The Crystal Structure of Palmitoyl Protein Thioesterase-2 (PPT2) Reveals the Basis for Divergent Substrate Specificities of the Two Lysosomal Thioesterases, PPT1 and PPT2*

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Guillermo Calero‡, Praveena Gupta§, M. Cristina Nonato‡, Sagun Tandel¶, Edward R. Biehl†, Sandra L. Hofmann§, and Jon Clardy‡‡

From the ‡Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853-1301, the §Department of Internal Medicine and the Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, Texas 75390-8593, and the ¶Department of Chemistry, Southern Methodist University, Dallas, Texas 75275

Mutations in palmitoyl protein thioesterase-1 (PPT1) have been found to cause the infantile form of neuronal ceroid lipofuscinosis, which is a lysosomal storage disorder characterized by impaired degradation of fatty acid-modified proteins with accumulation of amorphous granular deposits in cortical neurons, leading to mental retardation and death. Palmitoyl protein thioesterase-2 (PPT2) is a second lysosomal hydrolase that shares a 26% identity with PPT1. A previous study had suggested that palmitoyl-CoA was the preferred substrate of PPT2. Furthermore, PPT2 did not hydrolyze palmitate from the several S-palmitoylated protein substrates. Interestingly, PPT2 deficiency in a recent transgenic mouse model is associated with a form of neuronal ceroid lipofuscinosis, suggesting that PPT1 and -2 perform non-redundant roles in lysosomal thioester catabolism. In the current paper, we present the crystal structure of PPT2 at a resolution of 2.7 Å. Comparisons of the structures of PPT1 and -2 show very similar Architectural features; however, conformational differences in helix α1 lead to a solvent-exposed lipid-binding groove in PPT1. The limited space between two parallel loops (β3-αA and β8-αF) located immediately above the lipid-binding groove in PPT2 restricts the binding of fatty acids with bulky head groups, and this binding groove is significantly larger in PPT1. This structural difference accounts for the ability of PPT2 to hydrolyze an unbranched structure such as palmitoyl-CoA but not palmitoylcyistine or palmitoylated proteins. Furthermore, differences in fatty acid chain length specificity of PPT1 and -2, also reported here, are explained by the structure and may provide a biochemical basis for their non-redundant roles.

Neuronal ceroid lipofuscinosis (NCL) is a family of autosomal recessive neurodegenerative disorders characterized clinically by impaired vision, mental retardation, ataxia, and seizures. Histopathological findings of NCL include accumulation of autofluorescent material and neuronal depletion of the brain cortex accompanied by astrocytosis (1). Traditionally NCLs are classified as infantile, late infantile, juvenile, and adult based on the age of the patient and severity of symptoms at the onset and by differences in morphology of the lysosomal storage material (granular osmiophilic deposits in infantile NCL, curvilinear deposits in late infantile NCL, and fingerprint inclusions in juvenile NCL) (2). Genetic studies have shown that mutations in the gene encoding palmitoyl protein thioesterase-1 (PPT1) are the cause of infantile NCL, which is the severe form of NCL (3). PPT1 has also been associated with some cases of late infantile and juvenile NCL as well (4, 5). Biochemical experiments have revealed that PPT1 possesses thioesterase activity against fatty acid thioesters with 14–18 carbons, including palmitoyl-CoA, and fatty acid-modified proteins, such as H-Ras/p21 and other proteins involved in cell signaling (6, 7).

A second lysosomal enzyme with thioesterase activity was identified in our laboratory and was designated palmitoyl protein thioesterase-2 (PPT2). Enzymatic studies revealed that PPT2 has thioesterase activity against palmitoyl-CoA, but it is unable to remove palmitate groups from several S-palmitoylated proteins (8). Full-length PPT2 consists of 302 amino acids and is only 26% identical in amino acid sequence to PPT1. Residues 1–27 at the N terminus constitute a signal peptide that is cleaved from the mature protein (8). RNA blot hybridization of human tissues has shown that PPT2 is found in variable amounts with notably high expression in muscle. PPT2 expression is diffuse throughout the brain with particularly high levels in the granular layer of the cerebellum and moderate expression in the pons, hippocampus, and dentate gyrus (9). On the other hand, high levels of expression of PPT1 are seen in the Purkinje cells of the cerebellum, the thalamus, and the hippocampus as well as the pons and various nuclei of the medulla. An alternatively spliced form of PPT2, in which 46 novel amino acids replace 47 amino acids from the C terminus, has been identified (8). This alternatively spliced version of PPT2 is catalytically inactive because it lacks His-289, which is one of the residues of the catalytic triad.

