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Alteration of the Fatty Acid Substrate Specificity of Lysophosphatidate Acyltransferase by Site-Directed Mutagenesis

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The JC201 strain of *Eschericia coli* contains a temperature-sensitive lesion in lysophosphatidate acyltransferase (LPAT) activity. The LPAT gene from JC201 was isolated by PCR and a single mutant nucleotide, adenine-440, was identified by DNA sequence analysis. Site-directed mutagenesis converted the mutant adenine-440 back to the native guanine-440 nucleotide. The restored LPAT gene rescued JC201 cells at the non-permissive temperature. The fatty acid substrate specificity of LPAT from *Eschericia coli* was altered by site-directed mutagenesis of a single amino acid in the restored LPAT gene. Threonine-122 of LPAT was changed to alanine or leucine. A change from threonine-122 to alanine increased the substrate specificity *in vitro* for oleoyl-CoA and linoleoyl-CoA; whereas a change to leucine increased the substrate specificity for lignoceroyl-CoA.

The fatty acid composition of glycerolipids is a target for molecular engineering due to its economic importance and has correspondingly generated much research [reviewed in: 1-4]. Glycerolipid fatty acids are a major factor, for example, in the commercial usefulness of seed oils and in nutritional concerns such as hypercholesterolemia. Considerable progress in the bioengineering of the fatty acid composition of plant seed oil glycerolipids has come about through the application of transgenic technologies [4-9].

The biochemical and physical properties of glycerolipids are related to a triad of structural properties of its fatty acids. These properties are, the carbon chain-length, the degree of unsaturation, and the stereospecific attachment of the fatty acids to the glycerol backbone [1, 3]. The fatty acyl moieties attached to the glycerol backbone are not distributed randomly. The arrangement of fatty acids are related to the activities of the membrane-bound acyltransferases, especially that of lysophosphatidate acyltransferase (LPAT; 1-acyl-*sn*-glycerol-3-phosphate acyltransferase, EC2.3.1.51) [1-6, 10-20]. LPAT catalyzes the esterification of fatty acids to the *sn*-2 position of 1-acyl-*sn*-glycerol-3-phosphate. Although the fatty acid substrate specificities of LPAT from many sources have been characterized, the structural determinants of LPAT function are not well understood. These determinants, once elucidated, would be useful targets for augmenting LPAT fatty acid substrate specificity by molecular biological techniques.

The sequence of several LPAT genes and their deduced primary protein structures are known [6, 7, 17-19, 21-26]. An *E.schericia coli* (*E. coli*) strain, JC201, which has a temperature-sensitive deficiency in LPAT activity, was used to clone the LPAT gene, plsC, from *E. coli* by complementation of the JC201 phenotype [21]. An LPAT-like acyltransferase gene isolated from yeast, SLC1, also complements the JC201 strain [23]. A mutation in the SLC1 gene complemented a yeast strain deficient in sphingolipid production. The mutation was a Leu replacement of Gln-132 in the SLC1 gene product. Introduction of the Leu resulted in phospholipids with fatty acids of 26 carbon chain-lengths attached to the *sn*-2 position of the glycerol backbone as opposed to the usual chain lengths of 16 or 18 carbons. The mutations in the plsC gene of JC201 and the SLC1 gene of yeast provided targets for examining the structural integrity and fatty acid substrate specificity of LPAT.

In this study, the JC201-plsC gene was isolated by PCR followed by site-directed mutagenesis to convert JC201-plsC to the native plsC structure. Also, the amino acid in *E. coli* LPAT complementary to the mutation in the yeast SLC1 gene was changed by site-directed mutagenesis which resulted in altering the fatty acid substrate specificity of LPAT *in vitro*. The results of this report potentially allows the finding of the yeast LPAT mutation to be extended to other organisms for the purpose of engineering the fatty acid specificity of LPAT.

