Complete Amino Acid Sequence of the Thioesterase Domain of Chicken Liver Fatty Acid Synthase†

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ABSTRACT: The complete amino acid sequence of thioesterase domain of chicken liver fatty acid synthase has been determined by sequencing peptides produced by trypsin, *Staphylococcus aureus* V8 protease, and cyanogen bromide cleavage. The thioesterase domain consists of 300 amino acid residues. All of the tryptic peptides of the thioesterase domain were isolated and sequenced, except the segment covered from position 109 to position 124. Peptides resulting from digestion by *Staphylococcus aureus* V8 protease and cyanogen bromide cleavage filled the missing part and overlapped the complete sequence of the entire thioesterase domain. The NH_2 terminus of the thioesterase domain was determined to be lysine by sequencing the whole domain up to 20 residues while the COOH terminus was identified as serine through carboxyl peptidase Y cleavage. The active site of the thioesterase domain of chicken fatty acid synthase was suggested to be the serine on position 101 according to its homology with other serine-type esterases and proteases which have a common structure of -Gly-X-Ser-Y-Gly- with the variable amino acids X and Y disrupting the homology.

Chicken fatty acid synthase is a homodimer of a subunit protein of M_r 260 000. The subunit consists of a single multifunctional protein containing a site for the attachment of the prosthetic group 4′-phosphopantetheine and the six catalytic activities necessary for the cyclic addition of 2-carbon units to the growing acyl chain, as well as a thioesterase activity required for the release of the completed product palmitate (Wakil et al., 1983; Mattick et al., 1983a). It was previously shown that the synthase polypeptide may be cleaved by α-chymotrypsin into fragments with M_r of 230 000 and 33 000. The 230-kDa[1] fragment contains the NH_2 terminus and all the catalytic activities required for fatty acid synthesis, while the 33-kDa fragment contains the COOH terminus and the thioesterase activity of the fatty acid synthase (Mattick et al., 1983a,b). The chymotryptic digestion pattern suggested the possibility of a simple and rapid procedure for the isolation of the thioesterase domain from the remainder of the complex. By employing such a procedure, we have isolated the thioesterase in a highly purified state and determined its amino acid sequence. The information thus obtained was important in our isolation and characterization of the genomic clone coding for part of this domain of the chicken fatty acid synthase and in the establishment of the COOH-terminal region of the thioesterase domain and the 3′ noncoding region of this complex gene (Kasturi et al., 1988). In this paper we present the complete amino acid sequence of the thioesterase component of chicken fatty acid synthase.

Materials and Methods

Chicken Fatty Acid Synthase. The enzyme was isolated and purified from the livers of young adult chickens (White Leghorn) as described previously (Arslanian & Wakil, 1975). The purity was verified by SDS–polyacrylamide gel electrophoresis, and the specific activity ranged from 1.5 to 1.7 μmol of NADPH oxidized min^{-1} (mg of protein)$^{-1}$.

Reagents. TPCK-treated trypsin was supplied by Worthington Biochemicals; *Staphylococcus aureus* V8 protease was obtained from Miles Biochemicals. Polyamide thin-layer sheets for DABITC manual sequencing and sequencing reagents were bought from Pierce Chemical Co., while acetonitrile and methanol for HPLC were obtained from Burdick and Jackson. Vydac C_4 and C_{18}, IBM ODS, and Spherisorb ODS columns (25 × 4.6 cm) were purchased from Cell Associates, Houston, TX. Amino acid analyzer reagents were purchased from Dionex. An Aquapore RP-300, a C_8 column (30 × 2.1 mm), and reagents used in the gas-phase protein sequences were obtained from Applied Biosystems. All chemicals used were of the highest reagent grade available.

Preparation of the Thioesterase Fragment of Fatty Acid Synthase. Proteolysis of the synthase and preparation of the 33-kDa chymotryptic fragment were performed essentially as described earlier (Mattick et al., 1983a). The thioesterase recovered after ammonium sulfate precipitation was dissolved in 0.1 M ammonium bicarbonate with a pH of 8.0. The homogeneity of the thioesterase fragment was checked by SDS–polyacrylamide gel electrophoresis (Mattick et al., 1983a).

