Vol. 103, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1981 Pages 377-382

PRIMARY STRUCTURE OF A CHYMOTRYPTIC PEPTIDE CONTAINING THE "ACTIVE SERINE" OF THE THIOESTERASE DOMAIN OF FATTY ACID SYNTHASE

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Received September 5,1981

SUMMARY: "Active serine" of the thioesterase domain of fatty acid synthase from the goose uropygial gland was selectively labeled with [1,3-^{3}H]diisopropylfluorophosphate and the chymotryptic peptide containing this active serine was purified to homogeneity by a combination of gel filtration, cation exchange chromatography and high performance liquid chromatography. The primary structure of this active site peptide, Ser-Phe-Gly-Ala-Cys-Val-Ala-Phe, is remarkably homologous to the "active serine" containing peptide of human plasmin.

INTRODUCTION

Fatty acid synthase from vertebrates is a multifunctional enzyme which catalyzes the synthesis of n-fatty acids from malonyl-CoA and methyl-branched fatty acids from methylmalonyl-CoA (1,2). The overall synthesis of fatty acids involves seven sequential reactions. Previous studies have shown that fatty acid synthase from the goose uropygial gland consists of two 250 K dalton peptides each containing one each of 4'-phosphopantetheine, keto-reductase, enoyl reductase and thioesterase domains suggesting that the monomers are identical (3,4,5,6,7). Conclusive evidence for the identity of the monomers and elucidation of structure-function relationship of this complex multifunctional enzyme require knowledge of the amino acid sequence of the 250 K dalton peptides and especially that of the seven active sites. One of the seven active sites involves "active serine" in the thioesterase domain, which releases the completed acyl chains from the synthase and this serine can be selectively labeled with [1,3-^{3}H]diisopropylfluorophosphate (DFP). In this

DFP, diisopropylfluorophosphate; HPLC, high performance liquid chromatography; SP-Sephadex, Sulphopropyl sephadex.

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paper we report the purification of a chymotryptic peptide containing the labeled serine and the primary structure of this active site peptide. The sequence of this peptide, Ser-Phe-Gly-Ala-Cys-Val-Ala-Phe, is similar to the "active serine" containing peptides of other "active serine" hydrolases and remarkably homologous to the "active serine" containing peptide of human plasmin (9). This is the first report of the primary structure of a thioesterase active site from any source.

MATERIALS AND METHODS

Labeling of the Thioesterase Active Site with [1,3-^{3}H]DFP: Thioesterase (10.2 mg/ml) from fatty acid synthase (8) was treated with 15 μM [1,3-^{3}H]-DFP (3.3 Ci/mmole; Amersham Searle Corp.) for 2 hr at 30°C in 0.1 M sodium phosphate buffer (pH 7.0) containing 0.5 mM dithioerythritol. Unlabeled DFP (Sigma Chemical Co.) was then added to obtain a final concentration of 5 mM, and the mixture was incubated for an additional 2 hr before extensive dialysis against distilled water and lyophilization.

Chymotryptic Digestion: [1,3-^{3}H]DFP-labeled protein (198 mg) was oxidized with performic acid (10) and incubated with 4 mg chymotrypsin (A_4, bovine pancreas, Boehringer Mannheim) in 10 ml of 0.2 M NH_4HCO_3 (pH adjusted to 7.3 with glacial acetic acid) for 4 hr at 30°C and subsequently 4 mg of chymotrypsin was added and the mixture was incubated overnight.

Purification of the [1,3-^{3}H]DFP-Peptide: Two Sephadex G-25 (superfine) columns (2.5 x 115 cm) were equilibrated with 0.2 M NH_4HCO_3 (pH 7.3) and half of the chymotryptic digest was applied to each column. The peptides were eluted with the same buffer at a flow rate of 32 ml/hr. The radioactive fractions were pooled and lyophilized. Active site peptide obtained from the gel filtration step was dissolved in 18 ml of 0.02 M ammonium acetate, pH 3.8 (pH adjusted with formic acid), and applied on a SP-Sephadex column (2.2 x 40 cm) equilibrated with the same buffer. The bound peptides were eluted at a flow rate of 30 ml/hr with a linear gradient of 0.02 M-1M of ammonium acetate, pH 3.8. The radioactive fractions were pooled and lyophilized. Final purification of the active site peptide was carried out by HPLC using a C_{18} μBondapak column (3.9 x 300 mm; Waters Associates) that was equilibrated with 0.1% phosphoric acid. A gradient of increasing acetonitrile concentration was used at a flow rate of 1.0 ml/min to elute the peptides. Radioactive fractions were pooled, lyophilized and desalted using a Sephadex G-25 superfine column (1.5 x 110 cm) equilibrated with distilled water.

Determination of N-Terminus: N-terminal residue was dansylated and the dansylated peptide was hydrolyzed as described (11). The dansylated amino acids were identified by thin layer chromatography (12) and by HPLC (13).

Amino Acid and Sequence Analysis: Peptide was hydrolyzed under reduced pressure with 6N HCl at 110°C for 24 hr and the hydrolysate was analyzed on a Beckman Model 121 MB amino acid analyzer. Sequence analysis was performed with a Beckman Model 890C sequencer using Beckman program number 121078 with Polybrene (3 mg). PTH amino acids were identified by HPLC (14), and either gas liquid chromatography (15) or thin layer chromatography (16).