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MATERIALS AND METHODS

Materials. Custom oligonucleotide primers and Taq polymerase were purchased from GibCO-BRL. The plasmid pBluescriptKSII, the Quick Change site-directed mutagenesis kit, and XL1-Blue bacterial cells, were purchased from Stratagene. Restriction endonucleases were purchased from New England Biolabs. Fatty acyl-CoAs (palmitoyl, 16:0; stearoyl, 18:0; behenoyl, 22:0; lignoceroyl, 24:0; oleoyl, 18:1Δ9; linoleoyl, 18:2Δ9,12; linolenoyl, 18:3Δ9,12,15) and 1-oleoyl-*sn*-glycerol-3-phosphate, were purchased from Sigma. The BCA protein assay kit was purchased from Pierce. Radiolabeled [1-C^{14}]-palmitoyl-CoA, 55 mCi/mmol, was purchased from Amersham. The 60Å silica gel TLC plates were purchased from Whatman.

General methods. Routine molecular biological techniques were according to standard protocols [27]. An MJ Research thermocycler was used for all PCR reactions. The PCR reaction parameters were: 35 cycles (94°C, 1 min; 50°C, 30 sec; 72°C, 30 sec), 2 units Taq polymerase, 1X Taq polymerase buffer, 50 pmol each primer, 0.8 mM dNTP, 1 mM $MgCl_2$, 1 μg plasmid or genomic DNA, in a final volume of 50 μl. DNA sequence analysis was conducted by automated sequencing with ABI PRISM Dye Terminator Cycle Sequencing and the Ready Reaction Kit, the sequence reaction products were monitored on an ABI PRISM 377 DNA Sequencer, and the sequence data was analyzed using the ABI PRISM A Sequencing 2.1.1 software. Transformation of JC201 cells by electroporation was with a Bio-Rad Gene Pulser and manufacturer's protocol. Detection and quantitation of 1-oleoyl-2-[C^{14}]-palmitoyl-*sn*-glycerol-3-phosphate was with a Fuji Fujix BAS1000 Bio-imaging Analyzer and phosphoimager screens.

Isolation of the JC201-plsC gene. The JC201-plsC gene fragment containing the entire coding sequence of LPAT was isolated from JC201 genomic DNA by PCR using primers constructed from the known *E. coli* plsC gene sequence [21]. The primers used were, forward, TAAGAATTCAGGTGACGTACAATG (*EcoRI*), and reverse, TAAGGTACCGTGCAGGAAAGTGTTC (*KpnI*). Genomic DNA from JC201 was prepared according to Current Protocols [28]. A BamHI site approximately in the middle of the plsC coding region [21] (Figure 1) was utilized to subclone the amplified JC201-plsC gene fragment in two parts. The amplified JC201-plsC gene fragment was digested with KpnI, followed by BamHI and EcoRI. The two halves of the JC201-plsC gene were subcloned into the corresponding sites of pBluescriptIIKS. The plasmids were named, pplsCEB containing the EcoRI-BamHI portion, and pplsCBK containing the BamHI-KpnI portion.

The JC201-plsC coding region was reconstructed by subcloning the portion of the plsC gene from pplsCBK into the BamHI and XbaI sites of pplsCEB. The BamHI-KpnI portion of the plsC gene in pplsCBK was amplified by PCR. The primers used were, forward, TAAAAAGAGCTTGCTGTGGATCCCC (*BamHI*), and reverse, ACCCTCTAGAAAGTGTTCCCCGCAAAAATAC (*XbaI*). The amplified product was digested with BamHI and XbaI and subcloned into the corresponding sites of pplsCEB. The resulting plasmid contained the entire structural sequence of the JC201-LPAT protein and was named pplsC6.

Site-directed mutagenesis. Mutagenesis was accomplished with the Quick Change kit according to the manufacturer's protocol. Conversion of the adenine-440 of JC201-plsC in pplsC6 to guanine-440 by site-directed mutagenesis restored the native plsC gene structure. The primers were, forward, CCACCTTTGGGCATATGTTTGGCCG, and reverse, AAACATATGGCCCAAAGGTGGCCACAT. The resulting plasmid with the native LPAT structural gene was named pplsC6.3.

