Regulation of Phosphatidate Phosphatase Activity from the Yeast *Saccharomyces cerevisiae* by Phospholipids†

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ABSTRACT: Regulation of *Saccharomyces cerevisiae* membrane-associated phosphatidate phosphatase (3-*sn*-phosphatidate phosphohydrolase, EC 3.1.3.4) activity by phospholipids was examined using purified enzyme and Triton X-100/phospholipid-mixed micelles. Anionic phospholipids activated phosphatidate phosphatase activity whereas zwitterionic phospholipids had a slight inhibitory effect on activity. Cardiolipin ($A_{0.5}$ = 1.9 mol %), CDP-diacylglycerol ($A_{0.5}$ = 2.6 mol %), and phosphatidylinositol ($A_{0.5}$ = 5.5 mol %) were the most potent anionic phospholipid activators. Enzyme activation by cardiolipin (n = 2.8), CDP-diacylglycerol (n = 2.1), and phosphatidylinositol (n = 3.3) followed positive cooperative kinetics. A kinetic analysis was performed to determine the mechanism of phosphatidate phosphatase activation by anionic phospholipids. The dependence of phosphatidate phosphatase on phosphatidate was cooperative ($n \sim 2.2$) in the absence and presence of phospholipid activators. Cardiolipin, CDP-diacylglycerol, and phosphatidylinositol were mixed competitive activators of phosphatidate phosphatase activity. The major effect of the activators was to cause a decrease in the K_m for phosphatidate. Sphinganine, a positively charged sphingoid base, inhibited phosphatidate phosphatase activity and antagonized the activation of the enzyme by cardiolipin and phosphatidylinositol. Sphinganine caused an increase in the cooperativity of cardiolipin activation, but had little effect on the $A_{0.5}$ value for cardiolipin. On the other hand, sphinganine had little effect on the cooperativity of phosphatidylinositol activation, but caused an increase in the $A_{0.5}$ value for phosphatidylinositol. The activation constants for cardiolipin, CDP-diacylglycerol, and phosphatidylinositol were within the range of their cellular concentrations. These results suggested that the activation of phosphatidate phosphatase activity by anionic phospholipids may be physiologically relevant.

Phosphatidate (PA)[1] phosphatase (3-*sn*-phosphatidate phosphohydrolase, EC 3.1.3.4) catalyzes the dephosphorylation of PA, yielding DG and P_i (Smith et al., 1957). In the yeast *Saccharomyces cerevisiae*, PA phosphatase catalyzes the committed step in the synthesis of the major membrane phospholipids PE and PC (Carman & Henry, 1989; Carman, 1989; Paltauf et al., 1992) through a DG-dependent pathway (Figure 1). The DG derived from PA is used with CDP-ethanolamine and CDP-choline to form PE and PC, respectively (Kennedy & Weiss, 1956). The utilization of the DG-dependent pathway for PC synthesis is primarily used by wild-type cells grown in the presence of choline (Carman & Henry, 1989; Carman, 1989; Paltauf et al., 1992). This pathway becomes more important for PC synthesis when the enzymes in the CDP-DG-dependent pathway (Figure 1) are repressed (Homann et al., 1985; Klig et al., 1985, 1988; Poole et al., 1986; Bailis et al., 1987; Carson et al., 1982; 1984; Overmeyer & Waechter, 1991; Lamping et al., 1991; Gaynor et al., 1991; Yamashita et al., 1982; Yamashita & Oshima, 1980; Waechter & Lester, 1973) or defective (Atkinson et al., 1980a,b; Trotter & Voelker, 1995; Trotter et al., 1995; Kodaki & Yamashita, 1987, 1989; Summers et al., 1988; McGraw & Henry, 1989). PA phosphatase also catalyzes the committed step in the synthesis of triacylglycerols (Figure 1).

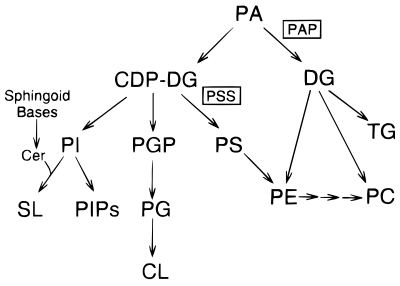

FIGURE 1: Phospholipid biosynthetic pathways in *S. cerevisiae*. The pathways shown include the relevant steps discussed in the text. More compressive pathways which include lipid and water-soluble intermediates of phospholipid metabolism may be found in Carman and Henry (1989) and Paltauf et al. (1992). Abbreviations: PA, phosphatidate; DG, diacylglycerol; TG, triacylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; CDP-DG, CDP-diacylglycerol; PS, phosphatidylserine; PGP, phosphatidylglycerophosphate; PG, phosphatidylglycerol; CL, cardiolipin; PI, phosphatidylinositol; PIPs, polyphosphoinositides; SL, sphingolipids; Cer, ceramide; PAP, PA phosphatase; PSS, PS synthase.

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[1] Abbreviations: PA, phosphatidate; DG, diacylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; CDP-DG, CDP-diacylglycerol; PS, phosphatidylserine; CL, cardiolipin; PI, phosphatidylinositol.

