Characterization of the Acyl CoA Synthetase Activity of Purified
Murine Fatty Acid Transport Protein 1

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Running title: FATP1 Acyl CoA Synthetase Activity

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Summary

Fatty acid transport protein 1 (FATP1) is an ~63 kDa plasma membrane protein that facilitates the influx of fatty acids into adipocytes as well as skeletal and cardiac myocytes. Previous studies with FATP1 expressed in COS1 cell extracts suggested that FATP1 exhibits very long chain acyl CoA synthetase activity and that such activity may be linked to fatty acid transport. To address the enzymatic activity of the isolated protein, murine FATP1 and acyl CoA synthetase 1 (ACS1) were engineered to contain a C-terminus myc-his tag, expressed in COS1 cells via adenoviral-mediated infection and purified to homogeneity using nickel affinity chromatography. Kinetic analysis of the purified enzymes was carried out for long chain palmitic acid (C16:0) and very long chain lignoceric acid (C24:0) as well as for ATP, and CoA. FATP1 exhibited similar substrate specificity for fatty acids 16 to 24 carbons in length while ACS1 was 10-fold more active on long chain fatty acids relative to very long chain fatty acids. The very long chain acyl CoA synthetase activity of the two enzymes was comparable as were the K_m values for both ATP and Coenzyme A. Interestingly, FATP1 was insensitive to inhibition by triacsin C whereas ACS1 was inhibited by micromolar concentrations of the compound. These data represent the first characterization of purified FATP1 and indicate that the enzyme is a broad substrate specificity acyl CoA synthetase. These findings are consistent with the hypothesis that that fatty acid uptake into cells is linked to their esterification with Coenzyme A.
**Introduction**

The mechanism by which fatty acids enter cells has been the source of considerable debate. Like other hydrophobic compounds, fatty acids are able to cross membranes by passive diffusion in a protein independent mechanism (1,2) driven by the concentration gradient on either side of the membrane. Whether passive diffusion within the biological context is sufficient to supply the amount of fatty acids required for tissues with high lipid flux remains to be determined. A second view contends that fatty acids traverse the plasma membrane via specific protein-mediated transporters (3,4). Studies in multiple tissue types, including cardiomyocytes and adipocytes (5,6), support the hypothesis that fatty acid transport occurs by a saturable, protein-mediated mechanism and several candidate proteins have been identified.

A variety of proteins have been implicated in protein mediated FA import. These include the fatty acid binding protein from the plasma membrane (FABP<sub>PM</sub>), the fatty acid translocase (FAT / CD36), as well as the fatty acid transport protein family of molecules (FATP) reviewed in (7). FATP1 is a member of a large family of related proteins from diverse organisms that increase fatty acid import when expressed in cultured cells (8,9). In mammalian cells, FATP isoforms 1-6 have been identified based on sequence similarity and have distinct tissue-specific distributions of expression (9). The yeast homologue (Fat1p) has been shown by genetic and biochemical analyses to be required for fatty acid uptake (10).

FATP1 shares sequence similarity with the family of acyl CoA synthetases and has been demonstrated to increase very long chain acyl CoA synthetase activity of extracts when expressed in COS1 cells (11). FATP1 contains two functional motifs, motif 1 that contains a putative site for the binding of ATP (via serine 250) that is part of the acyl CoA synthetase reaction mechanism and motif 2 that is proposed to be a ligand (fatty acid) binding domain (11-
13). Over expression of FATP1 in HEK 293 cells results in an increased rate of fatty acid influx for both C18:1 and C24:0 which are channeled to triacylglycerol biosynthesis (14). As such, fatty acid transport protein 1 has been proposed to be a bifunctional protein capable of facilitating transbilayer movement of fatty acids and their metabolic activation (esterification) with coenzyme A.