The fatty acid substrate specificity of the *E. coli* LPAT was altered by changing Thr-122 of the native LPAT structure to Ala or Leu by site-directed mutagenesis. For the conversion to Ala, the primers were, forward, AGAAACAATCGCGCTAAAGCTCACGGCACCATTGCGGA, and reverse, GCCGTGAGCTTTAGCGCGATTGTTTCTGTCGATCAATA; and, for the conversion to Leu, the primers were, forward, AGAAACAATCGCCTTAAAGCTCACGGCACCATTGCGGA, and reverse, GCCGTGAGCTTTAAGGCGATTGTTTCTGTCGATCAATA. The plasmids were named pplsC6.3A and pplsC6.3L for the amino acid changes of Thr-122 to Ala and Leu, respectively. In all cases, successful mutagenesis was verified by automated DNA sequence analysis.

Complementation of the JC201 phenotype. The functional competence of the restored native LPAT structural gene of pplsC6.3 was verified by complementation of the JC201 phenotype. JC201 cells were transformed with 100 ng of pplsC6 or pplsC6.3 by electroporation according to the manufacturer's protocol. Transformants were selected on LB-ampicillin plates at RT overnight. Ampicillin-selected colonies were streaked in duplicate onto fresh LB-ampicillin plates and incubated overnight with one duplicate at 30°C and the other at 42°C. Survivors at the non-permissive temperature of 42°C indicated a functional, non-lethal LPAT.

Bacterial membrane preparation. The fatty acid substrate specificity of LPAT in XL1-Blue cells transformed with pplsC6.3, pplsC6.3A and pplsC6.3L were examined *in vitro* with isolated membrane fragments. Bacterial membranes were prepared from overnight cultures by a modification of the protocol described by Coleman [29]. All manipulations were carried out at 4°C. Cells were collected from 50 ml cultures by centrifugation at 12,000 × g for 10 min. The cells were resuspended in 35 mls of 50 mM Tris-HCl (pH7.5), 0.25 M sucrose and 1 mM EDTA. The resuspended cells were sheared with a French Press at 16,000 PSI. The sheared cells were centrifuged at 12,000 × g for 10 min to remove particulate matter and bacterial membranes were collected from the supernatant by centrifugation at 100,000 × g for 90 min. The pelleted membrane fragmants were rinsed twice and then resuspended with 50 μl of 50 mM Tris-HCl (pH7.5). Protein assays of the membrane preparations were by the BCA method according to the manufacture's protocol.

LPAT assay. Assay of LPAT activity was by a modification of the protocol described by Morand [20]. The LPAT assay reaction mixture was: 100 mM Tris-HCl (pH9.0), 0.5 mM $MgCl_2$, 100 μM 1-oleoyl-*sn*-glycerol-3-phosphate, 5 μM [1-C^{14}]-palmitoyl-CoA, and 5 μM fatty acyl-CoA [palmitoyl, stearoyl, behenoyl, lignoceroyl, oleoyl, linoleoyl, linolenoyl], 25 μg bacterial membrane protein, in a final volume of 50 μl. The reactions were initiated with addition of the bacterial membranes and incubated at 35°C for 2 min. Following the 2 min incubation, 2 × 10 μl samples were removed immediately and spotted onto silica gel TLC plates. The TLC plates were developed in a mixture of chloroform/methanol/acetic acid/water (50/37.5/3.5/2, vol/vol/vol/vol). The reaction products were detected on phophoimager screens exposed for 16 hrs to the TLC plates and quantitated with a bio-imaging analyzer according to the manufacture's procedures. Relative mobilities of the acyltransferase products on the TLC plates were verified by the lack of detectable product in the control incubations. Control incubations were conducted in the absence of the 1-oleoyl-*sn*-glycerol-3-phosphate.