Carboxymethylation. Reduction and carboxymethylation of the protein were carried out according to the method of Anfinsen and Haber (1961).

Enzymatic Hydrolysis. The carboxymethylated protein (5 mg/mL, 0.1 M NH_4HCO_3, pH 8.0) was digested with TPCK-treated trypsin and *S. aureus* V8 protease (enzyme: substrate ratio 1:50 w/w) at 37 °C. Complete digestion with trypsin and *S. aureus* V8 protease required 5 and 24 h, respectively (Wittmann-Liebold & Grener, 1980). The hydrolysates were analyzed by HPLC.

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[1] Abbreviations: kDa, kilodalton(s); TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; DABITC, *p*-[[*p*-(dimethylamino)-phenyl]azo]phenyl isothiocyanate; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; DABTH, (dimethylamino)-azobenzene isothiohydantoin; SDS, sodium dodecyl sulfate; ACP, acyl carrier protein.

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Cyanogen Bromide Cleavage. Reduced and alkylated thioesterase (13.5 mg) was dissolved in 70% formic acid and excess cyanogen bromide (8:1 w/w) and left to react at room temperature for 4 h. The hydrolysate was then diluted in water (1:1) and concentrated in a Speedvac concentrator (Gross & Witkop, 1962). In experiments where the thioesterase was not reduced and alkylated prior to cyanogen bromide treatment, 5 mg of thioesterase was treated for 24 h at 37 °C with 5 mg of cyanogen bromide in 70% formic acid. The excess cyanogen bromide and formic acid were removed by lyophilization.

Peptide Separation. Peptide separations were performed by HPLC (Kratzin et al., 1980, 1983; Yang et al., 1981), with a Shandon ODS, IBM ODS, and a Vydac C_{18} column (250 × 4.6 mm) operated at 1.5 mL/min and 50 °C. The tryptic and staphylococcal digests were separated with a TFA buffer system [0.1% TFA in water as the starting buffer (A) and 0.08% TFA in 95% acetonitrile and 5% water as a second buffer (B)]. A linear gradient of buffer B was increased from 0 to 70% in 60 min. Peptides were detected by their absorbance at 220 nm. The eluant under each peak was collected manually and dried by a Speedvac. Manual amino acid sequencing was performed to verify the purity of each peak fraction (Chang et al., 1978; Yang, 1979). The pure peptides were then sequenced. The partially purified fractions, however, were rechromatographed with an IBM ODS column (250 × 4.6 mm), using an ammonium acetate buffer system with a linear gradient of a starting buffer of 0.025 M ammonium acetate, pH 6.0 (buffer C), and 40% 0.05 M ammonium acetate, pH 6.0, and 60% acetonitrile (buffer D). The fractions with the peptide peaks were collected and identified.

Amino Acid Analysis. The thioesterase or pure peptides were first hydrolyzed in 150 μL of a solution of 0.125% phenol in 6 N HCl and incubated for 24 h in evacuated, sealed tubes at 110 °C. The samples were then dried and prepared for amino acid analysis, according to the method of Stein and Moore (1954), with a Durrum Model D500 amino acid analyzer. The program that was run on the analyzer was a standard 90-min procedure provided by Dionex.

Sequencing. Sequence determination of all peptides was performed according to the method of Chang et al. (1978) with a modified Edman reagent, DABITC. DABTH-amino acids were identified by thin-layer chromatography (TLC) (Chang et al., 1978; Yang, 1979) or HPLC (Yang & Wakil, 1984). The sequences of all large peptides were determined with a gas-phase protein sequencer (Applied Biosystems) equipped with a Model 120A PTH analyzer (Hewick et al., 1981; Hunkapiller, 1986).

The COOH-terminal sequence of the protein or peptide was determined by carboxypeptidase Y digestion (Hayashi, 1977). The peptide was dissolved in 0.1 M pyridine–acetate buffer with a pH of 6.0 and incubated with carboxypeptidase Y. At different times, aliquots were withdrawn and analyzed for the release of amino acids.