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Two membrane-associated forms (104 and 45 kDa) of PA phosphatase have been purified and characterized from *S. cerevisiae* (Lin & Carman, 1989; Morlock et al., 1991). The biochemical properties of these PA phosphatases are similar except that the 104-kDa enzyme has a higher turnover number (Lin & Carman, 1989, 1990; Morlock et al., 1991). Both PA phosphatase isoforms are regulated similarly by growth phase (Morlock et al., 1991), sphingoid bases (Wu et al., 1993), and nucleotides (Wu & Carman, 1994). On the other hand, the 104- and 45-kDa forms of PA phosphatase are regulated differentially by inositol supplementation (Morlock et al., 1988, 1991) and phosphorylation (Quinlan et al., 1992). Inositol supplementation induces the expression of 45-kDa PA phosphatase but has no effect on the expression of the 104-kDa enzyme (Morlock et al., 1991). cAMP-dependent protein kinase phosphorylates and activates the 45-kDa enzyme but has no effect on 104-kDa PA phosphatase (Quinlan et al., 1992). Since the substrate and product of the PA phosphatase reaction are found at branch points in the pathways leading to the synthesis of phospholipids (DG-dependent and CDP-DG-dependent) and triacylglycerols (Figure 1), it would be expected that the enzyme should play a role in the regulation of these lipids. Indeed, the regulation of PA phosphatase activity by growth phase, inositol, phosphorylation, sphingoid bases, and nucleotides correlates with changes in the synthesis of phospholipids and triacylglycerols (Hosaka & Yamashita, 1984; Taylor & Parks, 1979; Morlock et al., 1988; Quinlan et al., 1992; Wu et al., 1995; Wu & Carman, 1994).

Whereas phospholipids play a role as structural components of membranes, they also function as cofactors and activators of several membrane-associated enzymes (Hjelmstad & Bell, 1991a). Because PA phosphatase plays an important role in the regulation of phospholipid synthesis, we questioned whether phospholipids played a role in the regulation of PA phosphatase activity. We examined the effects of phospholipids on PA phosphatase activity using pure enzyme and Triton X-100/phospholipid-mixed micelles. Anionic phospholipids activated PA phosphatase activity with CL, CDP-DG, and PI being the most potent. Kinetic analyses showed that CL, CDP-DG, and PI were mixed competitive activators of PA phosphatase activity. Sphinganine, a positively charged sphingoid base, inhibited PA phosphatase activity and diminished the activation of activity by anionic phospholipids. The role of lipid effectors on the regulation of PA phosphatase activity is discussed in relation to overall phospholipid metabolism.

EXPERIMENTAL PROCEDURES

Materials. All chemicals were reagent grade. Lipids were purchased from Sigma and Avanti Polar Lipids. Triton X-100 and bovine serum albumin were purchased from Sigma. CDP-DG was synthesized from dioleoyl-PA as previously described (Carman & Fischl, 1992). *Escherichia coli* DG kinase was from Lipidex Inc. [γ-^{32}P]ATP was purchased from Du Pont−New England Nuclear, and scintillation counting supplies were purchased from National Diagnostics.

Purification of PA Phosphatases. The 104- and 45-kDa forms of PA phosphatase were purified to near-homogeneity as previously described (Lin & Carman, 1989; Morlock et al., 1991). The specific activities of the 104- and 45-kDa enzymes were 15 and 2.3 μmol min^{-1} mg^{-1}, respectively.

PA Phosphatase Assay and Protein Determination. PA phosphatase activity was measured by following the release of water-soluble [^{32}P]P_i from chloroform-soluble [^{32}P]PA (20 000 cpm/nmol) at 30 °C (Carman & Lin, 1991). The standard reaction mixture contained 50 mM Tris−maleate buffer (pH 7.0), 10 mM 2-mercaptoethanol, 2 mM $MgCl_2$, 1 mM Triton X-100, 0.1 mM PA, and 6 ng of PA phosphatase in a total volume of 0.1 mL. All assays were conducted in triplicate with an average standard deviation of ±5%. Enzyme assays were linear with time and protein concentration. One unit of PA phosphatase activity was defined as the amount of enzyme that catalyzed the formation of 1 μmol of product per minute. Specific activity was defined as units per milligram of protein. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as the standard.

Preparation of ^{32}P-Labeled PA. [^{32}P]PA was enzymatically synthesized from DG and [γ-^{32}P]ATP using *E. coli* DG kinase (Walsh & Bell, 1986a) as described previously (Lin & Carman, 1989).

Preparation of Triton X-100/Lipid-Mixed Micelles. Lipids in chloroform were transferred to a test tube, and solvent was removed *in vacuo* for 40 min. Triton X-100/lipid-mixed micelles were prepared by adding various amounts of a 5% (w/v) solution of Triton X-100 to the dried lipids. After the addition of Triton X-100, the mixture was vortexed. The surface concentration of lipids in mixed micelles was varied by the addition of Triton X-100. The total lipid concentration in Triton X-100/lipid-mixed micelles did not exceed 15 mol % to ensure that the structure of the mixed micelles were similar to the structure of pure Triton X-100 (Lichtenberg et al., 1983; Robson & Dennis, 1983). The uniformity of the Triton X-100/lipid-mixed micelles was determined by gel filtration chromatography (Bae-Lee & Carman, 1990; Lin & Carman, 1990). The mole percent of a lipid in a mixed micelle was calculated using the formula:

$$\text{mol \%}_{\text{lipid}} = \{[\text{lipid(bulk)}]/([\text{lipid(bulk)}] + [\text{Triton X-100}])\} \times 100$$

Analysis of Kinetic Data. Kinetic data were analyzed according to the Michaelis−Menten and Hill equations using the EZ-FIT Enzyme Kinetic Model Fitting Program (Perrella, 1988).

