Mutating His^{29}, His^{125}, His^{133} or His^{158} abolishes glycosylphosphatidylinositol-specific phospholipase D catalytic activity

Nandita S. RAIKWAR, Rosario F. BOWEN and Mark A. DEEG[1]

Department of Medicine and Department of Biochemistry and Molecular Biology, Indiana University School of Medicine and the Department of Veterans Affairs, Richard L. Roudebush VAMC, Indianapolis, IN 46202, U.S.A.

Glycosylphosphatidylinositol (GPI)-specific phospholipase D (GPI-PLD) specifically cleaves GPIs. This phospholipase D is a secreted protein consisting of two domains: an N-terminal catalytic domain and a predicted C-terminal β-propeller. Although the biochemical properties of GPI-PLD have been extensively studied, its catalytic site has not been identified. We hypothesized that a histidine residue(s) may play a critical role in the catalytic activity of GPI-PLD, based on the observations that (i) Zn^{2+}, which utilizes histidine residues for binding, is required for GPI-PLD catalytic activity, (ii) a phosphohistidine intermediate is involved in phospholipase D hydrolysis of phosphatidylcholine, (iii) computer modelling suggests a catalytic site containing histidine residues, and (iv) our observation that diethyl pyrocarbonate, which modifies histidine residues, inhibits GPI-PLD catalytic activity. Individual mutation of the ten histidine residues to asparagine in the catalytic domain of murine GPI-PLD resulted in three general phenotypes: not secreted or retained (His^{56} or His^{88}), secreted with catalytic activity (His^{34}, His^{81}, His^{98} or His^{219}) and secreted without catalytic activity (His^{29}, His^{125}, His^{133} or His^{158}). Changing His^{133} but not His^{29}, His^{125} or His^{158} to Cys resulted in a mutant that retained catalytic activity, suggesting that at least His^{133} is involved in Zn^{2+} binding. His^{133} and His^{158} also retained the biochemical properties of wild-type GPI-PLD including trypsin cleavage pattern and phosphorylation by protein kinase A. Hence, His^{29}, His^{125}, His^{133} and His^{158} are required for GPI-PLD catalytic activity.

Key words: β-propeller domain, catalytic activity, glycosylphosphatidylinositol, histidine, mutagenesis, phospholipase D.

INTRODUCTION

Numerous extracellularly oriented proteins are anchored to the plasma membrane via a GPI (glycosylphosphatidylinositol) anchor. GPI-anchored proteins are widely distributed in eukaryotic organisms and have been identified in every major tissue and cell type examined [1]. These functionally diverse GPI-anchored proteins include complementary regulatory proteins, cell adhesion molecules, ectoenzymes, lymphocyte differentiation antigens, tumour markers and cell-surface receptors [2]. GPI anchors can be cleaved by specific phospholipases resulting in the release of the protein from the plasma membrane [3]. The only mammalian phospholipase cloned to date is GPI-PLD (glycosylphosphatidylinositol-specific phospholipase D; EC 3.1.4.50), an 813–816 amino acid protein found in nearly every cell type [4,5]. *In vivo*, either endogenous or overexpressed GPI-PLD cleaves and releases GPI-anchored proteins [6,7].

GPI-PLD comprises two functional domains: an N-terminal catalytic domain (amino acids 1–276) [8] and a predicted C-terminal β-propeller (amino acids 376–816) [9]. β-Propellers are involved in intra- and inter-molecular interactions and in some proteins serve as catalytic sites [10,11]. The β-propeller domain of GPI-PLD plays an important role in its catalytic activity as evidenced by the following: (i) the β-propeller is required for maximal catalytic activity [8], (ii) a C-terminal antibody inhibits GPI-PLD catalytic activity [12], and (iii) deleting the ten C-terminal amino acids leads to an inactive enzyme probably from the unravelling of the β-propeller [13].

Although the biochemical properties of GPI-PLD have been extensively characterized, its catalytic site has not been identified. One possibility is that the catalytic site involves a Zn^{2+}-binding site since Zn^{2+} is required for GPI-PLD catalytic activity [14]. This raises the possibility that a histidine residue is critical for GPI-PLD activity as this residue is present in numerous Zn^{2+}-binding sites [15,16]. Histidine residues could also play a direct role in the catalytic step as a phosphohistidine intermediate. This occurs in the hydrolysis of phosphatidylcholine by phosphatidylcholine phospholipase D [17]. Finally, a computer model has identified a distant relationship between GPI-PLD and bacterial phosphatidylcholine phospholipase C. This model predicts a trimetal catalytic site in GPI-PLD, which utilizes five histidine residues for co-ordinating Zn^{2+} binding [18].

