Escherichia coli Thioesterase I, Molecular Cloning and Sequencing of the Structural Gene and Identification as a Periplasmic Enzyme*

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Hyesoon Cho† and John E. Cronan, Jr.,‡§
From the Departments of †Microbiology and ‡Biochemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

The structural gene for Escherichia coli thioesterase I (called tesA) has been cloned by use of sequence data obtained from the purified protein. The tesA gene was mapped at 530 kilobase pair of the E. coli physical map (minute 11.6 of E. coli genetic map). The DNA sequence of the tesA gene was obtained and the deduced protein sequence showed that thioesterase I consists of 182 amino acids and has a molecular mass of 20.5 kDa. Comparison of the DNA and protein sequence data suggested that a leader sequence of 26 amino acid residues is cleaved from the primary translation product, and this processing was confirmed by NH₂-terminal sequencing of the primary translation product synthesized in vitro. These data predicted that thioesterase I (long believed to be a cytoplasmic protein) is exported to the cell periplasm, a prediction supported by release of the enzyme from cells upon osmotic shock. The TesA protein sequence does not exhibit any significant overall sequence similarity with other known proteins, although the sequence does contain two small sequence elements found in several other thioesterases. One of these elements is a sequence similar to the serine esterase active sites found in serine proteases and four other thioesterases. A serine residue within this TesA element was shown to be covalently labeled with [³H] diisopropyl fluorophosphate, a potent inhibitor of TesA activity. The second sequence element is a histidine-containing sequence found close to the carboxy terminus of the protein. In the carboxyl termini of the four known active serine thioesterases. The physiological role of thioesterase I is unclear. A strain carrying a null mutation of the tesA gene was constructed and found to have no growth phenotype. Moreover, a strain carrying a plasmid that gave massive overproduction of TesA (approximately 100-fold higher than that of the wild type) also grew normally. In addition a strain containing double null mutations in both tesA and tesB (the structural gene for E. coli thioesterase II) also failed to display any growth phenotype. Analysis of the fatty acid compositions of phospholipid, lipid A, and lipoprotein of the above strains showed no significant changes from a wild type strain.

Thioesterases I and II of Escherichia coli catalyze the hydrolytic cleavage of fatty acyl-coenzyme A (CoA) thioesters in vitro. These enzymes also cleave fatty acyl-acyl carrier protein (ACP) thioesters but at 10³ to 10⁴ lower rates than acyl-CoA esters of the same length (Spencer et al., 1978). Thioesterase I was reported as a protein of 22,000 Da that hydrolyzes only long chain acyl thioesters (C₁₀₋₁₈) and is sensitive to serum esterase inhibitors (Barnes et al., 1968). Thioesterase II is a tetramer of 127,400 Da with a broader substrate specificity (C₅₋₁₈) and is insensitive to the serum esterase inhibitors (Bonnet et al., 1972; Barnes, 1975; Naggert et al., 1991).

Thioesterases of E. coli, unlike their eukaryotic counterparts several of which have a clear chain-terminating function in de novo biosynthesis of fatty acids, currently lack an assigned function in lipid metabolism. It has been suggested that these enzymes may be involved in the cleavage of fatty acyl-ACPs to yield free fatty acid for membrane-bound acyl-ACP synthetase (Monson et al., 1980) or in allowing utilization of exogenously supplied fatty acyl-CoA for phospholipid synthesis (Greenaway et al., 1983). However, these ideas are suspect since the acyl-ACP substrates are unexpectedly resistant to hydrolysis by both E. coli thioesterases (Spencer et al., 1987), and fatty acyl-CoAs are known to be good substrates for glycerol-3-phosphate acyltransferase (Cronan, 1984). The presence of these constitutive enzymes has been an enigma, since they do not seem to be required for fatty acid synthesis and would seem potentially harmful to cells when function of the β-oxidation system is required for growth. It has been also suggested that these enzymes may function as acyltransferases in vitro and that hydrolysis assayed in vitro is due to the absence of the physiological acceptor molecules (Cronan et al., 1987). Indeed, a thioesterase of the luminescent bacterium, Vibrio harveyi has been shown to acylate various hydroxyl group containing molecules in vitro (Byers and Meighen, 1985).

