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Genetic and Biochemical Characterization of an *Escherichia coli* K-12 Mutant Deficient in Acyl-Coenzyme A Thioesterase II

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Mutants of *Escherichia coli* deficient in thioesterase II activity were isolated by taking advantage of the fact that thioesterase I specifically hydrolyzes long-chain (C_{12} to C_{18}) acyl coenzyme A (CoA) esters but is unable to cleave the short-chain substrate decanoyl-CoA. One of these lesions (designated *tesB1*) reduces thioesterase II activity to about 10% of the normal level. The mutant enzyme activity was abnormally labile to temperature, but it was normal in all the other characteristics examined (pH optimum, K_m for decanoyl-CoA, molecular weight). The level of thioesterase I activity was unaffected by the *tesB1* lesion. The *tesB* locus was mapped with a closely linked Tn*10* insertion. *tesB* was mapped to minute 10 of the *E. coli* linkage map, close to the *lon* locus. The clockwise gene order is *lon tesB acrA dnaZ*. The *tesB* mutation is recessive. We found no phenotype for the mutation. The fatty acid compositions of the phospholipids, lipid A, and lipoprotein components are normal in thioesterase II mutants. These data show that thioesterases I and II of *E. coli* are encoded by different genetic loci and strongly suggest that *tesB* is the structural gene for thioesterase II.

Cell-free extracts of *Escherichia coli* contain two distinct soluble enzymes that catalyze the hydrolytic cleavage of naturally occurring thioesters. Two enzymes, thioesterases I and II (we adopted the nomenclature of Barnes [3] for reasons discussed by Spencer et al. [29]), have been purified to homogeneity (5, 7). Thioesterase I is a low-molecular-weight (22,000) protein that is sensitive to diisopropyl fluorophosphate, whereas thioesterase II is an enzyme of higher molecular weight (122,000) composed of four identical subunits, which is insensitive to the inhibitor. The two enzymes also differ in their substrate specificities (4, 5, 7). Thioesterase I is specific for long-chain (C_{12} to C_{18}) acyl coenzyme A (CoA) esters and cannot cleave short-chain (C_6 to C_{10}) acyl-CoA esters or 3-hydroxyacyl-CoA esters, whereas thioesterase II has broader substrate specificity. Thioesterase II cleaves short- and long-chain (C_6 to C_{18}) acyl-CoA esters as well as 3-hydroxyacyl-CoA esters. Both enzymes hydrolyze long-chain thioesters of acyl carrier protein at substantially lower rates than acyl-CoA esters of the same chain length (29).

Long-chain acyl thioesterases occur in a wide variety of organisms, and the properties of several of these proteins have been investigated (8, 11, 19, 23, 28). The physiological role of these enzymes is, however, still unclear. In *E. coli*, an intracellular pool of acyl-CoA esters, the preferred substrates of thioesterases I and II, has not been detected (26). It has been proposed that thioesterases may function as acyltransferases and, in the absence of physiological acceptor(s), the acyl group may be transferred to water (7). Indeed, a thioesterase of the luminescent bacterium *Vibrio harveyi* has recently been shown to acylate various hydroxyl group-containing molecules in vitro (9). Lipid A and lipoproteins of *E. coli* have *O*-acyl and *N*-acyl substituents. The mechanism whereby these molecules are acylated is unknown and could be a site of thioesterase action.

We took a genetic approach toward delineating the role of thioesterases in *E. coli* lipid metabolism. We report isolation and characterization of a mutant deficient in thioesterase II activity. Available evidence suggests that the mutation is at a locus (designated *tesB*) that encodes thioesterase II.

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MATERIALS AND METHODS

Bacterial strains and genetic techniques. All of the bacteria used were derivatives of *E. coli* K-12 and are described in Table 1 and Fig. 1. P1 transductions were performed with a strain of P1 *vir* (15) except that, when strain ML37 was used as the donor, P1 Cm *clr*100 was used as described by Miller (22). Tn*10* insertions linked to the *tesB* locus by P1 cotransduction were isolated by the method of Chang and Cronan (10). A phage Mu lysate of a pool of these Tn*10* insertions was then used to isolate a Tn*10* insertion closely juxtaposed to *tesB* (14). Strain JL4 was isolated in this manner.

