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Roles of Ser^{101}, Asp^{236}, and His^{237} in catalysis of thioesterase II and of the C-terminal region of the enzyme in its interaction with fatty acid synthase

(site-directed mutagenesis/fatty acids)

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ABSTRACT **Thioesterase II (TE II), present in specialized tissues, catalyzes the chain termination and release of medium-chain fatty acids from fatty acid synthase [FAS; acyl-CoA:malonyl-CoA *C*-acyltransferase (decarboxylating, oxoacyl- and enoyl-reducing and thioester-hydrolyzing), EC 2.3.1.85]. We have expressed rat mammary gland TE II in *Escherichia coli* and created several site-directed mutants. Replacing both Ser^{101} and His^{237} with Ala yielded inactive proteins, suggesting that these residues are part of the catalytic triad as in FAS thioesterase (TE I). Mutating the conserved Asp^{236} or modifying it with Woodward's reagent K caused partial loss (40%) of TE II activity and reduced reactivity of Ser^{101} and His^{237} toward their specific inhibitors, phenylmethylsulfonyl fluoride and diethylpyrocarbonate, respectively. These results suggested that Asp^{236} enhances, but is not essential for, the reactivity of Ser^{101} and His^{237}. Mutation analyses revealed that, at the C terminus, Leu^{262} is critical for TE II to interact with FAS. Hydrophobic interactions seem to play a role, since the interaction of TE II with FAS is enhanced by polyethylene glycol but reduced by salt. The Ser^{101} and His^{237} mutants and a synthetic C-terminal decapeptide did not compete in the interaction. These results suggest that a TE II–acyl FAS complex forms first, which then is stabilized by the interaction of the hydrophobic C terminus of TE II with FAS, leading ultimately to hydrolysis and release of fatty acid.**

Fatty acid synthase [FAS; acyl-CoA:malonyl-CoA *C*-acyltransferase (decarboxylating, oxoacyl- and enoyl-reducing and thioester-hydrolyzing), EC 2.3.1.85] catalyzes the synthesis of long-chain fatty acids from acetyl CoA, malonyl CoA, and NADPH. The native enzyme is a homodimer of a multifunctional protein (M_r, 274,510) (1, 2). Each protein has a site for the prosthetic group, 4′-phosphopantetheine (acyl carrier protein), to which the growing acyl groups are bound, and contains the six catalytic activities required for chain elongation and reduction and a thioesterase activity (TE I) for chain termination and release of free acid. The product of synthesis is primarily palmitate with stearate and myristate as minor components, each reflecting the substrate specificity of TE I for acyl CoA derivatives (2, 3). In some specialized tissues, such as the lactating mammary gland of nonruminant animals (4) and the uropygial gland of waterfowl (5), shorter chain acids (C_8, C_{10}, and C_{12}) are also produced due to the presence of yet another thioesterase, medium-chain *S*-acyl FAS thioesterase (TE II), which is not part of FAS. TE II has broad substrate specificity and interacts with FAS to hydrolyze the fatty acyl thioesters before they mature to longer-chain acids. The cDNA sequences of the TE IIs of the rat mammary gland (6) and of the uropygial gland of mallard ducks (7) have been reported. We recently showed by site-directed mutagenesis that the Ser^{101} and His^{274} of TE I are important in its mechanism of action (2, 3). The amino acid sequences of TE I and TE II show little or no similarity, except for the active site motifs. Recently, Witkowski *et al.* (8) reported that modification of the cysteine thiol near the C terminus of TE II (Cys^{256}) or loss of the C-terminal dipeptide reduces the interaction and activity of TE II with FAS but not with acyl-CoA model substrates. To understand the importance of the active-site sequences and how TE II interacts with FAS, we performed site-directed mutagenesis of residues at the catalytic sites and C terminus of TE II. Herein, we present the results of these studies.

EXPERIMENTAL PROCEDURES

Materials and Methods. FAS from rat livers (9) and native TE II from lactating rat mammary glands (10) were prepared as described.

