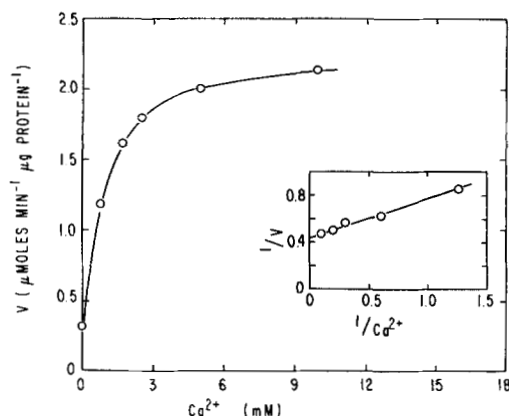


FIG. 3.  $Ca^{2+}$  dependence of phospholipase  $A_2$  activity towards egg phosphatidylcholine. Standard assay conditions were employed, except for the  $Ca^{2+}$  variation indicated.

were either added to the pH-stat vessel directly or, in some cases, all three substances were first homogenized together. The reactants were brought to assay temperature and pH under nitrogen or argon. Then the enzyme, contained in a 10- $\mu$ l micropipette, was added to the reaction vessel to initiate the reaction. There was no observable hydrolysis of substrate without the addition of enzyme.

Initial rates were obtained from the slopes of the recorder output as shown in Fig. 1, when dipalmitoyl glycerophosphorylcholine was used as substrate. When egg phosphatidylcholine was used as substrate, the amount of base added decreased somewhat from linearity with time, so that initial rates were calculated from the amount of base added after 2 min. The results reported in some experiments are the averages of duplicate determinations; the average reproducibility for these points is about  $\pm 5\%$ , although individual points may vary by greater amounts. It should be noted that at low Triton concentrations, the electrodes do not respond as consistently as under standard conditions, causing less reproducibility in the results.

## RESULTS

### Assay

The output of a typical pH-stat experiment using dipalmitoyl glycerophosphorylcholine as substrate is shown in Fig. 1; the amount of base added was linear with time. Using this assay, maximal activity was obtained around 40°C and about pH 8, and the activity was proportional to added protein over the range 0.05–0.8  $\mu$ g. The products of the reaction are lysophosphatidylcholine and free fatty acid, as shown in Fig. 2. Added  $Ca^{2+}$  was required for maximal activity and the  $K_m$  was about 1 mM, as shown in Fig. 3; residual activity

TABLE 1. Cation dependence of phospholipase A<sub>2</sub> activity towards egg phosphatidylcholine<sup>a</sup>

Addition	Ca <sup>2+</sup>	V
		$\mu\text{moles min}^{-1} \mu\text{g protein}^{-1}$
None	+	1.2
	-	0.5
1 mM EDTA	+	1.2
	-	0.03

<sup>a</sup> Standard assay conditions were employed, except for the additions and deletions noted. Averages of duplicate determinations are reported.

could be eliminated without added Ca<sup>2+</sup> by the addition of EDTA, as shown in Table 1.

### Triton X-100/phosphatidylcholine dependence

Enzyme activity was dependent on the addition of Triton X-100, as shown in Fig. 4; under the conditions of this experiment, an apparent  $K_m$  for Triton X-100 can be determined and it was about 2 mM. The dependence of activity on the substrate phosphatidylcholine concentration was determined in two ways. First, Triton X-100 concentration was held constant at a concentration about 6 times the apparent  $K_m$  determined in the experiment shown in Fig. 4. The results are shown in Fig. 5, and the Lineweaver-Burk plot of these data is included in Fig. 7. In another experiment, the molar ratio of Triton to phospholipid was kept constant at 2:1, and the saturation kinetics shown in Fig. 6 were observed. The apparent  $K_m$  for phosphatidylcholine under both conditions is between 2 and 5 mM. As shown in Fig. 4, Triton X-100 was necessary for the activity of this enzyme; however, at high concentrations of Triton X-100 an apparent inhibition of activity occurred, as shown in Fig. 7.