Nomenclature. The purified peptides are numbered in the order in which they occur, starting from the NH_2 terminus of the parent peptide (33-kDa fragment), and are designated as follows: T, tryptic peptide; S, staphylococcal protease peptide; CN, cyanogen bromide peptide.

Results

The amino acid composition of thioesterase is shown in Table I. The complete amino acid sequence of thioesterase was achieved through the alignment of tryptic, staphylococcal, and cyanogen bromide peptides. Most of the tryptic peptides were isolated and identified except the region covered from position 109 to position 124. The amino acid sequences obtained from other digests, such as cyanogen bromide and staphylococcal protease, were employed to fill in for the missing regions and facilitated the correct alignment of the complete sequence of the thioesterase domain of chicken liver fatty acid synthase.

Table I: Amino Acid Composition of the Thioesterase Domain

amino acid	no. of residues based on hydrolysis	no. of residues based on sequence
Cys[a]	7.00	7
Asp	23.78	24
Thr	13.64	14
Ser	20.96	21
Glu	38.06	38
Pro	13.63	14
Gly	21.74	21
Ala	29.76	30
Val	13.60	14
Met	3.60	4
Ile[b]	18.34	19
Leu	33.66	34
Tyr	13.75	14
Phe	10.15	11
His	10.46	11
Lys	13.64	14
Arg	10.50	10
total	300	300

[a] Cys was determined as cysteic acid. [b] The value for isoleucine was 72-h hydrolysate.

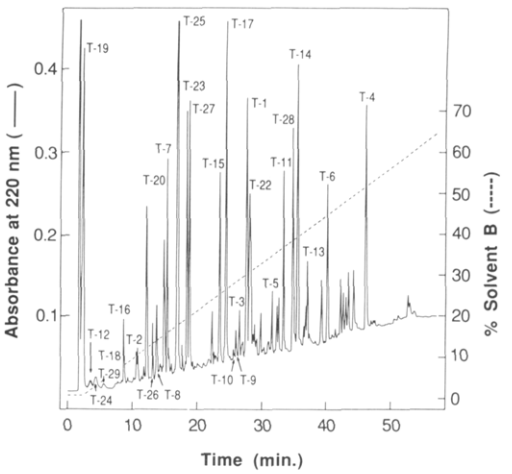

FIGURE 1: HPLC separation of peptides from tryptic digest of thioesterase. The sample was injected into a Vydac C_{18} column, and the peptides were eluted by a linear gradient of buffers A (0.1% TFA in water) and B (0.08% TFA in 95% acetonitrile and 5% water) from 0% to 70% in 60 min. All peptides were collected and subjected to amino acid analyses. The labeled peaks correspond to the amino acid compositions given in Table I of the supplementary material (see paragraph at end of paper regarding supplementary material).

Tryptic Peptides. The HPLC chromatogram of the tryptic peptides is shown in Figure 1. The TFA buffer system provided a good separation of most of the liberated peptides and thus made the direct analysis of most of the fragments possible. Some peptides required further chromatography in order to obtain pure peptides for sequence analyses. This was accomplished by rechromatography on either an IBM ODS or a Shandon ODS column as described under Materials and Methods. Peptides T8 and T10 were byproducts of further digestion of the thioesterase domain by chymotrypsin as reported previously (Mattick et al., 1983a). Twenty-nine tryptic peptides of the thioesterase domain were purified and identified. The amino acid sequences of the peptides are shown in Figure 2, and the labeled peaks in the chromatogram of