TE II was assayed by one or more of the following methods. (*i*) Hydrolysis of decanoyl CoA. The reaction mixture (1.0 ml) contained 40 mM Tris·HCl (pH 7.5), 1 mM EDTA, 20 μM decanoyl CoA, 0.2 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), and TE II (≈6 μg). CoASH release was followed spectrophotometrically at 412 nm (2). (*ii*) Hydrolysis of [1-^{14}C]palmitoyl CoA. The reaction mixture and assay were the same as described (3). (*iii*) The interaction of TE II with FAS was measured by the reactivation of FAS in which TE I activity had been inhibited with phenylmethylsulfonyl fluoride (PMSF) (10). The assay mixture contained PMSF-treated FAS (85 μg), 50 μM acetyl CoA, 100 μM malonyl CoA, 300 μM NADPH, and 50 mM phosphate buffer (pH 7.5). The reaction was started by adding TE II (1–6 μg) and was followed spectrophotometrically at 340 nm (10).

Binding of the [1-^{14}C]palmitoyl group to TE II was analyzed as reported (3). The protein concentration was determined spectrophotometrically by using an $A_{280}^{1\%}$ of 8.5 for TE II and of 10.2 for FAS (9, 10) or by using a Bio-Rad protein assay kit according to the manufacturer's protocol. Woodward's reagent K was purchased from Aldrich. DEAE-Bio-Gel was purchased from Bio-Rad. All chemicals were of reagent grade and were obtained from commercial suppliers.

Treatment of TE II with Woodward's Reagent K. Protein samples (250 μg) in 0.5 ml of 20 mM Tris·HCl (pH 7.5) were treated with various amounts of Woodward's reagent K (20–400 nmol) at 25°C for 30 min (11), and excess reagent was removed by filtration on a PD-10 (Pharmacia) column. The extent of protein modification was calculated from the absorbance at 340 nm (ε = 7000 $M^{-1}\cdot cm^{-1}$), protein concentration, and an assay for TE II activity (11).

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Abbreviations: FAS, fatty acid synthase; TE I, thioesterase component of FAS; TE II, medium-chain *S*-acyl FAS thioesterase; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); PMSF, phenylmethylsulfonyl fluoride; DEPC, diethylpyrocarbonate.

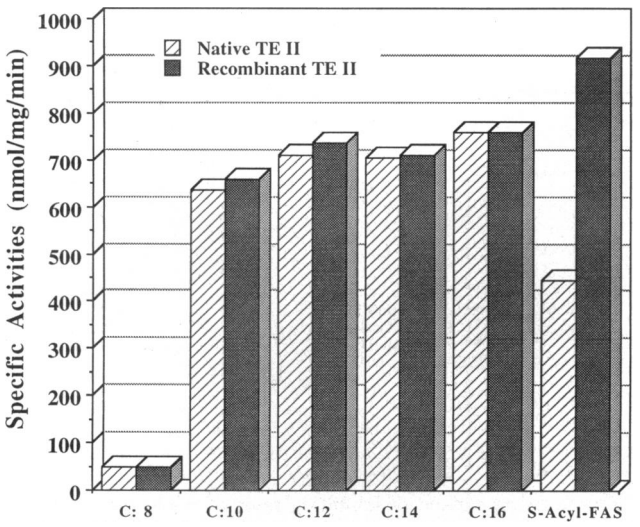

FIG. 1. Relative activities of native and recombinant TE II with acyl-CoA derivatives of various chain lengths or *S*-acyl FAS as substrates. The following concentrations of acyl CoA were used: C_8, 40 μM; C_{10}, 20 μM; C_{12}, 10 μM; C_{14}, 10 μM; C_{16}, 5 μM. In the *S*-acyl FAS assay, PMSF-inhibited FAS was used as described.