To characterize the FATP1 acyl CoA synthetase reaction, murine FATP1 was engineered to contain a C-terminus myc-his tag, expressed in COS1 cells, and purified to homogeneity by nickel affinity chromatography. Additionally, the well-studied long chain acyl CoA synthetase 1 (ACS1) was cloned, expressed, and purified similarly in order to make a comparative analysis. Kinetic analysis of purified FATP1 and ACS1 was carried out for two model fatty acid substrates (C16:0 and C24:0), ATP, and CoA. These data lay the foundation for the biochemical dissection of FATP1-dependent fatty acid uptake and metabolic activation and provide evidence consistent with the model for vectoral acylation of fatty acids at the plasma membrane.
Experimental Procedures

Reagents—[3H] palmitic acid and [3H] lignoceric acid were obtained from American Radiochemicals Company. All non-labeled fatty acid was obtained from NuChek Prep., Inc., Elysian, MN. Triacsin C was obtained from BIOMOL. Cell culture reagents were obtained from GIBCO. All other reagents were of analytical grade and obtained from Sigma Chemical Co., St. Louis, MO.

Generation of FATP1 and ACS1 Recombinant Adenovirus—A recombinant adenovirus expressing both the green fluorescent protein (controlled by the cytomegalovirus (CMV) promoter) and either murine FATP1 or ACS1 were constructed by recombination in Escherichia coli using the methods described by He and colleagues (15). The resulting construct was recombined into pADEasy in E. coli BJ5183 cells recreating the replication-deficient adenovirus genome. Linear constructs of the recombinant adenovirus were transfected (LipofectAMINE; GIBCO-BRL, Gaithersburg, MD) into 293 cells (American Type Culture Collection, Manassas, VA) to allow packaging and amplification of the adenovirus. Large-scale adenovirus preparations from twenty 10-cm plates of infected cells were propagated until approximately 50% of the cells lysed. The cells and media were collected and the remaining cells lysed by three freeze/thaw cycles. The medium was centrifuged at 20,000 xg for 10 min to pellet the cellular debris and the supernatant containing virus particles was recovered and frozen in aliquots at –70°C.

Protein expression of Recombinant FATP1 and ACS1 Proteins in COS1 cells—FATP1 and ACS1 his tagged proteins were expressed in COS1 cells using adenovirus infection. Prior to infection, COS1 cells were plated in 10-cm plates and grown to approximately 80% confluence
at 37\(^\circ\) C in a 5 \% CO\(_2\) incubator. For infection, the effective concentration of infectious adenoviral particles was experimentally determined by monitoring green fluorescent protein expression and COS1 cell viability seventy-two hours post infection. Adenovirus particles that yielded ~90-100 \% infection were delivered in 8 mL of DMEM supplemented with 10 \% FBS per plate. Seventy-two hours post-transfection, cells were harvested by centrifugation, immediately frozen and stored at -70\(^\circ\) C.