In order to determine if histidine plays a role in the catalytic activity of GPI-PLD with a long-term goal of identifying a catalytically inactive mutant without loss of structural features, we individually mutated the ten histidine residues in the catalytic domain to asparagine and examined the mutant protein for catalytic activity and known biochemical properties of wild-type GPI-PLD, including trypsin cleavage and phosphorylation by protein kinase A. Mutating His^{29}, His^{125}, His^{133} or His^{158} to asparagine abolished catalytic activity while retaining the biochemical properties of wild-type GPI-PLD.

MATERIALS AND METHODS

DEPC (diethyl pyrocarbonate) inactivation of GPI-PLD activity

GPI-PLD was purified approx. 5000-fold from human and mouse serum as previously described [19]. To determine the effect of DEPC on GPI-PLD activity, 5 ng of purified human GPI-PLD

Abbreviations used: DEPC, diethyl pyrocarbonate; GPI, glycosylphosphatidylinositol; GPI-PLD, GPI-specific phospholipase D; NP40, Nonidet P40.

[1] To whom correspondence should be addressed, at Endocrinology 111E, Indiana University, 1481 W. 10th St., Indianapolis, IN 46202, U.S.A. (email mdeeg@iupui.edu).

was diluted 100-fold in 50 mM sodium acetate (pH 4.5) and incubated with the desired concentration of DEPC for 1 h on ice. GPI-PLD activity was determined as previously described [12]. Briefly, 10 μl of the DEPC-treated GPI-PLD was mixed with 90 μl of reaction buffer [20 mM Hepes, pH 7.0, 2 mM $CaCl_2$ and 0.5 %, v/v, NP40 (Nonidet P40), containing approx. 10 000 c.p.m. of the [^{3}H]myristate membrane form of the variant surface glycoprotein], and incubated at 37 °C as indicated in the Figure legends. The reaction was stopped by adding 0.5 ml of NH_4OH-saturated n-butanol and the radioactivity in the upper organic phase was quantified by liquid-scintillation counting. Recovery of the product, phosphatidic acid, was > 98 % (results not shown). To determine if DEPC inhibition was reversed by hydroxylamine, GPI-PLD was treated with 50 mM DEPC, as described above, and then incubated for up to 40 min. At 30 min, 500 mM hydroxylamine (50 mM final concentration) or reaction buffer was added and the reaction stopped at the desired time as described above.

Mutation of histidine residues in GPI-PLD

His^{29}, His^{34}, His^{56}, His^{81}, His^{88}, His^{98}, His^{125}, His^{133}, His^{158} and His^{219} of murine pancreatic GPI-PLD [5] were individually mutated to asparagine or cysteine residues as indicated using Altered Sites® II *in vitro* mutagenesis system (Promega, Madison, WI, U.S.A.). Histidine was mutated to asparagine because asparagine provides a polar amide group that does not participate in Zn^{2+} binding and was used to study the catalytic site of phosphatidylcholine phospholipase D [17,20]. All the mutations were verified by sequencing (Biochemistry and Biotechnology Facilities, Indiana University). Additional nucleotides that were not reported in our original murine pancreatic GPI-PLD cDNA sequence due to a sequencing error were identified [5]. These nucleotides corresponded to four additional amino acids (Ile, Glu, Gln and Gly) after Gly^{136} and matched those for the murine liver GPI-PLD reported by others [21,22]. Wild-type and mutated GPI-PLD cDNAs were subcloned into the expression vector pcDNA3.1 (Invitrogen, Carlsbad, CA, U.S.A.) at the EcoRI and XbaI sites. COS-I cells (A.T.C.C., Rockville, MD, U.S.A.) (60 mm dishes) were transiently transfected with wild-type or each mutant (5 μg of plasmid cDNA) using 20 μg of Lipofectamine™ (Invitrogen) in Opti-MEM® I (Reduced Serum Medium) according to the manufacturer's methods. After 24 h, the medium was replaced with Dulbecco's modified Eagle's medium containing 100 mg/dl of glucose with 0.5 mg/ml of fatty acid-free BSA (Sigma–Aldrich, St. Louis, MO, U.S.A.). After an additional 24 h, the medium was removed and centrifuged for 5 min (200 ***g***) at 4 °C to remove any suspended cells/debris. The cells were harvested and sonicated in ice-cold PBS containing 0.1 % (v/v) NP40, 1 mM benzamidine, 5 μg/ml leupeptin, 0.2 mM PMSF and 5 μg/ml aprotinin. Lysates were centrifuged (16 000 ***g***) at 4 °C for 10 min. GPI-PLD activity was determined in both the medium and the cell lysates as described above except that the incubation time was increased to 1 h and the final NP40 concentration was 0.01 % (v/v). Proteins in the medium were precipitated with ice-cold acetone and then separated by SDS/PAGE (7 % polyacrylamide). GPI-PLD mass was analysed by Western blotting using anti-GPI-PLD^{771} antibody as previously described [12].