In order to elucidate the in vivo role(s) of the E. coli thioesterases, we previously cloned the tesB gene encoding thioesterase II and mutants lacking or overexpressing the enzyme activity were constructed and studied (Narashimhan et al., 1986; Naggert et al., 1991). In this paper, we report the cloning and genomic mapping of the tesA gene encoding E. coli thioesterase I by use of a reverse genetic strategy and demonstrate that this enzyme is located in the cellular periplasm. The TesA gene as well as mutants deficient in thioesterase I activity have been characterized. The effects of overexpression of the tesA gene have also been studied. We have also identified the "active site serine" of thioesterase I by

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† To whom correspondence should be addressed: Dept. of Microbiology, University of Illinois, 131 Burrill Hall, 407 S. Goodwin Ave., Urbana, IL 61801. Tel.: 217-244-3466; Fax: 217-244-6997.

‡ The abbreviations used are: ACP, acyl-acyl carrier protein; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; bp, base pair(s); kbp, kilobase pair(s); ORF, open reading frame.

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radiochemical modification to gain insight into the catalytic mechanism of the enzyme.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, and Media—**E. coli K-12 strain UB1005 was mutagenized by UV to purify thioesterase I. For PCR, chromosomal DNA was prepared from strain MC4100 (araD139 ΔlacU169 strA). NMS22 (hasd5 Δlac-proAB supE thi F' proAB lacPZM15) was used for DNA sequencing. LE392 (hasd supF54 supE metB lacI galX) was the host strain used for phenotypic studies and lipid analyses. For labeling studies, a fabD mutation was introduced by phage P1 transduction by use of a linked Tn10 insertion into LE392.

Plasmids, pTZ18U and 18R (Mead et al., 1986), and pK18 (Pridmore, 1987) were used to construct subclones for DNA sequencing and to overexpress thioesterase I under the control of lac promoter. They were also used for in vitro transcription and translation of thioesterase I. RB (g/liter, trp 10, yeast extract 1, NaCl 5) or 2XYT medium (g/liter, trp 16, yeast extract 10, NaCl 5) was used for routine growth of bacterial cultures unless otherwise specified.

**Thioesterase Assays—**Thioesterase I and II activities were measured spectrophotometrically by monitoring the increase in absorbance at 412 nm (Barnes, 1975). The assay for thioesterase I (per milliliter): potassium phosphate buffer (pH 7.4), 0.06 mmol; 5,5′-dithiobis(2-nitrobenzoic acid), 100 nmol; bovine serum albumin, 0.08 mg; palmitoyl-CoA, 14 nmol; and enzyme. The assay for thioesterase II (per milliliter): Tris-HCl buffer (pH 8.0), 0.1 mmol; 5,5′-dithiobis(2-nitrobenzoic acid), 100 nmol; decanoyl-CoA, 20 nmol; and enzyme. A unit of activity is the amount of enzyme catalyzing the cleavage of 1 nmol of acyl-CoA/min under the above conditions. The molar extinction coefficient of reduced 5,5′-dithiobis(2-nitrobenzoic acid) was taken to be 13,600.

**Purification of Thioesterase I—**Thioesterase I was purified by the modified method of Barnes and Waki (1986). The cell-free crude extract of E. coli strain UB1005 (160 g, wet weight) was prepared in 0.05 M potassium phosphate buffer (pH 7.4) containing RNase A (10 mg/ml) by sonication for 20 min at 4 °C. The extract was purified by two steps of column chromatography on DEAE-cellulose and one hydroxylapatite chromatographic step exactly as described previously.

The eluate from the hydroxylapatite column was then applied to a Mono Q HR 5/5 column (Pharmacia LKB Biotechnology Inc.) previously equilibrated with 20 mM Tris-HCl (pH 8.0) and subjected to fast protein liquid chromatography. The column was first washed with 20 mM Tris-HCl (pH 8.0) until all of the ultraviolet-absorbing material was eluted and then eluted with a linear gradient of NaCl, 0.0–0.08 M in 20 mM Tris-HCl. The final elution was done with a linear gradient of NaCl, 0.08 to 1 M in 20 mM Tris-HCl. Fractions of 0.5 ml were collected and assayed for thioesterase activity.

**Amino Acid Sequence Analysis—**Amino acid sequencing was performed by automatic Edman degradation in a gas-phase system (Applied Biosystem, 474A protein sequencer). For NH2-terminal sequencing, partially purified thioesterase I was resolved by SDS-PAGE and electrophoretically to a polyvinylidene fluoride membrane. The thioesterase I band was visualized with Fast Stain (Diversified Biotech) and subjected to on-membrane amino acid analysis. To obtain internal peptides the membrane segment containing thioesterase I was treated with CNBr as described in the user bulletin of Applied Biosystems (issue No. 36, March 21, 1988). The resulting peptide was treated with CNBr as described in the kit.