**Affinity Purification of Recombinant FATP1 and ACS1 Proteins**—COS1 cells expressing FATP1 or ACS1 were thawed in buffer A (150 mM Tris-HCl, pH 7.5, 250 mM sucrose, and 150 mM NaCl), subjected to five freeze/thaw cycles, and solubilized with 2 \% Triton X-100 for four hours at 4\(^\circ\) C. The soluble fraction was separated from debris by centrifugation at 100,000 xg for 1 hour at 4\(^\circ\) C, recovered, and glycerol was added to a final concentration of 20\%. For purification of recombinant proteins, the nickel affinity matrix beads (Qiagen) were equilibrated with buffer A plus 30 mM imidazole and the bound protein eluted with five column volumes of buffer A plus 150 mM imidazole. Eluates were pooled, aliquoted, and stored at -70\(^\circ\) C until use. FATP1 and ACS1 migrated as single bands on a 12 \% SDS-polyacrylamide gel and were judged to be at least 90\% pure by coomassie blue staining. The level of murine FATP1 enrichment obtained from the Ni\(^{2+}\) column purification was approximately 2000-fold, as assessed by comparison of the specific activity of the loaded fraction (0.5 nmol/min/mg for C16:0) to the eluted fraction (1000 nmol/min/mg for C16:0). To determine the amount of purified protein, samples were precipitated (16) and protein concentration determined by the bicinchoninic acid method (Pierce) with bovine serum albumin as the standard.
Fatty acyl CoA synthetase assays—Samples were assayed for acyl CoA synthetase activity by the conversion of \(^3\)H-palmitate or \(^3\)H-lignocerate to their CoA derivatives by a modified method from Nagamatsu et al. (17). All kinetic studies reported used 0.5-2 µg of purified FATP1 or ACS1 for 2 min at pH 7.5 and 30 mM NaCl in 250 µL of a buffer containing 20 µM fatty acid, 100 mM Tris-HCl (pH 7.5), 10 mM ATP, 5 mM MgCl\(_2\), 200 µM CoA, and 200µM dithiothreitol. Addition of enzyme purified in the presence of 2% Triton X-100 resulted in a final concentration of 0.4% Triton X-100 in all standard assays. Reactions were terminated with the addition of 1.25 mL of isopropenol:heptane:H\(_2\)SO\(_4\) (40:10:1, v/v/v), 0.5 mL of H\(_2\)O and 0.75 mL of heptane to facilitate organic phase separation. The aqueous phase was extracted three times with 0.75 mL of heptane to remove unreacted fatty acids and the radioactivity determined by liquid phase scintillation counting. The enzyme activity was stable in 20 % glycerol and was retained for 2 months without significant loss of activity when stored at -70\(^\circ\) C.

Competition studies—Due to the unavailability of commercially radiolabeled fatty acids, the lipid substrate specificity of the purified FATP1 was indirectly determined by competitive enzymatic inhibition analysis. In these studies, unlabelled fatty acids at a fixed concentration of 15µM was added to either \([^3\)H] palmitic acid or \([^3\)H] lignoceric acid and the rate of reaction monitored. A selectivity series for fatty preference was generated by determining the fraction of acyl CoA synthetase activity at \([S]_{0.5}\). The detergent-like effects of fatty acids are not significant since 0.4% Triton X-100 is present in the standard assays.
Results

Expression and Purification of Recombinant FATP1 Protein—Previous studies in cellular extracts have suggested that FATP1 is an acyl CoA synthetase (11). However, an alternate possibility is that FATP1 activates a latent, endogenous acyl CoA synthetase present in cells that results in increased fatty acid esterification. Alternatively, the fatty acid transport activity of FATP1 could lead to an influx of fatty acids that in turn generates signaling molecules that regulate the expression of an acyl CoA synthetase gene. To demonstrate that FATP1 exhibits intrinsic fatty acid CoA synthetase activity and to kinetically characterize the properties of such a reaction, murine FATP1 was purified and studied. To facilitate purification, FATP1 was C-terminal tagged with a myc epitope and a polyhistidine tract. The purification involved detergent extraction of FATP1 from the membrane with Triton X-100 and chromatography on Ni$^{2+}$-agarose resin. Figure 1 presents the SDS-PAGE analysis of fractions collected during a typical FATP1 purification. The purified enzyme was essentially homogenous as shown by SDS polyacrylamide gel electrophoresis, with an apparent molecular mass of ~68 kDa (Figure 1); a value that agrees well with the calculated molecular mass from the deduced amino acid sequence of the cDNA (63 kDa) plus that of the myc-his epitope and linker amino acids.

Optimization of the FATP1 acyl CoA synthetase reaction—Analysis of very long chain acyl CoA synthetase activity enriched from rat liver microsomes demonstrated an inhibitory effect of Triton X-100 on the activity (18). To determine whether FATP1 acyl CoA synthetase activity was sensitive to Triton X-100, purified enzyme was assayed with increasing concentrations of detergent. FATP1 activity (for both C16:0 and C24:0 esterification) was sensitive to Triton X-
100 in the reaction buffer (Figure 2). In order to establish standard reaction conditions, 0.4% Triton X-100 in the final assay buffer was adopted as routine. This level of Triton X-100 partially inhibited both FATP1 catalytic activity but was in a region of the progress curve that was relatively insensitive to small changes in detergent concentrations.