RESULTS

Effect of Phospholipids on PA Phosphatase Activity. The effect of phospholipids on PA phosphatase activity was examined using Triton X-100/phospholipid-mixed micelles. The nonionic detergent Triton X-100 is required to elicit a maximum turnover for PA phosphatase activity *in vitro* (Lin & Carman, 1989, 1990). The function of Triton X-100 in the assay system for PA phosphatase is to form a uniform mixed micelle with the substrate PA (Lin & Carman, 1990). The Triton X-100 micelle serves as a catalytically inert matrix in which PA is dispersed, preventing high local concentration of PA at the active site (Lin & Carman, 1990). In addition, this micelle system permitted the analysis of PA phosphatase activity in an environment which mimicked the physiological surface of the membrane (Dennis, 1983). In Triton X-100/phospholipid-mixed micelles, PA phosphatase activity follows surface dilution kinetics (Carman et al., 1995) where activity is dependent on both the bulk and surface

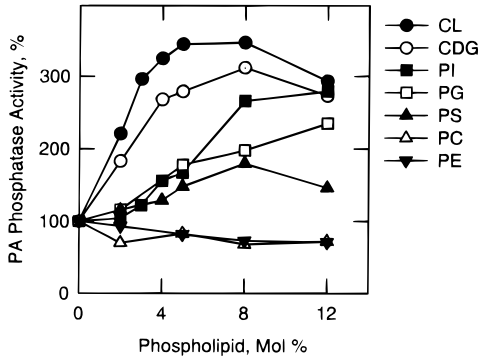

FIGURE 2: Effect of phospholipids on PA phosphatase activity. 104-kDa PA phosphatase activity was measured under standard assay condition with 3 mol % PA (bulk concentration of 0.1 mM) in the presence of the indicated surface concentrations of phospholipids. Abbreviations: CL, cardiolipin; CDG, CDP-diacylglycerol; PI, phosphatidylinositol; PG, phosphatidylglycerol; PS, phosphatidylserine; PC, phosphatidylcholine; and PE, phosphatidylethanolamine.

concentrations of PA (Lin & Carman, 1990). In these experiments, PA phosphatase activity was measured such that activity was only dependent on the surface concentration of PA (Lin & Carman, 1990). The concentrations of PA and other phospholipids were expressed as a surface concentration (in mol %) as opposed to bulk concentration since phospholipids form uniform mixed-micelles with Triton X-100 (Lichtenberg et al., 1983; Robson & Dennis, 1983).

104- and 45-kDa PA phosphatase activities were assayed in the presence of phospholipids found in *S. cerevisiae* (Paltauf et al., 1992). A surface concentration of PA (3 mol %) was used near its K_m value (Lin & Carman, 1990; Morlock et al., 1991) so we could simultaneously observe stimulatory or inhibitory effects of phospholipids on each activity. Figure 2 shows the effects of phospholipids on the 104-kDa PA phosphatase activity. The enzyme was activated by anionic phospholipids (CL, CDP-DG, PI, PG, and PS) and inhibited by zwitterionic phospholipids (PC and PE) in a dose-dependent manner. The most potent phospholipid activator was CL. Activation by CL followed positive cooperative kinetics ($n = 2.8$), and maximum activation (350%) occurred at 5 mol % (Figure 2). The concentration of CL which resulted in half-maximum activation ($A_{0.5}$) was 1.9 mol %. PA phosphatase activity was also stimulated in a cooperative manner by CDP-DG ($n = 2.1$) and PI ($n = 3.3$) (Figure 2). Maximum stimulation by CDP-DG (300%) and PI (280%) was obtained at 8 mol % and 12 mol %, respectively. The $A_{0.5}$ values for CDP-DG and PI were 2.6 mol % and 5.5 mol %, respectively. PA phosphatase activity was measured in the presence of various combinations of CL, CDP-DG, and PI at a concentration of 2 mol % for each phospholipid. The results of these experiments indicated that the phospholipid activators were not synergistic. Activation of PA phosphatase activity by PG and PS was relatively low and required relatively high concentrations of these phospholipids. Thus, PG and PS were not considered to be strong activators and were not pursued further. Likewise, the inhibition of activity by PC and PE was relatively small (35% or less), and these phospholipids were not considered to be strong inhibitors. The 45-kDa form of PA phosphatase was affected by phospholipids in a manner similar to that of the 104-kDa form of the enzyme (data not shown). Because the 104- and 45-kDa forms of PA phosphatase share similar