**Protein Expression Studies—**Purified thioesterase was used for in vitro transcription/translation using E. coli S30 extract (Promega). They were also used for overexpression studies. The pkH18 containing the tesA gene was used to purify the recombinant thioesterase I to near-homogeneity. As a control plasmid a 450-bp DNA fragment was deleted from the tesA gene of pkH63 by digesting with HindIII and AccI to give pkH64 (Fig. 1). It was shown that the 20 kDa band corresponding to thioesterase I disappeared in the control extract of the plasmid-containing strain.

**DNA Sequencing—**Subclones containing various sizes of the tesA fragments were generated by restriction enzyme digests with AccI, BssHI, EcoRI, and HincII (Fig. 1). Single strand phagemid DNA templates were prepared as described earlier (Mead et al., 1985). DNA sequencing was performed according to the chain-termination procedure of Sanger et al. (1977). The resulting sequencing reactions were then analyzed by autoradiography and by gel electrophoresis on polyacrylamide gels.

**Gene Mapping, Cloning, and Disruption—**The 440-bp DNA fragment from the PCR reaction was labeled (100 counts/minute/nmol) with [α-32P]ATP by the random primer-labeling method (Feinberg and Vogelstein, 1984) and used as a probe. A Nylon membrane containing a mininet (600 clones) of the Kohara genomic library (Kohara et al., 1987) was hybridized at 68 °C overnight and washed as described in Sambrook et al. (1989). DNAs from clones 304 and 156 were prepared as described in Sambrook et al. (1989). The 3.4-kbp PvuII DNA fragments generated from the clone 156 clone were then transduced into pS109 (to give pkH30) or PTZ718R (to give pkH32), respectively. The pkH30 and pkH32 were transformed into NMS22 to give HC30 and HC32, respectively.

**Protein Expression Studies—**HC32 DNA was digested with BstXI and treated with T4 DNA polymerase. The resulting, linearized DNA was then digested with BstYI and separated by agarose gel electrophoresis. A 720-bp restriction fragment containing the tesA gene including its ribosome-binding site was isolated and cloned into the Smal  and BamHI-digested PTZ18R (pHC61). The EcoRI and PstI-digested PTZ18R containing the 720-bp DNA fragment was cloned into EcoRI and PstI-digested pk18 (pHC63, Fig. 1). The pk18 containing the tesA gene was then transformed into strain S300 (pHC32) to give HC70 which has null mutations in both the tesA and tesB genes.
side was added to cultures of the strains containing tesA-overexpressing or control plasmid at a final concentration of 1 mM. Phospholipids were extracted from cell pellets by a modified method of Bligh and Dyer (1959). Fatty acid methyl esters were obtained by transesterification with 0.5 M sodium methoxide for 20 min at room temperature. They were analyzed by gas chromatography (Hewlett-Packard) on a prepacked capillary column. The run was started at 130 °C. The temperature was first raised to 170 °C at the rate of 2.5 °C/min and then to 230 °C at the rate of 5.0 °C/min.

The bound lipids (lipid A and lipoproteins) in the residue remaining after Bligh and Dyer extraction were washed four times with H2O/CHCl3/CH3OH (0.8:1:2 (v/v/v)) and hydrolyzed by heating in 0.5 M KOH at 70 °C for 16 h. After acidification to pH 2 with 1 N HCl, the fatty acids were extracted with n-hexane and dried under a stream of N2. Fatty acid methyl esters were then made by esterification with 6% KOH in methanol at 70 °C for 2 h and analyzed by gas chromatography as described above.

**Cellular Localization of Thioesterase I—** E. coli strain MG1655 was grown overnight at 37 °C in the presence of isopropyl-1-thio-β-D-galactopyranoside (at a final concentration of 1 mM). Cells were collected and then washed in cold 30 mM Tris-HCl (pH 8.0) three times. The cell pellets were resuspended in 14% sucrose, 30 mM Tris-HCl (pH 8.0) at room temperature. One ml of the sucrose buffer was used for 150 mg wet weight of cell pellet. One-hundredth volume of 0.5 M EDTA (pH 8.0) was added slowly to the cell suspension at 30 °C. Cells were collected at 20,000 × g for 20 min at room temperature and resuspended in ice-cold distilled water. The cell suspension was stirred on ice for 10 min and centrifuged at 20,000 × g for 20 min at 4 °C. Supernatant was collected and assayed for either thioesterase or β-galactosidase activities.