Cyanogen Bromide Cleavage of TE II. Recombinant TE II or Woodward's reagent K-modified TE II was treated with cyanogen bromide in 70% formic acid at 25°C for 24 h. The peptides were separated on a Vydac (Hesperia, CA) C18 reverse-phase HPLC column (5 μm; pore size, 300 Å; 0.46 × 25 cm) using a linear gradient (0–60%) of acetonitrile in 0.1% trifluoroacetic acid over 40 min and a flow rate of 0.6 ml/min. The peptides were collected and partially sequenced to identify them using an Applied Biosystems 470A sequencer.

Cloning, Expression, and Mutagenesis of TE II cDNA. Two oligonucleotides, one corresponding to the 5′ end of the rat TE II cDNA coding region and the other corresponding to the 3′ end (6), were synthesized. The first-strand cDNA was synthesized by using the 3′ oligonucleotide, total RNA isolated from lactating rat mammary glands, and reverse transcriptase as described (12). The PCRs were performed using the first-strand cDNA and the two oligonucleotides as described (13). In these reactions, the sequence of the 5′ primer used was modified slightly to introduce a *Bam*HI site for subsequent cloning and expression. The PCR-amplified 0.8-kb fragment was cloned into pUC18, and its sequence was verified. The TE II cDNA was cloned into a λPL promoter-based *Escherichia coli* expression vector, pOTs (14). The plasmid, pOTs-TE II, was expressed in *E. coli* strain AR68 (λeI857; protease deficient) as described (2, 3).

The *Hin*dIII/*Xba* I cDNA fragment (see Fig. 2) was cloned into M13mp18 vector and used for mutagenesis. The site-directed mutants were made as described (2, 3, 15).

RESULTS AND DISCUSSION

Isolation and Properties of TE II. Expression of pOTs-TE II in *E. coli* strain AR68 was performed as described for pOTs-acyl carrier protein-TE I (2). In a typical experiment, 20 liters of induced culture (30 g) was harvested, and the cell-free extract was fractionated with ammonium sulfate (2). The protein fraction that precipitated between 30% and 55% saturation was collected, dissolved in buffer A (20 mM Tris·HCl, pH 7.5/1 mM EDTA), dialyzed, and further fractionated on a DEAE-Bio-Gel column (3 × 30 cm) using a linear salt gradient (0–200 mM NaCl) of 200 ml each in buffer A. Fractions containing TE II activity were pooled, and the proteins were precipitated with $(NH_4)_2SO_4$ (0–60% saturation), redissolved in buffer A, and fractionated on a Sephadex G-75 column (5 × 100 cm). Active fractions were pooled and further purified by using a DEAE-5PW-FPLC column (0.75 × 7.5 cm) and a linear salt gradient (0–200 mM NaCl) of 30 ml each in buffer A. The enzyme was homogeneous by SDS/PAGE criteria and homologous with native TE II (M_r, 29,000) as shown by Western blotting using antibodies raised against native rat TE II (data not shown). Sequence analyses of the N and C termini (16) indicated that the recombinant protein was full length as expected from the cDNA sequence (6), except for the N-terminal changes described in Fig. 2. Comparative analyses of specific activities of recombinant TE II with those of native TE II showed no significant differences in substrate specificity when acyl-CoA derivatives were used (Fig. 1). However, the rate of hydrolysis of the acyl groups from *S*-acyl FAS (measured by the reactivation of PMSF-inhibited FAS by recombinant TE II) was nearly twice that of native TE II (Fig. 1). This aspect is addressed in detail below.

Site-Directed Mutagenesis of TE II. The serine esterases function through a catalytic triad mechanism involving Ser, His, and Asp (17). The amino acid sequences containing these residues are highly conserved in thioesterases (2), and, as shown in studies with TE I, the conserved Ser^{101} and His^{274} are necessary for enzymatic activity (3, 18). To extend these observations to the mechanism of action of TE II, we performed site-directed mutagenesis of the enzyme. Although TE II has hardly any homology with TE I, it has the conserved residues Ser^{101}, Asp^{236}, and His^{237}, which we mutated as indicated in Fig. 2.