Targeted deletions in either PPT1 or -2 genes in mice cause...
Crystal Structure of Palmitoyl Protein Thioesterase-2

EXPERIMENTAL PROCEDURES

Protein Expression and Purification—Human PPT2 was overexpressed using an insect cell-baculovirus expression system. Protein was secreted into the cell medium at an approximate concentration of 0.5 mg/liter. Cell media was adjusted to 25 mM HEPES, pH 7.0, and 2 mM NaCl and loaded into a butyl-Sepharose (Amersham Biosciences) column. A gradient from 2 to 500 mM NaCl was used to elute the protein, which was subsequently dialyzed against a solution containing 25 mM HEPES, pH 7.0, and 50 mM NaCl, and loaded onto an ion exchange Q-Sepharose (Amersham Biosciences) column. A gradient from 50 to 400 mM NaCl was used to elute the protein, which was subsequently dialyzed against a solution containing 25 mM HEPES, pH 7.0. The final PPT2 preparation was assessed to be >95% pure by SDS-PAGE.

Crystallographic and Structure Determination—PPT2 crystals were obtained at 4 °C by the sitting drop vapor diffusion method. Two microliters of protein (15 mg/ml) were mixed with an equal volume of a solution containing 2 mM ammonium sulfate, 100 mM sodium cacodylate (pH range 5.5–6.5), and 8% methyl-pentanediol to produce crystals diffracting to a resolution of 2.7 Å. All diffraction experiments were performed using synchrotron radiation at the Cornell High Energy Synchrotron Source (CHESS). An oscillation step of 1° was used throughout, and the crystal-to-detector distance was set to 200 mm. Raw reflection intensities were reduced using Denzo and Scalepack (22). The space group was determined to be P6122. The unit cell dimensions are a = b = 148.54 Å, c = 152.39 Å. The structure of PPT2 was solved using PPT1 (Protein Data Bank code 1EI9, Ref. 10) as a search model for molecular replacement (23). After rigid body refinement (23), the model was subject to several cycles of simulated annealing (23) and model building using O (24). The crystallographic Rwork and Rfree values are 24.2 and 22.1%, respectively (40–2.7 Å). Other data collection and refinement statistics are shown in Table I. Figures were generated with Bobscript (25) and SPOCK2 (26) and rendered using RASTER3D (27).

Synthesis of S-Palmitoyl-N-acetylcysteine and S-Palmitoyl-N-acetyl-O-carboxymethyl-cysteine—S-Palmitoyl-N-acetylcysteine was synthesized from palmitoyl chloride and N-acetylcysteine in the presence of triethylamine. Briefly, N-acetylcysteine in dichloromethane was cooled to 0 °C, triethylamine was added and stirred for 5 min, and palmitoyl chloride was added dropwise under stirring for 30 min at room temperature. The reaction mixture was concentrated under vacuum and purified by high performance liquid chromatography. N-Acetyl-cysteine methyl ester was synthesized from N-acetylcysteine and diazomethane in ether at room temperature, and the reaction was monitored by gas chromatography/mass spectrometry. S-Palmitoyl-N-acetyl-O-carboxymethyl-cysteine was synthesized from N-acetylcysteine methyl ester and palmitoyl chloride as described above. The compounds were verified by gas chromatography/mass spectrometry. [3H]Palmitate-labeled H-Ras was synthesized by metabolic labeling of H-Ras in the insect cell-baculovirus system as described previously (6).

RESULTS AND DISCUSSION

Overall Structure of PPT2—The atomic structure of PPT2 (residues 35–302) consists of three domains: an α-β hydrolase domain, a lipid binding domain (or cap domain), and an accessory domain (Fig. 1). Residues 35–145, 202–240, and 280–308 conform to an α-β hydrolase fold consisting of six parallel β strands, β3–8, arranged in the order β4–β3–β5–β6–β7–β8 (Fig. 1). The numbering of the β strands and α helices corresponds to the nomenclature of the canonical α-β hydrolase fold (14). The β strands are partially surrounded by four α helices, αA, αB, αC, αD, and αF. Compared with the canonical α-β hydrolase

<table>
<thead>
<tr>
<th>Table I</th>
<th>Data collection and refinement statistics</th>
</tr>
</thead>
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<td>Data collection</td>
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<tr>
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<tr>
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<tr>
<td>Redundancy</td>
<td>6.5</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>100</td>
</tr>
</tbody>
</table>

**Rwork** = Σ∥Fcalc∥−|Fobs∥/Σ|Fobs∥, where (I) = average intensity obtained from multiple observations of symmetry-related reflections.

