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LIPID PHOSPHATE PHOSPHATASES FROM *SACCHAROMYCES CEREVISIAE*

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Abstract

DPP1-encoded and *LPP1*-encoded lipid phosphate phosphatases are integral membrane proteins in the yeast *Saccharomyces cerevisiae*. They catalyze the Mg^{2+} -independent dephosphorylation of bioactive lipid phosphate molecules such as diacylglycerol pyrophosphate and phosphatidate. These enzymes possess a three-domain lipid phosphatase motif that is localized to the hydrophilic surface of the membrane. The lipid phosphate phosphatase activities of *DPP1*-encoded and *LPP1*-encoded enzymes are measured by following the release of water-soluble radioactive inorganic phosphate from chloroform-soluble radioactive lipid phosphate substrate following a chloroform/methanol/water phase partition. The *DPP1*-encoded enzyme, commonly referred to as diacylglycerol pyrophosphate phosphatase, is purified from wild-type *S. cerevisiae* membranes by detergent solubilization with Triton X-100 followed by chromatography with DEAE-cellulose (DE₅₃), Affi-Gel blue, hydroxylapatite, and Mono Q. The purification scheme yields an essentially homogeneous enzyme preparation that is stable for several years upon storage at -80° . The properties of the *DPP1*-encoded and *LPP1*-encoded lipid phosphate phosphatase enzymes are summarized.

1. INTRODUCTION

Lipid phosphate phosphatase in the yeast *Saccharomyces cerevisiae* catalyzes the Mg^{2+} -independent dephosphorylation of bioactive lipid phosphate molecules such as diacylglycerol pyrophosphate (DGPP) and phosphatidate (PA) (Furneisen and Carman, 2000; Toke *et al.*, 1998, 1999a; Wu *et al.*, 1996) (reactions 1 and 2):

1. Diacylglycerol pyrophosphate $\rightarrow$ Phosphatidate + P_i
2. Phosphatidate $\rightarrow$ Diacylglycerol + P_i

Essentially all of the lipid phosphate phosphatase activities in *S. cerevisiae* are encoded by the *DPP1* (Toke *et al.*, 1998) and *LPP1* (Toke *et al.*, 1999a) genes, with the former gene being the major contributor of this enzyme (Toke *et al.*, 1999a). The *DPP1*- and *LPP1*-encoded enzymes are integral membrane proteins with six transmembrane-spanning regions; they are localized to the vacuole (Han *et al.*, 2001, 2004) and Golgi (Huh *et al.*, 2003) compartments of the cell, respectively. The lipid phosphate phosphatase enzymes possess a three-domain lipid phosphatase motif that is localized to the hydrophilic surface of the membrane (Han *et al.*, 2004; Stuke and Carman, 1997; Toke *et al.*, 1998, 1999a,b). This catalytic motif consists of the consensus sequences KxxxxxxRP (domain 1)—PSGH (domain 2)—SRxxxxxHxxxD (domain 3). The conserved arginine residue in domain 1 and the conserved histidine residues in domains 2 and 3 are essential for catalytic activity (Stuke and Carman, 1997; Toke *et al.*, 1999b; Zhang *et al.*, 2000).

The substrate specificity of the lipid phosphate phosphatases suggests that these enzymes are involved in signaling events rather than in phospholipid synthesis (Brindley *et al.*, 2002; Sciorra and Morris, 2002). The lipid phosphate phosphatase enzymes may play a role in signal transduction by terminating signaling events of lipid phosphates. Because the products of the

lipid phosphate phosphatases are also bioactive lipid molecules, they can initiate signal transduction by producing signaling molecules. Thus, the regulation of lipid phosphate phosphatase activities is likely to modulate the balance of the signaling molecules that are substrates and products in their reactions. The *DPP1*- and *LPPI*-encoded PA phosphatase activities are not responsible for *de novo* lipid synthesis; the *PAH1*-encoded Mg^{2+} -dependent PA phosphatase is the responsible enzyme for this function in *S. cerevisiae* (Carman and Han, 2006; Han *et al.*, 2006).