Characterization of GPI-PLD mutants

To examine protein kinase A phosphorylation of wild-type and mutated GPI-PLD, conditioned media (~ 10 ml) from transfected COS-I cells were concentrated approx. 25-fold using an Amicon Ultra (Millipore, Billerica, MA, U.S.A.) with a 100 kDa molecular mass cut-off and washed five times with 5 ml of 20 mM Tris (pH 7.5) and 50 mM NaCl. The 100 kDa cut-off was chosen to minimize the amount of BSA in the concentrate. The amount of GPI-PLD in the concentrated medium was estimated by Western blotting using purified murine serum GPI-PLD as the standard. Secreted GPI-PLD was phosphorylated by protein kinase A (Calbiochem, San Diego, CA, U.S.A.) using an equivalent amount of GPI-PLD from the conditioned medium of COS-I cells transfected with wild-type or mutated GPI-PLD as previously described [23]. Phosphorylated proteins were separated by SDS/PAGE (7 % polyacrylamide) and visualized by autoradiography.

Trypsin cleavage of secreted GPI-PLD was examined by using conditioned medium prepared as described above and was incubated with or without trypsin (4 μg/ml for 15 min) as previously described [23]. Fragments were separated by SDS/PAGE (7–15 % polyacrylamide) and fragments containing the C-terminal of GPI-PLD were identified with anti-GPI-PLD^{771} antibody by Western blotting as previously described [23].

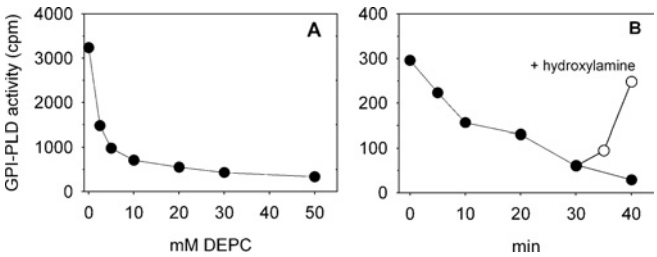

Figure 1 DEPC inhibits GPI-PLD activity

(**A**) Concentration dependence of DEPC inhibition of GPI-PLD activity. GPI-PLD was pretreated with DEPC concentrations ranging from 0 to 50 mM for 60 min, and then GPI-PLD activity was determined as described in the Materials and methods section. (**B**) Time course of DEPC inhibition of GPI-PLD activity. GPI-PLD was treated with 50 mM DEPC for 0–40 min. At 30 min, GPI-PLD activity was determined with (○) and without (●) the addition of hydroxylamine as described in the Materials and methods section. Results shown are mean of duplicate determinations and are representative of three independent experiments.

RESULTS AND DISCUSSION

Identification of histidine residues required for GPI-PLD catalytic activity

As an initial approach to determine if histidine residue(s) play a role in GPI-PLD catalytic activity, we examined the effect of DEPC on GPI-PLD activity since DEPC modifies histidine and lysine residues. DEPC inhibited GPI-PLD catalytic activity in a concentration- and time-dependent manner (Figure 1). Modification of histidine but not lysine residues can be reversed by hydroxylamine [24]. To differentiate a histidine versus lysine residue modification, GPI-PLD was incubated with DEPC for 30 min and then incubated with or without hydroxylamine for 5–10 min. Hydroxylamine reversed DEPC inhibition of GPI-PLD catalytic activity (Figure 1B). These results suggest that a histidine modification accounts for the DEPC inhibition of GPI-PLD catalytic activity.