Media. Rich broth contained tryptone (10 g/liter), NaCl (5 g/liter), and yeast extract (1 g/liter). Rich agar media was rich broth plus 1.5% agar. Minimal medium was medium E (31) or M9 (22) supplemented with thiamine (1 mg/liter) and glucose or glycerol (0.4%). L-Amino acids (50 mg/liter)–casein hydrolysate (0.1%)–nucleic acid bases (25 mg/liter)–streptomycin sulfate (200 mg/liter)–tetracycline hydrochloride (10 mg/liter)–sodium ampicillin (100 mg/liter)–isopropylthiogalactoside (1 mM) was added to the media as indicated.

Strains carrying the *acrA* mutation were scored by their inability to grow on rich broth agar containing methylene blue (50 mg/liter) (12, 24). The temperature-sensitive *adk* and *dnaZ* strains were scored by their inability to grow on rich broth agar plates at 42°C.

Chemicals. *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine was purchased from Aldrich Chemical Co., Inc. Palmitoyl-CoA and decanoyl-CoA were products of P-L Biochemicals, Inc. Methylene blue was from Fisher Scientific Co. DEAE-cellulose DE-52 was purchased from Whatman, Inc. All other chemicals were of the purest grade available. Sodium [1-^{14}C]acetate was purchased from New England Nuclear Corp.

3-Hydroxymyristoyl-CoA was synthesized by the reaction of the mixed anhydride of 3-hydroxymyristic acid and ethyl chloroformate with the lithium salt of CoA. The mixed

TABLE 1. Bacterial strains

Strain	Relevant markers	Source (reference)[a]
CQ(pJH2)	*thi ara leu purE gal trp his argG rpsL xyl ilv met lacI*^{q} (pJH2)	R. Young (16)
X478	*thi-1 proC32 purE42 leuB6 metE70 trpE38 lysA23 rpsL109 lacZ36 ara-14 xyl-5 mtl-1 tsx-67 azi-6 supE45 tonA23*	CGSC
N43	*acrA lac ara mal xyl mtl*(?) *gal str*	(24)
N2484	*acrA adk lac mtl xyl gal str*	(25)
SG1095	*lon-146*::ΔTn*10*	S. Gottesman
AX727	*dnaZ2016 thi lac str*	CGSC 5445
W3747	F13 *argF*$^+$ *lac*$^+$ *tsx-69 purE*$^+$/*metB1 relA1 spoT1* deletion corresponding to F13	CGSC 5218
KL 16-99	Hfr PO45 of KL16 *recA1 relA1*(?) *thi-1 deoB13*(?) λ$^-$	CGSC
Hfr strains H, C, P801, P4X	Hfr (Fig. 1)	K. B. Low (20)
JL3	*tesB1 env*(?)[b] of CQ(pJH2)	This work[c]
JL4	*zbb*::Tn*10 tes*$^+$ of JL3	This work
ML27	*zbb*::Tn*10 tesB1 env*(?) of CQ(pJH2)	P1 transduction from strain JL4
ML28	Tet^s of ML27	By fusaric acid (21)
ML33	*zbb*::Tn*10 tesB1 adk*$^+$ *env*(?) of N2484	This work
ML37	*lon-146*::ΔTn*10 tesB1* of ML28	This work
ML38	*recA1 lys*$^+$ of X478	X478 × KL16-99
ML43	*zbb*::Tn*10 tesB1 env*(?) of X478	This work
ML45	*zbb*::Tn*10* of AX727	This work
ML48	*zbb*::Tn*10 tesB1 env*(?)$^+$ *dnaZ*$^+$ of AX727	This work
ML49	*zbb*::Tn*10 env*(?)$^+$ *dnaZ*$^+$ of AX727	This work
ML51	*recA1 lys*$^+$ of ML43	This work

[a] Strains designated CGSC were obtained from the *E. coli* Genetic Stock Center, Yale University, New Haven, Conn., through the kindness of B. Bachmann.
[b] *env*(?) refers to an uncharacterized mutation that confers supersensitivity to crystal violet (10 to 100 mg/liter), novobiocin (50 mg/liter), and sodium dodecyl sulfate (0.3%)–disodium EDTA (1 mM) and therefore could be an envelope defect. *env*(?)$^+$ refers to the wild-type allele.
[c] Plasmid pJH2 is an Ap^r derivative of pBR322 which carries the *S*, *R*, and R_z genes of phage lambda. The *S* gene carries the *Sam7* lesion.

anhydride was synthesized essentially as described by Santaniello et al. (27) except that the reaction was allowed to proceed at 0°C for 60 to 90 min. The solvents were blown off in a stream of nitrogen. Thioester formation was carried out as described by Goldman and Vagelos (17).