Smith and coworkers (8) showed that modification of Cys^{256} by thionitrobenzoate or loss of the C-terminal Leu-Thr peptide significantly reduces the interaction of TE II with its natural substrate acyl FAS. To understand the nature of these interactions and the role of some amino acid residues at the C terminus of TE II, we performed site-directed mutagenesis in this region (Fig. 2). All mutations were confirmed by nucleotide sequencing (data not shown). The plasmids containing each of these mutations were expressed in *E. coli* strain AR68, and the mutant proteins were isolated. During

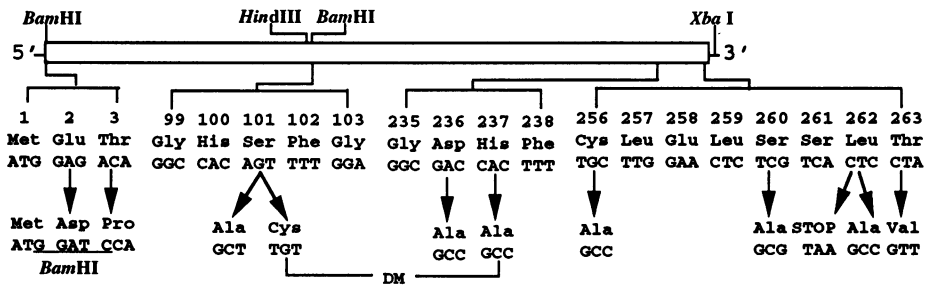

FIG. 2. Summary of TE II site-directed mutagenesis and analysis of the purified mutant proteins by SDS/PAGE. Position and codon of both wild-type and mutant proteins are shown. For cloning purposes, nucleotides at the 5′ end were altered as indicated. These alterations resulted in modification of 2 amino acid residues at the N terminus of recombinant TE II. A double mutant (DM) was also constructed by replacing Ser^{101} with Cys and His^{237} with Ala.

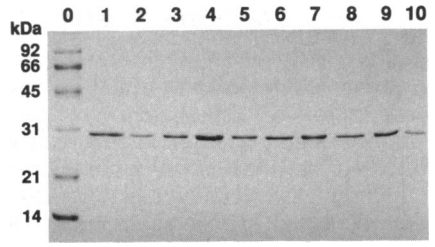

FIG. 3. SDS/PAGE analysis of purified mutant TE II. Lane 0, standard proteins of molecular masses indicated; lanes 1–10, mutant proteins Ser[101]-Ala, Ser[101]-Cys, Asp[236]-Ala, His[237]-Ala, Ser[101]-Cys/His[237]-Ala, Cys[256]-Ala, Ser[260]-Ala, Leu[262]-Stop, Thr[263]-Val, and Leu[262]-Ala, respectively.

purification, the inactive protein was followed solely by its immunoreactivity with anti-TE II antibodies as analyzed by Western blotting. The isolated mutant proteins were essentially homogeneous as judged by the SDS/PAGE analysis shown in Fig. 3.

Characterization of Ser[101], Asp[236], and His[237] Mutants. As shown in Table 1, the proteins containing the Ser[101]-Ala or the His[237]-Ala mutation were inactive, thus reaffirming the roles of the active Ser[101] and His[237] in catalysis of TE II as in TE I (3, 18) and in the active serine esterases (17). Replacing Ser[101] with Cys yielded a mutant enzyme (thiol-TE II) that retained considerable catalytic activity and displayed properties of a cysteine thioesterase similar to those obtained with thiol-TE I (3). As expected, the thiol-TE II was inactivated by thiol-alkylating agents such as iodoacetamide but was insensitive to inhibition by PMSF. Replacing His[237] with Ala abolished activity in both the wild-type enzyme and thiol-TE II, a finding that is consistent with the role of His[237] as a general base for abstracting a proton from the Ser-OH or, in the deacylation step, from the attacking water molecule (3).