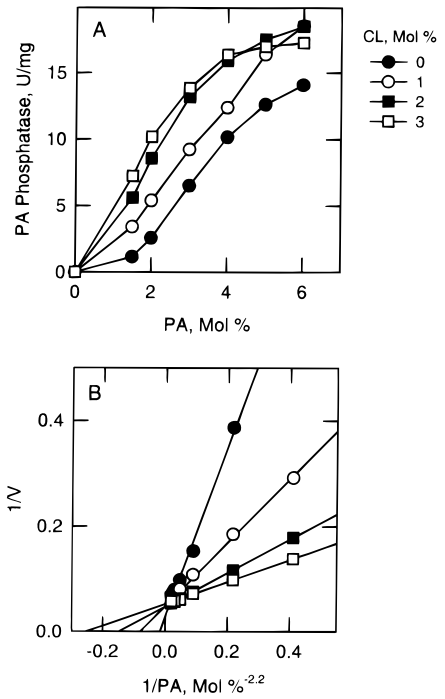

FIGURE 3: Effect of CL on the kinetics of PA phosphatase activity. Panel A: 104-kDa PA phosphatase activity was measured as a function of the surface concentration (mol %) of PA (bulk concentration of 0.1 mM) at the indicated set surface concentrations (mol %) of CL. Panel B: reciprocal plot of the data in panel A where the PA concentration was raised to the Hill number of 2.2. The lines drawn in panel B were a result of a least-squares analysis of the data.

enzymological properties (Morlock et al., 1991), the 45-kDa enzyme was not examined further.

Effect of Phospholipid Activators on the Kinetics of PA Phosphatase Activity. A detailed kinetic analysis was performed on PA phosphatase to explore the mechanism of phospholipid activation on enzyme activity. The dependence of PA phosphatase activity on PA was examined in the absence and presence of various set concentrations of CL (Figure 3A), CDP-DG (Figure 4A), and PI (Figure 5A). As previously described (Wu et al., 1993; Wu & Carman, 1994), PA phosphatase activity exhibited positive cooperative kinetics ($n = 1.9$) with respect to the surface concentration of PA. PA phosphatase activity was stimulated by CL, CDP-DG, and PI in a dose-dependent manner at each PA concentration. Moreover, the kinetics of activity with respect to PA followed cooperative kinetics where the Hill number at each concentration of the respective phospholipid activators did not vary significantly from a value of 2. The data for CL (Figure 3B), CDP-DG (Figure 4B), and PI (Figure 5B) were transformed to double-reciprocal plots where the PA concentration was raised to the average Hill number of 2.2 (Segel, 1975). These analyses resulted in a family of lines which intersected just to the right of the $1/V$ intercept (Figures 3B, 4B, and 5B). These patterns of lines were indicative of CL, CDP-DG, and PI being mixed competitive activators of PA phosphatase activity. The major effect of the activators was to cause a decrease in the apparent K_m value for PA. This indicated that the mechanism of activation involved an increase in the enzyme's affinity for PA. The activators also had a small effect on the apparent V_{max} value.

The mechanism of PA phosphatase activation by CL, CDP-DG, and PI was complex. Various replots of the data

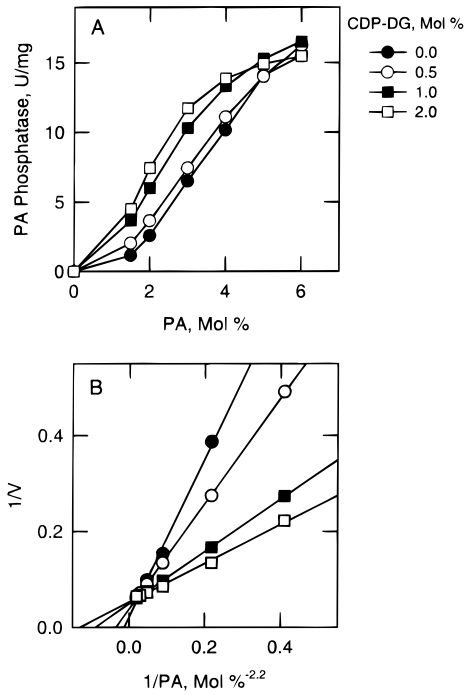

FIGURE 4: Effect of CDP-DG on the kinetics of PA phosphatase activity. Panel A: 104-kDa PA phosphatase activity was measured as a function of the surface concentration (mol %) of PA (bulk concentration of 0.1 mM) at the indicated set surface concentrations (mol %) of CDP-DG . Panel B: reciprocal plot of the data in panel A where the PA concentration was raised to the Hill number of 2.2. The lines drawn in panel B were a result of a least-squares analysis of the data.