The *DPP1*-encoded enzyme (Toke *et al.*, 1998), commonly referred to as DGPP phosphatase, has been purified to homogeneity by standard protein purification procedures (Wu *et al.*, 1996). We describe here the purification of the enzyme.

2. PREPARATION OF RADIOLABELED SUBSTRATES

$[\beta\text{-}^{32}\text{P}]\text{DGPP}$ is synthesized enzymatically from PA and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ using *Catharanthus roseus* PA kinase as described by Wu *et al.* (1996). $[\text{}^{32}\text{P}]\text{PA}$ is synthesized enzymatically from diacylglycerol using *Escherichia coli* diacylglycerol kinase (Walsh and Bell, 1986) as described by Lin and Carman (1989). The labeled substrates are purified by thin-layer chromatography on potassium oxalate-treated silica gel 60 plates using the solvent system chloroform/acetone/methanol/glacial acetic acid/water (50:15:13:12:4) (Wissing and Behrbohm, 1993).

3. ASSAY METHODS

Lipid phosphate phosphatase activities are measured for 20 min at 30° by following the release of water-soluble $[\text{}^{32}\text{P}]\text{P}_i$ from chloroform-soluble $[\gamma\text{-}^{32}\text{P}]\text{DGPP}$ (10,000 cpm/nmol) or $[\text{}^{32}\text{P}]\text{PA}$ (10,000 cpm/nmol) (Wu *et al.*, 1996). The reaction mixture for DGPP phosphatase activity contains 50 mM citrate buffer (pH 5.0), 0.1 mM DGPP, 2 mM Triton X-100, 10 mM 2-mercaptoethanol, and enzyme protein in a total volume of 0.1 ml. The reaction mixture for PA phosphatase activity contains 50 mM Tris-maleate buffer (pH 6.5), 0.1 mM PA, 1 mM Triton X-100, 2 mM Na_2EDTA , 10 mM 2-mercaptoethanol, and enzyme in a total volume of 0.1 ml. The reactions are terminated by the addition of 0.5 ml of 0.1 N HCl in methanol. Chloroform (1 ml) and 1 M MgCl_2 (1 ml) are added, the system is mixed, and the phases are separated by 2 min of centrifugation at 100g. Ecoscint H (4 ml) is added to a 0.5-ml sample of the aqueous phase, and radioactivity is determined by scintillation counting. All enzyme assays are conducted in triplicate. A unit of enzymatic activity is defined as the amount of enzyme that catalyzes the formation of 1 μmol of product/min. Specific activity is defined as units per milligram of protein. Protein concentration is determined by the method of Bradford (1976) using bovine serum albumin as the standard.

4. GROWTH OF YEAST

Strain *MATa ade5* (Culbertson and Henry, 1975), which shows normal regulation of phospholipid metabolism (Greenberg *et al.*, 1982; Klig *et al.*, 1985, 1988; Poole *et al.*, 1986), is used for purification of the *DPP1*-encoded DGPP phosphatase. Cultures are maintained on YEPD medium (1% yeast extract, 2% peptone, 2% glucose) plates containing 2% Bactoagar. For enzyme purification, cells are grown in YEPD medium at 30° to late exponential phase, harvested by centrifugation, and stored at -80° as described previously (Fischl and Carman, 1983).

5. PURIFICATION PROCEDURE

All steps are performed at 5°.

5.1. Preparation of cell extract

The cell extract is prepared from 200 g (wet weight) of cells by disruption with glass beads with a Bead-Beater (Biospec Products) in buffer A (50 mM Tris-maleate [pH 7.0], 1 mM Na₂EDTA, 0.3 M sucrose, 10 mM 2-mercaptoethanol, and 0.5 mM phenylmethanesulfonyl fluoride) (Fischl and Carman, 1983). Unbroken cells and glass beads are removed by centrifugation at 1500g for 5 min.