There are ten histidine residues in the N-terminal catalytic domain at positions 29, 34, 56, 81, 88, 98, 125, 133, 158 and 219. In order to identify the histidine(s) residues required for GPI-PLD activity, we utilized a site-directed mutagenesis approach to individually change the histidine residues to asparagine. Transiently expressing wild-type GPI-PLD in COS-I cells increases media- and cell-associated GPI-PLD activity and mass by more than 50-fold compared with endogenous GPI-PLD (results not shown). Mutating the individual histidine residues in the catalytic domain in GPI-PLD resulted in three general phenotypes: not secreted

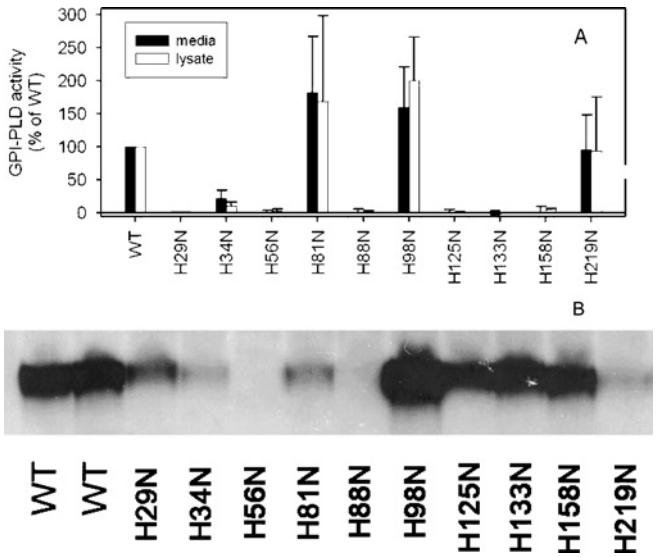

Figure 2 Mutating His[29], His[125], His[133] and His[158] abolishes GPI-PLD activity

His[29], His[34], His[56], His[81], His[88], His[98], His[125], His[133], His[158] or His[219] of mouse GPI-PLD was individually mutated to an asparagine residue as described in the Materials and methods section. COS-I cells were transiently transfected with wild-type (WT) or each mutant (5 μg of plasmid DNA) using Lipofectamine™ (20 μg). After 24 h, GPI-PLD catalytic activity was determined in the medium and cell lysate (**A**) or GPI-PLD mass in the medium by Western blotting (**B**) as described in the Materials and methods section. Absolute GPI-PLD activity in the medium and cell lysates from cells transfected with wild-type GPI-PLD was 140 ± 118 ($n = 6$) and 43 ± 20 ($n = 6$) c.p.m. $\cdot$ h^{-1} $\cdot$ (mg of protein)$^{-1}$ respectively. Results in (**A**) are from three independent experiments performed in triplicate. Results in (**B**) are representative of three experiments.

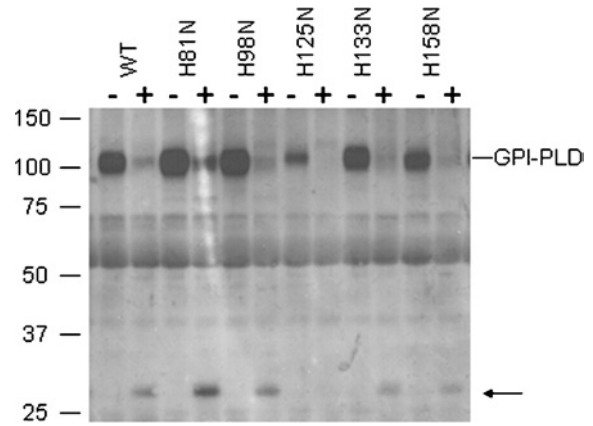

Figure 3 Trypsin fragmentation of GPI-PLD mutants

Conditioned media from COS-I cells transfected with wild-type (WT) GPI-PLD or one among H81N, H98N, H125N, H133N and H158N GPI-PLD were concentrated and treated with (+) or without (−) trypsin as described in the Materials and methods section. Proteins and fragments were separated by SDS/PAGE (7–15 % polyacrylamide) and a C-terminal epitope of GPI-PLD was visualized by Western blotting with anti-GPI-PLD[771] as described in the Materials and methods section. Molecular mass standards are indicated in kDa. The 27 kDa fragment is indicated by the arrow. Results are representative of three independent experiments.

or retained, secreted with catalytic activity and secreted without catalytic activity.

