Cloning, Expression, and Characterization of a Human 4'-Phosphopantetheinyl Transferase with Broad Substrate Specificity*

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A single candidate 4’-phosphopantetheine transferase, identified by BLAST searches of the human genome sequence data base, has been cloned, expressed, and characterized. The human enzyme, which is expressed mainly in the cytosolic compartment in a wide range of tissues, is a 329-residue, monomeric protein. The enzyme is capable of transferring the 4’-phosphopantetheine moiety of coenzyme A to a conserved serine residue in both the acyl carrier protein domain of the human cytosolic multifunctional fatty acid synthase and the acyl carrier protein associated independently with human mitochondria. The human 4’-phosphopantetheinyl transferase is also capable of phosphopantetheinylation of peptideyl carrier and acyl carrier proteins from prokaryotes. The same human protein also has recently been implicated in phosphopantetheinylation of the α-aminoacidopentane semialdehyde dehydrogenase involved in lysine catabolism (Praphanphonj, V., Sacksteder, K. A., Gould, S. J., Thomas, G. H., and Geraghty, M. T. (2001) Mol. Genet. Metab. 72, 336–342). Thus, in contrast to yeast, which utilizes separate 4’-phosphopantetheine transferases to service each of three different carrier protein substrates, humans appear to utilize a single, broad specificity enzyme for all postranslational 4’-phosphopantetheinylation reactions.

The fatty acid synthases (FASs)† associated with the soluble cytoplasm of yeast and animal cells comprise large multifunctional polypeptides that contain all of the catalytic components required for the synthesis of long-chain fatty acids from malonyl-CoA de novo. These multifunctional polypeptides are commonly referred to as type I FASs. The animal FASs consist of two identical polypeptides of approximately 2500 residues (αy), whereas the yeast FAS comprises six copies each of two non-identical polypeptides (αyβy; α = 1845, β = 1887 residues) (1, 2). In most bacteria and in plant plastids, the various catalytic components of the FASs are present on separate discrete polypeptides and are commonly referred to as type II FASs (3). An essential component of both type I and type II systems is a small molecular mass domain/poly peptide known as an acyl carrier protein (ACP) that is posttranslationally modified, by insertion of a 20-Å-long phosphopantetheinyl moiety, derived from CoA, to a positionally conserved serine residue (4). The terminal sulfhydryl of the phosphopantetheinyl moiety provides the site of covalent attachment of the substrates and the growing fatty acyl chain, so that the phosphopantetheine plays an essential role as a “swinging arm” in the translocation of intermediates between different catalytic sites of the FASs (5). ACPs fulfill a similar role in the type I and type II polyketide synthases (PKSs) found mainly in bacteria and fungi that are capable of elaborating a broad range of secondary metabolites (6, 7).

Fungi (6, 7), animal, and plant cells also contain a type II FAS system in their mitochondria. The role of the mitochondrial FAS is not well established, but it has been suggested that, at least in fungi and plants, they may serve to provide octanolate, the precursor of lipic acid and/or long-chain fatty acids that are used in the remodeling of mitochondrial phospholipids (7–10). The ACP component of the mitochondrial FAS appears to be associated with the respiratory complex I in animals and in Neurospora crassa (11, 12).

Phosphopantetheinylated carrier proteins also play an essential role as components of the non-ribosomal peptide synthases found in microorganisms that are responsible for producing a variety of short peptides containing both proteogenic and unusual amino acids (13). The nonribosomal peptide synthases also comprise multifunctional polypeptides in which the role of the carrier protein domain is to translocate amino acyl moieties from an adenylation domain to a condensation domain, where formation of a new peptide bond takes place (14).

Enzymes capable of phosphopantetheinylating carrier proteins involved in the biosynthesis of fatty acids, polyketides, and peptides have been identified and characterized from a variety of sources. Many organisms have more than one phosphopantetheine transferase (PPTase), and different PPTases commonly are utilized to service carrier proteins associated with FASs and non-ribosomal peptide synthases in the same species (4, 15). Yeast utilizes separate PPTases for phosphopantetheinylation of the ACP domain of the cytosolic type I FAS and the mitochondrial type II ACP. Remarkably, the former is actually a constituent domain of the α-subunit of the type I cytosolic FAS, so that this protein is capable of self-phosphopantetheinylation (16), whereas the latter is a separate PPTase that acts only on the mitochondrial ACP (17).