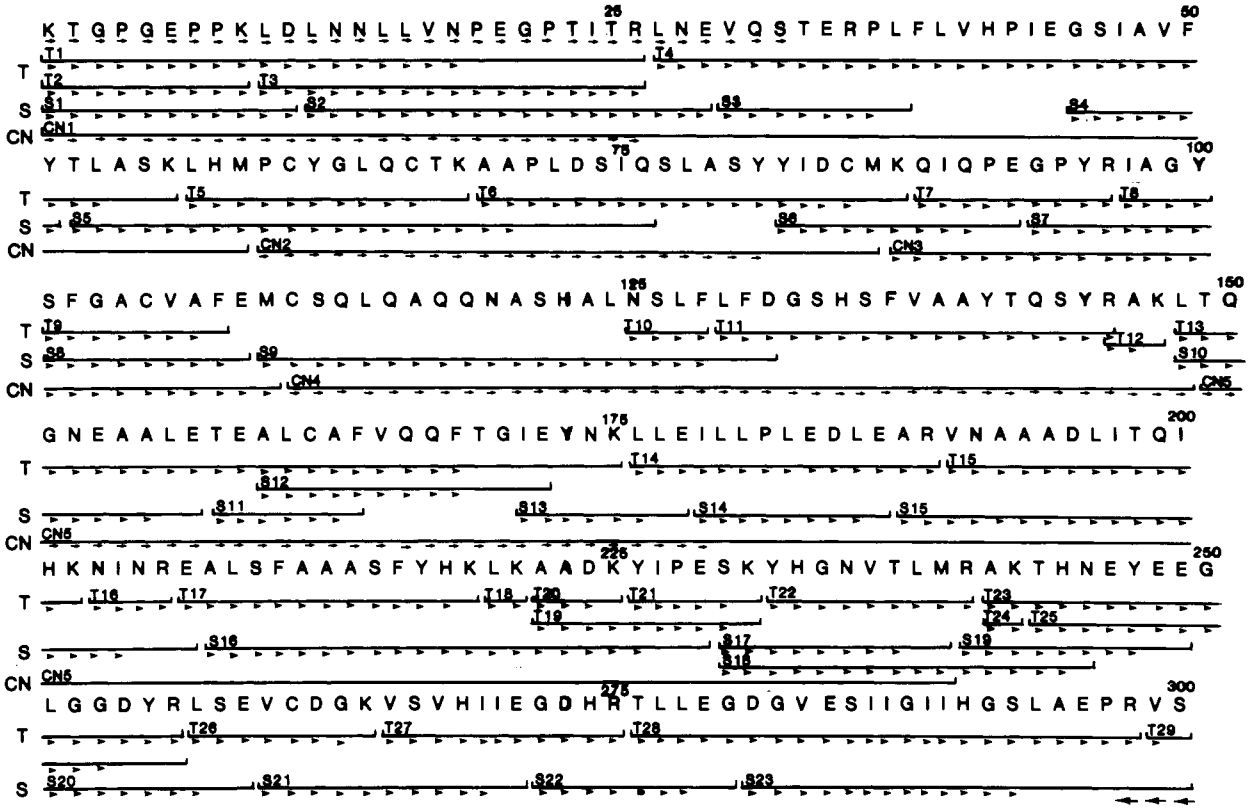

FIGURE 2: Summary of the proof of the sequence of the thioesterase domain (33 kDa) of chicken liver fatty acid synthase. Abbreviations: CN, cyanogen bromide fragment; S, *S. aureus* V8 protease peptide; T, tryptic peptide. The peptides obtained from each digestion were numbered sequentially from the NH_2 terminus. (arrowhead) DABTIC manual sequence analysis; (→) gas-phase sequence analysis; (←) carboxypeptidase Y analysis.

Figure 1 correspond to the amino acid sequence and composition given in Figure 2.

Staphylococcal Peptides. The peptides generated from *S. aureus* V8 protease degradation of the thioesterase were resolved by HPLC with a Shandon ODS column. The solvents employed were the same as those used for the separation of the peptides from the tryptic digests. Twenty-three peptides resulting from *S. aureus* V8 protease digestion were purified and identified. The various fractions were collected, and their purity was assessed by manual sequencing. The pure peptides were used for further sequencing. Others were further purified by rechromatography on HPLC in a manner similar to that used for the purification of tryptic peptides with the systems described under Materials and Methods, and the products were analyzed on HPLC (data not shown).