Mutating His[56] or His[88] resulted in a GPI-PLD that was neither secreted nor accumulated in the cellular lysates (Figure 2). This suggests that these mutations resulted in a misfolded protein that was degraded intracellularly. Hence, His[56] and His[88] may play a role in maintaining the conformation of the catalytic domain. Histidine residues are known to play many different roles in maintaining the structure of proteins. Histidine may be involved in maintaining conformation by Zn^{2+} binding [25,26] or participating in hydrogen bonding or salt bridging to stabilize secondary structures or interaction between domains [27,28]. Since these mutants were not secreted, it is likely that there was a considerable misfolding of these particular mutations of GPI-PLD. One possibility is that His[56] and His[88] play a role in stabilizing the interaction of the catalytic and β-propeller domains of GPI-PLD, which is required for optimal catalytic activity [8,14]. His[88] is also predicted to be one of the five histidine residues in the GPI-PLD catalytic site co-ordinating Zn^{2+} binding [18]. Altering His[88] to Asn results in a new N-glycosylation site. Misfolding may occur if this site is N-glycosylated. Our results are consistent with this role of His[88] in this model. These mutations were not characterized further.

Mutating His[34], His[81], His[98] or His[219] resulted in a secreted GPI-PLD with catalytic activity (Figure 2), although the amount of secreted GPI-PLD varied. These results suggest that these particular histidine residues may not play important roles in the catalytic activity of GPI-PLD but may influence its conformation and therefore were not further characterized.

Mutating His[29], His[125], His[133] or His[158] resulted in a GPI-PLD that was secreted but did not have any catalytic activity (Figure 2). This result suggests that these mutations produced a properly folded, secreted GPI-PLD that lacks catalytic activity. These mutations were further characterized by comparing their biochemical properties with wild-type GPI-PLD. Since H29N (His[29] $\rightarrow$ Asn) was only weakly secreted, it was not characterized further.

Characterization of wild-type and H125N, H133N and H158N mutated GPI-PLD

Some of the known biochemical properties of GPI-PLD include cleavage by trypsin, generating specific fragments [8,23], and phosphorylation by protein kinase A [23,29]. To determine if mutating His[125], His[133] or His[158] altered any of these properties, we used secreted GPI-PLD from COS-I cells transfected with H125N, H133N or H158N GPI-PLD using wild-type, H81N and H98N as controls.

Trypsin cleaves GPI-PLD to generate specific fragments [23]. Utilizing an antibody that recognizes a C-terminal epitope, trypsin cleaves murine GPI-PLD, generating a 27 kDa fragment (Figure 3). Mutating His[81], His[98], His[133] or His[158] did not alter the generation of the 27 kDa fragment (Figure 3). Tryspin did not generate this 27 kDa fragment from the H125N mutant. This suggests that the conformation of the C-terminal of these mutants may be altered enough to affect trypsin cleavage. A change in the conformation of the C-terminus would also affect binding of the antibody that was used to determine the GPI-PLD mass, which helps to identify the C-terminal of GPI-PLD (residues 771–790). This would explain the apparent lower mass of secreted GPI-PLD determined by Western blotting when COS cells are transfected with H125N (Figures 2–5).

Protein kinase A phosphorylates GPI-PLD [23,29]. Protein kinase A phosphorylated a secreted protein from COS-I cells transfected with wild-type or H81N, H98N, H125N, H133N or H158N. It is likely that this protein is GPI-PLD since it was not present in conditioned media from cells transfected with empty vector (results not shown) and has the same molecular mass as the phosphorylated purified murine serum GPI-PLD (results not shown). These results suggest that mutating His[125], His[133] or His[158] does not alter protein kinase A phosphorylation of GPI-PLD. The properties of the various GPI-PLD mutants are summarized in Table 1.

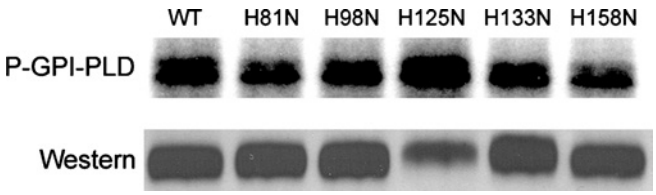

Figure 4 Phosphorylation of GPI-PLD mutants

Proteins in conditioned media from COS-I cells transfected with wild-type (WT) or GPI-PLD mutants were phosphorylated with protein kinase A and separated by SDS/PAGE (7% polyacrylamide) as described in the Materials and methods section. Phosphorylated proteins were visualized by autoradiography. GPI-PLD mass was identified by Western blotting as described in the Materials and methods section.