RESULTS AND DISCUSSION

The isolated JC201-plsC gene fragment spanned nucleotides 279-1096 of the native plsC gene (Figure 1). The forward primer had been designed to create an inframe fusion protein with the plasmid derived β-galactosidase gene. Sequence analyses of both DNA strands of each of the subcloned plsC fragments of pplsCEB and pplsCBK revealed one nucleotide difference from the coding sequence of the native plsC gene. Adenine-440 was identified in the JC201-plsC sequence instead of guanine-440 in the native plsC sequence, which created a Glu in place of the Gly-39 [21]. Also, sequence analysis of pplsCEB showed one of three guanines, guanine-759, -760, or -761, was deleted from the

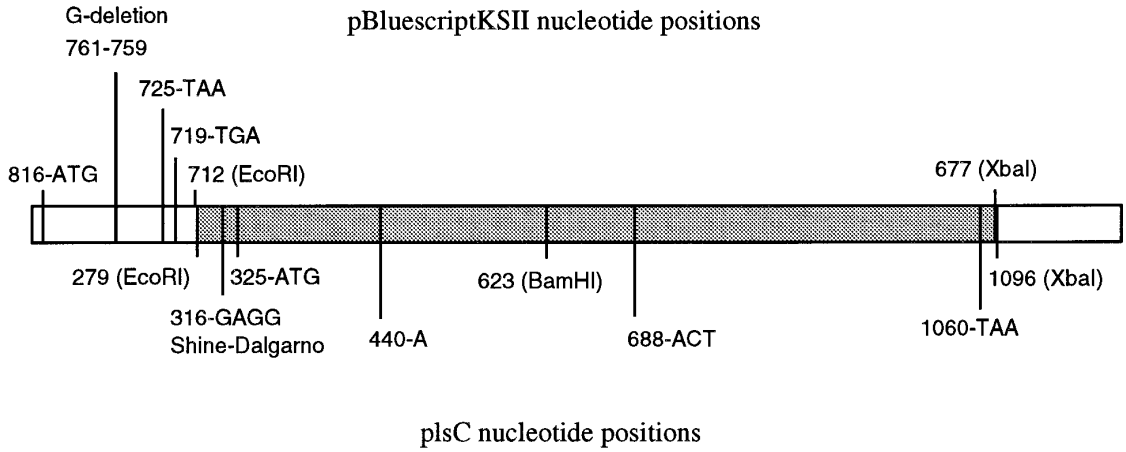

FIG. 1. Plasmid construct of pplsC6. The subcloned *E. coli* plsC gene (shaded area) is flanked by the β-galactosidase region of the plasmid, pBluescriptKSII (anti-coding strand reference sequence, Stratagene, 1995-1996 catalog). The β-galactosidase translation start site is ATG-816. One of the three guanines, 761-759, is deleted in the β-galactosidase sequence which created two stop codons, TAA-725 and TGA-719, via a frame-shift. Positions 712 and 677 are the EcoRI and XbaI sites, respectively, of pBluescriptKSII. The plsC gene fragment from nucleotides 279 to 1096 is subcloned into the EcoRI and XbaI sites of pBluescriptKSII. The LPAT translation start site is ATG-325 and the stop site is TAA-1060. The putative Shine-Dalagarno sequence is GAGG-316. Position 440 is the mutant adenine in the JC201-plsC gene sequence. The Thr codon, ACT-688, is the target for alteration of the LPAT fatty acid substrate specificity *via* site-directed mutagenesis.

β-galactosidase coding region (pBluescriptKSII anti-coding strand as the reference sequence, Stratagene). Consequently, two translation stop codons were created for β-galactosidase, TAA-719 and TGA-712. It is not known if the deletion of the guanine was derived from bacterial activity or from a Taq polymerase error. The putative ribosome binding site for the plsC gene [21] was retained in pplsCEB.