Mutant isolation. Strain CQ(pJH2) was grown in rich broth containing ampicillin until log phase and then mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine at a final concentration of 0.1 mg/ml for 30 min at 33°C (1). The cells were collected, washed once, suspended in rich broth, and grown overnight. Only 4% of the mutagenized cells survived, and 2% of the survivors were unable to ferment either lactose or maltose. The mutagenized cells were diluted and plated on rich broth containing ampicillin, and single colonies were isolated and screened for thioesterase II activity as described below.

Screening for thioesterase mutants was done in strips of 12 polystyrene test tubes (0.8 by 4 cm) (Labsystems, Inc.). The tubes were sterilized by exposure to 5 min of microwave radiation (2.45 gHz). A colony was inoculated into a test tube containing half-strength rich broth containing ampicillin, streptomycin, and isopropylthiogalactoside. After the culture was grown overnight at 33°C, the cells were lysed by two freeze-thaw cycles (13). The test tube strips were incubated at 42°C for 2 h and then at room temperature for 30 min. Each test tube received 0.02 ml of a reaction mix containing Tris hydrochloride (pH 8.0), 0.02 mmol; 5,5'-dithiobis-(2-trinitrobenzoic acid) (DTNB), 20 nmol; and decanoyl-CoA, 6.6 nmol. After 60 min at room temperature, the intensity of yellow color in the tubes was visually compared with tubes containing unmutagenized cells. Tubes lacking decanoyl-CoA were used to correct for the color produced by reaction of DTNB with the thiol groups of proteins and medium components, whereas uninoculated tubes were included to correct for spontaneous hydrolysis of decanoyl-CoA. Reduced intensity of yellow color compared with controls indicated a possible thioesterase mutant. We performed the same assay in 96-well microtiter plates to isolate Tn*10* insertions near the *tesB* lesion, except that the absorbance in each well was read with a Uniskan photometer (Eflabs) with a 414-nm filter.

We found that microtiter plate assays were not sufficiently reproducible to accurately score cotransduction frequencies. For this purpose, cells from 5-ml overnight cultures were collected by centrifugation at 4,000 × *g* for 10 min at room temperature. The cell pellets were drained well, and the cells were suspended in 0.5 ml of 50 mM Tris hydrochloride (pH 8.0) containing disodium EDTA (20 mM), lysozyme (1 mg/ml), and pancreatic RNase A (10 mg/liter). After incubation at 37°C for 15 min to allow lysozyme action, the cells were lysed by two freeze-thaw cycles. The lysed cells were then incubated at 42°C for 60 min to allow nuclease action. The debris was removed by centrifugation for 15 min at 12,000 × *g* in a microcentrifuge at 4°C. The cell-free extracts were stored at 4°C.

Thioesterase II activity was assayed spectrophotometrically by the method of Barnes (3). The assay for thioesterase I plus II contained (per milliliter): potassium phosphate buffer (pH 7.4), 0.06 mmol; DTNB, 100 nmol; bovine serum albumin, 0.08 mg; palmitoyl-CoA, 14 nmol; and enzyme. The assay for thioesterase II contained (per milliliter): Tris hydrochloride buffer (pH 8.0), 0.1 mmol; DTNB, 100 nmol; decanoyl-CoA, 20 nmol; and enzyme. Reduction of DTNB by the CoA liberated in the thioesterase reaction was measured at 412 nm. Initial rates were measured in a recording spectrophotometer (The Perkin-Elmer Corp.). A unit of enzyme activity was defined as the amount of enzyme catalyzing the cleavage of 1 μmol of acyl-CoA per min under the above conditions. The molar extinction coefficient of reduced DTNB was taken to be 13,600.