PPTases have been classified into three groups, based on sequence and structural similarity and substrate specificity (15). Members of the first group, typified by the AcpS of Esch-
erichia coli, are characterized by ~120 residues long, function as homotrimers, and have fairly narrow substrate specificities limited to the ACPs of type II FAS and PKS systems (18). Members of the second group, typified by the Sfp of Bacillus subtilis, are usually at least 240 residues long, function as homotrimers, and have very broad substrate specificities that include carrier proteins associated with non-ribosomal peptide synthetases, FASs, and PKSs (18, 19). Membership in the third group is reserved for those PPTases that are attached covalently to type I FASs, such as that associated with the yeast cytosolic FAS (16). The PPTases involved in phosphopantetheinylation of the ACPs involved in fatty acid synthesis in animals have not yet been identified. Thus, the objective of this study was to identify the PPTase(s) responsible for posttranslational modification of the ACP domain of the type I cytosolic FAS and the ACP associated with the type II mitochondrial FAS in humans. EXPERIMENTAL PROCEDURES Cloning, Expression, and Purification of Human PPTase—Based on the available sequence information (accession nos. GI 71085636, GI 9295190, and EST cDNA ATCC 1840095), PCR primers (Table I) were designed and used to amplify full-length (~1 kb) DNA from human liver and brain cDNA libraries (Clontech, Palo Alto, CA). The PCR procedure employed was essentially as described previously (20). To improve the specificity of the PCR, two sets of primers were used to amplify cDNA. The first round of PCR, with primer pair T4/B3 and template DNA from the two human cDNA libraries, resulted in the amplification of an expected sized (~1 kb) product, indicating the presence of PPTase transcripts in both the libraries. The amplified DNA from the first round PCR reaction was then used as template for the second round, using a nested PPTase-specific primer pair T2/B1. Again, an amplified product of ~1 kb was obtained using DNA template arising from both human cDNA libraries. The amplified fragment from the two sources was purified, using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA), digested at KpnI and PstI sites that had been engineered into the amplification primers T2 and B1, respectively, and cloned into the E. coli expression vector pQE80 L (Qiagen). To facilitate purification of the encoded proteins by metal ion affinity chromatography, a cleavable His6 tag was engineered at the start of the cloned cDNAs. Authenticity of the cloned PCR fragments was confirmed by DNA sequencing using vector-specific primers. The sequences of the amplified cDNAs from the two libraries were found to be identical. The amino acid sequence of the encoded protein is shown in Fig. 1. Protein expression from the cloned cDNA was attempted in E. coli DH5α and BL21-codon plus strains; however, most of the expressed PPTase was found to be present in inclusion bodies. To overcome this problem, the cDNA encoding the putative human PPTase in pQE80 L was reamplified using PPTase primers T9 and B5 and cloned into a modified baculoviral transfer vector, pFast Bac 1 (Qiagen). The putative N-terminal targeting sequence for the ACPmit, which was deleted for expression from the cloned cDNA. Authenticity of the cloned, amplified PCR fragments was confirmed by DNA sequencing using vector-specific primers. The clones derived from liver and brain libraries had identical sequences. The encoded amino acid sequence is shown in Fig. 1. The recombinant plasmid was expressed in the E. coli BL21-codon plus strain. Cells were grown at 37 °C in LB medium to an optical density of 0.5–0.7 and induced with 1 mM IPTG for 3 h. Cells were suspended in 0.25 M potassium phosphate buffer, pH 7, containing 10% glycerol and protease inhibitors, and lysed using a French pressure cell press. The lysate was centrifuged at 50,000 × g for 1 h at 4 °C. The recombinant ACP was purified from the soluble extract of E. coli cells, by a combination of nickel ion affinity chromatography and gel filtration. The soluble extract was applied to a nickel-nitrioltriacetic acid column equilibrated with 0.25 M potassium phosphate buffer, pH 7, and 10% glycerol. The column was then washed sequentially with the equilibration buffer containing 50, 100, and 250 mM imidazole. Fractions containing ACPmit (eluting at 100 mM imidazole) were pooled, concentrated using a Vivaspin concentrator, and chromatographed on a column of Biogel P30 (40 × 1.4 cm column eluted with 0.1 M potassium phosphate buffer, pH 7, at 13 mL/h, 20 °C). Cloning, Expression, and Purification of the Human Mitochondrial ACP (ACPmit)—Based on BLAST searches of the human genome sequence data base, using authentic type I and type II ACPs, we identified a putative ACPmit sequence that was represented in two human EST IMAGE clones 4799845 and 4816243. Sequence alignments with authentic type II ACP sequences indicated that these ESTs likely encoded a full-length human ACPmit, together with an N-terminal extension of ~67 residues that could constitute a mitochondrial targeting sequence. Specific primers, ACPmit T1/B1 (Table I) with appropriate restriction sites, were designed, and the cDNA encoding the putative ACPmit was amplified using the PCR, essentially as described previously (20). The amplified fragments from the two sources were purified using a QIAquick PCR purification kit (Qiagen), then cleaved at the introduced KpnI and PstI sites and cloned into the E. coli expression vector pQE80 L (Qiagen). To facilitate purification of the encoded protein by metal ion affinity chromatography, a cleavable His6 tag was engineered at the start of the ACPmit. Authenticity of the cloned, amplified fragments was confirmed by DNA sequencing using vector-specific primers. The sequences of the amplified fragments from the two IMAGE clones were found to be identical. The sequence of the encoded protein is shown in Fig. 1. The cloned cDNA was expressed in E. coli
BL21-codon plus strain. Cells were grown at 37 °C in LB medium to a density equivalent to A600 of 0.5–0.7 and induced with 1 mM IPTG for 3 h. However, no recombinant protein could be detected. Induction of protein expression resulted in rapid cessation of cell growth, suggesting that expression of this protein interferes with the host cell metabolism. Lowering the IPTG concentration or growth temperature, reducing the induction time, or using alternative host strains and expression vectors did not improve expression.