Adenine-440 was presumed responsible for the temperature sensitive lesion in the LPAT activity of JC201. It was necessary to reconstruct a functional LPAT gene and then convert the mutant adenine-440 to the native guanine-440 by site-directed mutagenesis to test this supposition. Although the expression of the cloned plsC gene in a plasmid vector rescues the JC201 phenotype, continued propagation of the plasmid with the plsC gene and expression of the *E. coli* LPAT in JC201 and other *E. coli* strains has proven to be problematic and often lethal (Dr. J. Coleman, personal communication; our work, data not shown). Thus, it was necessary also to construct a plasmid containing the plsC gene which permitted expression of the acyltransferase at non-lethal levels. It was reasoned that pplsCEB, which contained the amino-terminus of the acyltransferase, would not form a fusion protein with β-galactosidase because of the two stop codons introduced by the deletion of the single guanine in the β-galactosidase gene. Furthermore, it was assumed that a reconstructed JC201-plsC LPAT structural sequence utilizing pplsCEB would not produce the acyltransferase at lethal levels since the translation start of the plsC gene was now separated from the β-galactosidase promoter by an additional 77 nucleotides from the now truncated β-galactosidase. Hence, the portion of the acyltransferase gene in pplsCBK was amplified by PCR and subcloned into the BamHI and XbaI sites of pplsCEB. The new plasmid with the reconstructed JC201-LPAT structural sequence was named pplsC6 (Figure 1).

The native plsC structural gene sequence was restored by converting the mutant adenine-440 to guanine-440 by site-directed mutagenesis. The plasmid, pplsC6.3, contained the native LPAT protein structure and was tested by complementation of the JC201 phenotype as described by Coleman [21]. Plasmids pplsC6 and pplsC6.3 confered ampicillin resistence to the JC201 cells after transformation when grown at the permissive temperature of 30°C; however, only pplsC6.3 was able to rescue the JC201 strain at the non-permissive temperature of 42°C (data not shown). It was concluded that adenine-440 of the JC201-plsC gene is responsible for the JC201 temperature sensitive lesion in the acyltransferase activity. Additionally, it was concluded that pplsC6 and pplsC6.3 produced a functional *E. coli* LPAT at non-lethal levels.

Protein engineering of enzymatic function by site-directed mutagenesis methodology requires judicial selection of target amino acids. The primary structure of a protein is to be changed without gross alteration of the tertiary structure and enzymatic function. Fortunately, a target for changing the fatty acyl-CoA substrate specificity of LPAT was identified by the mutation in the SLC1 gene of yeast. Table 1 shows the amino acid sequences of the LPAT proteins in the region corresponding with Gln-132 of the yeast enzyme. Thr-122 of the *E. coli* LPAT sequence corresponds with Gln-132 in yeast. It was hypothesized that altering Thr-122 of

TABLE 1

Amino Acid Sequences of LPATs Surrounding Threonine-122 of *E. coli*

E. coli	117- D R N N R T K A H G T -127
H. influenza	116- D R E N R T K A H N T -126
Yeast	127- D R S K R Q E A I D T -137
Coconut	174- D R S N P S A A I E S -184
L. douglasii (pLAT2)	153- D R S N P A A A I Q S -163
L. alba	153- D R S N P A A A I Q S -163
Maize (pMAT1)	141- E R S - W A K D E K T -150
Human	144- N R Q R S S T A M T V -154
Mouse	145- D R K R T G D A I S V -155
C. elegans	142- D R Y N R E R A M A S -152

Note. Threonine-122 of *E. coli* LPAT [21] corresponds with the mutation of Gln-132 to Leu of the yeast LPAT responsible for the incorporation of 26 carbon fatty acid chain-lengths into yeast phospholipids [23]. Other LPAT sequences are: coconut [17], maize [18], *Limnanthes douglasii* [19], *Limnanthes alba* [6], *Haemophilus influenza* [22], human [24], mouse [25] and *Caenorhabitis elegans* [26].

the *E. coli* LPAT sequence would influence the fatty acid substrate specificity of the acyltransferase. Thr-122 of pplsC6.3 was altered by site-directed mutagenesis to test the hypothesis.