Characterization of thioesterase activity. Strains carrying pJH2 were grown overnight in rich broth containing ampicil-

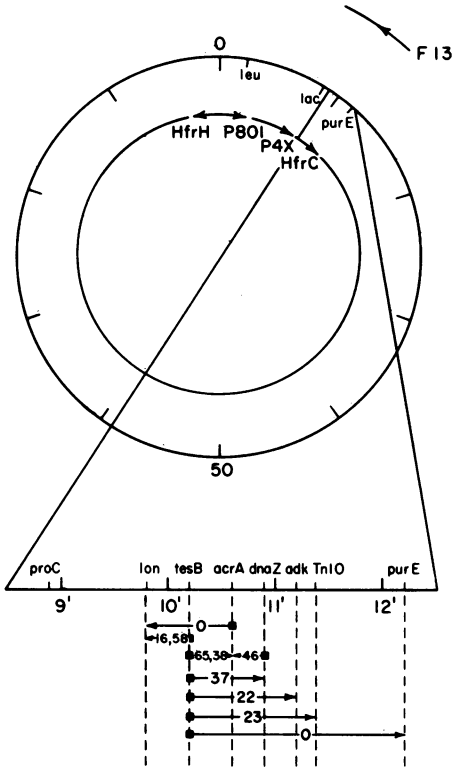

FIG. 1. Relevant portion of the *E. coli* genetic map. The linkages are given as average cotransduction frequencies. The heads of the arrows represent the selected markers. The map is a modification of that of Bachmann (2).

lin and isopropylthiogalactoside. The cells were suspended in 10 mM potassium phosphate buffer (pH 7.4) containing RNase A (10 mg/liter) (6 ml of buffer per g [wet weight]) and lysed as described earlier. The suspension was centrifuged at 40,000 × *g* for 70 min at 4°C. The supernatant was stored at 4°C and used for further characterization. All subsequent steps were performed at 4°C. The thioesterases were separated on a column of DEAE-cellulose exactly as described by Barnes et al. (4). Molecular weights were estimated by gel filtration on a column of Sepharose 4B (2.2 by 50 cm) in 10 mM potassium phosphate buffer (pH 7.4).

Lipid analyses. The total amounts of fatty acids in free and bound cellular lipids were measured by labeling cells with [1-^{14}C]acetate. Cells were grown at 37°C to a density of 5 × 10^8 cells per ml in medium M9-glycerol supplemented with [1-^{14}C]acetate (1 mCi/liter; 57.6 mCi/mmol). The cells were chilled, collected by centrifugation, and washed twice with cold 0.9% NaCl before analysis.

The fatty acid compositions of cellular lipids were analyzed in cells from overnight cultures grown in rich broth at 37°C. Phospholipids were extracted from cell pellets (from 3-ml cultures) by the method of Bligh and Dyer (6). Labeled phospholipids were dried, dissolved in toluene-ethanol (80:20), and counted in Econofluor (New England Nuclear). 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 on a column (1/8 in. by 10 ft) of 10% diethylene glycol succinate on Chromosorb-WAW (80/100 mesh) at 170°C.

The lipids in the residue remaining after Bligh and Dyer extraction were termed bound lipids and consisted of lipid A and lipoprotein. The residue (from 3-ml cultures for labeled cells, 25-ml cultures for fatty acid composition) was washed four times with water-$CHCl_3$-CH_3OH (0.8:1:2 [vol/vol]) and hydrolyzed by heating with 0.5 M KOH at 70°C for 16 h. After acidification to pH 2 with dilute HCl, the fatty acids were extracted with petroleum ether-ether (1:1) or *n*-hexane. Labeled fatty acids were dried and counted as described above. For analysis, the fatty acids were esterified by heating at 70°C with 6% HCl in methanol for 1 h and analyzed by gas chromatography on a column (1/8 in. by 6 ft) of 10% SP2330 on Chromosorb WAW (100/120 mesh). The run was started at 125°C, and after 3 min the temperature was raised to 165°C at the rate of 2°C per min.

Murein-lipoprotein sacculi were made from labeled cells from 27-ml cultures by boiling cell pellets in 5 ml of 4% sodium dodecyl sulfate (18). The sacculi were pelleted by centrifugation at 171,000 × *g* for 2 h. They were washed once with 5 ml of boiling 4% sodium dodecyl sulfate and then three times with 5 ml of water (until no soluble radioactivity was present in the wash). The sacculi were hydrolyzed with 6 N HCl in a sealed ampoule at 110°C for 17 h. Fatty acids were extracted into *n*-hexane, dried, and counted as described above. The aqueous phase was neutralized with NaOH and counted in PCS solubilizer (Amersham Corp.).