In TE II, Asp[236] is the most conserved Asp residue and, therefore, is a good candidate for mutagenesis (2). As in papain (19), this Asp is adjacent to the active His[237], whereas in trypsin (20, 21) the Asp is located in sequences separate from the active His. Replacing the putative Asp[236] with alanine decreased the activity of TE II by ≈40% (Table 1). The decrease in activity is relatively marginal and raises some doubt about the role of this residue in catalysis. Nevertheless, mutating Asp[236] altered several properties of TE II. The wild-type TE II exhibited maximum activity at pH 7.0, whereas the Asp[236]-Ala mutant showed the highest activity between pH values of 7 and 9.5. Similar broadening of the pH-dependence profile was observed with the His[237]-Ala mutant protein, even though the activity was far less (3 orders of magnitude) than the wild type (data not shown). In addition, the reactivities of the hydroxyl group of Ser[101] toward PMSF and of His[237] toward diethylpyrocarbonate (DEPC) were significantly reduced in the Asp[236]-Ala mutant protein compared to the wild-type enzyme (Fig. 4). The relative decrease in the rate of inactivation of the Asp[236]-Ala TE II mutant, therefore, may be due to decreased basicity of His[237], leading to reduced nucleophilicity of the active site Ser-OH (20, 21). The decreased rate also may be due to conformational changes at the active site.

Table 1. Specific activities of recombinant wild-type and mutant TE II proteins assayed with decanoyl CoA and by reactivation of PMSF-inhibited FAS (PMSF-FAS)

Recombinant TE II	Specific activity, nmol·min^{-1}·mg^{-1}	
	Decanoyl CoA	PMSF-FAS
Wild-type	660	918
Ser[101]-Ala	<2	<1
Ser[101]-Cys	317	230
Asp[236]-Ala	406	550
His[237]-Ala	<5	<5
Ser[101]-Cys and His[237]-Ala	<2	<2
Cys[256]-Ala	650	805
Ser[260]-Ala	675	935
Leu[262]-Ala	620	79
Leu[262]-stop codon	618	68
Thr[263]-Val	638	991

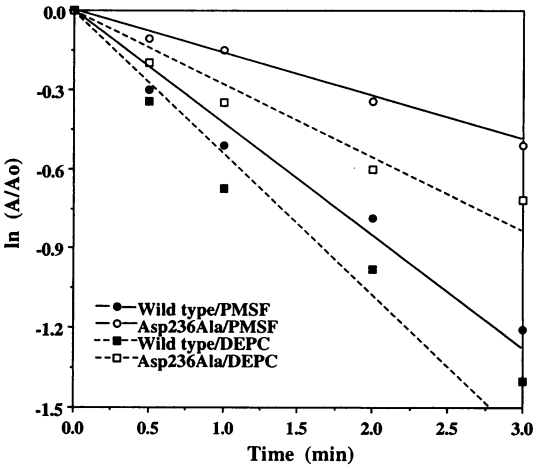

FIG. 4. Comparison of inhibition rates of wild-type TE II and Asp[236]-Ala TE II after treatment with PMSF or DEPC. TE II [50 μg in 0.2 ml of Tris·HCl (50 mM, pH 7.4)] was treated with 1 mM PMSF at 25°C and at the times indicated. Aliquots were withdrawn and assayed for activity with decanoyl CoA used as a substrate. Treatment with DEPC was the same as with PMSF, except that DEPC (0.5 mM) was substituted for PMSF using Tris·HCl (50 mM, pH 6.0) and the incubation temperature was 4°C. A/Ao is the fraction of activity remaining at the times indicated.

When TE II was treated with Woodward's reagent K at the molar ratios of 1:2, 1:4, 1:8, 1:12, and 1:20, the numbers of carboxyl groups modified were estimated to be 1, 2, 4, 8, and 12, respectively. The activity of the modified TE II decreased as more carboxyl groups reacted with reagent K (Fig. 5). The decrease in TE II activity (≈32%) that occurred when one carboxyl group was modified (Fig. 5) was similar to that