Thr-122 of the *E. coli* LPAT sequence (Figure 1) was changed to Ala or Leu. Ala was chosen as a representative LPAT sequence from plants (Table 1). An Ala within the LPATs of *Limnanthes alba, Limnanthes douglasii,* and maize occupies the position corresponding to Thr-122 of *E. coli* LPAT, whereas the sequence from coconut has a Ser. Leu was chosen since it was the mutation in the yeast SLC1 sequence which appeared responsible for the fatty acids of 26 carbon chain-lengths at the *sn*-2 position of yeast phospholipids [23].

It is customary to test the function of an enzyme in bacteria by complementation expriments with a strain deficient in the enzymatic function as demonstrated by the experiments to clone the plsC gene [21]. However, the XL1-Blue strain commonly used for recombinant DNA work was chosen to avoid problems associated with utilizing JC201. Continued propagation of a plasmid with the plsC gene in JC201 elicited various undesired recombination events (data not shown). These recombinations could not be controlled when using JC201.

The *E. coli* strain XL1-Blue was transformed with the control plasmid, pplsC6.3, and the experimental plasmids, pplsC6.3A or pplsC6.3L. Table 2 shows the specific activities of LPAT from membrane preparations of transformed XL1-Blue cells. The specific activities are comparable to previously published values for *E. coli* [21, 30]. Cells with pplsC6.3 gave the highest rate at 1.40 nmol palmitate incorporated mg^{-1} min^{-1}. Cells with pplsC6.3A and pplsC6.3L gave rates of 1.19 and 1.12 nmol palmitate incorporated mg^{-1} min^{-1}, respectively. Introduction of Ala or Leu into the *E. coli* LPAT structure apparently decreased the specific activity approximately 18% compared with the rates from

TABLE 2

Specific Activity of LPAT *in vitro* from Membranes of XL1-Blue Cells

Plasmid	Rate
pplsC6.3	1.40 ± 0.06
pplsC6.3A	1.19 ± 0.01
pplsC6.3L	1.12 ± 0.10

Note. Rates are: nmol palmitate incorporated mg^{-1} min^{-1}. Results are from 2 experiments each with 2 replicas (± SD).

the native structure. It is unknown, however, what the relative contributions to the LPAT rates are genome or plasmid derived.

The effect of the amino acid changes on LPAT fatty acid substrate specificty were examined by competition experiments between [C^{14}]-palmitoyl-CoA and an equimolar concentration of other unlabeled fatty acyl-CoA substrates. The results of the competition experiments are shown in Table 3. The results are expressed as relative rates of [C^{14}]-palmitate incorporation normalized to 1.00 for the equimolar mix of [C^{14}]-palmitoyl-CoA and unlabeled palmitoyl-CoA. The relative rates of palmitate incorporation derived from cells transformed with pplsC6.3 show no significant differences in the presence of the saturated fatty acyl-CoA substrates: palmitoyl, stearoyl, behenoyl, and lignoceroyl. The relative rates of palmitate incorporation were lowered to values of 0.27, 0.26, and 0.12, in the presence of the unsaturated fatty acyl-CoA substrates: oleoyl, linoleoyl, and linolenoyl, respectively. These results were expected since palmitate is found esterified almost exclusively to the *sn*-1 position of the glycerol-3-phosphate backbone of *E. coli* phospholipids, while the unsaturated fatty acids, *cis*-vaccenate and palmitoleate, are esterified predominantly to the *sn*-2 position [30].

TABLE 3

Relative Rates of [C^{14}]Palmitate Incorporation by LPAT *in vitro* from Membranes of XL1-Blue Cells

	pplsC6.3	pplsC6.3A	pplsC6.3L
Palmitoyl-CoA (16:0)	1.00 ± 0.03	1.00 ± 0.02	1.00 ± 0.07
Stearoyl-CoA (18:0)	0.89 ± 0.05	0.92 ± 0.09	1.10 ± 0.1
Behenoyl-CoA (22:0)	0.96 ± 0.04	0.90 ± 0.01	1.10 ± 0.09
Lignoceroyl-CoA (24:0)	0.93 ± 0.08	0.80 ± 0.12	0.25 ± 0.05
Oleoyl-CoA (18:1)	0.27 ± 0.01	0.17 ± 0.03	0.21 ± 0.04
Linoleoyl-CoA (18:2)	0.26 ± 0.001	0.08 ± 0.05	0.22 ± 0.02
Linolenoyl-CoA (18:3)	0.12 ± 0.05	0.15 ± 0.003	0.14 ± 0.03