Cyanogen Bromide Peptides. The cyanogen bromide peptides were separated by HPLC in a manner similar to that used for the tryptic peptides. A sample of CNBr peptides obtained from reduced and alkylated thioesterase was applied on a Vydac C_4 (25 × 0.46 cm) column, and the peptides were eluted by a linear gradient of buffers A and B from 10% to 65% at a flow rate of 1.5 mL/min. Peptides CN1, CN2, CN3, and CN4 were isolated in pure form, and their amino acid compositions were determined. They cover the amino acids of positions 1–148. The cyanogen peptides CN1, CN2, and CN3 were sequenced up to 25, 21, and 21 residues, respectively. As shown in Figure 2, they contained tryptic peptides T1 to T9. Peptide CN4 was sequenced entirely and provided the overlap sequences for peptides S9 and T10 to T12. The cyanogen peptide CN5 fractionated on HPLC with a retention time of 33–35 min. However, a narrow fraction with a retention of 33–34 min was collected and rechromatographed on HPLC with an ABI C_8 column. A peptide representing CN5 was obtained as a single peak, and its amino acid composition was determined and sequenced up to 32 residues. The results showed that the CN5 peptide contained peptides T13 to T22 (Figure 2) and CN5 was not generated by cleavage of a methionine residue between them for no such residue exists. However, they may have been derived by cleavage of the peptide bond between residues 148 and 149 during the reduction–alkylation step prior to cyanogen cleavage of the thioesterase domain since no such cleavage occurs if the protein was treated with cyanogen bromide in the absence of the reduction–alkylation step. In the latter case, the peptides obtained after cyanogen cleavage (see Materials and Methods) were fractionated by HPLC on a Vydac C_4 column (25 × 0.46 cm) operated at 50 °C and a flow rate of 0.9 mL/min with a linear gradient of buffers A and B from 10% to 60%. The fractions that eluted between 26 and 35 min were pooled and lyophilized. The residue was oxidized with performic acid (Hirs, 1956) and rechromatographed as above. The peptide eluting at 26 min, represented by a single peak, was collected, and the amino acid sequence was determined by a gas-phase protein sequencer up to 45 residues. The results confirmed the sequence of amino acid residues 111–155; hence, this peptide contained the CN4 and CN5 peptides shown in Figure 2.

The last cyanogen bromide fragment containing residues 240–300 was separated from the other cyanogen peptides by HPLC by eluting around 50% solvent B. Since this region was identified very well by the staphylococcal and tryptic peptides, this CN fragment was not studied further.

Alignment of Peptides. As indicated in Figure 2, the amino acid sequence was primarily found by aligning overlapping peptides of the tryptic, staphylococcal, and cyanogen bromide digests. The NH_2 terminus of thioesterase was confirmed by sequencing the intact protein with the DABITC method. The COOH terminus was confirmed by the result of carboxy-

Table II: Comparison of "Active Serine" Peptides

Thioesterase of chicken fatty acid synthase	G P Y R I A G Y S F G A C V A F E M C S
Thioesterase of goose fatty acid synthase[a]	S F G A C V A F
S-acyl thioesterase of rat mammary gland[b]	K A F A F F G H S F G S Y I A L I T A L
S-acyl thioesterase of goose uropygial gland[c]	K P F A L F G H S F G S F V S Y A L A V
Malonyl/palmitoyl transacylase of yeast fatty acid synthase[d]	A D A T F A G H S L G E Y A A L A S L A
Acetyl transacylase of yeast fatty acid synthase[d]	Y L K G A T G H S Q G L V T A V A I A E
Plasmin, human[e]	G T D S C Q G D S G G P L V C F E K D K
Trypsin, bovine[f]	G K D S C Q G D S G G P V V C S G K L Q
Carboxyl esterase, bovine[g]	G E S A G A E S

[a] Poulose et al., 1981. [b] Randhawa & Smith, 1987. [c] Poulose et al., 1985. [d] Chirala et al., 1987. [e] Wiman, 1977. [f] Walsh et al., 1964. [g] Augusteyn et al., 1969.

peptidase Y digestion of thioesterase and the fatty acid synthase subunit (Mattick et al., 1983a).