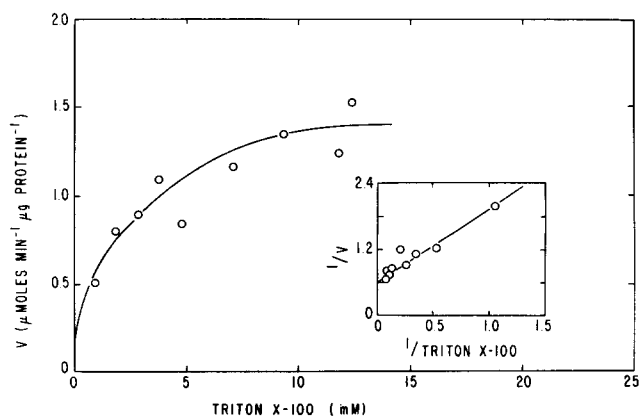


FIG. 4. Triton X-100 dependence of phospholipase A<sub>2</sub> activity towards egg phosphatidylcholine. Standard assay conditions were employed, except for the Triton X-100 variation indicated.

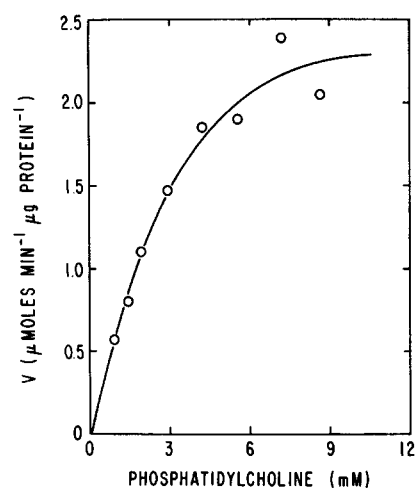


FIG. 5. Concentration dependence of phospholipase A<sub>2</sub> activity towards egg phosphatidylcholine. Standard assay conditions were employed, except for the phospholipid variation indicated.

The above experiments involving Triton X-100 were conducted with egg phosphatidylcholine as substrate; the same sort of activation and inhibition is illustrated with dipalmitoyl glycerophosphorylcholine as substrate in Fig. 8. With 6 mM dipalmitoyl glycerophosphorylcholine, an increase in the phospholipase A<sub>2</sub> activity was observed with increasing concentrations of Triton X-100 until about 12 mM; as the concentration of Triton was further increased, the activity diminished progressively. With either phospholipid, it is apparent that maximal enzymatic activity is obtained at about 12 mM Triton X-100 (Figs. 4 and 8), a molar ratio of 2:1 Triton/phospholipid. This is consistent with the above finding (Fig. 6) that at a constant molar ratio of 2:1 Triton/phospholipid, enzyme activity follows typical Michaelis-

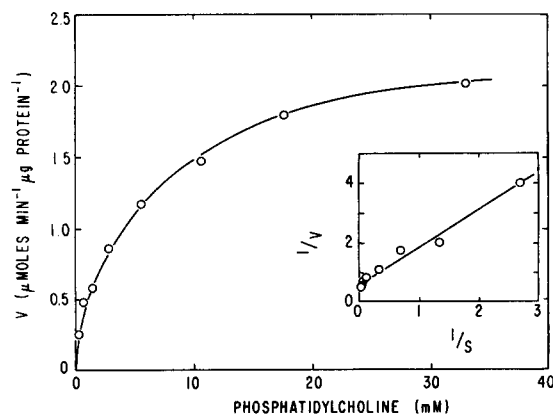


FIG. 6. Phospholipase A<sub>2</sub> activity towards egg phosphatidylcholine at a constant molar ratio of Triton X-100 to phospholipid. Standard assay conditions were employed, except that the molar ratio of Triton/phospholipid was maintained at 2:1 as the phospholipid concentration was varied.

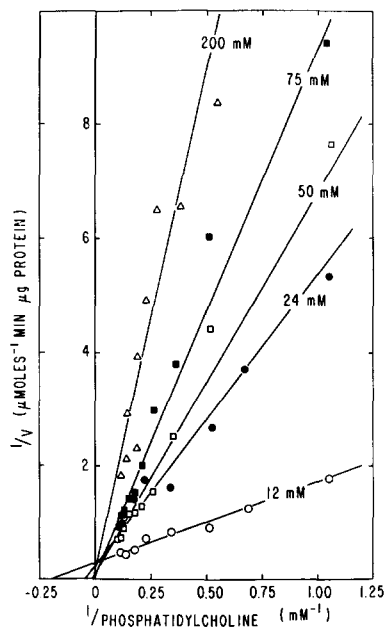


FIG. 7. Inhibition by Triton X-100 of phospholipase  $A_2$  activity towards egg phosphatidylcholine. Standard assay conditions were employed, except for the variation with phospholipid at the Triton X-100 concentrations indicated.