**Rfree** = Σ∥Fcalc∥−|Fobs∥/Σ|Fcalc∥ for 5% of the data.

Root mean square deviations.

Thioesterase Assays—A colorimetric assay (7, 11) was used to monitor the hydrolysis of fatty acid from fatty acyl CoAs S-palmitoyl-N-acetylcysteine and S-palmitoyl-N-acetyl-O-carboxymethyl-cysteine. Briefly, 300-μl assay mixtures contained 100 mM sodium acetate, pH 5.2, 1 mM EDTA, 0.2 mM 5,5′-dithio-bis[2-nitrobenzoic acid], 40 μM or varying concentrations as needed) of fatty acyl substrate, and 0.3–0.4 μg of purified recombinant human PPT1 or -2. Reactions were monitored by absorbance at 412 nm, and calculations were performed based upon the molar extinction coefficient of 2-nitro-5-thiobenzoate (1.36 × 10⁵ liters/mmol/cm). Hydrolysis of [3H]palmitoyl-H-Ras was determined as described by Camp and Hofmann (6). Hydrolysis of the fluorescent substrate 4-methylumbelliferonyl-β-S-palmitoyl-β-g-glucoside was performed by the method of van Diggelen et al. (12) and was quantified using a standard curve of increasing concentrations of 4-methylumbelliferone (Sigma).

Site-directed Mutagenesis, COS Cell Transfection, and Analysis of PPT2 Mutants—The plasmid pCMV5-hPPT2 (8) was used as a template to construct mutant plasmids using a commercially available mutagenesis kit (QuickChange, Stratagene). Oligonucleotides (38-mer) with desired mutations were used in an extension reaction using the vector and Ffu Turbo DNA polymerase in a thermocycler. Wild-type parental strands were digested with DpnI, and mutated DNA was transformed into XLI-Blue-competent cells. All mutations were confirmed by sequencing the entire insert to verify the presence of the mutation and the absence of extraneous mutations. Simian COS-1 cells were maintained in 60-mm dishes and transiently transfected with 1 μg of plasmid DNA in the presence of 6 μl of FuGENE 6 reagent (Roche Applied Science). The cells were harvested 60–72 h post-transfection and were sonicated immediately in homogenization buffer containing 50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, and a protease inhibitor mixture (2 μg/ml leupeptin, 1 μg/ml pepstatin, and 2 mM phenylmethylsulfonyl fluoride). The homogenates were spun at 12,000 × g for 10 min, and supernatants were assayed for palmitoyl-CoA hydrolase using [3H]palmitate-labeled palmitoyl-CoA as described by Das et al. (13). Aliquots were also subjected to SDS-PAGE and immunoblotting with anti-PPT2 antibodies to analyze PPT2 expression levels (8).

J. A. Christopher, SPOCK at quorum.tamu.edu/spock/
fold, PPT2 lacks the first two antiparallel β strands β1 and -2. The α-β hydrolase fold constitutes the core of PPT2 and includes the catalytic triad formed by Ser-111, His-283, and Asp-228 (Fig. 1). Two intramolecular disulfide bonds, Cys-109 to Cys-115 and Cys-277 to Cys-295, help stabilize the hydrolase core. The first one, Cys-109 to Cys-115, links the position of the sharp "elbow" that contains the nucleophile residue Ser-111 (Fig. 1). The second disulfide bond, Cys-277 to Cys-295, secures the position of the catalytic His by linking β8 to αF and fixing the position of the loop joining these two secondary structure elements. Interestingly, the position of the catalytic histidine residue (His-286) in Pseudomonas cepacia lipase (15) is fixed by an octahedrally coordinated Ca²⁺ ion bridging helix αE with the loop that contains His-286.

The lipid binding domain constitutes an insertion to the α-β fold and is located between strand β6 and helix αD (Fig. 1). This domain consists of a series of small α helices, α4–9 (residues 136–208), which forms a binding pocket for the substrate. The pocket is composed of a groove (α5–9, residues 154–208) covered by a lid (α4, residues 147–153). An intramolecular disulfide bridge, Cys-165 to Cys-176, stabilizes the floor of the groove.

The accessory domain (residues 234–280) (Fig. 1) is also an extension of the α-β hydrolase fold and replaces helix αE in the canonical fold. It is formed by helices α10–13 and a β hairpin (βa and βb). The function of this small domain is unknown.