Discussion

The fatty acid synthase is a complex system that catalyzes the de novo synthesis of long-chain fatty acids (palmitate) from acetyl-CoA and malonyl-CoA. The complex requires seven different catalytic activities plus an acyl carrier protein (ACP). In bacteria and plant chloroplasts the activities exist as discrete monofunctional proteins (Wakil et al., 1983). However, in yeast and other fungi the partial activities are contained within two polypeptides, a trifunctional subunit (α) and pentafunctional protein (β). Whereas in animal cells, all activities are associated with a single multifunctional protein of M_r 260000. A functional fatty acid synthase consists of two multifunctional proteins arranged in a head-to-tail configuration (Wakil et al., 1983). This arrangement is essential for the chain elongation and hence the synthesis of long-chain fatty acids. It has been shown that the main product of the animal fatty acid synthase is free palmitic acid, and more recently, there is evidence that the thioesterase activity of the complex is responsible for the chain termination and release of free palmitate (Singh et al., 1984). The ease with which this activity is removed from the dimer of the multifunctional proteins made it possible for us to study its structure and gain further insight into the structure of the multifunctional subunit.

The preparation of thioesterase was simplified by the fact that the treatment of fatty acid synthase with chymotrypsin cleaves the enzyme into two fragments of M_r 230000 and 33000, respectively. The latter fragment can be isolated in its pure state and shown to contain the thioesterase activity as well as the COOH terminus of the fatty acid synthase. Amino acid sequence analysis of this domain was carried out, and its primary structure was aligned according to sequences obtained from its tryptic, staphylococcal, and cyanogen bromide peptides. The amino acid sequence presented in Figure 2 shows that the thioesterase domain of the chicken liver fatty acid synthase consists of 300 amino acid residues; its NH_2 terminus is lysine, while its COOH terminus is serine. The sequence lacks an additional four residues, -Val-Arg-Glu-Gly-, predicted by the genomic DNA sequence (Kasturi et al., 1988). This difference may be due to proteolysis of the synthase during its isolation or of the thioesterase during its cleavage by chymotrypsin. The amino acid composition of thioesterase contains no tryptophan, on the basis of 4 M methanesulfonic acid analysis and the amino acid sequence presented in this study.

The active serine of thioesterase from chicken fatty acid synthase is located in position 101. The amino acids near this active serine have the sequence -Gly-Tyr-Ser-Phe-Gly-, which is basically the same motif as -Gly-X-Ser-Y-Gly- that characterizes the serine proteases (Barker & Dayhoff, 1972), the yeast acetyl and malonyl palmitoyltransacylases of yeast synthase (Chirala et al., 1987), the active site of goose fatty acid synthase thioesterase (Poulose et al., 1981), and the thioesterases II (or medium-chain *S*-acyl fatty acid synthase thioester hydrolase) of mallard uropygial gland (Poulose et al., 1985) and rat mammary gland (Randhawa & Smith, 1987) (Table II). However, comparison of the overall sequence of the thioesterase domain of chicken liver fatty acid synthase with that of thioesterases II of mallard uropygial gland and rat mammary gland did not show significant homology. Also, a computer search did not identify any other protein with significant overall homology with the chicken synthase thioesterase domain.

The hydropathic profile of the chicken thioesterase domain was calculated by the algorithm of Kyte and Doolittle (1982) as shown in Figure 3A. A similar profile was also calculated for the thioesterases II of mallard uropygial gland and rat mammary gland (Figure 3B,C). Comparison of the three hydropathic profiles shows that the active serine residue is located near a hydrophobic domain (Figure 3) and that the thioesterase domain of the chicken liver fatty acid synthase has more hydrophobic regions than the thioesterases II of the specialized glands of mallard and rat. The latter two thioesterases II are not covalently linked to the fatty acid synthase of their respective glands but are able to interact with the synthases causing the release of medium-chain fatty acids from the 4′-phosphopantetheine prosthetic groups. The thioesterase domain of chicken liver fatty acid synthase, on the other hand, is an integral part of the synthase subunit and capable of releasing palmitic acid as the major acid with some myristic and stearic acids as minor acids. Cleavage of the synthase with chymotrypsin separates the thioesterase and makes it unable to interact with synthase, and therefore, it cannot release the palmitate from the 4′-phosphopantetheine moiety (Singh et