Menten kinetics with regard to substrate dependence and a  $K_m$  for substrate could be determined. After the phospholipid-Triton mixtures were prepared, activity was measured at various times over a 24-hr period, and this activity did not change significantly. Thus, the dispersion was stable with respect to time as measured in enzymatic assay.

#### Temperature dependence

The temperature dependence of phospholipase  $A_2$  with both dipalmitoyl glycerophosphorylcholine and egg phosphatidylcholine as substrates over the range of 20–50°C is shown in Fig. 9. The activity with both

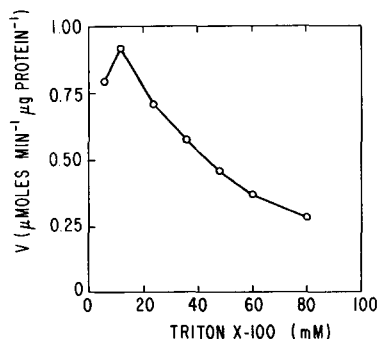


FIG. 8. Triton X-100 dependence of phospholipase  $A_2$  activity towards dipalmitoyl glycerophosphorylcholine. Standard assay conditions were employed, except for the Triton X-100 variation indicated. Averages of duplicate determinations are reported.

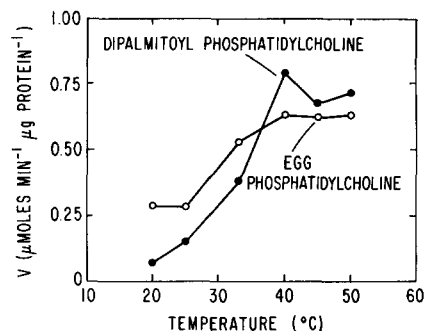


FIG. 9. Temperature dependence of phospholipase  $A_2$  activity towards both egg phosphatidylcholine and dipalmitoyl glycerophosphorylcholine. Standard assay conditions were employed, except for the temperature variation indicated. Averages of duplicate determinations are reported.

substrates was similar at high temperatures, but the activity towards dipalmitoyl glycerophosphorylcholine fell off much more dramatically than that towards egg phosphatidylcholine at low temperature. The slightly lower values for egg phosphatidylcholine at high temperature may be due to the slight lack of linearity with time of the egg phosphatidylcholine compared with the dipalmitoyl glycerophosphorylcholine, which would cause slightly lower initial rates to be measured for the egg phosphatidylcholine as indicated in Materials and Methods.

## DISCUSSION

### Phospholipase $A_2$ activity

Phospholipase  $A_2$  has been traditionally assayed by the hemolysis of red blood cells (13, 20), the decrease in acyl ester bonds using hydroxamic acid (14, 21), turbidometric measurements (22), chromatographic analysis of the products (23), manometric measurements of  $CO_2$  produced from bicarbonate (24), extraction followed by titration of the free fatty acids released (6, 25), or direct titration of the products to a fixed end point using an indicator dye (5). However, in order to obtain more meaningful kinetic information, we have used an automatic recording pH-stat procedure so that the time course of the reaction could be continuously monitored during every kinetic determination and at the same time the pH could be kept constant. The pH-stat technique without the continuously recording feature has been used previously (8, 26), particularly with "soluble" short-chain phospholipids (8, 9). Also, the use of a recording pH meter to measure the initial rate of pH change has recently been described (27). The recording pH-stat procedure described here for use with "insoluble" phospholipids in the presence of Triton X-100 provides many advantages over other

assay techniques, but it does require the maintenance of electrode sensitivity throughout the reaction in the presence of protein, detergent, and phospholipid; this somewhat difficult task has been achieved through the controlled use of Clorox washes as described in Materials and Methods.