**RESULTS**

**Purification and Amino Acid Sequencing of Thioesterase I—** Thioesterase I was purified approximately 320-fold from E. coli UB1005 by a modification of the method of Barnes and Wakil as described under "Experimental Procedures." We then labeled the thioesterase I protein by treatment of a partially purified enzyme preparation with radioactively labeled DFP, a potent inhibitor of thioesterase I. DFP is known to covalently modify a specific serine residues (active site serine) of other serine esterase enzymes such as some proteases and thioesterases. We used this modification to identify the thioesterase I protein band among those seen upon SDS-PAGE of the partially purified thioesterase I preparation. The radioactive inhibitor labeled a protein band of approximately 20 kDa, the molecular mass expected, if the protein is monomeric (Barnes and Wakil, 1988). However, since DFP is known to react with other E. coli cellular proteins such as serine proteases, we confirmed the identification by showing that the DFP-labeled band comigrated with thioesterase I activity upon electrophoresis under non-denaturing conditions (Hames and Rickwood, 1982). The protein band thus identified as thioesterase I was then electroblotted onto a polyvinylidene fluoride membrane for NH2-terminal amino acid microsequencing as well as for internal peptide sequencing.

**PCR-assisted Cloning of the tesA Gene—** The high degree of codon degeneracy of the amino acid residues of the NH2-terminal sequence of thioesterase I suggested that design of a specific oligonucleotide probe to screen genomic libraries would be problematic. We therefore, sought to obtain the sequence of an internal peptide to allow the use of PCR to isolate a unique internal DNA segment for use as a hybridization probe. A sample of thioesterase I was digested with cyanogen bromide and the resulting peptides separated by reverse-phase high performance liquid chromatography. One of the fractions giving a symmetrical elution profile was then...
sequenced. Unfortunately, the amino acid sequence obtained was ambiguous, presumably due to contamination with other peptides. However, there seemed to be a dominant sequence and a “best guess” sequence was then used to design an oligonucleotide complementary to the putative antisense strand assuming (from cyanogen bromide cleavage) that a methionine residue preceded the determined sequence. We also assumed that the first alanine residue of the obtained NH₂-terminal sequence was preceded by an initiator methionine that had been removed by NH₂-terminal methionyl protease (Hirel et al., 1989) and then designed an oligonucleotide complementary to the sense strand following the recommendations of Martin et al. (1985). Segments of E. coli chromosomal DNA between the two primers was then amplified using two primers in a PCR. Four amplified DNA fragments resulted (data not shown) which were cloned and partially sequenced. One 440-bp fragment was shown to contain a part of the tesA gene by agreement with the determined amino terminus. This DNA fragment was then used as a probe to screen the Kohara E. coli genomic minibank (Kohara et al., 1987). Two overlapping minibank clones, λ154 (7C12) and λ155 (8E4), were hybridized to the probe (data not shown). Further Southern blot analysis (data not shown) on DNA fragments of the two clones confirmed that both clones contained the tesA gene and that the tesA gene was located within the approximately 6-kbp overlapping region of the two λ clones (location at 530 kbp and min 11.6 of E. coli physical and genetic maps, respectively, data not shown). The tesA-containing 6-kbp EcoRI and 3.4-kbp PslII fragments of clone λ155 (8E4) were then subcloned into pSU19 (to give pHC30) and pTZ21R (to give PHC32), respectively. Strains carrying both subclones, H30C and H32 showed approximately 30–60-fold increases in thioesterase I activity compared to a wild type strain. It should be noted that this PCR approach was forgiving of errors in amino acid sequence analysis and codon assignment in that both our primers were flawed. The amino-terminal primer was flawed by inclusion of a 5’-methionine codon and incorrect choice of two leucine codons. Moreover, the 5’ half of the primer designed from the putative internal peptide sequence was incorrect since the last 4 residues of our best guess sequence were incorrect. Our success can be attributed to the rules used to design the probes in which nucleotides were chosen such that mismatch destabilization of base pairing between the primers and chromosomal DNA would be minimized (Martin et al., 1985; Aboul et al., 1985). This strategy resulted in primers that formed a sufficient number of stable base pairs with the template to allow PCR amplification. The 3’ ends of the NH₂-terminal and internal primers had 12 and 10 bases, respectively, and were base-paired to the template without destabilizing mismatches.