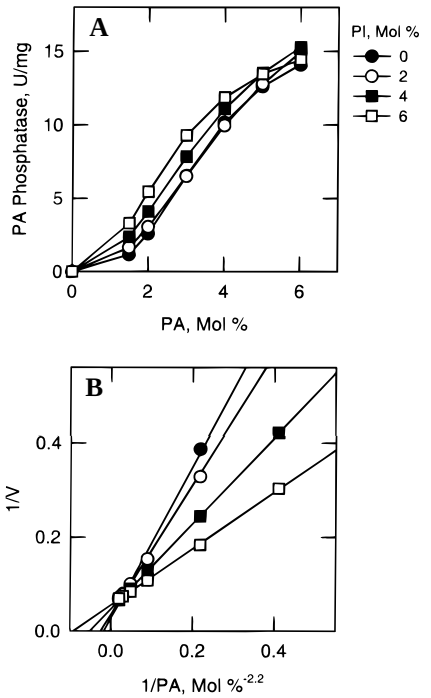

FIGURE 5: Effect of PI on the kinetics of PA phosphatase activity. Panel A: 104-kDa PA phosphatase activity was measured as a function of the surface concentration (mol %) of PA (bulk concentration of 0.1 mM) at the indicated set surface concentrations (mol %) of PI . Panel B: reciprocal plot of the data in panel A where the PA concentration was raised to the Hill number of 2.2. The lines drawn in panel B were a result of a least-squares analysis of the data.

from Figures 3, 4, and 5 according to equations described by Segel (1975) for enzyme activators were nonlinear. The

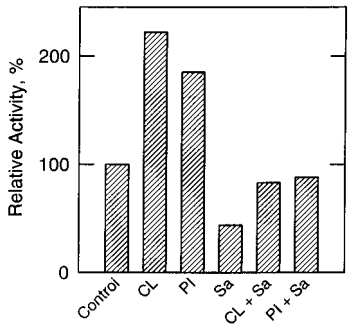

FIGURE 6: Effect of sphinganine on the activation of PA phosphatase activity by phospholipids. 104-kDa PA phosphatase activity was measured with 3 mol % PA in the absence and presence of CL (2 mol %), PI (6 mol %), and sphinganine (4 mol %) as indicated. Abbreviations: CL, cardiolipin; PI, phosphatidylinositol; Sa, sphinganine.

cooperative dependence of PA phosphatase activity on PA and the cooperative dependence of activation by phospholipid activators contributed to this complexity. This precluded the determination of dissociation constants for these phospholipids. We determined, however, $A_{0.5}$ values for CL (1.9 mol %), CDP-DG (2.6 mol %), and PI (5.5 mol %) from an analysis of the data from Figure 2 according to the Hill equation. These values were useful for comparing the potency of the phospholipid activators.

Effect of Sphinganine on the Activation of PA Phosphatase Activity by Phospholipid Activators. Sphingoid bases such as sphingosine, sphinganine, and phytosphingosine are parabolic competitive inhibitors of PA phosphatase (Wu et al., 1993). Sphinganine and phytosphingosine are precursors of inositol-containing sphingolipids in *S. cerevisiae* (Wells & Lester, 1983; Pinto et al., 1992). Since sphingoid bases are positively charged lipids and the phospholipid activators were negatively charged lipids, we questioned what effect a combination of these lipids would have on PA phosphatase activity. For these studies, we examined two phospholipid activators (CL and PI) and one sphingoid base (sphinganine). As described above, the addition of 2 mol % CL and 6 mol % PI stimulated PA phosphatase activity 222% and 190%, respectively (Figure 6). As previously described (Wu et al., 1993), 4 mol % sphinganine inhibited 60% of PA phosphatase activity (Figure 6). When 4 mol % sphinganine was added to the assay system with either 2 mol % CL or 6 mol % PI, about 85% of the control PA phosphatase activity was obtained (Figure 6). In other words, sphinganine prevented the activation of activity by CL and PI. Thus, these lipid effectors were antagonistic.

We examined the mechanism of sphinganine's antagonism of PA phosphatase activation by CL and PI. The dependence of PA phosphatase activation on CL (Figure 7) and PI (Figure 8) was measured in the absence and presence of 4 mol % sphinganine. CL and PI activated PA phosphatase activity in the presence of sphinganine. However, the activation of activity was diminished by sphinganine at each CL (Figure 7) and PI (Figure 8) concentration. Sphinganine caused an increase in the cooperativity of activation by CL and a small change in the $A_{0.5}$ value for CL. The Hill numbers for CL in the absence and presence of sphinganine were 2.8 and 6, respectively, and the $A_{0.5}$ values for CL in the absence and presence of sphinganine were 1.9 and 2.3 mol %, respectively. Sphinganine did not significantly affect the Hill number for PI but did cause an increase in the $A_{0.5}$ value for

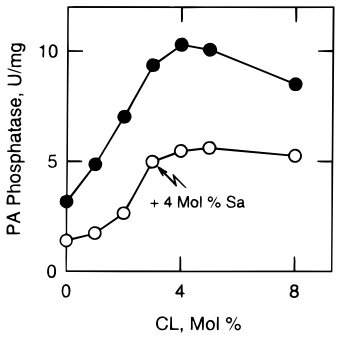

FIGURE 7: Effect of sphinganine on the kinetics of PA phosphatase activation by CL. 104-kDa PA phosphatase activity was measured with 3 mol % PA as a function of the surface concentration (mol %) of CL in the absence and presence of 4 mol % sphinganine. Abbreviations: CL, cardiolipin; Sa, sphinganine.

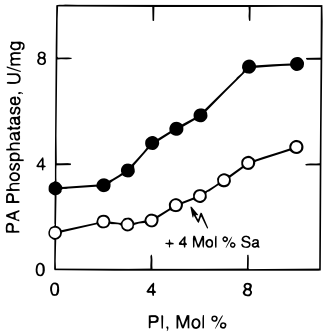

FIGURE 8: Effect of sphinganine on the kinetics of PA phosphatase activation by PI. 104-kDa PA phosphatase activity was measured with 3 mol % PA as a function of the surface concentration (mol %) of PI in the absence and presence of 4 mol % sphinganine. Abbreviations: PI, phosphatidylinositol; Sa, sphinganine.

