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LSDP5 is a PAT protein specifically expressed in fatty acid oxidizing tissues

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Abstract

The PAT family (originally named for *P*erilipin, *A*DFP and *T*IP47) now includes four members: Perilipins, ADFP, TIP47 and S3-12. Significant primary sequence homology and the ability to associate with lipid storage droplets (LSDs) are well conserved within this family and across species. In this study, we have characterized a novel PAT protein, *l*ipid *s*torage *d*roplet *p*rotein *5* (LSDP5) of 463 residues. A detailed sequence analysis of all murine PAT proteins reveals that LSDP5, TIP47 and ADFP share the highest order of sequence similarity, whereas perilipin and S3-12 have more divergent carboxyl- and amino-termini, respectively. Ectopically-expressed YFP-LSDP5 or flag-LSDP5 fusion proteins associate with LSDs. In accord with recent published data for perilipin, forced expression of LSDP5 in CHO cells inhibits lipolysis of intracellular LSDs. The LSDP5 gene is primarily transcribed in cells that actively oxidize fatty acids, such as heart, red muscle and liver. Expression of LSDP5 is stimulated by ligand activation of peroxisomal proliferator-activated receptor alpha (PPARα), and significantly reduced in liver and heart in the absence of this transcription factor. PPARα is generally required for regulation of fatty acid metabolism during fasting, but fasting induces LSDP5 mRNA in liver even in the absence of PPARα.

Keywords: LSDP5; Adipophilin; ADFP; S3-12; Perilipin; TIP47; PAT; TAG; Fatty acid; PPAR

1. Introduction

Most mammalian cells are able to store triacylglycerols (TAG)[1], cholesterol esters or other lipids in relatively small (<1 μm diameter) lipid storage droplets (LSDs) which can be used as an energy source or for membrane biogenesis [1]. For decades, these LSDs have been viewed as simple lipids reservoirs, but this view has changed mainly due to the discovery of a family of structurally related LSD binding proteins. Perilipins [2–4] were the first proteins that were experimentally demonstrated to associate with the LSD surface. Soon thereafter, the previously cloned mouse adipose differentiation-related protein (ADFP) [5] was found on LSDs in many cells [6] and tissues [7]. Two other proteins, tail-interacting protein of 47 kDa (TIP47)/placental tissue protein 17 (pp17) and S3-12 were subsequently cloned [8–10] and reported to bind to LSDs [11,12]. Structurally and functionally conserved family related proteins are also found in non-mammalian species such as *Drosophila melanogaster* and *Dictyostelium discoideum* [13]. Proteomics studies of LSDs from various cells demonstrate that PAT proteins (named after *P*erilipin, *A*DFP and *T*IP47) are among the most abundant proteins on the surface of LSDs [14–17].

Perilipin, ADFP and TIP47 exhibit high sequence identity within an amino-terminal PAT-1 domain and weaker homology

Abbreviations: ADFP, adipose differentiation-related protein; ATGL, adipocyte triaclyl glycerol lipase; CMC, carboxymethyl-cellulose; FA, fatty acid; HSL, hormone-sensitive lipase; LSD, lipid storage droplets; LSDP5, lipid storage droplet protein 5; OA, oleic acid; PPAR, peroxisomal proliferator-activated receptor; PPRE, peroxisomal proliferator response element; TIP47, tail-interacting protein of 47 kDa; TAG, triacylglycerol; WAT, white adipose tissue

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in the central and carboxyl-terminal PAT-2 domain [13,18]. The amino-terminal segment of S3-12 shares limited identity to the PAT-1 domain, but the remaining protein shares significant sequence homology to ADFP and TIP47 in the carboxyl-terminus [10,19] and is considered as a peripheral member [13,18]. All of the above proteins contain putative 11-mer helical repeats in the central sequence, which are also found in other lipid associated proteins such as synucleins, apolipoproteins, phosphate cytidyltransferases and dehydrins [20]. Although yet to be experimentally proven, it is likely that LSD targeting of PAT proteins is facilitated by these amphipathic helical repeats.

The tissue distribution of the PAT proteins is well characterized. TIP47 and ADFP are both expressed ubiquitously. Whereas TIP47 mRNA is expressed at similar levels in most tissues examined [19,21], ADFP is transcriptionally regulated by fatty acids (FAs) [22] and fasting [21], leading to mRNA enrichment in FA metabolizing organs [6,19]. Expression of perilipin is largely confined to adipose and steroidogenic cells [2,3,23]. S3-12 protein expression is likely restricted to adipose cells [10,12], even though high levels of S3-12 mRNA has been found in skeletal muscle and heart [19].