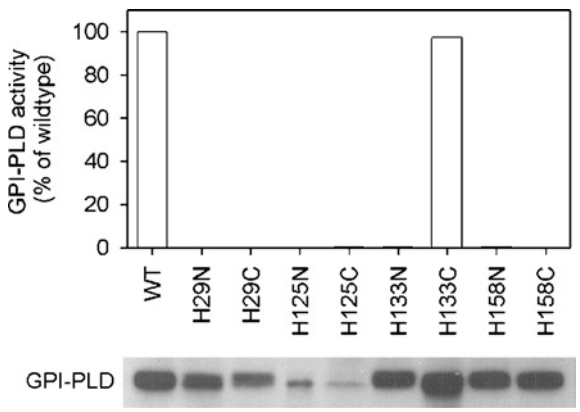

Figure 5 Effect of Cys substitution for His^{29}, His^{125}, His^{133} and His^{158} on GPI-PLD secretion and activity

His^{29}, His^{125}, His^{133} and His^{158} were individually mutated to Cys, expressed in COS cells and GPI-PLD activity and mass determined as described in Figure 1. Results are representative of three independent experiments.

Table 1 Summary of biochemical properties of wild-type and mutated GPI-PLDs

Biochemical properties of the mutant GPI-PLD generated are summarized. H133N and H158N are in italic to emphasize that these mutants have all the properties of wild-type GPI-PLD without catalytic activity. n.d., not determined; Y, yes; N, no.

Mutation	Secreted	Catalytically active	Trypsin (27 kDa fragment)	Phosphorylated
Wild-type	Y	Y	Y	Y
H29N	Y	N	n.d.	n.d.
H34N	Y	Y	n.d.	n.d.
H56N	N	N	n.d.	n.d.
H81N	Y	Y	Y	Y
H88N	N	N	n.d.	n.d.
H98N	Y	Y	Y	Y
H125N	Y	N	N	Y
H133N	*Y*	*N*	*Y*	*Y*
H158N	*Y*	*N*	*Y*	*Y*
H219N	Y	Y	Y	n.d.

Although it is clear that mutating His^{29}, His^{125}, His^{133} or His^{158} resulted in a catalytically inactive protein, what role these histidine residues play in GPI-PLD activity and/or structure is unknown. One of these histidine residues could participate in catalysis by serving as a phosphohistidine intermediate, as in phosphatidylcholine phospholipase D, but the primary sequence near these amino acids does not match the catalytic motif $H(X)K(X)_4D$ in the phospholipase D superfamily [30,31]. This suggests that GPI-PLD utilizes a different catalytic mechanism although further experimentation is required. Computer modelling has identified a distant relationship between GPI-PLD and bacterial phosphatidylcholine phospholipase C, which predicts involvement of Asp^{68} and Glu^{85} in co-ordinating a nucleophilic water molecule for GPI-PLD-mediated hydrolysis of GPIs [18]. Mutating His^{88} to Asn may interfere with this role for Glu^{85}.

This model also predicts the involvement of five histidine residues (His^{29}, His^{88}, His^{125}, His^{133} and His^{158}) in co-ordinating three bound Zn^{2+} atoms. Our results are in good agreement with the prediction that His^{29}, His^{125}, His^{133} or His^{158} could be involved in co-ordinating Zn^{2+} binding. To determine if these histidine residues are involved in Zn^{2+} binding, we mutated His^{29}, His^{125}, His^{133} and His^{158} to Cys. Since cysteine is involved in some Zn^{2+}-binding sites, we hypothesize that if the histidine residue was involved in Zn^{2+} binding, mutating the residue to cysteine would not result in the loss of catalytic activity. Although H29C, H125C and H158C were secreted, these mutants did not have any catalytic activity (Figure 5). In contrast, H133C did retain its catalytic activity (Figure 5), consistent with the hypothesis that His^{133} is involved in Zn^{2+} binding.

In summary, mutating histidine residues in the catalytic domain of GPI-PLD results in mutants that are not secreted and are probably misfolded and degraded (H56N and H88N), are secreted and have catalytic activity (H34N, H81N, H98N and H219N) or are secreted but lack catalytic activity (H29N, H125N, H133N and H158N). These results are in good agreement with the predicted catalytic site of GPI-PLD. Further experiments are required to confirm the role of these histidine residues in GPI-PLD catalytic activity. Since H133N and H158N GPI-PLD retain the tested biochemical properties of wild-type GPI-PLD, these mutants can be used to determine if GPI-PLD has properties independent of its catalytic activity. Both lipoprotein lipase and heparin lipase cleave the triacylglycerol present in triacylglycerol-rich lipoproteins in serum and act as bridging proteins to enhance binding of these triacylglycerol-rich lipoprotein to hepatocytes independent of their catalytic activity [32,33]. Hence, it is conceivable that GPI-PLD could serve as a bridging protein that could occur via the β-propeller domain.

These studies were supported by Indiana University.