5.2. Preparation of microsomal membranes

Microsomal membranes are isolated from the cell extract by differential centrifugation (Fischl and Carman, 1983). They are washed, resuspended in buffer B (50 mM Tris-maleate [pH 7.0], 10 mM MgCl₂, 10mM 2-mercaptoethanol, 20% glycerol, and 0.5 mM phenylmethanesulfonyl fluoride), and frozen at -80° until used for purification.

5.3. Preparation of Triton X-100 extract

Microsomal membranes are suspended in buffer B containing 1% Triton X-100 at a final protein concentration of 10 mg/ml. The suspension is incubated for 1 h on a rotary shaker at 150 rpm. After the incubation, the suspension is centrifuged at 100,000g for 1.5 h to obtain the Triton X-100 extract (supernatant).

5.4. DE₅₃ (DEAE53-cellulose) chromatography

A DE₅₃ column (2.5 × 20.5 cm) is equilibrated with buffer C (50 mM Tris-maleate [pH 7.0], 10 mM MgCl₂, 10mM 2-mercaptoethanol, 20% glycerol, and 1% Triton X-100). The Triton X-100 extract is applied to the column at a flow rate of 60 ml/h. The column is washed with 1 column volume of buffer C followed by elution of DGPP phosphatase activity in 6-ml fractions with 9 column volumes of a linear NaCl gradient (0–0.25 M) in buffer C. The peak of DGPP phosphatase activity elutes from the column at the beginning of the NaCl gradient. The most active fractions containing activity are pooled and used for the next step in the purification scheme.

5.5. Affi-Gel blue chromatography

An Affi-Gel blue column (2.0 × 16 cm) is equilibrated with buffer C. The DE₅₃-purified enzyme is applied to the column at a flow rate of 30 ml/h. The column is washed with 1 column volume of buffer C followed by 2 column volumes of buffer C containing 0.3 M NaCl. DGPP phosphatase activity is eluted from the column in 3.5-ml fractions with 10 column volumes of a linear NaCl gradient (0.3–0.9 M) in buffer C. The peak of DGPP phosphatase activity elutes from the column at a NaCl concentration from 0.3 to 0.4 M. The most active fractions are pooled, and the enzyme preparation is desalted by dialysis against buffer D (10 mM potassium phosphate [pH 7.0], 10 mM MgCl₂, 10mM 2-mercaptoethanol, 20% glycerol, and 1% Triton X-100).

5.6. Hydroxylapatite chromatography

A hydroxylapatite column (1.5 × 8.5 cm) is equilibrated with buffer D. The desalted Affi-Gel blue-purified enzyme is applied to the column at a flow rate of 20 ml/h. The column is washed with 1 column volume of buffer D, and DGPP phosphatase activity is eluted from the column in 2-ml fractions with 20 column volumes of a linear potassium phosphate gradient (10×150 mM) in buffer D. Two peaks of DGPP phosphatase activity elute from the column. The first peak (peak I) of activity elutes from the column at the beginning of the gradient, and the second peak (peak II) of activity elutes at a phosphate concentration of about 24 mM. The most active fractions from each peak are pooled and dialyzed against buffer C.

5.7. Mono Q I chromatography

A Mono Q column (0.5×5 cm) is equilibrated with buffer C. The hydroxylapatite-purified enzyme from peak I is applied to the column at a flow rate of 24 ml/h. The column is washed with 2 column volumes of buffer C. DGPP phosphatase activity is eluted from the column in 1-ml fractions with 40 column volumes of a linear NaCl gradient ($0-0.3$ M) in buffer C. The peak of DGPP phosphatase activity elutes from the column at a NaCl concentration of about 0.12 M. Fractions containing activity are pooled and stored at -80° . The purified enzyme is stable for at least 5 years.