The amino acid sequence of PPT2 contains five potential N-linked glycosylation sites at residues Asn-60, -190, -206, -245, and -289. Of these potential sites we can see clear electron density for a molecule of N-acetylglucosamine (NAG) covalently linked to Asn-190. The color code used in this figure will be used throughout.

The catalytic triad of PPT2. a, the electron density 2Fo − Fc map calculated at 2σ using crystallography NMR software (23); b, mutation of catalytic triad residues in human PPT2 abrogates palmitoyl-CoA hydrolase activity of the enzyme. Wild-type human PPT2 was expressed in a transient transfection of COS1 cells, and cell lysates were assayed for palmitoyl-CoA hydrolase activity. Expression levels and glycosylation patterns of wild-type and mutant enzymes were virtually identical as confirmed by immunoblotting (data not shown).

The positions of the residues of the catalytic triad with respect to each other are critical for the catalytic activity of the α-β hydrolase fold. A network of hydrogen bonds among the residues of the catalytic triad and with neighboring residues appears to help organize the catalytic site of PPT2. The catalytic triad residues form hydrogen bonds with each other in the fashion expected (Fig. 2a) (Ser-111 (OG)-His-283 (NE2) = 2.71 Å and Asp-228 (OD2)-His-283 (ND1) = 2.95 Å). Among the hydrogen bonds between the catalytic triad residues and the neighboring residues are His-283 (N)-Tyr-110 (OH) (2.61 Å), which cross-links the loop containing His-283 with β5 containing Ser-111. In addition, Ser-111 makes hydrogen bonds with the carbonyl oxygen of Leu-133 and the amide nitrogen of Gly-112 (Ser-111 (N)-Leu-133 (O) = 2.94 Å and Ser-111 (N)-Gly-112 (N) = 3.35 Å). Also part of the network with neighboring residues are the hydrogen bonds that the carbonyl oxygen atoms of Asp-228 make with the main chain amide nitrogen atoms of Val-229, Ile-230, and Trp-286 (Asp-228 (OD1)-Val-229 (N) = 3.00 Å, Asp-228 (OD1)-Ile-230 (N) = 2.87 Å, and Asp-228 (OD2)-Trp-286 (N) = 2.51 Å) and with the hydroxyl oxygen of Gly-112.

"elbow" and has the unfavorable geometry (Ramachandran plot) of ϕ = 45°, ψ = -128°, which is a characteristic of this feature. The sequence motif surrounding the nucleophilic serine (Ser-111) shared by most lipases and thioesterases, ψ-X-ϕ-X-Gly-X-Ser-X-Gly-X-Gly-X-ψ (where ψ is any hydrophobic residue), is fairly conserved in PPT2. Two substitutions (indicated in bold), Cys-109 for Gly and Cys-115 for ψ, alter the formation of an intramolecular disulfide bridge that plays an important role in stabilizing Ser-111 (see above).
atom of Ser-136 (Asp-228 (OD2)-Ser-136 (OG)) with a low B-factor (16 Å²) forms a hydrogen bond with Ser-111 (OG) (2.86 Å) and with the amide nitrogen atoms of Gln-116 (3.07 Å) and Leu-41 (3.16 Å). This hydrogen bond network has His-283 as the common vertex of two linked triangles. The vertices of the first triangle are Ser-111, Tyr-110, and His-283; the vertices of the second triangle are Asp-228, Trp-286, and His-283.

Structure Comparisons between PPT2 and PPT1—Although the sequence identity between PPT2 and PPT1 is only 18% (Fig. 3a), their three-dimensional structures are very similar with an overall root mean square difference for Cα atoms of 1.25 Å (Fig. 3b). The spatial relation of the catalytic triad with respect to the elements of the fold and the positions that the residues hold with respect to each other and with other residues is well preserved between the two proteins. The catalytic residues in PPT1, Ser-115, Asp-233, and His-289, have an average root mean square difference for their Cαs of 0.3, 0.7, and 1.08 Å, respectively, with the corresponding residues of PPT2. The hydrogen bonds within the catalytic triad are also present in PPT1. The hydrogen bonds from Ser-115 to the Gly-116 amide nitrogen Ser-115 (O)-Gly-116 (N) (3.2 Å) and to the Val-139 carbonyl oxygen Ser-115 (N)-Val-139 (O) (3.1 Å) are conserved between the two structures.