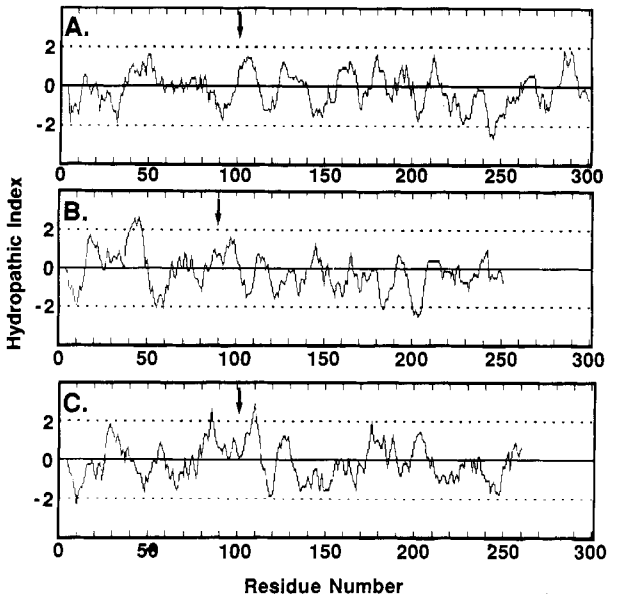

FIGURE 3: Hydrophobic profile of the amino acid sequences of (A) the thioesterase domain of chicken liver fatty acid synthase, (B) the thioesterase II of mallard uropygial gland (Poulose et al., 1985), and (C) the thioesterase II of rat mammary gland (Randhawa & Smith, 1987) according to the algorithm of Kyte and Doolittle (1982). The arrows indicate the positions of the active seryl sites of the thioesterases.

al., 1984). As shown in Figure 3A, there are eight hydrophobic regions in the thioesterase domain of the chicken liver fatty acid synthase, and the active serine is located within a domain of a prominent hydrophobic character. The presence of these multiple hydrophobic regions within this molecule may be important for the interaction of this enzyme with the hydrocarbon chain of the product, a palmitoyl group, prior to its hydrolysis, hence dictating its specificity for long-chain acids. These hydrophobic regions are not as prominent in thioesterases II and may be the reason why these enzymes are specific for the release of medium-chain acids. Also, the lower hydrophobic characters of thioesterases II as compared to that of the chicken thioesterase (Figure 3) may contribute to their ease of interaction with the synthase at the ACP site, which has been predicted for *Escherichia coli* ACP (Rock & Cronen, 1979; Mayo et al., 1983; Mayo & Prestegard, 1985), animal synthase, and yeast synthase ACP regions (unpublished data) to be hydrophilic. Clearly, further structural and physical information is needed in order to verify and support these predictions.

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SUPPLEMENTARY MATERIAL AVAILABLE

Four figures showing further purification of tryptic peptides as exemplified by rechromatography of peptides T9 and T19 on HPLC and HPLC separation of peptides from staphylococcal protease digest and cyanogen bromide cleavage products of thioesterase, as well as the rechromatography of CN5 fraction on HPLC, and three tables containing the amino acid compositions of peptides obtained from cleavage of thioesterase by trypsin, staphylococcus protease, and cyanogen bromide (11 pages). Ordering information is given on any current masthead page.

REFERENCES

Anfinsen, C., & Haber, E. (1961) *J. Biol. Chem. 236*, 1361–1363.

Arslanian, M. J., & Wakil, S. J. (1975) *Methods Enzymol. 35*, 59–65.

Augusteyn, R. C., DeJersey, J., Webb, E. C., & Zerner, B. (1969) *Biochim. Biophys. Acta 171*, 128–137.

Barker, W. C., & Dayhoff, M. O. (1972) in *Atlas of Protein Sequence and Structure*, Vol. 5, pp 53–66, National Medical Research Foundation, Washington, DC.