5.8. Mono Q II chromatography

A second Mono Q column (0.5×5 cm) is equilibrated with buffer C. The hydroxylapatite-purified enzyme from peak II is applied to the column at a flow rate of 24 ml/h. The column is washed with 2 column volumes of buffer C. DGPP phosphatase activity is eluted from the column in 1-ml fractions with 40 column volumes of a linear NaCl gradient ($0-0.3$ M) in buffer C. The peak of DGPP phosphatase activity elutes from the column at the beginning of the NaCl gradient. Fractions containing activity are pooled and stored at -80° . The purified enzyme is completely stable for at least 5 years.

5.9. Enzyme purity

Mono Q I chromatography of the hydroxylapatite peak I enzyme results in isolation of an apparent homogeneous protein preparation as shown by SDS-PAGE. The minimum subunit molecular mass of the purified protein is 34 kDa. Mono Q II chromatography of the hydroxylapatite peak II DGPP phosphatase results in isolation of a protein preparation that contains a major protein doublet migrating at a molecular mass of 34 kDa. This DGPP phosphatase preparation also contains some minor protein contaminants. Overall, the hydroxylapatite peak I DGPP phosphatase is purified 33,333-fold over the cell extract with an activity yield of 0.73% to a final specific activity of 150 $\mu\text{mol}/\text{min}/\text{mg}$ (Table 17.1). The hydroxylapatite peak II DGPP phosphatase is purified 24,666-fold over the cell extract to a final specific activity of 111 $\mu\text{mol}/\text{min}/\text{mg}$ with an activity yield of 4% (Table 17.1).

5.10. Identification of DPP1 and LPP1 genes

Protein sequencing analysis has shown that DGPP phosphatase enzymes purified from Mono Q I and from Mono Q II are the same. The differences in chromatographic behavior of the two DGPP phosphatase preparations might be because of a post-translational modification(s). However, studies to address this hypothesis have not been pursued. The N-terminal amino acid sequence (MNR VSFIKTPFNIGAKWRLE) and two internal amino acid sequences (QPVEGLPLDTLFTAK and FPPIDDPLPFKPLMD) derived from the pure DGPP phosphatase enzyme have been used to identify the *DPP1* gene in the *Saccharomyces* Genome Database (Toke *et al.*, 1998).

The *LPP1* gene has been identified in the yeast database because its deduced protein sequence shows homology to the *DPP1*-encoded protein (Toke *et al.*, 1999a). The expression of *DPP1* and *LPP1* genes on multicopy plasmids in *S. cerevisiae* results in enrichment of their encoded enzymes of 10- and 13-fold, respectively (Toke *et al.*, 1998, 1999a). The expression of *DPP1* and *LPP1* in baculovirus-infected Sf9 insect cells provides 500- and 200-fold enrichments of the *DPP1*- and *LPP1*-encoded lipid phosphate phosphatase enzymes, respectively, when compared with that found in wild-type yeast cell extracts (Toke *et al.*, 1998, 1999a). Accordingly, the overexpression of *DPP1*- and *LPP1*-encoded lipid phosphate phosphatase enzymes either in yeast or in insect cells should facilitate the purification of these enzymes.