The FATP1 acyl CoA synthetase activity was optimized with respect to several standard reaction parameters for both long chain (C16:0) and very long chain (C24:0) fatty acids. As shown in Figure 3, the activity was dependent on, and proportional to, the amount of enzyme added to the reaction (Figure 3A) as well as time of reaction (Figure 3B). In addition, the pH sensitivity of the reaction was evaluated (Figure 3C), as was the influence of ionic strength (Figure 3D). The purified FATP1 has broad pH dependence with 6.5-8.0 being generally optimal and there was little effect on enzymatic activity when the NaCl concentration was varied from 30 mM to 150 mM, however the activity was reduced by 80% at 500 mM. Importantly, optimization utilizing C16:0 as a substrate yielded results that were essentially identical to those obtained with C24:0 as a substrate.

**Kinetic properties of FATP1**—The fatty acid esterification properties of purified FATP1 were measured for two model lipid substrates as well as for CoA and ATP. The apparent $K_m$ values of the purified enzyme were determined for palmitic acid (C16:0), lignoceric acid (C24:0), ATP, and CoA at 37°C (Figure 4). FATP1 demonstrated high affinity toward its substrates and co-substrates exhibiting a $K_m$ of 21 µM for C16:0, 13 µM for C24:0, 850 µM for ATP, and 8.3 µM for CoA (Table I). It should be stressed that because these studies are done in the presence of detergent, the free concentration of fatty acids cannot be determined and the $K_m$ values reported for each fatty acid represent apparent values assuming all lipid was available to the enzyme.
Since the solubility of lignoceric acid and palmitic acid vary greatly, this assumption is likely to be incorrect, but represents an experimentally tractable method for analyzing the data and comparing one enzyme to another. A catalytic constant of $1.5 \text{s}^{-1}$ at $37^\circ\text{C}$ was calculated from the molecular mass of the purified enzyme (68 kDa) and the maximal specific activity of 1000 nmol/ min/ mg for C16:0.

The kinetic properties of purified ACS1 protein were evaluated and compared to those for FATP1. The kinetic profile obtained in this study closely resembled that for both purified native ACS1 and flag-tagged ACS1 (ref. 15 and Figure 4). Purified his-tagged ACS1 demonstrated the expected high affinities toward C16:0, ATP, and CoA, with $K_m$ values of 33 µM, 320 µM, and 6.4 µM respectively (Table I). Interestingly, ACS1 also utilized very long chain fatty acids (C24:0) with a $K_m$ of 18 µM. The maximal velocity of ACS1 for C16:0 was about 3200 nmol/ min/ mg, but was greatly reduced for C24:0 to 240 nmol/ min/mg. Consistent with the reduced velocity for very long chain fatty acids, ACS1 was 10-fold more active toward C16:0 than C24:0 as demonstrated by $V_{\text{max}}/K_m$ values of 100 and 15 respectively. FATP1 exhibited similar activities toward C16:0 ($V_{\text{max}}/K_m$ of 6) and increased specificity for C24:0 ($V_{\text{max}}/K_m$ of 17).

**Fatty Acid Specificity of FATP1**—Because a large number of different fatty acids are not commercially available in radiolabeled form, the fatty acid substrate specificity of the purified FATP1 was indirectly determined by a competitive enzymatic inhibition by the addition of unlabelled fatty acids in the reaction in addition to either $[^3\text{H}]$ palmitic acid or $[^3\text{H}]$ lignoceric acid (Figure 5). Esterification of labeled lignoceric acid was effectively inhibited by the addition of 15 µM of various long chain fatty acids with the rank order of C18:1 > C20:4 >
C16:0 > C12. Similarly, the conversion of [3H] palmitate to palmitoyl-CoA was decreased by the addition of very long chain fatty acids with the rank order of $C_{22}:0 = C_{20}:0 > C_{24}:0 > C_{16}:0$.