Salach et al. (27, 28) purified several forms of *Naja naja* phospholipase A<sub>2</sub> which reacted similarly, and our enzyme displayed the same pH optimum, Ca<sup>2+</sup> dependence, and effect of EDTA as they found. The temperature optimum was not reported by Salach et al. (28), but our finding of a maximum at about 40°C and the other properties mentioned above are generally similar to findings for phospholipase A<sub>2</sub> from other sources (8, 10, 26). However, our studies at 40°C revealed that both egg phosphatidylcholine and dipalmitoyl glycerophosphorylcholine reacted at similar rates. This was in apparent contradiction to the report of Salach et al. (7) that saturated egg phosphatidylcholine reacted at about 1/8 the rate of natural egg phosphatidylcholine (and as low as 1/30 for some forms of the enzyme [28]) and that dipalmitoyl glycerophosphorylcholine reacted 1/2 to 1/8 as fast as egg phosphatidylcholine (28). For this reason the experiment reported in Fig. 9 was conducted, and this revealed that the temperature behavior of phospholipase A<sub>2</sub> was quite different towards each of the two substrates. Presumably, the poorer reactivity towards dipalmitoyl glycerophosphorylcholine at low temperatures reflects the phase change (29, 30) that occurs for this phospholipid between the 25°C assay of the Salach (28) study and the 40°C assay of this study. Our preliminary evidence using nuclear magnetic resonance techniques suggests differential temperature behavior for these two phospholipids even in the presence of Triton X-100.<sup>2</sup> Thus, a determination of the temperature behavior of the enzyme must be viewed with caution until the effect of temperature on the physical state of the phospholipid substrate in the presence of Triton X-100 is understood more precisely.

### Function of Triton X-100

Triton X-100 is a polydisperse preparation of *p-t*-octylphenoxypolyethoxyethanols consisting of oxyethylene chain lengths averaging 9–10 oxyethylene units. However, the general physical properties of this detergent are similar to those of the pure homogeneous compound having a chain length of 9 or 10 units (31). Although we will speak of Triton X-100 as though it were a single chemical species for purposes of our discussion, its polydispersity should be kept in mind.

Triton X-100 in aqueous solution forms micelles consisting of about 100–160 monomers corresponding to a molecular weight of about 63,000–105,000 determined by ultracentrifugation (32) and light-scattering techniques (33–35). The CMC of pure Triton X-100 is about 0.3 mM (36), and although the CMC (and micellar size) may be somewhat different in the presence of Ca<sup>2+</sup> and at the pH and the temperature of our assays (31, 37), the studies reported here are all conducted at concentrations considerably above the CMC. Thus, it is reasonable to assume that practically all of the Triton molecules are in the form of micelles.

Phospholipids such as egg phosphatidylcholine and dipalmitoyl glycerophosphorylcholine in aqueous dispersion in the concentration range of our studies do not exist as either monomers or micelles (of the detergent type), but rather as large aggregates in the form of bilayers. Detergents are generally thought to solubilize phospholipids by forming mixed phospholipid–detergent micelles; mixed micelles of certain ionic detergents, such as sodium cholate, and phosphatidylcholine have been studied somewhat (38). However, little is known about mixed micelles of the nonionic detergent Triton X-100 and phosphatidylcholine. Using nuclear magnetic resonance techniques, we have found that the addition of Triton X-100 micelles to phosphatidylcholine bilayers in the concentration range of the enzymatic studies reported here results in the formation of phospholipid–Triton structures in which the physical state of the phospholipid is dramatically altered;<sup>1</sup> we will refer to these structures as mixed micelles. It appears that these changes are maximum when the molar ratio of Triton/phospholipid is in the range of 2:1, and additional Triton X-100 above this molar ratio appears to cause no further dramatic change in the physical state of the phospholipid. For purposes of discussion at this time, we will assume that the mixed micelles, at least at molar ratios of Triton to phospholipid much greater than 2:1, are generally similar in size and shape to pure Triton micelles. Thus, the addition of pure Triton to a solution of mixed micelles should produce new mixed micelles of a correspondingly greater number and containing a correspondingly higher Triton-to-phospholipid ratio.