6. PROPERTIES OF *DPP1*- AND *LPP1*-ENCODED LIPID PHOSPHATE PHOSPHATASES

Properties of the *DPP1*-encoded lipid phosphate phosphatase have been examined using purified enzyme (Toke *et al.*, 1998, 1999a; Wu *et al.*, 1996), whereas properties of the *LPP1*-encoded lipid phosphate phosphatase have been examined using membranes isolated from Sf9 insect cells (Furneisen and Carman, 2000). These enzymes do not have a divalent cation requirement for activity (Furneisen and Carman, 2000; Wu *et al.*, 1996); this is consistent with the enzymes being members of the superfamily of enzymes possessing the lipid phosphatase motif (Han *et al.*, 2004; Stuke and Carman, 1997; Toke *et al.*, 1999b). The kinetic properties of the lipid phosphate phosphatase enzymes utilizing DGPP and PA are summarized in Table 17.2. The specificity constant ($V_{\max}/K_m$) of the *DPP1*-encoded enzyme for DGPP is 10-fold greater than that of PA (Wu *et al.*, 1996). DGPP is a very potent inhibitor of the PA phosphatase activity of the *DPP1*-encoded enzyme, whereas PA does not inhibit the DGPP phosphatase activity of the enzyme (Wu *et al.*, 1996). The specificity constant of the *LPP1*-encoded enzyme for PA is slightly higher (1.2-fold) than that for DGPP (Furneisen and Carman, 2000). DGPP and PA inhibit PA phosphatase and DGPP phosphatase activities, respectively, of the *LPP1*-encoded enzyme with equal potencies (Furneisen and Carman, 2000). The affinity (reflected in K_m value) of the *LPP1*-encoded enzyme for PA and DGPP as substrates is greater than the affinity of the *DPP1*-encoded enzyme for these substrates. Purified *DPP1*-encoded lipid phosphate phosphatase also utilizes lysoPA (Dillon *et al.*, 1996), phosphatidylglycerophosphate (Dillon *et al.*, 1996), sphingoid base phosphates (Dillon *et al.*, 1997), and isoprenoid phosphates (Faulkner *et al.*, 1999) as substrates. The *LPP1*-encoded lipid phosphate phosphatase will also utilize lysoPA as a substrate (Furneisen and Carman, 2000). Although these enzymes utilize a variety of lipid phosphate substrates, only DGPP and PA have been shown to be substrates *in vivo* (Toke *et al.*, 1999a).

DPP1- and *LPP1*-encoded enzymes differ with respect to their sensitivity to thioreactive compounds. In particular, the *LPP1*-encoded enzyme is potently inhibited by *N*-ethylmaleimide (Furneisen and Carman, 2000), whereas the *DPP1*-encoded enzyme is insensitive to this compound (Wu *et al.*, 1996). This difference in *N*-ethylmaleimide sensitivity may be based on the fact that the *LPP1*-encoded enzyme has 10 cysteine residues, whereas the *DPP1*-encoded enzyme contains only 3.

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REFERENCES

- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem* 1976;72:248–254. [PubMed: 942051]
- Brindley DN, English D, Pilquil C, Buri K, Ling ZC. Lipid phosphate phosphatases regulate signal transduction through glycerolipids and sphingolipids. *Biochim. Biophys. Acta* 2002;1582:33–44. [PubMed: 12069808]
- Carman GM, Han GS. Roles of phosphatidate phosphatase enzymes in lipid metabolism. *Trends Biochem. Sci* 2006;31:694–699. [PubMed: 17079146]
- Culbertson MR, Henry SA. Inositol requiring mutants of *Saccharomyces cerevisiae*. *Genetics* 1975;80:23–40. [PubMed: 1093935]
- Dillon DA, Chen X, Zeimet GM, Wu W-I, Waggoner DW, Dewald J, Brindley DN, Carman GM. Mammalian Mg^{2+} -independent phosphatidate phosphatase (PAP2) displays diacylglycerol pyrophosphate phosphatase activity. *J. Biol. Chem* 1997;272:10361–10366. [PubMed: 9099673]