REFERENCES

1 Low, M. G. and Brodbeck, U. (1999) Enzymes cleaving the phosphodiester bond in the GPI anchor. In GPI-anchored Membrane Proteins and Carbohydrates (Hoessli, D. C. and Ilangumaran, S., eds.), pp. 167–186, R.G. Landes, Austin
2 Xie, M. and Low, M. G. (1994) Expression and secretion of glycosylphosphatidylinositol-specific phospholipase D by myeloid cell lines. Biochem. J. **297**, 547–554
3 Low, M. G. (2000) Structure and function of GPI-specific phospholipases. In PNH and the GPI-linked Proteins (Young, N. G. and Moss, J., eds.), pp. 239–268, Academic Press, San Diego, CA
4 Stadelmann, B., Zurbiggen, A. and Brodbeck, U. (1993) Distribution of glycosylphosphatidylinositol-specific phospholipase D mRNA in bovine tissue sections. Cell Tissue Res. **274**, 547–552
5 LeBoeuf, R. C., Caldwell, M., Guo, Y., Metz, C., Davitz, M. A., Olson, L. K. and Deeg, M. A. (1998) Mouse glycosylphosphatidylinositol-specific phospholipase D (Gpld1) characterization. Mam. Gen. **9**, 710–714
6 Brunner, G., Metz, C. N., Nguyen, H., Gabrilove, J., Patel, S. R., Davitz, M. A., Rifkin, D. B. and Wilson, E. L. (1994) An endogenous glycosylphosphatidylinositol-specific phospholipase D releases basic fibroblast growth factor-heparan sulfate proteoglycan complexes from human bone marrow cultures. Blood **83**, 2115–2125
7 Khan, W. N. and Hammarstrom, S. (1991) Biosynthesis of carcinoembryonic antigen (CEA) gene family members expressed in human tumor cell lines: evidence for cleavage of the glycosyl phosphatidyl inositol (GPI) anchor by GPI-PLC and GPI-PLD. Biochem. Int. **25**, 723–731

8 Heller, M., Bütikofer, P. and Brodbeck, U. (1994) Generation by limited proteolysis of a catalytically active 39-kDa protein from the 115-kDa form of phosphatidylinositol-glycan-specific phospholipase D from bovine serum. Eur. J. Biochem. **224**, 823–833

9 Springer, T. A. (1997) Folding of the N-terminal, ligand-binding region of integrin alpha-subunits into a beta-propeller domain. Proc. Natl. Acad. Sci. U.S.A. **94**, 65–72

10 Li, D. and Roberts, R. (2001) WD-repeat proteins: structure characteristics, biological function, and their involvement in human diseases. Cell. Mol. Life Sci. **58**, 2085–2097

11 Pons, T., Gomez, R., Chinea, G. and Valencia, A. (2003) Beta-propellers: associated functions and their role in human diseases. Curr. Med. Chem. **10**, 505–524

12 Deeg, M. A. and Bowen, R. F. (1999) Midportion antibodies stimulate glycosylphosphatidylinositol-specific phospholipase D activity. Arch. Biochem. Biophys. **370**, 278–284

13 Stadelmann, B., Butikofer, P., Konig, A. and Brodbeck, U. (1997) The C-terminus of glycosylphosphatidylinositol-specific phospholipase D is essential for biological activity. Biochim. Biophys. Acta **1355**, 107–113

14 Li, J.-Y., Hollfelder, K., Huang, K.-S. and Low, M. G. (1994) Structural features of GPI-specific phospholipase D revealed by proteolytic fragmentation and Ca^{2+} binding studies. J. Biol. Chem. **269**, 28963–28971

15 Newton, A. L., Sharpe, B. K., Kwan, A., Mackay, J. P. and Crossley, M. (2000) The transactivation domain within cysteine/histidine-rich region 1 of CBP comprises two novel zinc-binding modules. J. Biol. Chem. **275**, 15128–15134

16 Schwabe, J. W. and Klug, A. (1994) Zinc mining for protein domains. Nat. Struct. Biol. **1**, 345–349

17 Gottlin, E. B., Rudolph, A. E., Zhao, Y., Matthews, H. R. and Dixon, J. E. (1998) Catalytic mechanism of the phospholipase D superfamily proceeds via a covalent phosphohistidine intermediate. Proc. Natl. Acad. Sci. U.S.A. **95**, 9202–9207

18 Rigden, D. J. (2004) A distant evolutionary relationship between GPI-specific phospholipase D and bacterial phosphatidylcholine-preferring phospholipase C. FEBS Lett. **569**, 229–234