Both PPT1 and PPT2 have a lipid-binding groove leading away from the active site. This groove, which holds the fatty acid portion of the thioester to be cleaved, is very similar in both proteins (Fig. 4a). Although some residue types are not the same, the positions are very similar. The conserved residues Leu-161, Tyr-178, and Trp-179 have root mean square Cα deviations of 0.9, 0.5, and 0.6 Å, respectively. The Ala-171/Ala-183 pair in PPT1 is substituted in PPT2 by Cys-165/Cys-176 and has root mean square Cα deviations of 0.8 and 0.5 Å, respectively. The two cysteine residues form a disulfide bridge located 3.8 Å below the plane of the palmitate observed in PPT1. The substitution of Ile-175 in PPT2 for the polar residue Gln-182 in PPT1 would not be expected to affect lipid binding because Gln-182 makes contacts with the lipid through its Cβ and Cγ atoms. Despite the fact that we have not yet obtained the structure of PPT2 in complex with a substrate (e.g. palmitate) or an inhibitor, the overlap between the two structures shows that the palmitate in PPT1 fits almost perfectly inside

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**Fig. 3. Sequence and structure alignments.** a, sequence alignment of PPT2 versus PPT1 calculated using Mulalign (20) and rendered using Alscript (21). Identical residues are highlighted in yellow. Catalytic residues are indicated with green arrows. b, superimposition of the Cα trace between PPT2 (blue) and PPT1 (red) using LSQMAN (28).
Contrary to the similarities observed in the binding groove, superposition of the two structures at the β6-α4 loop in PPT2 (β6-α4 in PPT1) shows striking differences. As illustrated in Fig. 4b, the main chain of loop β6-α4 in both proteins runs almost parallel to the palmitate. At Pro-150 the β6-α4 loop of PPT1 makes a right angle turn in a direction opposite to the binding groove, and as a result it is exposed to solvent. We refer to this as the open conformation (Fig. 5b). Moreover, the position of this loop is stabilized by a disulfide bridge formed by Cys-152 to Cys-160 and by a hydrogen bond Arg-151 (NE)-Thr-
In contrast, the $\beta_6\alpha_4$ loop of PPT2 turns roughly 60° (Fig. 4a) toward the binding groove, allowing helix $\alpha_4$ to form the lid of a binding pocket (Fig. 5a). This change to a closed conformation buries a surface area of 1250 Å$^2$. To accommodate a hydrophobic substrate the inner surface of helix $\alpha_4$ is lined with hydrophobic residues and makes weak contacts with residues from the binding groove (Leu-149 (CD1)-Leu-161 (CD1), Leu-152 (CD1)-Ile-175 (CD1), and Phe-153 (CZ)-Phe-173 (CE2)). None of the residues of helix $\alpha_4$ make hydrogen bonds with nearby residues, and helix $\alpha_4$ is described as the lid of the active site.

Previous experiments in the laboratory had shown that PPT1 and -2 had thioesterase activity against many palmitate substrates but that there was a group of substrates (palmitate bound to the CAAX box of H-Ras for example) for which PPT2 had no thioesterase activity (8). To study the substrate specificity more carefully and to find possible PPT2 substrates for which PPT1 has no activity, we performed a series of experiments in which either the fatty acid head group or fatty acid chain length was varied.

Probing Thioester Head Groups—The data in Table II show that both PPTs have similar activity against linear molecules such as S-palmitoyl-CoA or S-palmitoyl-N-acetylcysteamine; however, the activity of PPT2 against substrates with "branched" or bulky head groups, such as S-palmitoyl-H-Ras, S-palmitoyl-N-acetyl-O-carboxymethyl-cysteine, or 4-methylumbelliferyl-6-S-palmitoyl-β-D-glucopyranoside, was almost undetectable (Table II). The structures of PPT1 and -2 help explain the head group specificity. In both structures, the $\beta_3\alpha_4$ loop and the $\beta_8\alpha_F$ loop are parallel to each other and are directly above the lipid binding site; therefore, an intact substrate must fit between these two loops. As shown in Fig. 6a, the separation between these loops is larger in PPT1 than in PPT2 by roughly 8 Å (on average). Hence PPT2 cannot bind lipid groups with branched side chains because there is simply not enough space in this region to accommodate them. The presence of a vicinal disulfide bridge (Cys-45 to Cys-46) in the $\beta_3\alpha_4$ loop of PPT1 (Fig. 6a) suggests that the wider separation in PPT1 is enforced by this disulfide bond.