- Dillon DA, Wu W-I, Riedel B, Wissing JB, Dowhan W, Carman GM. The *Escherichia coli* *pgpB* gene encodes for a diacylglycerol pyrophosphate phosphatase activity. *J. Biol. Chem* 1996;271:30548–30553. [PubMed: 8940025]
- Faulkner AJ, Chen X, Rush J, Horazdovsky B, Waechter CJ, Carman GM, Sternweis PC. The *LPP1* and *DPP1* gene products account for most of the isoprenoid phosphatase activities in *Saccharomyces cerevisiae*. *J. Biol. Chem* 1999;274:14831–14837. [PubMed: 10329682]
- Fischl AS, Carman GM. Phosphatidylinositol biosynthesis in *Saccharomyces cerevisiae*: Purification and properties of microsome-associated phosphatidylinositol synthase. *J. Bacteriol* 1983;154:304–311. [PubMed: 6300035]
- Furneisen JM, Carman GM. Enzymological properties of the *LPP1*-encoded lipid phosphatase from *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 2000;1484:71–82. [PubMed: 10685032]
- Greenberg M, Goldwasser P, Henry SA. Characterization of a yeast regulatory mutant constitutive for inositol-1-phosphate synthase. *Mol. Gen. Genet* 1982;186:157–163. [PubMed: 7050628]
- Han G-S, Johnston CN, Carman GM. Vacuole membrane topography of the *DPP1*-encoded diacylglycerol pyrophosphate phosphatase catalytic site from *Saccharomyces cerevisiae*. *J. Biol. Chem* 2004;279:5338–5345. [PubMed: 14630917]
- Han G-S, Johnston CN, Chen X, Athenstaedt K, Daum G, Carman GM. Regulation of the *Saccharomyces cerevisiae* *DPP1*-encoded diacylglycerol pyrophosphate phosphatase by zinc. *J. Biol. Chem* 2001;276:10126–10133. [PubMed: 11139591]
- Han G-S, Wu W-I, Carman GM. The *Saccharomyces cerevisiae* lipin homolog is a Mg^{2+} -dependent phosphatidate phosphatase enzyme. *J. Biol. Chem* 2006;281:9210–9218. [PubMed: 16467296]
- Huh WK, Falvo JV, Gerke LC, Carroll AS, Howson RW, Weissman JS, O'Shea EK. Global analysis of protein localization in budding yeast. *Nature* 2003;425:686–691. [PubMed: 14562095]
- Klig LS, Homann MJ, Carman GM, Henry SA. Coordinate regulation of phospholipid biosynthesis in *Saccharomyces cerevisiae*: Pleiotropically constitutive *opi1* mutant. *J. Bacteriol* 1985;162:1135–1141. [PubMed: 3888957]
- Klig LS, Homann MJ, Kohlwein SD, Kelley MJ, Henry SA, Carman GM. *Saccharomyces cerevisiae* mutant with a partial defect in the synthesis of CDP-diacylglycerol and altered regulation of phospholipid biosynthesis. *J. Bacteriol* 1988;170:1878–1886. [PubMed: 2832385]
- Lin Y-P, Carman GM. Purification and characterization of phosphatidate phosphatase from *Saccharomyces cerevisiae*. *J. Biol. Chem* 1989;264:8641–8645. [PubMed: 2542283]
- Poole MA, Homann MJ, Bae-Lee M, Carman GM. Regulation of phosphatidylserine synthase from *Saccharomyces cerevisiae* by phospholipid precursors. *J. Bacteriol* 1986;168:668–672. [PubMed: 3023284]
- Sciorra VA, Morris AJ. Roles for lipid phosphate phosphatases in regulation of cellular signaling. *Biochim. Biophys. Acta* 2002;1582:45–51. [PubMed: 12069809]
- Stukey J, Carman GM. Identification of a novel phosphatase sequence motif. *Protein Sci* 1997;6:469–472. [PubMed: 9041652]
- Toke DA, Bennett WL, Dillon DA, Chen X, Oshiro J, Ostrander DB, Wu W-I, Cremesti A, Voelker DR, Fischl AS, Carman GM. Isolation and characterization of the *Saccharomyces cerevisiae* *DPP1* gene encoding for diacylglycerol pyrophosphate phosphatase. *J. Biol. Chem* 1998;273:3278–3284. [PubMed: 9452443]
- Toke DA, Bennett WL, Oshiro J, Wu WI, Voelker DR, Carman GM. Isolation and characterization of the *Saccharomyces cerevisiae* *LPP1* gene encoding a Mg^{2+} -independent phosphatidate phosphatase. *J. Biol. Chem* 1999a;273:14331–14338. [PubMed: 9603941]
- Toke DA, McClintick ML, Carman GM. Mutagenesis of the phosphatase sequence motif in diacylglycerol pyrophosphate phosphatase from *Saccharomyces cerevisiae*. *Biochemistry* 1999b;38:14606–14613. [PubMed: 10545184]
- Walsh JP, Bell RM. sn-1, 2-diacylglycerol kinase of *Escherichia coli*: Mixed micellar analysis of the phospholipid cofactor requirement and divalent cation dependence. *J. Biol. Chem* 1986;261:6239–6247. [PubMed: 3009449]
- Wissing JB, Behrbohm H. Diacylglycerol pyrophosphate, a novel phospholipid compound. *FEBS Lett* 1993;315:95–99. [PubMed: 8380145]