To overcome this difficulty, the ACPmit gene was re-amplified using two sets of primers, ACPmitT/B2/B2 (contains the putative mitochondrial targeting sequence) and ACPmitT3/B2 (no targeting sequence) (see Table I). The two amplified fragments were cloned into a modified baculoviral expression plasmid. Restriction sites or restriction site-specific overhangs are underlined. Sequences. Bold letters indicate engineered start/stop codons. The restriction sites were used to clone individual fragments into pQE80L or modified Fast Bac 1 plasmid. Restriction sites or restriction site-specific overhangs are underlined.

For purification of the cytosolic form, insect cells were homogenized and centrifuged at 4 °C in accession no. GI 7106836, for ACPfas specific primers base pairs are according to GI 915391, while for ACPmit the numbers are according EST clone IMAGE-5287610.

**TABLE I**

| Primers | Sequence | Location* |
|---------|----------|-----------|
| PPTase T2 | 5'-atcatggtaccATGGTTTCTGCAGAAGATTTCT | 167-191 bp |
| PPTase T4 | 5'-ATAGGGCGGTCGCTCGTTCAGT | 141-164 bp |
| PPTase B1 | 5'-atatggtcagTCAGACTTTGTTACATTCTGTAT | 1073-1096 bp |
| PPTase B3 | 5'-ATITCCCCTGTTATACGAGGATTAT | 1096-1122 bp |
| PPTase T9 | 5'-cataacgcgccgGATACGATATCCCCAACGACGGA | 5-28 bp |
| PPTase B5 | 5'-gctttgcacgccCATAGTTCTTCATCTTG | 1074-1096 bp |
| ACPfas T | 5'-tttcaggtaccAGGACAGGACGCCAAGGGA | 6472-6494 bp |
| ACPfas B | 5'-tatataaatcctcCTCTGGGCTGCGGATCC | 6716-6738 bp |
| ACPmit T1 | 5'-atcatggtaccATGGCCGTCGCTCGTTCCTTA | 15-35 bp |
| ACPmit B1 | 5'-atatggtcagTTATCATATACTATCTCTTATCTG | 460-485 bp |
| ACPmit T2 | 5'-ctatttacagccATGGCCGTCGCTCTGTCCTTCAGCCTAT | 15-41 bp |
| ACPmit T3 | 5'-catttgacgccatGATACGATATCCCCAACGACGGA | 216-237 bp |
| ACPmit B2 | 5'-ctatttacagccTTATACGATATCCCCAACGGGTA | 461-482 bp |
| TEV linker | 5'-cttgacgattacagatatatcctacctacacgga | | |
| Tev T | | | |
| Tev B | 5'-cctggaaaaatacaggttttctgctggtagatatcagaggta | | |