RESULTS

Isolation and characterization of a *tesB* mutant. Strain CQ(pJH2) was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and approximately 2,000 mutagenized colonies were lysed by exploitation of the bacteriophage lambda genes carried on the plasmid (13). The lysates were then screened for thioesterase II activity as described in Materials and Methods. Several mutant candidates were obtained and further characterized by assay of their thioesterase II activities in crude extracts. One strain, JL3, had about 10% of the activity of the parent strain. We characterized the lesion in this mutant, which we designated *tesB1* (for thioesterase II).

Crude extracts of strain JL3 or ML27 (and of all other

TABLE 2. Thioesterase activities of various strains

Expt no. and strain(s)	Genotype	Total thioesterase activity[a] (palmitoyl-CoA) in mU/mg of protein	Thioesterase II activity (decanoyl-CoA) in mU/mg of protein
1			
CQ(pJH2)	Wild type	24.3	11.0
ML27	*tesB1*	10.7	2.2
CQ(pJH2) and ML27[b]		19.4	6.8
2			
CQ(pJH2)	Wild type	31.8	10.6
JL3	*tesB1*	13.3	1.1
JL4	*tesB*$^+$ of JL3	29.4	11.1
3			
CQ(pJH2)	Wild type	37.5	13.3 (8.9)[c]
ML27	*tesB1*	15.8	1.9 (1.8)[c]

[a] Total thioesterase activity is the sum of the activities of enzymes I and II.
[b] Equal amounts of proteins from the two extracts were mixed and assayed.
[c] Values in parentheses represent specific activity with 20 μM 3-hydroxymyristoyl-CoA as the substrate. Assay conditions were identical to those used for decanoyl-CoA.

TABLE 3. Properties of DEAE-cellulose-purified thioesterases

Thioesterase and strain	Concn (M) of NaCl required for elution	Sp act with palmitoyl-CoA (mU/mg)	Sp act with decanoyl-CoA (mU/mg)	Mol wt (10^3)	K_m for decanoyl-CoA (μM)
I					
CQ(pJH2)	0	287	<0.04	25	
ML27	0	379	<0.04	33	
II					
CQ(pJH2)	0.25	36.7	20.7	125	7.1
ML27	0.25	3.4	2.4	138	8.3

tesB1 strains) tested contained 10 to 20% of the thioesterase II activity and about 50% of total thioesterase (I plus II) activity of isogenic wild-type strains (Table 2). The thioesterase II activity of strain JL3 had the same K_m for decanoyl-CoA (Table 3) and pH optimum (pH 8.5) as the parental strain CQ(pJH2). Further enzyme characterization was done on strain ML27, in which the *tesB1* lesion had been moved into an unmutagenized background.

The residual thioesterase II activity in strain ML27 was more heat labile than the thioesterase II activity of the parental strain (Fig. 2). When crude extracts from the parent and mutant strains were mixed before heating (or if heated extracts were mixed), the effects were additive, indicating that the low activity and temperature sensitivity of the mutant thioesterase II was not due to its interaction with some other component of crude extracts. The heat stabilities of total thioesterase activities in crude extracts of strain CQ(pJH2) and the *tesB* mutant, strain ML27, were essentially the same, since the small difference observed in total activity (assayed with palmitoyl-CoA) can be attributed to loss of mutant thioesterase II activity on heating.

To provide better evidence that *tesB1* affected only thioesterase II, we separated the thioesterases from strains CQ(pJH2) and ML27 on columns of DEAE-cellulose (Fig. 3). The peak of thioesterase I activity was unaltered in strain ML27, whereas the peak of thioesterase II activity was greatly diminished (both peaks eluted at the usual salt concentration). The specific activity of thioesterase I in the DEAE-cellulose eluate was comparable in both strains, whereas the specific activity of thioesterase II in strain ML27 extracts was 10 to 20% of that of strain CQ(pJH2) when assayed with palmitoyl-CoA, decanoyl-CoA, or 3-hydroxymyristoyl–CoA (Tables 2 and 3; data not shown). Similar results were found with extracts of the original mutant, strain JL3. The DEAE-cellulose-purified mutant thioesterase II was more temperature sensitive than the corresponding wild-type enzyme, in agreement with the results of the crude extracts (Fig. 2 and data not shown) and was also more labile on storage. Gel filtration showed that the thioesterases of both strains had molecular weights (Table 3) similar to those reported earlier (5, 7).

Mapping of the *tesB* gene. A Tn*10* insertion closely linked to the *tesB* gene was obtained as described in Materials and Methods. This insertion (designated *zbb*::Tn*10*) was 20 to 30% linked to *tesB1* by P1 cotransduction.