- Wu W-I, Liu Y, Riedel B, Wissing JB, Fischl AS, Carman GM. Purification and characterization of diacylglycerol pyrophosphate phosphatase from *Saccharomyces cerevisiae*. *J. Biol. Chem* 1996;271:1868–1876. [PubMed: 8567632]
- Zhang QX, Pilquil CS, Dewald J, Berthiaume LG, Brindley DN. Identification of structurally important domains of lipid phosphate phosphatase-1: Implications for its sites of action. *Biochem. J* 2000;345 (Pt 2):181–184. [PubMed: 10620492]

Table 17.1
Purification of *DPP1*-encoded DGPP phosphatase from *Saccharomyces cerevisiae*^a

Purification step	Total units (μmol/min)	Protein (mg)	Specific activity (units/mg)	Yield (%)	Purification (-fold)
1. Cell extract	49.4	10,957.6	0.0045	100	1
2. Microsomes	27.4	2,312.8	0.0118	55.5	2.6
3. Triton X-100	16.2	803.2	0.0201	32.8	4.5
4. DE53	5.6	15.41	0.363	11.3	80.6
5. Affi-Gel blue	5.5	2.62	2.10	11.1	466.6
6a. Hydroxylapatite	0.51	0.143	3.49	1.0	775.5
6b. Hydroxylapatite	2.36	0.185	12.7	4.7	2,822
7. Mono Q I	0.36	0.0024	150	0.73	33,333
8. Mono Q II	2.0	0.018	111	4.0	24,666

^aData from Wu *et al.* (1996).

Table 17.2
Kinetic constants for *DPP1*- and *LPPI*-encoded lipid phosphate phosphatases

<i>DPP1</i> -encoded lipid phosphatase ^a				<i>LPPI</i> -encoded lipid phosphatase ^b			
Substrate or inhibitor	$V_{\max}$ (units/ mg)	K_m (mol%)	$V_{\max}/K_m$ (units/ mg/mol%)	K_i (mol%)	$V_{\max}$ (units/ mg)	K_m (mol%)	$V_{\max}/K_m$ (units/ mg/mol%)
DGPP	172	1.1, 1.1, 1.1	313	0.35 ^d	0.244	1.1, 1.1, 1.2	3.5
PA	70	2.2	32	NI ^e	0.210	0.07, 0.05	4.2
							0.12 ^d 0.12 ^f

^aData for the *DPP1*-encoded enzyme were taken from Wu *et al.* (1996).
^bData for the *LPPI*-encoded enzyme were taken from Fumeisen and Carman (2000). Because the *LPPI*-encoded enzyme is not pure, the specificity constants reported for the enzyme cannot be compared with those reported for the *DPP1*-encoded enzyme.
^cUnit of activity defined as $\mu\text{mol}/\text{min}$.
^dInhibitor constant with respect to PA as a substrate.
^eNI, not inhibitory.
^fInhibitor constant with respect to DGPP as a substrate.