* The base pair numbers for PPTase T9 correspond to Tev T linker; numbers for other PPTase-specific primers are according to PPTase sequence in accession no. GI 7106836, for ACPfas specific primers base pairs are according to GI 915391, while for ACPmit the numbers are according EST clone IMAGE-5287610.
sisted laser desorption ionization plate. Analysis was performed on a Voyager-DE™ STR matrix-assisted laser desorption ionization time-of-flight biospectrometry workstation (Applied Biosystems, Foster City, CA) in a positive linear mode. Mass scale was externally calibrated utilizing bovine insulin and horse heart myoglobin. Singly charged ions were used for a molecular mass determination. Reported masses represent averages of the results of six acquisitions, and they have been determined within a standard deviation of 1–2 Da.

**Tissue Specificity of Expression of Human PPTase**—A 32P-labeled probe was synthesized by the random priming method using [α-32P]dCTP (3000 Ci/mmol), a PCR-amplified PPTase template cDNA, and a NEBlot kit (New England Biolabs, Beverly, MA). A human multiple tissue Northern blot (Clontech) was probed with the purified radiolabeled probe (~2 × 10⁶ dpm/blot), using ExpressHyb hybridization solution (Clontech). The amount of poly(A)⁺ RNA in each lane of the Clontech blot (~2 μg) has been adjusted to obtain a consistent signal for a housekeeping gene (β-actin) across all lanes. The blot was analyzed using a phosphorimager (Storm 840, Amersham Biosciences).

**Subcellular Fractionation**—Cells were harvested by centrifugation at 600 × g for 5 min, washed with phosphate-buffered saline, and resuspended in 5 volumes of mitochondrial isolation buffer (210 mM mannitol, 70 mM sucrose, 5 mM HEPES-KOH, pH 7.35, 1 mM EDTA) containing a protease inhibitor mixture (EDTA-free protease inhibitor cocktail, Roche Applied Science). The cellular suspension was disrupted, using a Teflon/glass homogenizer, and centrifuged at 800 × g for 5 min at 4 °C to remove cell debris. The supernatant was then centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was collected and further centrifuged at 104,000 × g for 30 min at 4 °C, and the mitochondrial pellet was washed twice with isolation buffer and resuspended in 100 μl of the same buffer. The mitochondria were then ruptured by three cycles of freezing and thawing and the addition of Lubrol (0.1 mg/mg of protein). The 104,000 × g supernatant (cytosol) was collected, and the pellet was resuspended in 200 μl of isolation buffer. Protein concentrations of all three subcellular fractions were determined by the Bradford assay and the fractions stored at −80 °C.

The activity of citrate synthase, a mitochondrial matrix enzyme, was assayed (22) in each subcellular fraction to assess the possibility that mitochondria had been damaged during preparation of the homogenate.

**RESULTS**

**Cloning and Expression of a Human PPTase**—Initial BLAST searches of the GenBank™ sequence data base, using the sequences of various PPTases of the small molecular mass PPTase type, did not reveal a likely candidate for a human PPTase enzyme. However, BLAST searching with a PPTase probe sequence of the high molecular weight type, namely the B. subtilis surfactin synthetase-activating enzyme, Sfp, revealed a candidate human PPTase sequence that was subsequently used to derive full-length cDNAs, by PCR, from human liver and brain cDNA libraries and from a human EST cDNA clone. The putative human PPTase cDNAs obtained from the three sources had identical DNA sequences. When expressed in E. coli, this cDNA generated the expected protein, but it was recovered entirely in inclusion bodies. Expression as a baculoviral vector in S9 cells, however, yielded a soluble protein of the expected molecular mass (~39 kDa, including His₆ and TEV protease recognition site) that could be readily purified to homogeneity by a combination of nickel-nitrilotriacetic acid affinity chromatography and anion exchange HPLC (Fig. 2A).