PI. The $A_{0.5}$ values for PI in the absence and presence of sphinganine were 5.5 and 8.3 mol %, respectively.

DISCUSSION

In this report, we carried out systematic kinetic experiments using well-defined Triton X-100/phospholipid-mixed micelles and showed that PA phosphatase activity was regulated by anionic phospholipids. Although all of the anionic phospholipids examined in this study activated PA phosphatase activity, there was a specificity for activation. CL, CDP-DG, and PI were the most potent phospholipid activators whereas PG and PS were much less potent. CL, CDP-DG, and PI activated PA phosphatase activity by increasing the enzyme's affinity for its substrate PA. In a previous study (Lin & Carman, 1989), we showed that PA phosphatase activity was inhibited by CDP-DG. This discrepancy can be explained by the fact that only one concentration of CDP-DG was tested and the conditions for enzyme assay did not take into account the kinetics of PA phosphatase in Triton X-100/phospholipid-mixed micelles (Lin & Carman, 1990).

Activation of PA phosphatase activity by CL, CDP-DG, and PI followed cooperative kinetics. PA phosphatase activity also followed cooperative kinetics with respect to PA when measured in the absence and presence of the phospholipid activators. The cooperative behavior observed in the kinetic experiments could have many causes. Mosior and McLauglin (1992) have analyzed the quantitative effects of surface charge on the cooperative binding of peptides and proteins to membrane bilayers. They conclude that cooperativity is induced by the additional electrostatic term in the binding energy as well as the reduced dimensionality. The surface charge density of the Triton X-100/lipid-mixed micelle surface could be responsible for the cooperativity observed here for PA phosphatase.

Table 1: Activation Constants and Cellular Concentrations of Phospholipids

activator	$A_{0.5}$	cellular concn[a] (mol %)
CL	1.9	3.1
CDP-DG	2.6	4.2
PI	5.5	12

[a] Data taken from Paltauf et al. (1992) for exponential phase wild-type cells grown in glucose-containing growth medium.

Based on $A_{0.5}$ values, CL and CDP-DG were more potent activators of PA phosphatase activity when compared with PI (Table 1). Thus, phospholipids with two negative charges were better activators when compared with phospholipids with one negative charge. In other words, enzyme activation was dependent on the surface charge density of the micelle. Importantly, the $A_{0.5}$ values for CL, CDP-DG, and PI were within the range of their cellular concentrations (Paltauf et al., 1992) (Table 1). Thus, the regulation of PA phosphatase activity by these phospholipid activators may be physiologically relevant.

The activation of PA phosphatase activity by CL and PI was antagonized by sphinganine, a positively charged sphingoid base. Sphinganine and other sphingoid bases are parabolic competitive inhibitors of PA phosphatase activity (Wu et al., 1993). The mechanism of the antagonistic effect that sphinganine had on the activation by CL and PI differed. Sphinganine caused an increase in the cooperativity of activation by CL, but had little effect on the $A_{0.5}$ value for CL. On the other hand, sphinganine had little effect on the cooperativity of activation by PI, but caused an increase in the $A_{0.5}$ value for PI. These differences may be attributed to the charge differences between CL and PI. The fact that anionic phospholipids activated PA phosphatase activity while cationic lipids inhibit activity (Wu et al., 1993) showed that the overall charge of the micelle surface contributed to the regulation of the enzyme.

Phospholipids regulate the activities of several enzymes of lipid metabolism (Hjelmstad & Bell, 1991a). These enzymes include phospholipid biosynthetic enzymes (Bae-Lee & Carman, 1990; Hjelmstad & Bell, 1991b; Nickels et al., 1994; Moritz et al., 1992), enzymes of neutral lipid metabolism (Bhat et al., 1994; Walsh & Bell, 1986a,b), lipid hydrolyzing enzymes (Hendrickson & Dennis, 1984; Jones & Carpenter, 1993), and enzymes involved in lipid signaling pathways (Bell & Burns, 1991). Most relevant to this study is the regulation of *S. cerevisiae* PS synthase activity by PA and CL (Bae-Lee & Carman, 1990). PS synthase is a highly regulated enzyme (Carman & Henry, 1989; Paltauf et al., 1992) that catalyzes the committed step in PE and PC synthesis via the CDP-DG-dependent pathway (Figure 1). Interestingly, PS synthase activity is activated by PA (Bae-Lee & Carman, 1990) (substrate for the PA phosphatase reaction) whereas PA phosphatase activity was activated by CDP-DG (substrate for the PS synthase reaction). Thus, the phospholipid substrate for the enzyme which catalyzes the committed step for the synthesis of PE and PC by one

pathway activates the enzyme which catalyzes the committed step for the synthesis of these phospholipids by the other pathway. CL also plays a role in the regulation of PA phosphatase and PS synthase activities. CL activated PA phosphatase activity whereas CL inhibits PS synthase activity (Bae-Lee & Carman, 1990). Thus, the enzymes that catalyze the committed steps in the DG- and CDP-DG-dependent pathways for PE and PC synthesis are regulated differentially by phospholipids.