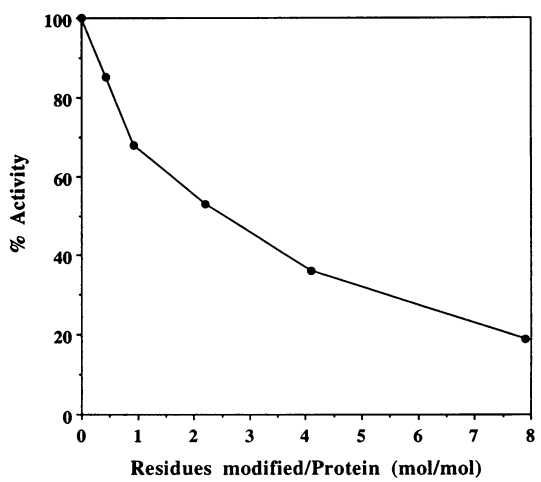

FIG. 5. TE II activity remaining after Woodward's reagent K-modified TE II (250 μg at a concentration of 0.5 mg/ml) was treated with various amounts of reagent K (see text) for 30 min at 25°C. Excess reagent K was removed, and the enzyme was assayed with decanoyl CoA used as a substrate. The number of carboxyl groups modified was calculated from absorbance at 340 nm (see *Experimental Procedures*).

obtained when Asp^{236} was replaced by Ala (Table 1). When the Asp^{236}-Ala mutant was treated with reagent K at a ratio of 1:2, the number of carboxyl groups modified was 0.15, with a 7% loss of activity as compared to 0.92 equivalent groups modified and a 32% loss of activity when the wild-type enzyme was treated under the same conditions. This observation suggested that Asp^{236} is the most active carboxyl group and the first modified by reagent K, even though there are 19 Asp and 15 Glu residues present in TE II. To demonstrate that Asp^{236} was the residue modified under these conditions, the reagent K-treated TE II was cleaved with cyanogen bromide, and the resulting mixture was fractionated by HPLC as described in *Experimental Procedures*. The elution patterns of the cyanogen bromide peptides of the untreated and treated TE IIs were essentially the same, except for one peptide that eluted at 15 min in the digest of the untreated enzyme and at 19.2 min in the digest of the reagent K-treated enzyme. Sequence analysis of these peptides showed that the untreated and the modified peptides are the same, since they matched the TE II sequence beginning at Leu^{233}-Pro-Gly-Asp-His^{237} in the wild-type enzyme and at Leu^{233}-Pro-Gly-Xaa-His^{237} in the treated enzyme, indicating that Asp^{236} is the first residue modified.

Similar studies with trypsin showed that the activity of trypsin decreased by 4 orders of magnitude when Asp^{102} was replaced with Ala or Asn (20, 21). The reactivities of Ser^{195} with diisopropylfluorophosphate and His^{57} with tosyl-L-lysine chloromethyl ketone were reduced 10^4-fold and 5-fold, respectively, in Asp^{102}-Asn mutants. In Asp^{158}-Ala and Asp^{158}-Asn mutants of papain (19), the activities of the enzyme were reduced 10^2-fold and 5-fold, respectively. In both trypsin (21) and papain (19), the residual activities of the mutant proteins showed broadening of pH activity profiles. The Asp^{236}-Ala TE II mutant manifested similar changes (albeit at different magnitudes) in reduced enzymatic activity, in broadening pH activity profile, and in reduced reactivity of Ser^{101} and His^{237} toward their specific inhibitors PMSF and DEPC, respectively (Fig. 4). These results suggested that if Asp^{236} in TE II plays a role in the catalytic process, this role may not be as crucial as in the proteases. Being in the active-site pocket with Ser^{101} and His^{237}, mutations of Asp^{236} could influence the catalytic process directly or indirectly by contributing to the stabilization of the imidazolium ion generated when the tetrahydral intermediate forms (3, 19).