Probing Fatty Acid Chain Length—Previous experiments have shown that PPT1 is able to hydrolyze long chain fatty acyl CoAs with an optimum fatty acid chain length of 14–18 carbons (7). In the experiment shown in Fig. 6b, we examined the catalytic activity of PPT1 and -2 as a function of fatty acid chain length (Fig. 6b). First of all, the activity of both proteins against 14–18 carbon fatty acids is similar. More importantly, PPT2 has significantly higher thioesterase activity against lipids with chain lengths of fewer than 10 carbons or lipids with very large chain lengths (18 or more carbon atoms). Comparison of the architecture of the binding site between the two proteins provides the structural basis for these data. Fig. 4a shows that PPT1 cannot bind lipid chains below 10 carbons because they are not long enough to make contacts with Trp-186, Gln-182, and Tyr-185, which probably hold the lipid firmly in the binding site. On the other hand, PPT2 can bind shorter lipids (above six carbons) because the lid has additional contacts that facilitate binding (Figs. 4b and 5b).

Conclusions—The crystal structure of PPT2 reveals that the core of the protein possesses an $\alpha_6\beta$ hydrolase fold with a catalytic triad formed by residues Ser-111, Asp-228, and His-283. Two disulfides help fix the position of Ser-111 and His-283. An extension to the $\alpha_6\beta$ hydrolase fold of six small $\alpha$ helices forms a lipid binding domain. The lipid binding domain of PPT2 forms a binding pocket for the fatty acid chain that consists of

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Relative Catalytic Efficiency (Km; Vmax)</th>
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<tbody>
<tr>
<td>S-palmitoyl-CoA</td>
<td></td>
<td>PPT1 1.0* (51 μM; 0.72U/mg)</td>
</tr>
<tr>
<td>S-Palmitoyl-N-acetylcysteamine</td>
<td></td>
<td>PPT2 1.8 (67 μM; 1.7 U/mg)</td>
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<tr>
<td>S-Palmitoyl N-acetyl-O-carboxymethyl-cysteine</td>
<td></td>
<td>PPT1 6.5 (34 μM; 3.2 U/mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPT2 6.3 (37 μM; 3.3 U/mg)</td>
</tr>
<tr>
<td>S-Palmitoyl H-Ras</td>
<td></td>
<td>PPT1 3.7 (48 μM; 2.5 U/mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPT2 &lt;0.1 (n.d.)</td>
</tr>
<tr>
<td>4-methylumbelliferyl-6-S-palmitoyl-β-D-glucopyranoside</td>
<td></td>
<td>PPT1 0.9 (14 μM; 0.18 U/mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPT2 &lt;0.1 (n.d.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPT1 5.9 (130 μM; 10.8 U/mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PPT2 0.26 (117 μM; 0.43 U/mg)</td>
</tr>
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</table>

* Relative catalytic efficiency is defined as $V_{max}/K_m$ relative to the value for PPT1 using S-palmitoyl-CoA as a substrate. One unit (U) is equal to one μmol of palmitate released per minute.
an α4 and a binding groove formed by α5-9. Residues lining the interior surface of the lid are hydrophobic and make weak contacts (≥3.5 Å) with residues in the binding groove.

The presence of a lid in PPT2 is worthy of comment as similar lid subdomains are found in the atomic structures of bacterial lipases. Topologically the bacterial lipases consist of a variant of the α/β hydrolase fold and a cap domain, which includes a lid subdomain (helices α4 and -5). The catalytic triad in bacterial lipases is buried (16, 17); the substrate gains access to the lid under the lid in PPT2 due to the restricted space between the β8-αF loop and β3-αF and β8-αF in PPT2 (steel blue) and PPT1 (red). The orientation used in this figure is the same orientation used in Fig. 4. The position of the palmitate residue corresponds to the position observed in the crystal structure of PPT1 (for review, see “Results and Discussion”) (10). b, fatty acid specificity of PPT1 and -2. Fatty acyl CoA of varying chain length were examined. Values shown are the average of three sets of experiments expressed as a percentage of maximum activity using C14:0 as a substrate for PPT1. Absolute maximal values were 1.0 and 2.1 µmol of acyl CoA hydrolyzed/min/mg of protein for PPT1 and -2, respectively.

Fig. 6. Fatty acid specificity. a, Corey-Pauling-Koltun and ribbons representation of a superimposition between residues in loops β3-αF and β8-αF in PPT2 (steel blue) and PPT1 (red). The orientation used in this figure is the same orientation used in Fig. 4. The position of the palmitate residue corresponds to the position observed in the crystal structure of PPT1 (for review, see “Results and Discussion”) (10).
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