Phospholipid content in *S. cerevisiae* can vary dramatically depending on culture conditions (Becker & Lester, 1977; Paltauf et al., 1992). Yet, the average charge of membrane phospholipids remains relatively constant (Becker & Lester, 1977; Paltauf et al., 1992). Cells make up for changes in phospholipids of one charge by causing parallel changes in phospholipids of another charge (Becker & Lester, 1977; Paltauf et al., 1992). The regulation of PA phosphatase and PS synthase activities by phospholipids may be one of several mechanisms by which *S. cerevisiae* maintains the charge of its membrane. For example, the activation of PA phosphatase by CL may increase PE, PC, and triacylglycerol content (neutral charge) while the inhibition of PS synthase by CL may decrease PS content (negative charge). At the same time, the inhibition of PS synthase would impact on the synthesis of PI and its derivatives (Kelley et al., 1988). Several phospholipid biosynthetic enzymes are regulated at the transcriptional level by inositol and other water-soluble phospholipid precursors (Carman & Henry, 1989; Paltauf et al., 1992) and at the biochemical level by phospholipids (Bae-Lee & Carman, 1990; Hjelmstad & Bell, 1991b; Nickels et al., 1994; Moritz et al., 1992), nucleotides (Buxeda et al., 1993; Wu & Carman, 1994), inositol (Kelley et al., 1988), and phosphorylation (Kinney & Carman, 1988; Quinlan et al., 1992). Thus, the regulation of phospholipid synthesis in *S. cerevisiae* is very complex, and all of these control mechanisms must be interrelated.

REFERENCES

Atkinson, K. D., Fogel, S., & Henry, S. A. (1980a) *J. Biol. Chem. 255*, 6653−6661.

Atkinson, K. D., Jensen, B., Kolat, A. I., Storm, E. M., Henry, S. A., & Fogel, S. (1980b) *J. Bacteriol. 141*, 558−564.

Bae-Lee, M., & Carman, G. M. (1990) *J. Biol. Chem. 265*, 7221−7226.

Bailis, A. M., Poole, M. A., Carman, G. M., & Henry, S. A. (1987) *Mol. Cell. Biol. 7*, 167−176.

Becker, G. W., & Lester, R. L. (1977) *J. Biol. Chem. 252*, 8684−8691.

Bell, R. M., & Burns, D. J. (1991) *J. Biol. Chem. 266*, 4661−4664.

Bhat, B. G., Wang, P., & Coleman, R. A. (1994) *J. Biol. Chem. 269*, 13172−13178.

Bradford, M. M. (1976) *Anal. Biochem. 72*, 248−254.

Buxeda, R. J., Nickels, J. T., Jr., & Carman, G. M. (1993) *J. Biol. Chem. 268*, 6248−6255.

Carman, G. M. (1989) in *Phosphatidylcholine metabolism* (Vance, D. E., Ed.) pp 165−183, CRC Press, Inc., Baca Raton, FL.

Carman, G. M., & Henry, S. A. (1989) *Annu. Rev. Biochem. 58*, 635−669.

Carman, G. M., & Lin, Y.-P. (1991) *Methods Enzymol. 197*, 548−553.

Carman, G. M., & Fischl, A. S. (1992) *Methods Enzymol. 209*, 305−312.

Carson, M. A., Atkinson, K. D., & Waechter, C. J. (1982) *J. Biol. Chem. 257*, 8115−8121.

Carman, G. M., Deems, R. A., & Dennis, E. A. (1995) *J. Biol. Chem. 270*, 18711−18714.

Carson, M. A., Emala, M., Hogsten, P., & Waechter, C. J. (1984) *J. Biol. Chem. 259*, 6267−6273.

Dennis, E. A. (1983) in *The enzymes* (Boyer, P. D., Ed.) pp 307−353, Academic Press, New York.

Gaynor, P. M., Gill, T., Toutenhoofd, S., Summers, E. F., McGraw, P., Homman, M. J., Henry, S. A., & Carman, G. M. (1991) *Biochim. Biophys. Acta 1090*, 326−332.

Hendrickson, H. S., & Dennis, E. A. (1984) *J. Biol. Chem. 259*, 5740−5744.

Hjelmstad, R. H., & Bell, R. M. (1991a) *Biochemistry 30*, 1731−1740.

Hjelmstad, R. H., & Bell, R. M. (1991b) *J. Biol. Chem. 266*, 4357−4365.

Homann, M. J., Henry, S. A., & Carman, G. M. (1985) *J. Bacteriol. 163*, 1265−1266.

Hosaka, K., & Yamashita, S. (1984) *Biochim. Biophys. Acta 796*, 110−117.

Jones, G. A., & Carpenter, G. (1993) *J. Biol. Chem. 268*, 20845−20850.

Kelley, M. J., Bailis, A. M., Henry, S. A., & Carman, G. M. (1988) *J. Biol. Chem. 263*, 18078−18085.

Kennedy, E. P., & Weiss, S. B. (1956) *J. Biol. Chem. 222*, 193−214.