Chang, Y. H., Brauer, D., & Wittmann-Liebold, B. (1978) *FEBS Lett. 93*, 205–214.

Chirala, S. S., Kuziora, M. A., Spector, D. M., & Wakil, S. J. (1987) *J. Biol. Chem. 262*, 4231–4240.

Gross, E., & Witkop, B. (1962) *J. Biol. Chem. 237*, 1856–1860.

Hayashi, R. (1977) *Methods Enzymol. 157*, 84–93.

Hewick, R. M., Hunkapiller, M. W., Hood, C. E., & Dreger, W. J. (1981) *J. Biol. Chem. 256*, 7990–7997.

Hirs, C. H. W. (1956) *J. Biol. Chem. 219*, 611–621.

Hunkapiller, M. W. (1986) *Users Manual, Applied Biosystems Model 120A PTH Analyzer*, Applied Biosystems, Foster City, CA.

Kasturi, R., Chirala, S., Pazirandeh, M., & Wakil, S. J. (1988) *Biochemistry* (following paper in this issue).

Kratzin, H., Yang, C. Y., Krusone, J., & Hilschmann, N. (1980) *Hoppe-Seyler's Z. Physiol. Chem. 361*, 1591–1598.

Kratzin, H., Yang, C. Y., Goetz, H., Thiines, F. P., Kruse, T., Egert, G., Pauly, E., Koelbel, S., McLaughlin, L., & Hilschmann, N. (1983) in *Modern Methods in Protein Chemistry* (Tschesche, H., Ed.) pp 207–227, de Gruyter, Berlin and New York.

Kyte, J., & Doolittle, T. F. (1982) *J. Mol. Biol. 157*, 105–132.

Mattick, J. S., Nickless, J., Mizugaki, M., Yang, C.-Y., Uchiyama, S., & Wakil, S. J. (1983a) *J. Biol. Chem. 258*, 15300–15304.

Mattick, J. S., Tsukamoto, Y., Nickless, J., & Wakil, S. J. (1983b) *J. Biol. Chem. 258*, 15291–15299.

Mayo, K. H., & Prestegard, J. H. (1985) *Biochemistry 24*, 7834–7838.

Mayo, K. H., Tyrell, P. M., & Prestegard, J. H. (1983) *Biochemistry 22*, 4485–4493.

Poulose, A. J., Rogers, L., Cheesbrough, T. M., & Kolattukudy, P. E. (1985) *J. Biol. Chem. 260*, 15953–15958.

Poulose, A. J., Rogers, L., & Kolattukudy, P. E. (1981) *Biochem. Biophys. Res. Commun. 103*, 377–382.

Randhawa, Z. I., & Smith, S. (1987) *Biochemistry 26*, 1365–1373.

Rock, C. O., & Cronen, J. E. (1974) *J. Biol. Chem. 254*, 9778–9785.

Singh, N., Wakil, S. J., & Stoops, J. K. (1984) *J. Biol. Chem. 259*, 3605–3611.

Stein, W. H., & Moore, S. (1954) *J. Biol. Chem. 211*, 893–898.

Wakil, S. J., Stoops, J. K., & Joshi, V. C. (1983) *Annu. Rev. Biochem. 52*, 537–579.

Walsh, K., & Neurath, H. (1964) *Proc. Natl. Acad. Sci. U.S.A. 52*, 884–889.

Wiman, B. (1977) *Eur. J. Biochem. 76*, 129–137.

Wittmann-Liebold, B., & Greuer, B. (1980) *FEBS Lett. 121*, 105–112.

Yang, C. Y. (1979) *Hoppe-Seyler's Z. Physiol. Chem. 360*, 1673–1675.

Yang, C. Y., & Wakil, S. J. (1984) *Anal. Biochem. 137*, 54–57.

Yang, C. Y., Pauly, E., Kratzin, H., & Hilschmann, N. (1981) *Hoppe-Seyler's Z. Physiol. Chem. 362*, 1131–1146.