Analysis of the purified protein by gel filtration under non-denaturing conditions revealed that the enzyme is a monomer (Fig. 3). The typical yield of purified protein was 5 mg/15 g of cells.

**Cloning and Expression of the ACP Domain of the Human Cytosolic FAS**—A cDNA encoding the ACP domain of human FAS (ACP₉₋₁₀ residues 2117–2205) was amplified from a human liver cDNA library, cloned into the pQE80 expression plasmid, and its sequence verified. When transfected into E. coli, this cDNA was expressed as a soluble protein (typically 4–8 mg/liter of culture medium) that was readily purified to homogeneity by a combination of nickel affinity chromatography and gel filtration (Fig. 2B). Analysis by electrospray mass spectrometry confirmed that, as anticipated, the ACP₉₋₁₀ was expressed as the apo form, lacking the phosphopantetheine moiety (Table II). Most of the ACP₉₋₁₀ purified as a monomer, but a small portion had a mass corresponding to that of a covalently linked dimer. In the presence of reducing agents, the dimeric form was converted to the monomeric 13,067-Da form (Table II), suggesting that the single cysteine residue present near the C terminus (Cys-2200) may be involved in inter-polypeptide disulfide formation.

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s9 cells. SDS-PAGE of the protein that had been partially purified by nickel-nitrotriacetic acid affinity chromatography revealed the presence of two molecular species that subsequently could be separated by anion exchange chromatography (Fig. 4). The molecular masses of two species differed by 341 Da, corresponding exactly to the theoretical mass difference of the apo and holo forms of the ACPmit.

Activity of Human PPTase toward the Apo-ACP Domain from the Cytosolic FAS—The mass of apo-ACPfas was increased by 341 Da after treatment with CoA, Mg2+, and human PPTase. This is the anticipated increase that would result from the transfer of the 4-phosphopantetheine moiety from CoA to Ser-2154 of the ACPmit. With an excess of PPTase enzyme, the apo-ACPfas could be quantitatively converted to the holo form. The human PPTase, in common with its prokaryotic counterparts, appears capable of transferring the 4-phosphopantetheine moiety from CoA to apo-ACP, regardless of whether the CoA is presented in the free thiol form or as an acetyl thioester. Thus, when CoA is replaced by [1-14C]acetyl-CoA, the radiola-

The discrepancy between theoretical and experimentally determined mass (42-44 Da) is most likely the result of N-acetylation or carbamoylation of the ACPmit.

Activity of Human PPTase toward the Apo-ACP Domain from the Mitochondrial FAS—The ability of human PPTase to phosphopantetheinylate two heterologous carrier proteins was also tested using two apo carrier proteins from bacilli: ACP-A, which is involved in fatty acid synthesis in B. subtilis and TycC3-PCP, the third PCP domain of the Bacillus brevis tyrosine synthetase 3 (21). Both were phosphopantetheinylated by the human enzyme; indeed, the affinities of the enzyme for the human ACPmit and PCPwere very similar (Table III). Ranked according to catalytic efficiency, the human PPTase showed preference for human ACPfas > human ACPmit > PCP. B. brevis > ACP. B. subtilis.

Tissue Distribution and Subcellular Location of Human PPTase—The human PPTase appears to be expressed in a wide range of tissues, including heart, skeletal muscle, brain, pancreas, kidney, and liver, and thus is clearly not confined to lipogenic tissues (Fig. 7).

Activity of endogenous PPTase could be measured in extracts of cultured human cell lines, using the standard radiochemical assay with added apo-ACPmit as the acceptor. More than 90% of the activity was recovered in the cytosolic fraction derived from both SKBr3 and HepG2 cells. In contrast, 91% of the mitochondrial matrix enzyme citrate synthase was recovered in the mitochondrial fraction, indicating that the PPTase recovered in the cytosol could not have resulted from mitochondrial damage during tissue homogenization (details not shown).