Conjugational mating of strain JL4 (which has markers spaced around the chromosome) with HfrC showed that the

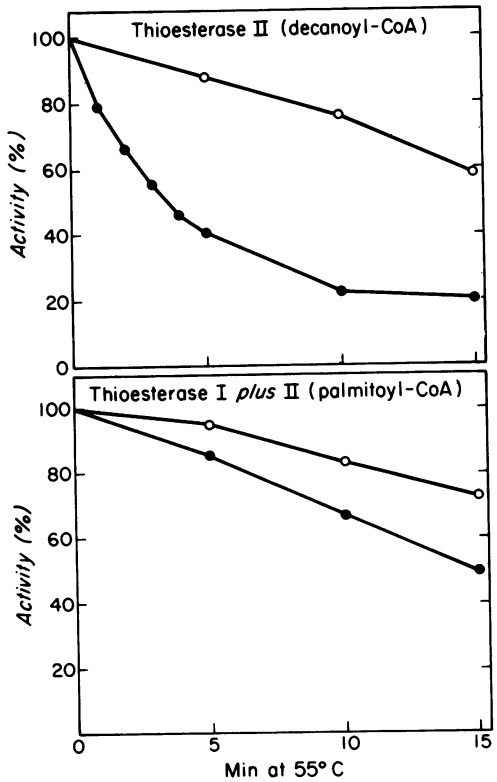

FIG. 2. Temperature sensitivity of thioesterase II in the *tesB* mutant. Crude extracts adjusted to 10 mg of protein per ml were heated at 55°C. Symbols: ○, wild type; ●, mutant.

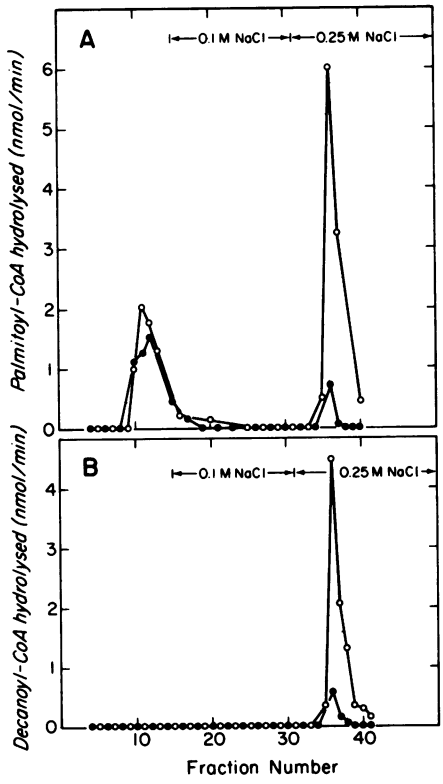

FIG. 3. DEAE-cellulose column chromatography. Protein samples (30 mg) were loaded on a column (1.5 by 13 cm) in 10 mM potassium phosphate buffer (pH 7.4), and 4-ml fractions were collected. Symbols: ○, wild type; ●, mutant.

TABLE 4. Cotransductional mapping

Cross no.	P1 donor and relevant marker	Recipient	Marker selected	No. of colonies scored	% Cotransductional frequency
1	ML27 *zbb*::Tn*10*	N2484 *adk*	Tet^r	276	78
2	ML27 *zbb*::Tn*10*	N2484 *acrA*	Tet^r	279	28
3	ML27 *zbb*::Tn*10 purE*	N2484	Tet^r	279	3
4	ML27 *zbb*::Tn*10 tesB*	N2484	Tet^r	43	23
5	ML27 *zbb*::Tn*10*	N2484 *adk*	*adk*$^+$	280	81
6	ML27	N2484 *adk acrA*	*adk*$^+$	280	31
7	ML27 *purE*	N2484 *adk*	*adk*$^+$	280	<2
8	ML27 *tesB*	N2484 *adk*	*adk*$^+$	45	22
9	N2484	ML27 *tesB purE*	*pur*$^+$	42	<2
10	N2484	ML27 *zbb*::Tn*10 purE*	*pur*$^+$	221	<0.5
11	N2484 *adk*	ML27 *purE*	*pur*$^+$	221	<0.5
12	N2484 *acrA*	ML27 *purE*	*pur*$^+$	137	<0.7
13	SG1095 *lon-146*::ΔTn*10*	ML28 *tesB*	Tet^r	43	16
14	ML27 *zbb*::Tn*10*	N43 *acrA*	*acr*$^+$	140	25
15	ML27 *tesB*	N43 *acrA*	*acr*$^+$	48	65
16	ML27 *zbb*::Tn*10*	AX727 *dnaZ*	*dna*$^+$	185	50
17	ML27 *tesB*	AX727 *dnaZ*	*dna*$^+$	46	37
18	ML27 *zbb*::Tn*10*	AX727 *dnaZ*	Tet^r	28	36
19	ML37 *lon*-146::ΔTn*10*	N43 *acrA*	Tet^r	200	<0.5
20	ML37 *tesB lon*-146::ΔTn*10*	N43	Tet^r	50	58
21	ML45 *dnaZ*	ML33 *acrA*	*acr*$^+$	100	46
22	ML45	ML33 *acrA tesB*	*acr*$^+$	50	38