Characterization of the tesA Gene and Its Product—Both strands of the ~1.2-kbp DNA fragment were completely sequenced and gave three possible open reading frames (ORFs) for the tesA gene, 207, 208, and 218 residues (Fig. 2). None of these possible amino termini corresponded to that determined for the purified enzyme suggesting the possibility of proteolytic processing of TesA during the purification procedure. This possibility was examined by sequencing the protein purified from an overproducing strain under strongly denaturing conditions. The proteins of growing cells of the overproducing strain were precipitated with 10% trichloroacetic acid, then separated by SDS-PAGE. The TesA band which was identified, collected by electroeluting, and sequenced gave the same sequence as that originally found. This result together with the DNA sequence suggested that the determined NH₂ terminus was not artifactual and that the mature thioesterase I resulted from in vivo processing of the primary translation product.

The tesA gene was transcribed and translated in vitro using an E. coli S30 extract in order to allow detection of the primary translation product. The tesA gene product made in in vitro was indeed of higher molecular mass (approximately 21 kDa) than that isolated from cells (approximately 19.8 kDa, Fig. 3). Consistent with removal of the NH₂-terminal 26 or 36 amino acid residues in vivo. In order to determine which of the possible ORFs encoded the primary translation product, the [³⁵S]methionine labeled tesA gene product obtained by in vitro transcription/translation was subjected to NH₂-terminal radiochemical microsequencing. Each cycle of the Edman degradation was collected and counted by a liquid scintillation spectrometry. Since the penultimate amino acids for the three ORFs are Leu, Met, and Asn, the possible initiation methionines should not be excised by the E. coli methionyl aminopeptidase (Hirel et al., 1989). Upon sequencing ³⁵S radioactivity was detected in the first and second cycles with only background radioactivity found in cycles 10 and 11 (data not shown). This result demonstrated that the 208 residue ORF is the primary translation product of the tesA gene. Thus, the initiation codon is centered at 457 bp of our DNA sequence (Fig. 2) and is preceded by a reasonable Shine-Dalgarno (ribosome binding) element located 8 bp upstream of the

![Fig. 2. Nucleotide sequence of the tesA gene and deduced amino acid sequence of E. coli thioesterase I.](image-url)
plasmid containing translation periplasmic protein by assaying for release of thioesterase I purified protein gave a value of 19,800. Barnes and Wakil the DNA sequence was 20,470 whereas SDS-PAGE of the that obtained by SDS-PAGE (21 kDa). Moreover, the molecular weight standards (molecular masses given); lane 1, crude extract of LE392/pHC63, a control plasmid; lane 2, crude extract of LE392/pHC61, a plasmid containing tesA clone; lanes 4 and 5, purified thioesterase I; lane 6, in vitro transcription/translation of pHG63; and lane 7, in vitro transcription/translation of pHG64. The arrow pointing toward lane 2 indicates overproduced thioesterase I in the crude extract containing pHG63 and one pointing toward lane 6 indicates thioesterase I made in vitro.

Identification of the Active Serine Residue—The deduced protein sequence of E. coli thioesterase I did not exhibit the Gly-X-Ser-X-Gly motif characteristically found in the DFP-sensitive animal and avian thioesterases (Fig. 4). Instead, TesA contains a related sequence Gly14-Asp-Ser-Leu-Ser-Ala-Gly14 (Fig. 2). It seemed possible that either Ser10 or Ser12 might be the site of DFP inhibition (the active site serine) found in the motif. This premise was supported by the presence of Gly-Ile-His sequence near the COOH terminus of the enzyme (either 143 or 145 amino acid residues downstream from the putative active site serine). A Gly-X-His sequence is typically found in the COOH -termini of the animal and avian thioesterases 133-170 residues downstream of the active site serine (Naggert et al., 1988). To test if either of these serine residues was the active site serine, we radiolabeled purified thioesterase I by treatment with [3H]DFP. Taking advantage of the fact that the Ser10 and Ser12 are close to the NH2 terminus of the mature protein, the labeled enzyme could be directly subjected to the Edman degradation. We found that significant amount of radioactivity was released in the 10th cycle (Fig. 5) with the expected carryover into cycle 11. Although a good yield of phenylthiohydantoin-serine resulted in cycle 12, this sample contained only a background level of radioactivity. Therefore, DFP labeling was specific to Ser10 indicating this residue to be the active site serine.