**DISCUSSION**

Only one putative PPTase could be found by sequence analysis of the human sequence data base. Cloning, expression, and characterization of this protein revealed that it is of the high molecular mass, monomeric type and has a broad specificity that includes not only the ACPs associated with both cytosolic and mitochondrial FAS systems in humans but also heterolo-
guous bacterial carrier proteins of both the ACP and PCP type. Similar BLAST searches of the Mus musculus, Drosophila melanogaster, and Caenorhabditis elegans data bases, using probes representative of all three classes of PPTase, gave similar results in that only the high molecular mass, monomorphic type of PPTase could be detected. These BLAST searches have been repeated as recently as April 2003. Thus, none of these animal species appears to have a PPTase of the type that typically phosphopantetheinylates ACPs associated with fatty acid and polyketide synthesizing systems in microorganisms.

In fungi, a key step in the biosynthesis of lysine is the reduction of α-aminoacidopropionate to the semialdehyde. The reaction requires two proteins, products of the LYS2 and LYS3 genes.
The LYS2 gene product is a 155-kDa multifunctional protein that contains an adenylation domain, PCP domain, and reductase domain, and the LYS5 gene product is a 31-kDa PPTase that catalyses conversion of the apo-PCP domain of the apo-PCP to the holo form (24). Although in humans lysine is an essential amino acid, a similar enzyme system apparently is involved in the degradation of lysine. Thus, conversion of apo-PCP to holo-PCP is catalyzed by apo-PCP dehydrogenase, an enzyme that contains a PCP-like domain that undergoes posttranslational phosphopantetheinylolation (25).

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FIG. 6. Phosphopantetheinylolation of human apo-ACP in vitro by human PPTase. Assays were performed as described under "Experimental Procedures." Reaction mixtures included 50 ng of PPTase, and the duration of the incubation was 2 min. The inset shows SDS-PAGE analysis of the apo-ACP substrate (lane 1) and the holo-ACP product (lane 2), together with molecular mass standards (lane 3).

FIG. 7. Tissue specificity of human PPTase expression. A 32P-labeled human PPTase probe was used to screen for expression of the mRNA in a human tissues using a multitissue Northern blot (Clontech).

### Table III

| (Apo) carrier protein | $K_m$ | $V_{max}$ | $k_{cat}/K_m$ |
|-----------------------|-------|-----------|---------------|
| Human ACM             | 3.1   | 288       | 3.6           |
| Human ACM mut         | 7.5   | 188       | 1.0           |
| B. brevis PCP         | 6.4   | 98        | 0.6           |
| B. subtilis ACP-A     | 21    | 24        | 0.05          |

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sequence encoded the PPTase responsible for phosphopantetheinylation of the human α-aminoadipate semialdehyde dehydrogenase (25). Northern analysis revealed that the human PPTase is expressed as a 3-kb RNA in a broad range of tissues, essentially as we report in this study. The human PPTase gene was mapped to chromosome 11q22.

Thus, in humans, a single PPTase appears to be responsible for posttranslational modification of carrier proteins in three different metabolic pathways: the cytosolic de novo fatty acid biosynthetic pathway, the mitochondrial fatty acid biosynthetic pathway, and the lysine degradative pathway. The human PPTase is located predominantly in the soluble cytoplasm, implying that this is the site of posttranslational modification of all three carrier proteins, regardless of their ultimate destination within the cell. Thus, the apo-ACP<sub>mut</sub> appears to be phosphopantetheinylated prior to importation into the mitochondria. The actual subcellular location of α-aminoadipate semialdehyde dehydrogenase is in doubt at present. Prapanthoj et al. (25) have suggested that the lysine degradation pathway is located in peroxisomes, although the α-aminoadipate semialdehyde dehydrogenase lacks the typical peroxisomal targeting sequence motif.