Tn*10* insertion was most closely (~30%) linked to the *leu* locus. Of the *leu*$^+$ recombinants obtained by conjugation with HfrH, 33% were Tet^s, whereas none of the *leu*$^+$ recombinants obtained in crosses between strain JL4 and Hfr strain P4X or P801 were Tet^s. These data indicated that the Tn*10* insertion in strain JL4 was located between the origins of Hfr strains P4X and P801, i.e., between minutes 6 and 12 of the *E. coli* genetic map.

No P1-mediated cotransduction of Tet^r with the *proAB*, *lacZ*, *proC*, or *purE* loci was observed. The Tn*10* insertion did cotransduce with the *adk* locus (80%). Further transduction experiments (Table 4) and three-factor crosses (Table 5) showed that the Tn*10* insertion was located between the *adk* and *purE* loci, very close to the *adk* locus. The *tesB* locus cotransduced with the *zbb*::Tn*10* insertion (23%) but not with *purE*. Further experiments (Table 4, Fig. 1) showed that *tesB* cotransduced with *lon* (16 to 58%), *acrA* (38 to 65%), and *dnaZ* (37%). The variations in values are probably due, at least in part, to the small numbers assayed. In a three-factor transductional cross of *acrA dnaZ tesB* with *acr*$^+$ as the selected marker, there was no clear four-crossover class (Table 5). These data are consistent with the gene order *lon tesB acrA dnaZ zbb*::Tn*10 pur*E.

Diploidy at the *tesB* locus. F13, which carries the region from 7 to 12 minutes of the *E. coli* chromosome (20), was transferred to strain ML51 (*tesB1 recA*) from strain W3747 by mating. Crude extracts of the resultant merodiploids had the same amount of thioesterase II activity as the isogenic wild-type strain ML38 (*recA*). Since ML51 carries *recA*, all cells in the diploid cultures were merodiploids, not recombinants, and were able to transfer *dnaZ* to strain AX727. These results indicated that the *tesB* gene is carried on F13 and that the *tesB* mutation is recessive to the wild-type allele.

Characterization of the *tesB1* mutant. Although the thioesterase II activity in *tesB1* strains were temperature sensitive, strains that carried the *tesB* gene grew normally at 42°C. These strains also showed no membrane defect resulting from the thioesterase deficiency. Strain ML27 was not leaky (as are lipoprotein mutants) when scored by the RNase leakage method (33). Both strains CQ(pJH2) and ML27 exhibited comparable sensitivities toward detergents (so-

TABLE 5. Three-factor crosses

Cross no.	Donor	Recipient	Marker selected	No. of colonies scored	Class	% Frequency
1	ML27 *zbb*::Tn*10*	N2484 *acrA adk*	Tet^r	179	*adk*$^+$ *acrA*$^+$	28
					adk$^+$ *acrA*	52
					adk acrA$^+$	0
					adk acrA	20
2	ML27 *zbb*::Tn*10 tesB*	N2484 *adk*	*adk*$^+$	45	Tet^r *tesB*	20
					Tet^r *tesB*$^+$	60
					Tet^s *tesB*	16
					Tet^s *tesB*$^+$	4
3	ML45 *dnaZ*	ML33 *acrA tesB*	*acrA*$^+$	50	*dnaZ*$^+$ *tesB*$^+$	8
					dnaZ$^+$ *tesB*	52
					dnaZ tesB$^+$	30
					dnaZ tesB	10

TABLE 6. Fatty acid analyses

Fatty acid composition[a]	Phospholipid (% by weight)[b]		Bound lipid (%)[b]	
	tesB$^+$	*tesB*	*tesB*$^+$	*tesB*
12:0			7	8
14:0	5	5	9	9
Unknown			8	9
16:0	43	42	5	5
16:1	6	7	3	3
17[c]	35	34		
18:0				
18:1	6	5		
19[c]	5	7		
3-OH-14:0			68	65

[a] Measured in strains CQ(pJH2) (*tesB*$^+$) and ML27 (*tesB*).