Physiological Role(s) of E. coli Thioesterases—In order to obtain a mutant lacking all thioesterase I activity, we constructed a plasmid containing the tesA gene having a 3-bp deletion and an insertion of 1.2-kbp neomycin phosphotransferase gene. Homologous recombination was then used to substitute this construct for the intact chromosomal tesA following transformation of a recBC sbcBC strain. Southern blot analysis and genetic studies confirmed the expected substitution had occurred (data not shown). It was also shown that the disrupted tesA-containing multicopy plasmid failed to produce more thioesterase I activity than the same strain lacking the plasmid. The tesA null mutation and the previ-
**E. coli Thioesterase**

<table>
<thead>
<tr>
<th>Thioesterase</th>
<th>DFP-Sensitive Ser</th>
<th>Spacer</th>
<th>C-Terminal His</th>
<th>Tail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thioesterase I of E. coli</td>
<td>LGDLSAGY</td>
<td>146 aa</td>
<td>GH</td>
<td>25 aa</td>
</tr>
<tr>
<td>Thioesterase II of rat</td>
<td>FGHRFGSYI</td>
<td>135 aa</td>
<td>GD</td>
<td>23 aa</td>
</tr>
<tr>
<td>Thioesterase II of duck</td>
<td>FGHRFGSFV</td>
<td>135 aa</td>
<td>GN</td>
<td>25 aa</td>
</tr>
<tr>
<td>Thioesterase of rat fatty acid synthase</td>
<td>AGYGFGACV</td>
<td>172 aa</td>
<td>GD</td>
<td>30 aa</td>
</tr>
<tr>
<td>Thioesterase of chicken fatty acid synthase</td>
<td>AGYGFGACV</td>
<td>172 aa</td>
<td>GD</td>
<td>26 aa</td>
</tr>
<tr>
<td>Thioesterase of goose fatty acid synthase</td>
<td>GFGACV</td>
<td>?</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FIG. 4.** **Arrangement of sequence elements in known thioesterases.** Spacer denotes the number of amino acid residues between the underlined serine and the underlined histidine. Tail denotes the number of amino acid residues between the underlined histidine and the carboxyl terminus. The only amino acid sequence information known for the thioesterase of goose fatty acid synthase is the sequence of the peptide containing the active site serine. a, Randhawa et al., 1987; b, Poulouse et al., 1985; c, Naggert et al., 1988; d, Yang et al., 1988; e, Poulouse et al., 1981.

**FIG. 5.** **Edman degradation of [3H]DFP-labeled thioesterase I.** The single letter below each cycle number represents the one-letter symbol of the amino acid residue identified in each cycle.

<table>
<thead>
<tr>
<th>Number of cycle, identified residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
</tr>
<tr>
<td>Radioactivity (cpm)</td>
</tr>
<tr>
<td>Concentration of amino acid (pmole)</td>
</tr>
</tbody>
</table>

**TABLE II**

**Distribution of enzyme activities**

The activities shown are normalized to 1 ml of cells (due to the differing protein concentrations of the fractions). Periplasm denotes the supernatant of the osmotic shock protocol whereas total denotes the activity of a total cell extract of a sample of cells not subjected to osmotic shock. For thioesterase and β-galactosidase activities, increases in absorbance at 412 and 420 nm/min/1 ml of cell culture were measured, respectively. Each value represents an average of two independent experiments. LE392 derivatives were used for this experiment.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Thioesterase*</th>
<th>β-Galactosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Periplasm Total</td>
<td>Periplasm Total</td>
</tr>
<tr>
<td>Wild type</td>
<td>0.04 0.17</td>
<td>0.02 3.26</td>
</tr>
<tr>
<td>ΔtesB::cml</td>
<td>0.05 0.05</td>
<td>0.02 3.67</td>
</tr>
</tbody>
</table>

*Thioesterase activity is the sum of thioesterase I and II in the wild type strain and thioesterase I in the ΔtesB::cml strain.
The amino acid sequence of *E. coli* thioesterase I is the first reported for a non-vertebrate serine thioesterase. Despite the evolutionary distance the *E. coli* enzyme has an overall organization similar to that of the thioesterase II enzymes of mammals and birds which act to prematurely terminate fatty acid chain growth to give short chain length fatty acids needed in certain specialized tissues. All three enzymes are small monomeric proteins of 20–29 kDa with the active site serine located within the NH₂-terminal end of the protein and a Gly-X-His sequence located 21–25 residues from the COOH terminus (135–146 residues from the active site serine). A similar arrangement of sequence elements is seen for the thioesterase domain located at the COOH terminus of the multidomain fatty acid synthase of mammals and birds except that the spacings are slightly larger.