Kinney, A. J., & Carman, G. M. (1988) *Proc. Nat. Acad. Sci. U.S.A. 85*, 7962−7966.

Klig, L. S., Homann, M. J., Carman, G. M., & Henry, S. A. (1985) *J. Bacteriol. 162*, 1135−1141.

Klig, L. S., Homann, M. J., Kohlwein, S., Kelley, M. J., Henry, S. A., & Carman, G. M. (1988) *J. Bacteriol. 170*, 1878−1886.

Kodaki, T., & Yamashita, S. (1987) *J. Biol. Chem. 262*, 15428−15435.

Kodaki, T., & Yamashita, S. (1989) *Eur. J. Biochem. 185*, 243−251.

Lamping, E., Kohlwein, S. D., Henry, S. A., & Paltauf, F. (1991) *J. Bacteriol. 173*, 6432−6437.

Lichtenberg, D., Robson, R. J., & Dennis, E. A. (1983) *Biochim. Biophys. Acta 737*, 285−304.

Lin, Y.-P., & Carman, G. M. (1989) *J. Biol. Chem. 264*, 8641−8645.

Lin, Y.-P., & Carman, G. M. (1990) *J. Biol. Chem. 265*, 166−170.

McGraw, P., & Henry, S. A. (1989) *Genetics 122*, 317−330.

Moritz, A., DeGraan, P. N. E., Gispen, W. H., & Wirtz, K. W. A. (1992) *J. Biol. Chem. 267*, 7207−7210.

Morlock, K. R., Lin, Y.-P., & Carman, G. M. (1988) *J. Bacteriol. 170*, 3561−3566.

Morlock, K. R., McLaughlin, J. J., Lin, Y.-P., & Carman, G. M. (1991) *J. Biol. Chem. 266*, 3586−3593.

Mosior, M., & McLauglin, S. (1992) *Biochim. Biophys. Acta 1105*, 185−187.

Nickels, J. T., Jr., Buxeda, R. J., & Carman, G. M. (1994) *J. Biol. Chem. 269*, 11018−11024.

Overmeyer, J. H., & Waechter, C. J. (1991) *Arch. Biochem. Biophys. 290*, 511−516.

Paltauf, F., Kohlwein, S. D., & Henry, S. A. (1992) in *The molecular and cellular biology of the yeast Saccharomyces: Gene expression* (Jones, E. W., Pringle, J. R., & Broach, J. R., Eds.) pp 415−500, Cold Spring Harbor Laboratory, Cold Spring Harbor.

Perrella, F. (1988) *Anal. Biochem. 174*, 437−447.

Pinto, W. J., Wells, G. W., & Lester, R. L. (1992) *J. Bacteriol. 174*, 2575−2581.

Poole, M. A., Homann, M. J., Bae-Lee, M., & Carman, G. M. (1986) *J. Bacteriol. 168*, 668−672.

Quinlan, J. J., Nickels, J. T., Jr., Wu, W., Lin, Y.-P., Broach, J. R., & Carman, G. M. (1992) *J. Biol. Chem. 267*, 18013−18020.

Robson, R. J., & Dennis, E. A. (1983) *Acc. Chem. Res. 16*, 251−258.

Segel, I. H. (1975) *Enzyme kinetics. Behavior and analysis of rapid equilibrium and steady-state enzyme systems*, John Wiley and Sons, New York.

Smith, S. W., Weiss, S. B., & Kennedy, E. P. (1957) *J. Biol. Chem. 228*, 915−922.

Summers, E. F., Letts, V. A., McGraw, P., & Henry, S. A. (1988) *Genetics 120*, 909−922.

Taylor, F. R., & Parks, L. W. (1979) *Biochim. Biophys. Acta 575*, 204−214.
Trotter, P. J., & Voelker, D. R. (1995) *J. Biol. Chem. 270*, 6062−6070.
Trotter, P. J., Pedretti, J., Yates, R., & Voelker, D. R. (1995) *J. Biol. Chem. 270*, 6071−6080.
Waechter, C. J., & Lester, R. L. (1973) *Arch. Biochem. Biophys. 158*, 401−410.
Walsh, J. P., & Bell, R. M. (1986a) *J. Biol. Chem. 261*, 6239−6247.
Walsh, J. P., & Bell, R. M. (1986b) *J. Biol. Chem. 261*, 15062−15069.
Wells, G. B., & Lester, R. L. (1983) *J. Biol. Chem. 258*, 10200−10203.
Wu, W.-I., & Carman, G. M. (1994) *J. Biol. Chem. 269*, 29495−29501.
Wu, W., Lin, Y.-P., Wang, E., Merrill, A. H., Jr., & Carman, G. M. (1993) *J. Biol. Chem. 268*, 13830−13837.
Wu, W.-I., McDonough, V. M., Nickels, J. T., Jr., Ko, J., Fischl, A. S., Vales, T. R., Merrill, A. H., Jr., & Carman, G. M. (1995) *J. Biol. Chem. 270*, 13171−13178.
Yamashita, S., & Oshima, A. (1980) *Eur. J. Biochem. 104*, 611−616.
Yamashita, S., Oshima, A., Nikawa, J., & Hosaka, K. (1982) *Eur. J. Biochem. 128*, 589−595.

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