As with the other kinetic analysis, due to the differing solubilities of the various competing fatty acids, true $K_i$ values cannot be determined and a simple selectivity series is presented. These results are consistent with a model for FATP1 having a single fatty acid binding domain that is utilized for esterification of both long and very long chain fatty acids.

**FATP1 is subject to feedback inhibition**—Reports of the cellular concentration of long chain acyl CoA esters vary between 5 and 160 µM (19,20). To determine whether FATP1 is regulated by feedback inhibition, increasing concentrations of palmitoyl-CoA were titrated into the standard reaction conditions (Figure 6) and the activity of FATP1 evaluated. At a concentration of 10 µM palmitoyl-CoA, the FATP1 acyl CoA synthetase reaction was inhibited by ~20-25%, while 100 µM inhibited the reaction by greater than 60% for both C16:0 and C24:0. Though to a slightly less degree, a similar inhibition was observed for ACS1.

**FATP1 is insensitive to Triacsin C Inhibition**—Triacsin C has been reported to be a potent competitive inhibitor of ACS1 and ACS4 (21). However, studies in crude cellular extracts suggested FATP1 may not be similarly affected (14). To test whether triacsin C inhibits purified FATP1, various concentrations were added to the standard reaction mixture (Figure 7) and the conversion of C16:0 and C24:0 evaluated. Surprisingly, triacsin C had no effect on FATP1 acyl CoA synthetase activity while ACS1 was inhibited in the reported dose-dependent manner with an IC$_{50}$ of 1 µM. Troglitazone at concentrations up to 10 µM inhibited FATP1-dependent acylation of C16:0 or C24:0 by only 20% (data not shown).
Discussion

Initial studies to identify proteins that facilitate fatty acid transport into mammalian cells focused on a protein's ability to bind fatty acids. A plasma membrane fatty acid binding protein (FABP<sub>PM</sub>) was purified by oleate-agarose affinity chromatography from hepatocytes, adipocytes, intestinal epithelial cells and cardiomyocytes and proposed to play a role in fatty acid import (22). Partial protein sequencing of FABP<sub>PM</sub> revealed identity with mitochondrial aspartate aminotransferase (mAspAT), furthermore antibodies to mAspAT were able to inhibit uptake of oleate into 3T3-L1 adipocytes, further supporting the conclusion that these proteins are identical (23). CD36, also known as fatty acid translocase (FAT), was identified based on its ability to bind a cross-linking, sulfonsuccinimidyl fatty acid derivative (24). Influx of fatty acid analogs is reduced in the heart, skeletal muscle, and adipose tissues in CD36-null mice (25). CD36 also functions as a multi-ligand scavenger receptor, binding oxidized low-density lipoproteins, thrombospondin, and collagen. The additional functions of FABP<sub>PM</sub> and FAT complicate their interpretation and role in fatty acid uptake.

The FATP family of proteins was originally identified by the ability to facilitate fatty acid uptake using a fluorescent fatty acid internalization assay (8). Sequence analysis of cloned FATP family members reveal two sequence motifs indicative of acyl CoA synthetases. Subsequent studies have demonstrated that the over expression of FATP1, FATP2, or FATP4 in COS1 cells increase cell associated acyl CoA synthetase activities (11,26,27). Moreover, mutation of serine 250 to alanine significantly reduced fatty acid uptake suggesting that esterification of fatty acids is coupled to influx (28). The current study was undertaken to evaluate the catalytic properties of the FATP1 acyl CoA synthetase reaction. Here we demonstrate that purified FATP1 has an
intrinsic acyl CoA synthetase activity with broad specificity for both long and very long chain fatty acids. FATP1 is a low velocity enzyme compared to ACS1, the traditional enzyme believed to esterify fatty acids broadly in cells. While ACS1 prefers C16:0 over C24:0, FATP1 has comparable specific activities toward C16:0 and C24:0. This observation with purified FATP1 was masked in previous studies utilizing COS cell extracts due to a high level of endogenous long chain acyl CoA synthetase activity. The FATP1 $K_m$ values measured for CoA and ATP are similar to those measured for ACS1.