Similar mechanisms with reaction intermediates are activated when Ser^{101} is replaced by Cys. The turnover of the acyl enzyme intermediates of the wild-type enzyme or thiol-TE II occurs so fast that we detected little or no protein-bound radioactivity when [1-^{14}C]palmitoyl CoA was present (Table 2). However, the His^{237}-Ala mutant and the Ser^{101}-Cys/His^{237}-Ala double mutant, respectively, bound about 0.21 and 0.92 equivalents of the [^{14}C]palmitoyl group (Table 2). Adding DTNB to the reaction mixtures containing the His^{237}-Ala mutant further increased binding of the [^{14}C]palmitoyl group to TE II due to removal of the CoASH produced and shifting of the equilibrium in favor of acyl enzyme formation (Table 2). Binding of the palmitoyl group to the Ser^{101}-Ala and Ser^{101}-Cys/His^{237}-Ala mutants involved Ser^{101}-OH or Cys^{101}, since treating the two mutants with PMSF and iodoacetamide, respectively, reduced the binding to background levels (Table 2). These observations suggested that the Ser-OH of the wild-type enzyme and the Cys-SH of the thiol mutant protein can attack the substrate and form the acyl enzyme intermediate, even without His^{237}. More acyl enzyme intermediates were observed in the Ser^{101}-Cys/His^{237}-Ala double mutant than in the His^{237}-Ala mutant, which is consistent with the greater nucleophilicity of the thiol group over the hydroxyl group. These results indicated that the absence of His^{237} from both the thiol and Ser enzymes significantly reduced the breakdown of the acyl intermediate by eliminating the nucleophilic attack on the acyl enzyme intermediate by a water molecule, thereby causing the acyl enzyme intermediate to accumulate.

Table 2. Labeling of various TE II proteins with [1-^{14}C]palmitoyl CoA

Thioesterase	Palmitate bound to protein, mol/mol
Wild type	<0.05
Ser^{101}-Ala	<0.05
Ser^{101}-Cys	<0.05
His^{237}-Ala	0.21
His^{237}-Ala + DTNB	0.75
His^{237}-Ala + PMSF	<0.05
Ser^{101}-Cys and His^{237}-Ala	0.92
Ser^{101}-Cys and His^{237}-Ala + iodoacetamide	<0.05

TE II protein (100 μg) was labeled with [1-^{14}C]palmitoyl CoA and its radioactivity was measured (3).

Role of the C-Terminal Region of TE II in Its Interaction with FAS. As shown in Table 1, the recombinant TE II is as active on the model acyl CoA derivatives as the native enzyme but is twice as active as the native enzyme when assayed with its natural substrate, acyl FAS. When Cys^{256} was replaced with Ala (Fig. 2), the mutant TE II had full activities with acyl-CoA derivatives and with its natural substrate (Table 1). Regardless of the substrate used, treating Cys^{256}-Ala mutant protein with DTNB had no inhibitory effect on activity (data not shown). These results indicate that Cys^{256} is not critical for the catalytic mechanism of the enzyme or for its interaction with its natural substrate.

However, replacing the penultimate residue Leu^{262} with Ala or a stop codon (Fig. 2) totally obliterated TE II activity with the FAS but had no effect on TE II activity with the acyl-CoA model substrate (Table 1). Replacing Ser^{260} with Ala or the C-terminal Thr^{263} with Val (Fig. 2) did not affect TE II activities with either substrate. If anything, both mutant proteins showed slight, but significant, increases in activity with the *S*-acyl FAS (Table 1). These observations strongly suggested that the hydrophobic character of the C-terminal region of TE II, especially the penultimate Leu^{262} residue, is important for the interaction of TE II with FAS. The probability that the interaction of TE II with FAS is hydrophobic is further supported by the significant increase in the specific activity of TE II that occurred when polyethylene glycol was