[b] Fatty acid content was measured in strains ML49 (*tesB*$^+$) and ML48 (*tesB*) by using [1-^{14}C]acetate. Fatty acids of phospholipid accounted for 11% of total counts incorporated into trichloroacetic acid-precipitable material of both strains. Fatty acids of bound lipid accounted for 6 and 7%, respectively, of total counts incorporated into $CHCl_3$-CH_3OH-insoluble residue. Fatty acids of lipoprotein accounted for 3 and 4%, respectively, of total counts incorporated into murein-lipoprotein sacculi. Fatty acid composition of lipoprotein was measured in strains ML49 and ML48. The compositions of ester-linked lipoprotein fatty acids of both strains were similar and similar to their phospholipid fatty acid composition. Palmitic acid was the major amide-linked fatty acid.

[c] Cyclopropane derivative.

dium deoxycholate), dyes (methylene blue, eosin Y, acridine orange, acriflavine), and antibiotics (penicillin G, rifampin, nalidixic acid, chloramphenicol) known to be more lethal to cells with defective membranes (12, 30, 32). However, strains JL3 and ML27 and many other strains which inherited the *tesB1* lesion from these strains by cotransduction with *zbb*::Tn*10* are unusually sensitive to rich broth agar containing crystal violet (10 to 100 mg/liter), sodium dodecyl sulfate (0.3%)–EDTA (1 mM) at 37°C, or novobiocin (50 mg/liter). This supersensitivity was due to a mutation designated *env*(?) closely linked to the *acrA* and *tesB* loci but distinct from either locus and mapped between these two loci (as shown by P1 cotransduction and three-factor crosses; data not shown).

Assay of acylated molecules. If the thioesterases are, in fact, acyl transfer enzymes which, in the absence of the appropriate acceptor, transfer acyl groups to water, then mutants defective in thioesterase activity would be expected to be deficient in acylated cellular constituents. We therefore compared the fatty acid contents and compositions of acylated molecules of isogenic *tesB1* and *tesB*$^+$ strains.

We found no alterations in the amount or molecular species of the fatty acylated molecules of the mutant and wild-type strains (Table 6). The phospholipids, lipid A, and lipoprotein fractions from both strains were assayed both as to mass and acyl chain composition. The results indicated that reduction of thioesterase II activity by 90% does not significantly affect a known cellular acylation process.

DISCUSSION

The properties of the *tesB1* mutant argues strongly that thioesterases I and II of *E. coli* are distinct enzymes. Since the subunits composing thioesterase II were of a size (7) similar to the native molecular weight reported for thioesterase I (5), it was possible that the enzymes were related. However, *tesB1* strains possess altered thioesterase II activity, whereas the thioesterase I activity is unaltered.

It seems likely that the *tesB* locus is the structural gene for thioesterase II, since the mutant enzyme had decreased activity with all three substrates tested (decanoyl-CoA, 3-hydroxymyristoyl–CoA, and palmitoyl-CoA) and was abnormally thermolabile. The lesion is recessive to the wild-type allele and transductional introduction of the wild-type allele restores normal activity. However, since the *tesB1* mutation has no effect on growth, we were unable to isolate revertants of the mutational lesion.

The physiological function of thioesterase II remains an enigma. *tesB1* strains are 90% deficient in enzyme activity but have no obvious physiological or biochemical defect. Moreover, the increased thermolability of the residual enzyme activity causes no defect in growth at high temperatures. This lack of phenotype may be due to the residual thioesterase II activity remaining or to the presence of thioesterase I, which may substitute for thioesterase II (or both). We are currently attempting to isolate mutants deficient in both thioesterase activities. It should be noted that another gram-negative bacterium, *Rhodopseudomonas sphaeroides* has two thioesterase activities very similar to those of *E. coli* (8). The finding that two such dissimilar bacteria possess similar thioesterase complements suggests that these enzymes play an important but as yet not understood role.

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