The fundamental question arising from the observations that FATP1 functions in both fatty acid transport and fatty acid activation is whether fatty acid import is driven by this intrinsic acyl-CoA synthetase activity (vectoral acylation) or whether these activities are distinct and the enzyme is bifunctional. Using site-directed mutagenesis of the FATP1 orthologue in yeast, Fat1p, fatty acid uptake and fatty acid activation are generally, but not universally, coupled processes (29). Four $FAT1$ alleles distinguish fatty acid transport and very long chain acyl CoA synthetase activity suggesting separable functions. Interestingly, the $FAT1$ T260A mutant exhibited only moderately reduced fatty acid import and acyl CoA synthetase activity while the corresponding mutant in murine FATP1, T252G, eliminates fatty acid uptake. Further analysis of the molecular determinants that characterize the two activities will be needed to critically evaluate the relationship between transport and catalysis.

Recently, Zou et al. demonstrated in Saccharomyces cerevisiae, the yeast orthologs of FATP1 and ACS1, Fat1p and Faa1p or Faa4p, function cooperatively to form a physical complex and mediate the import of exogenous long chain fatty acids (30). This conclusion was supported by
several lines of biochemical evidence. Firstly, a C-terminal truncated peptide of Fat1p deficient in fatty acid transport exerted a dominant negative effect against long chain acyl CoA synthetase activity. Secondly, a fusion protein of Fat1p as the bait and Faa1p as the trap were active partners when tested in the yeast two-hybrid system. Finally, Fat1p co-immunoprecipitated with Faa1p and Faa4p when expressed together. Interestingly, FATP1 has been linked to ACS1 by both functional assays and cellular localization in adipocytes (8,31), suggesting they may also form a physical complex to facilitate fatty acid transport in mammalian cells through vectoral acylation. Preliminary experiments with FATP1 and ACS1 co-expressed into COS cells suggests no significant kinetic differences in FATP1 acyl CoA synthetase activity when assayed in the presence of Triacsin C (Hall and Bernlohr, unpublished).

In sum, these results demonstrate that FATP1 exhibits intrinsic acyl CoA synthetase activity and is a broad substrate enzyme. This report represents the first characterization of the enzymatic activity of the protein in purified form. For long chain fatty acids, the prototypical ACS1 enzyme exhibits a higher $V_{\text{max}} / K_m$ ratio while for very long chain FA the enzymes are comparable. This may suggest that ACS family members should functionally compensate for disruptions of FATP and that loss of the fatty acid transport protein would not be metabolically significant. However, striking evidence from FATP4 null mice (32) that exhibit a wrinkle-free phenotype reminiscent of essential fatty acid deficiency suggests unique specialized roles for the fatty acid transport proteins and that their physiological significance cannot be overlooked. Additional experimentation will be required to dissect the molecular roles of the FATP transport and esterification activity as well as their metabolic functions in vivo.
Acknowledgements

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Abbreviations:

The abbreviations used are: FATP, fatty acid transport protein; ACS, acyl CoA synthetase; FABP<sub>PM</sub>, fatty acid binding protein from the plasma membrane; FAT/CD36, fatty acid translocase / CD36 antigen;
Table I.