To date, the function of only one of the PAT proteins, perilipin, has been firmly established. The generation of perilipin null mice provided a strong basis for the functional studies. Disruption of the *Plin* gene results in a lean mice with a 70% decreased adipose tissue mass [24,25], largely due to changes in the behavior of its adipocytes. A lack of perilipin surrounding adipose LSDs leads to a constitutively high basal lipolysis, but also to a loss in the ability to respond to lipolytic stimuli. The control of basal versus stimulated lipolysis is normally controlled by the phosphorylation state of the perilipin protein [25–30], and the defect in response upon lipolytic stimuli in *Plin* null mice reflects the inability of lipases, such as hormone-sensitive lipase (HSL), to bind to the LSD [27]. To date, only fragmentary functional knowledge has been reported for the other PAT proteins. The large majority of publications report only detection of expression or changes in the expression of these genes in various cell types. Some recently emerging data using cultured cells show that ADFP protects LSDs from degradation [31], implying a more general role for the PAT proteins in inhibition of lipolysis. Expression of ADFP is important in liver, being a major LSD binding protein in mice during fasting [21]. A modestly lower hepatic TAG content in *Adfp* null mice is so far the only phenotype discovered by disruption of the *Adfp* gene [32]. Little is known about the S3-12 and TIP47 proteins in lipid metabolism, except for their known targeting to LSDs in cells cultured in the presence of FAs [11–13].

The transcriptional regulation of the PAT genes suggests that they are tightly linked to fatty acid metabolism. Several of the PAT genes are transcriptionally regulated by members of the peroxisomal proliferator-activated receptors (PPARs). The PPAR family consists of three isotypes PPARα, PPARβ/δ and PPARγ, that belong to a subfamily of nuclear receptors that heterodimerize with retinoid X receptors (RXRs) and regulate transcription by binding to specific PPAR response elements (PPREs) in the promoter region of target genes [33]. The PPARs are expressed in a tissue-specific manner: PPARγ is highly enriched in white adipose tissue (WAT) and macrophages [34], PPARα in liver and fatty acid metabolizing tissues, such as muscle, heart and kidney [35], whereas PPARβ/δ is more ubiquitously expressed [36]. Expression of the PPARs generally correlates well with the tissue expression profile of the PAT proteins. S3-12 [19] and perilipin [19,37–39] are regulated by PPARγ, ADFP by PPARα [21,40,41] and PPARβ/δ [42–44], whereas TIP47 seems not to be regulated by PPARs [19,21].

In this report we describe a fifth and novel member of the PAT family, with highest sequence similarity to TIP47 and ADFP. Like the other PAT proteins, LSDP5 binds to the surface of LSDs and protects them from lipolytic degradation. This novel PAT member is transcriptionally regulated by PPARα, and mainly expressed in fatty acid oxidizing cells such as heart, red muscle and liver. The transcriptional regulation of the *Lsdp5* gene is similar to the regulation of the *Adfp* gene. These two PAT proteins are co-regulated in heart and liver upon physiological changes such as fasting and re-feeding.

During our preparation of this manuscript, another research group published analysis of the similar protein, designated as MLDP of 448 residues [45]. In this paper we compare and contrast our analysis of the larger LSDP5 protein of 463 residues with those in the concurrent independent study.

2. Experimental procedures

2.1. Materials

Restriction enzymes were obtained from Promega (Madison, Wisconsin). Cell culture reagents, 4-chloro-6-(2,3-xylidino)-2-pyrimidinylthioacetic acid (WY-14643), Oil red O, forskolin, isobutylmethylxanthine (IBMX), oligonucleotides and chemicals were purchased from Sigma (St. Louis, Missouri). All cell culture plasticware was obtained from Corning incorporated (Corning, New York).

2.2. Identification, cloning and genomic analysis of Lsdp5 and the remaining PAT genes

A partial mouse LSDP5 protein sequence was identified using the highly conserved carboxyl-terminal motif found in the PAT proteins S3-12, ADFP and TIP47 [19,46] using protein-protein BLAST (Matrix: PAM30) [47]. The protein sequence was used in a further BLAST search using tblastn. These BLAST hits were used in nucleotide-nucleotide BLAST searches to obtain a full-length mouse LSDP5 cDNA sequence. The human and rat LSDP5 cDNA sequences were identified by BLAST using the mouse LSDP5 sequence.

Mouse and human LSDP5 cDNAs were cloned with RT-PCR using Omniscript RT kit (Qiagen, Valencia, CA) from mouse liver total RNA (C57BL/6J-strain) and human heart mRNA (Clontech, Mountain View, CA, #636532), followed by PCR amplification using PfuUltra (Stratagene, La Jolla, CA) with PCR settings as described [48]. Primers used are listed in Table 1. The amplified PCR products were cloned into pPCR-Script (Stratagene), sequenced (Macrogen, Korea) and found to correspond 100% to the predicted cDNA sequences.

Nucleotide–nucleotide BLAST against the genome sequence databases were used to determine the chromosomal location of LSDP5 in the human, mouse and rat genomes. The murine genomic organization of *M6prbp1*, *Lsdp5* and *S3-12* on chromosome 17 was determined by analyzing a BAC clone (bMQ217-F16 [49]) containing these PAT genes. Briefly, a successful gap-repair subcloning of individual PAT genes, as well as a joint subcloning of the *Lsdp5* and *S3-12* genes, confirms the presence of all PAT genes, as well as co-localization of the *Lsdp5* and *S3-12* genes within this single BAC clone. To determine the distance