Ser[10] of *E. coli* thioesterase I has the DFP reactivity expected for the active site serine, although the second glycline of the Gly-X-Ser-Gly motif is replaced by serine. Since glycine can play no chemical role in the hydrolization reaction and serine and glycine are both small residues, this substitution seems likely to be conservative. Indeed, when assayed on a common substrate (palmitoyl-CoA) *E. coli* thioesterase I is as potent an acyl-CoA hydrolase as the vertebrate enzymes. Moreover, the *E. coli* enzyme is more sensitive to the classic serine esterase inhibitor, DFP, than are the other enzymes.

Comparison of the *E. coli* thioesterase I sequence with the other known serine thioesterases shows another conserved feature, the presence of a small residue (Ala or Ser) 3 residues COOH-terminal to the active site serine. It should be noted that chemical modification and directed mutagenesis studies (Pazirandeh et al., 1991; Witkowski et al., 1992) of certain of these thioesterases indicate that both the DFP-sensitive serine and the downstream histidine are essential for enzyme activity. These data have led to the suggestion that these enzymes function via a Ser-Asp-His catalytic triad similar to that of serine proteases. This suggestion is quite reasonable, however, it should be noted that the arrangement of the thioesterase Ser and His elements is the opposite of that found in the proteases and no strong candidate for the essential acidic residue of the triad has been identified.

These considerations suggest that *E. coli* thioesterase I might play a role in modulating fatty acid chain length in *vivo*. However, the data reported in this paper eliminate this possibility. First, thioesterase I is located in the cellular periplasm whereas fatty acid synthesis occurs in the cytosol. Second, complete inactivation of the *tesA* gene results in no increase in fatty acid chain length in *vivo* (Tables III and IV). Third, no fatty acid chain length changes are seen in cells which contain greatly increased levels of thioesterase I (Tables III and IV).

A rationale for the presence of a thioesterase in the periplasm of *E. coli* is not obvious. The usual explanation for periplasmic hydrolytic enzymes is to allow scavenging of portions of metabolically useful molecules. For example phosphorylated metabolic intermediates can be hydrolyzed by periplasmic phosphatases to products that can then be transported across the cytoplasmic membrane. However, thioesters such as acyl-CoAs hydrolyze spontaneously in aqueous solution, especially at pH values greater than 7 and thus, enzymatic hydrolysis would not appear needed. Their instability together with the fact that acyl-CoAs are only found as metabolic intermediates suggests that the primary role of thioesterase I might be to hydrolyze substrates other than acyl-CoAs. Reasonable candidates for alternative physiologically relevant substrates for thioesterase I are not obvious. Thioesterase I only hydrolyzes long chain (>12) acyl chain substrates and is inactive both on shorter chain substrates and on long chain substrates that contain a 3-OH substituent. This narrow specificity suggests that any alternate substrate must also contain long chain acyl groups. Oxygen enzymes would seem the most reasonable alternate substrates, but the long
chain acylated molecules abundant in nature (e.g. glycerides) form large micelles at very low concentrations. Such micelles (Lugtenberg and Alphen, 1983; Nikaido and Vaara, 1987) should be unable to pass through the small pores of the E. coli outer membrane. Indeed, the primary function of the outer membrane of the enterobacteria such as E. coli is thought to be to protect the cytoplasmic membrane from surface active agents such as lipid micelles (Tanford, 1980). A possible clue to thioesterase I function may come from the photosynthetic bacterium, Rhodobacter sphaeroides, which has been reported to contain two thioesterases that seem very similar to those of E. coli (Boyce and Lueking, 1984). The lower molecular weight enzyme of R. sphaeroides is a DFP-sensitive enzyme having a charged amino acid in this region in unusual. To our knowledge only one other bacterium, a photosynthetic bacterium, has an arginine residue between the hydrophobic segment and the cleavage site. This feature is atypical, they seem unlikely to be important, since extensive mutagenesis studies of other periplasmic proteins have failed to demonstrate that the number or types of amino acids in either region has an essential role in protein export.

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REFERENCES
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