The situation in animals is in complete contrast to yeast, where PPTases representative of all three classes of PPTase are present. The first, PPTase 2, is a typical low molecular mass PPTase that appears to be responsible solely for phosphopantetheinylation of the mitochondrial apo-ACP (it is unclear at present whether the phosphopantetheinylation takes place in the mitochondria or in the cytosol, prior to entry of the ACP into the mitochondria). The second, LYS5, is a typical high molecular mass PPTase that appears to be responsible solely for phosphopantetheinylation of the carrier protein domain of α-aminoadipate reductase, the LYS2 gene product. The third PPTase is that found at the C terminus of the yeast FAS α-subunit, which is responsible for the phosphopantetheinylation of the apo-ACP domain of the FAS.

The phylogenetic relationships of the three classes of PPTases are illustrated in Fig. 8. All PPTases represented in the dendogram bear the characteristic signature of conserved residues: GxD...E..E...W/FL/xK/R/E'x(A/S)xK/#. Crystal structures for both the low molecular mass, trimeric type (27) and the high molecular mass, monomeric type (28) of PPTases have revealed that the acidic residues marked * are involved in binding of Mg<sup>2+</sup>; the lysine (#) residue binds the α-phosphate of CoA, and the two residues marked % form an internal salt bridge. As mentioned earlier, all of the animal PPTases found in the sequence data bases belong to the high molecular mass, monomeric type and are clustered in the upper half of the dendogram. The three yeast PPTases are more closely related to PPTases of other species than they are to each other. The PPTase involved in phosphopantetheinylation of the yeast mitochondrial apo-ACP appears to be of the low molecular mass, monomeric type, and that associated with the α-subunit of the yeast FAS also appears to be more closely related to the low molecular mass type. On the other hand, the yeast LYS5 PPTase is a member of the high molecular mass, monomeric class, more closely related to the animal PPTases, in agreement with the finding that the human PPTase is able to complement the yeast LYS5 mutation.

Although many other microorganisms also utilize more than one PPTase, especially for primary (low molecular mass, trimeric AcpS-type) and secondary (high molecular mass, monomeric Sfp-type) metabolism, the situation in humans is not unique. Recently, both prokaryotic and eukaryotic organisms have been identified that appear to use a single PPTase for servicing carrier proteins associated with both primary and secondary metabolic pathways. For example, Marahiel's team have reported that Pseudomonas aeruginosa harbors but one PPTase that is responsible for the phosphopantetheinylation of the ACP required for fatty acid biosynthesis and the PCPs required for biosynthesis of the non-ribosomally produced peptide siderophores pyoverdin and pyochelin (29). In common with the situation in humans, the PPTase of P. aeruginosa is of the high molecular mass, monomeric class and, of course, this enzyme too, displays a very broad substrate specificity. Based on sequence analysis, Marahiel and collaborators (29) also identified Haemophilus influenzae and Synechocystis PCC6803 as organisms that have “lost” their AcpS-type PPTase and likely rely on a Sfp-type PPTase, and they simulated this evolutionary loss in B. subtilis by disruption of the gene for AcpS; the AcpS mutant strain exhibited wild-type phenotype. More recently a PPTase has been identified in Aspergillus nidulans (cfuA2/npgA) that appears to be utilized in the phosphopantetheinylation of a number of different carrier proteins involved in various metabolic pathways including the PCP domains associated with the δ-(1-α-aminoadipyl)-1-cysteylnyl-β-valine synthase involved in penicillin biosynthesis, the polyketide synthase ACP involved in conidial pigment formation, the carrier protein domain of the α-aminoadipate reductase involved in lysine biosynthesis, and perhaps the mitochondrial ACP (30).

In conclusion, the apparent absence of a low molecular mass, trimeric type of PPTase in any of the animal sequence data bases, the broad substrate specificity of the human PPTase characterized in this study, together with the ability of this protein to complement the yeast LYS5 mutation, constitutes strong evidence that a single type of PPTase likely is responsible for servicing all carrier proteins in animals, the ACP<sub>mut</sub>, and that associated with α-aminoadipate semialdehyde dehydrogenase. Nevertheless, it is conceivable that an as yet undiscovered, novel type of PPTase may be responsible for phosphopantetheinylation of one or more of these carrier proteins and formal proof of this hypothesis will require phenotypic analysis of the effect of disruption of the human PPTase gene.
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