Note. Rates are: nmol [C^{14}]-palmitate incorporated mg^{-1} min^{-1} for [C^{14}]-palmitoyl-CoA + unlabeled fatty acyl-CoA, divided by nmol [C^{14}]-palmitate incorporated mg^{-1} min^{-1} for [C^{14}]-palmitoyl-CoA + unlabeled palmitoyl-CoA. Thus, the top row of rates utilizing unlabeled palmitoyl-CoA is 1.00 for each of the plasmids. Results are from 2 experiments each with 2 replicas (± SD).

When pplsC6.3A was used to transform XL1-Blue cells, no significant differences in the relative rates of palmitate incorporation were observed for all of the saturated fatty acyl-CoA substrates and linolenoyl-CoA compared with using pplsC6.3. However, the relative rates of palmitate incorporation were lowered in the presence of oleoyl-CoA and linoleoyl-CoA. The presence of oleoyl-CoA gave a relative value of 0.17 for the incorporation of palmitate which is 37% lower than that of 0.27 from pplsC6.3. The presence of linoleoyl-CoA gave a relative value of 0.08 which is 70% lower than that of 0.26 from pplsC6.3.

When pplsC6.3L was used to transform XL1-Blue cells, no significant differences in the relative rates of palmitate incorporation were observed for all of the unsaturated fatty acyl-CoA substrates, and the saturated substrates stearoyl-CoA and behenoyl-CoA compared with using pplsC6.3. However, the relative rate of palmitate incorporation was lowered in the presence of lignoceroyl-CoA. The presence of lignoceroyl-CoA gave a relative value of 0.25 for the incorporation of palmitate which is 74% lower than that of 0.93 from pplsC6.3.

It was concluded that the changes from the native Thr-122 to Ala in pplsC6.3A was responsible for an increased incorporation of oleate. Similarly, the change to Leu in pplsC6.3L was responsible for an increased incorporation of lignoceroate. Oleate (*cis*-9-octadecanoic acid) and lignoceroate are not found in *E. coli* [30]. The 18 carbon chain-length unsaturated fatty acid in *E. coli* is *cis*-vaccenate (*cis*-11-octadecanoic acid). The greater incorporation of oleate and linoleate *in vitro* reflect the specificity of LPAT from the plants for these substrates [10, 14]. Likewise, the greater incorporation of lignoceroate *in vitro* reflects the altered specificity of the yeast LPAT gene resulting in the incorporation of 26 carbon chain-length saturated fatty acid into its phospholipids [23]. These results demonstrate the potential application of bioengineering LPAT fatty acid substrate specificity to other organisms *via* site directed mutagenesis of the amino acid corresponding to Gln-132 of yeast. A related study reported a single amino acid change altered the fatty acid substrate specificity of lecithin cholesterol acytransferase [31].

It is not known if Thr-122 of the *E. coli* LPAT influences fatty acid substrate specificity by participation in the substrate binding site or by perturbation of the tertiary structure of the enzyme which alters the substrate binding domain. Table 1 includes LPAT sequences surrounding Thr-122 of *E. coli* from microbial, yeast, plant and animal sources. Eberhardt [24] performed a sequence analysis on the human LPAT gene to identify putative functional sites and regions. Ser-149 of human LPAT which corresponds to Thr-122 of *E. coli* was not identified with any potential function. However, Thr-122 of *E. coli* and the corresponding residues within the other sequences, is four residues away from a conserved Arg at position 118 of *E. coli*. Two other well conserved residues are Asp and Ala, at positions 117 and 124, respectively, of *E. coli*. The functional importance of these conserved residues with influencing the fatty acid substrate specificity of LPAT is unknown.

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