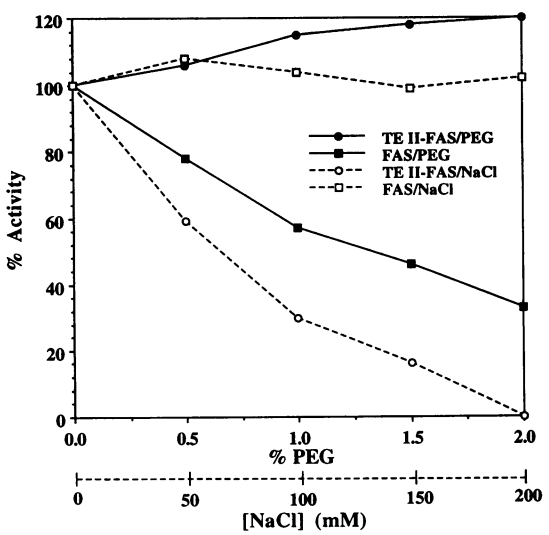

FIG. 6. Effect of polyethylene glycol (PEG) and NaCl on TE II reactivation of PMSF-treated FAS (TE II-FAS) and on FAS (see *Experimental Procedures*).

present during the reactivation of PMSF-inhibited FAS (Fig. 6). On the other hand, the presence of salt, such as NaCl, KCl, or $(NH_4)_2SO_4$, significantly reduced TE II activity with its natural substrate FAS (Fig. 6), even though none of these salts has an effect on the activity of FAS itself or on TE II when it is assayed with acyl CoA as a substrate.

The requirement for hydrophobic amino acids at the C-terminus of the TE II molecule, therefore, may be important in providing TE II the means to penetrate the hydrophobic pocket on FAS where the aliphatic fatty acyl chain is located in order for the molecule to carry out the release of fatty acids. When the sequence of the 10 amino acid residues at the C terminus of TE II (-Ile-Ala-Lys-Cys-Leu-Glu-Leu-Ser-Ser-Leu-Thr) was compared to that of TE I (-Ala-Glu-Pro-Arg-Val-Ser-Val-Arg-Glu-Gly), it became apparent that the relatively higher hydrophilicity of the peptide at the C terminus of TE I may be one reason why it cannot reassociate with the synthase complex after it is removed from FAS by chymotryptic digestion (1).

It has been suggested that the interaction of rat TE II with FAS is transient and requires an acyl chain linked to the 4′-phosphopantetheine (22). To understand this requirement, we carried out the following experiments: we added increasing amounts (up to 20-fold) of enzymatically inactive Ser^{101}-Ala, His^{237}-Ala, or Ser^{101}-Cys/His^{237}-Ala TE II mutants over wild-type recombinant TE II to the reactivation assay mixture containing PMSF-inhibited FAS and found that the rate of NADPH oxidation did not change. These results suggested that the substrate binding site of TE II is also important in its interaction with FAS. This conclusion was further substantiated by the inability of a synthetic decapeptide containing the C-terminal amino acid residues of TE II to compete with TE II for its anchoring site on FAS. Although the conformation of the C-terminal sequence of TE II and the synthetic peptide may be different, our results suggest the following model for TE II–FAS interaction: TE II interacts with FAS initially by binding to the intermediate acyl group bound to FAS. This interaction then leads to anchoring of the C-terminal end of TE II onto FAS, thereby stabilizing the TE II–acyl FAS transient complex. Such a mechanism may be physiologically significant because it favors TE II over TE I in interacting with FAS and releasing the intermediate-chain fatty acyl group. It is noteworthy that the Ser^{101}-Cys/His^{237}-Ala mutant TE II did not compete with wild-type TE II. Even though this mutant TE II can transfer the acyl group from FAS to its Cys-SH, it could not release the fatty acid and therefore became inactive as a thioesterase. Consequently, the mutant could not compete with TE II in the interaction of TE II with FAS after the first turnover. Although this mechanism may be functional in rat mammary gland, it may not be applicable to the TE II of goose uropygial gland, for, in the latter case, TE II binds to FAS, even when there are no growing acyl chains on FAS (23).

M.-H.T. submitted this work as part of a thesis presented to the Graduate School at Baylor College of Medicine in partial fulfillment of a Ph.D. We thank Dr. W.-Y. Huang for analyzing the cyanogen bromide-digested peptides and sequencing them, and we thank P. P. Powell for her editorial review. This study was supported in part by grants from the National Institutes of Health (GM-19091), the Clayton Foundation for Research, and the Robert A. Welch Foundation.

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