Comparison of kinetic constants for FATP1 and ACS1

Recombinant FATP1 and ACS1 were expressed, purified, and assayed for lignoceroyl-CoA and palmitoyl-CoA synthetase activities as described under “Experimental Procedures”. Data are representative of at least three individual trials ± standard deviation and should be considered apparent rather than true values due to the presence of detergent in the analysis. (*) denotes values determined using palmitic acid (C16:0) as substrate. The K_m values reported are apparent since 0.4% Triton X-100 is present under standard assay conditions.

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References

Figure legends:

**Figure 1. SDS-PAGE analysis of the FATP1 purification.** Lane 1, total crude extract; lane 2, soluble crude extract; lane 3, nickel affinity chromatography flow through; lanes 4-6, nickel column washes 1, 2 and 10, respectively; lane 7, nickel column elution 1; lane 8, pool of elute fractions from nickel column. Numbers on the y-axis represent molecular mass markers in kDa.

**Figure 2. FATP1 sensitivity to Triton X-100.** Purified FATP1 was assayed for acyl CoA synthetase activity with C16:0 (□) or C24:0 (■) in the presence of increasing concentrations of Triton X-100. The values are expressed as the average of duplicates ± standard deviation from a representative experiment of three independent determinations.

**Figure 3. FATP1 acyl CoA synthetase reaction conditions.** Panel A, acyl CoA synthetase activity for C16:0 (□) or C24:0 (■) as a function of FATP1. Panel B, time course of esterification by FATP1. Panel C, effect of pH on FATP1 acyl CoA synthetase activity. Panel D, effect of ionic strength on FATP1 acyl CoA synthetase activity. Data points are expressed as the average of duplicates ± standard deviation from a representative experiment of three independent determinations.
Figure 4. Substrate analysis for purified FATP1 (■) and ACS1 (□). Panel A, activity as a function of C24:0. Panel B, activity as a function of C16:0. Panel C, activity as a function of ATP. Panel D, activity as a function of CoA. Data points are expressed as the average of duplicates ± standard deviation from a representative experiment of three independent determinations.

Figure 5. Competition studies with various long chain and very long chain fatty acids.

Purified FATP1 was assayed for long chain or very long chain acyl CoA synthetase activity in the presence of indicated fatty acids at a concentration of 15 µM. In panel A, C16:0 (■) esterification was evaluated with the following competitor fatty acids: C24:0 (●), C22:0 (○), C22:0 (×), and C16:0 (△). In panel B, C24:0 (■) esterification was evaluated with C12:0 (○), C16:0 (▼), C18:1 (◆) and C20:4 (●). The values are expressed as the average of duplicates ± standard deviation from a representative experiment of three independent determinations.

Figure 6. Palmitoyl-CoA inhibits FATP1 activity. Purified FATP1 (■) and ACS1 (□) were assayed for C16:0 (Panel A.) and C24:0 (Panel B.) acyl CoA synthetase activity in the presence of increasing concentrations of palmitoyl-CoA. The values are expressed as the average of duplicates ± standard deviation from a representative experiment of three independent determinations. * refers to P < 0.05. # refers to P < 0.001
Figure 7. **FATP1 is insensitive to Triacsin C inhibition.** Purified FATP1 (circles) and ACS1 (squares) were assayed for C16:0 (closed symbols) or C24:0 (open symbols) acyl CoA synthetase activity in the presence of increasing concentrations of triacsin C. The values are expressed as the average of duplicates ± standard deviation from a representative experiment of three independent determinations.
Figure 2.

FATP1 Synthetase Activity (percent of initial) vs. Percent of Triton X-100.
Figure 3.

A. FATP1 Synthetase Activity (nmol/min) vs. Amount of FATP1 (μg)

B. FATP1 Synthetase Activity (nmol/mg) vs. Time (min)

C. FATP1 Synthetase Activity (nmol/min/mg) vs. pH

D. FATP1 Synthetase Activity (nmol/min/mg) vs. [NaCl] mM
Figure 4.

A.

B.

C.

D.
Figure 5.

A.

B.
Figure 7.
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