In the enzymatic studies reported here, the activity of phospholipase A<sub>2</sub> clearly depends on the concentration of Triton X-100 (Figs. 4 and 8), and maximal enzymatic activity may require a molar ratio in the range of 2:1 Triton/phospholipid (Fig. 6), but in fact, the activity is a complicated function of both phosphatidylcholine and Triton X-100 concentrations (Figs. 4–8). The most straightforward explanation for these results is that the physical state of the phospholipid must be altered by the addition of Triton X-100 in order for the phospho-

<sup>2</sup> Dennis, E. A., and A. A. Ribeiro. Unpublished experiments.

lipid substrate to be accessible to the enzyme. At a molar ratio of 2:1 Triton/phospholipid, most or all of the phospholipid may be in the altered physical state. Thus, maximal enzymatic activity is observed at a phospholipid concentration (and, consequently, a defined Triton X-100 concentration) which saturates the enzyme.

While the apparent activation of the enzyme by Triton X-100 appears to be an effect on the substrate phospholipid, as discussed above, the apparent kinetic inhibition of activity at high concentrations of Triton X-100 (Figs. 7 and 8) may be explained by one of two alternatives. 1) In the first case, it is assumed that in the concentration range of the inhibition experiments, all of the enzyme is bound to mixed phospholipid-Triton micelles even when the molar ratio of Triton to phospholipid is increased far beyond 2:1. However, once bound to a mixed micelle, the enzyme's activity actually depends on the amount of phospholipid available to it in the micelle. Therefore, at a saturating concentration of mixed micelles, further addition of Triton leads to a decrease in enzymatic activity. 2) Alternatively, if the enzyme does not bind to the mixed micelle, but rather only to the phospholipid in the mixed micelle, then additional Triton would cause a decrease in activity if it were to change the characteristics of the mixed micelle so as to place the phospholipid substrate in a less optimal physical arrangement in the mixed micelle. Although binding of the enzyme to the mixed micelle as described in the first alternative is a more straightforward possibility, further work is needed to distinguish between these two alternatives.

Similar kinetics for the apparent inhibition of triglyceride lipase activity in the presence of high concentrations of Triton X-100 have recently been reported by Kaplan and Teng (39), although their results are complicated by effects of aging of the Triton-triglyceride substrate. One possible explanation which they provide for the observed inhibition is that pure Triton X-100 micelles might compete with mixed micelles for the substrate binding site on the enzyme. However, it is unlikely that pure Triton micelles and pure mixed micelles would coexist in solution. Thus, if classical competitive inhibition by Triton X-100 is invoked to explain the observed inhibition, it must be made explicitly clear that it occurs by lowering the concentration of the lipid substrate in the mixed Triton-lipid micelle as stated in 1) above, rather than by competitive inhibition by either Triton monomers or Triton micelles. An alternative explanation offered by Kaplan and Teng (39) is that the fraction of added substrate which is in the form of effective substrate at equilibrium is inversely proportional to the Triton X-100 concentration. This is, in fact, the experimentally observed

result and is consistent with the two alternatives suggested above.

Presumably, the mixed phospholipid-Triton micelles formed on the addition of Triton X-100 micelles to phosphatidylcholine bilayers contain the phospholipid in a physical state similar (in terms of the enzyme) to that formed in micelles of phospholipids containing short-chain fatty acids which can serve as a substrate for phospholipase A<sub>2</sub> in the absence of detergents (8, 9). Thus, the use of this nonionic detergent provides a system whereby the Triton X-100 induces the proper surface for maximal enzymatic activity without the need for particularly synthesized short-chain phospholipids which form the correct micelles. Further work is underway to characterize the mixed phospholipid-Triton micelles more precisely.

Financial support was provided by the National Institutes of Health, grant NS-09326; the National Science Foundation, grant GB-19056; the American Cancer Society Institutional Research Grant IN-93; and a Merck Company Foundation "Faculty Development Award."

We wish to thank Dr. Beatriz M. Braganca for very generously supplying the enzyme used in these studies and Mrs. Karen Smith for technical assistance. Stimulating discussions with Dr. John Elovson were also most helpful.

Manuscript received 24 July 1972; accepted 30 October 1972.

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