19 Rhode, H., Schulze, M., Cumme, G. A., Gohlert, A., Blume, E., Bublitz, R., Schilling, K. and Horn, A. (2000) Glycosylphosphatidylinositol-specific phospholipase D of human serum activity modulation by naturally occurring amphiphiles. Biol. Chem. **381**, 471–485

20 Sung, T. C., Roper, R. L., Zhang, Y., Rudge, S. A., Temel, R., Hammond, S. M., Morris, A. J., Moss, B., Engebrecht, J. and Frohman, M. A. (1997) Mutagenesis of phospholipase D defines a superfamily including a trans-Golgi viral protein required for poxvirus pathogenicity. EMBO J. **16**, 4519–4530

21 Gregory, P., Ziemiecki, A., Zurcher, G., Brodbeck, U. and Butikofer, P. (2003) Anti-mouse GPI-PLD antisera highlight structural differences between murine and bovine GPI-PLDs. Biol. Chem. **384**, 1575–1582

22 Flores-Borja, F., Kieszkievicz, J., Church, V., Francis-West, P. H., Schofield, J., Rademacher, T. W. and Lund, T. (2004) Genetic regulation of mouse glycosylphosphatidylinositol-phospholipase D. Biochimie **86**, 275–282

23 Deeg, M. A. and Bowen, R. F. (2002) Phosphorylation decreases trypsin activation and apolipoprotein al binding to glycosylphosphatidylinositol-specific phospholipase D. Biochem. Cell. Biol. **80**, 253–260

24 Miles, E. W. (1977) Modification of histidyl residues in proteins by diethylpyrocarbonate. Methods Enzymol. **47**, 431–442

25 Guo, L., Groenendyk, J., Papp, S., Dabrowska, M., Knoblach, B., Kay, C., Parker, J. M., Opas, M. and Michalak, M. (2003) Identification of an N-domain histidine essential for chaperone function in calreticulin. J. Biol. Chem. **278**, 50645–50653

26 Saito, Y., Ihara, Y., Leach, M. R., Cohen-Doyle, M. F. and Williams, D. B. (1999) Calreticulin functions *in vitro* as a molecular chaperone for both glycosylated and non-glycosylated proteins. EMBO J. **18**, 6718–6729

27 Rodriguez-Crespo, I., Nishida, C. R., Knudsen, G. M. and de Montellano, P. R. (1999) Mutation of the five conserved histidines in the endothelial nitric-oxide synthase hemoprotein domain. No evidence for a non-heme metal requirement for catalysis. J. Biol. Chem. **274**, 21617–21624

28 Marti, D. N. and Bosshard, H. R. (2003) Electrostatic interactions in leucine zippers: thermodynamic analysis of the contributions of Glu and His residues and the effect of mutating salt bridges. J. Mol. Biol. **330**, 621–637

29 Civenni, G., Butikofer, P., Stadelmann, B. and Brodbeck, U. (1999) *In vitro* phosphorylation of purified glycosylphosphatidylinositol-specific phospholipase D. Biol. Chem. **380**, 585–588

30 Ponting, C. P. and Kerr, I. D. (1996) A novel family of phospholipase D homologues that includes phospholipid synthases and putative endonucleases: identification of duplicated repeats and potential active site residues. Protein Sci. **5**, 914–922

31 Koonin, E. V. (1996) A duplicated catalytic motif in a new superfamily of phosphohydrolases and phospholipid synthases that includes poxvirus envelope proteins. Trends Biochem. Sci. **21**, 242–243

32 Medh, J. D., Bowen, S. L., Fry, G. L., Ruben, S., Andracki, M., Inoue, I., Lalouel, J. M., Strickland, D. K. and Chappell, D. A. (1996) Lipoprotein lipase binds to low density lipoprotein receptors and induces receptor-mediated catabolism of very low density lipoproteins *in vitro*. J. Biol. Chem. **271**, 17073–17080

33 Merkel, M., Kako, Y., Radner, H., Cho, I. S., Ramasamy, R., Brunzell, J. D., Goldberg, I. J. and Breslow, J. L. (1998) Catalytically inactive lipoprotein lipase expression in muscle of transgenic mice increases very low density lipoprotein uptake: direct evidence that lipoprotein lipase bridging occurs *in vivo*. Proc. Natl. Acad. Sci. U.S.A. **95**, 13841–13846

Received 22 April 2005/2 June 2005; accepted 8 June 2005
Published as BJ Immediate Publication 8 June 2005, doi:10.1042/BJ20050656