Protein and Radioactivity Determination: Protein was determined by the method of Lowry et al. (17). Radioactivity was determined using a Packard Tricarb 460 CD liquid scintillation spectrometer using ScintiVerse (Fischer).

RESULTS AND DISCUSSION

Purification of the Thioesterase Active Site Peptide Containing "Active Serine": Limited trypsin treatment of fatty acid synthase from goose uropygial gland is known to release a 33 K dalton peptide containing the thioesterase activity (8). Such treatment of the synthase that was labeled with [1,3-^{3}H]DFP followed by gel filtration on Sephadex G-100 and SDS gel electrophoresis showed that at least 90% of the label was contained in the 33 K dalton thioesterase peptide (data not shown). This thioesterase segment was oxidized with performic acid, digested with chymotrypsin and the digest was subjected to gel filtration on Sephadex G-25 (Figure 1A). More than 70% of the applied radioactivity was eluted in one peak. Chromatography of this material on a SP Sephadex column revealed two radioactive peaks (Figure 1B). The first and the second peaks consisted of approximately 60% and 40% of the applied radioactivity, respectively. Peptides obtained from the two peaks were further fractionated by HPLC on a C_{18} μBondapak column. Analysis of the first peak, showed that more than 90% of the radioactivity was coincident with a single symmetric uv absorption peak (Figure 1C). Similar analysis of the second peak revealed the presence of seven radioactive peaks (data not shown), probably generated by the minor amounts of cleavages of the parent peptide by chymotrypsin. Dansylation of the HPLC-purified peptide from the first peak showed a single dansylated amino acid which was identified as dansyl-serine.

Primary Sequence: Amino acid composition of this peptide showed the predominance of hydrophobic residues (Table 1). Sequence determined by automated Edman degradation was Ser-Phe-Gly-Ala-Cys-Val-Ala-Phe. Repetitive yields in the first five cycles were >90% and dropped drastically in subsequent cycles as expected from a small hydrophobic peptide. However, the identification of the residues were unambiguous since 7 nmoles of the last residue (Phe) was obtained for identification. The radioactivity was released by the first

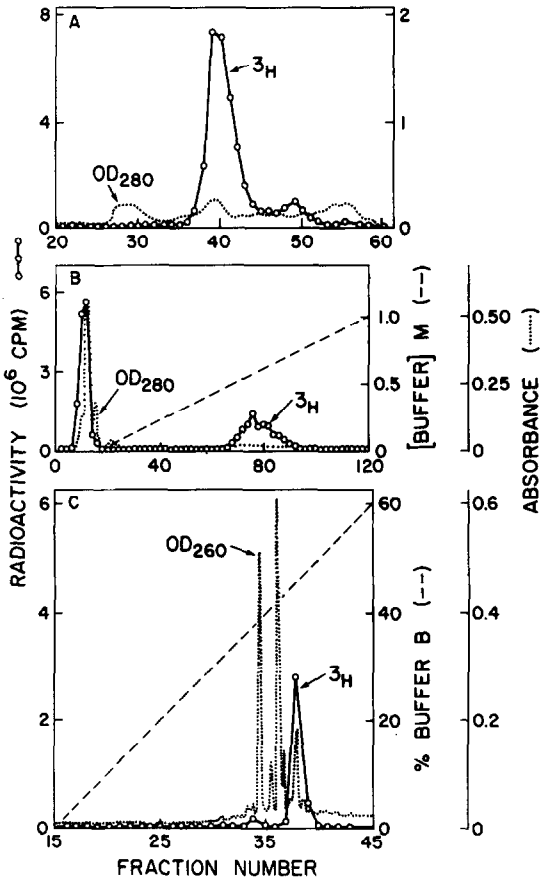

Figure 1. A) Gel filtration of the chymotryptic digest of [1,3-^{3}H]DFP labeled thioesterase fragment of fatty acid synthase on Sephadex G-25; fraction size, 8.5 ml.

B) SP Sephadex chromatography of the [1,3-^{3}H]DFP-peptide; fraction size, 8 ml.

C) HPLC of the [1,3-^{3}H]DFP-peptide. Buffer B contained 80% acetonitrile and 20% 0.2 M triethylammonium phosphate buffer, pH 3.2; fraction size, 1 ml.

cycle suggesting that the serine was the "active serine" present at the thioesterase domain of fatty acid synthase. Trypsin treatment of fatty acid synthase which was modified with [1,3-^{3}H]DFP yielded a 33000 dalton protein containing virtually all of the radioactivity and this protein, upon chymotrypsin treatment, gave rise to one major radioactive peptide. These results strongly suggest that each 250 K dalton peptide contains a

TABLE 1

Amino Acid Composition of the Thioesterase Active Site Peptide

Amino Acid	Mole%	Number of residues found in sequencing
Cys	13.1	1
Ser	8.2	1
Gly	12.8	1
Ala	22.8	2
Val	12.5	1
Phe	21.8	2

Amino acids representing <2% are not shown.

thioesterase domain and that the active sites of the thioesterase segment in both peptides are identical.

Some sequence homologies were observed with other "active serine" containing enzymes (Table 2). Glycine residue at the β position appears to be an invariant feature in these enzymes. The homology between the active site peptide from human plasmin and the present thioesterase of fatty acid synthase is remarkable. In view of the occurrence of many thioesterases in animal tissues it would be interesting to see whether the active sites of these enzymes are homologous.

TABLE 2

Comparison of "Active Serine" Peptides

Enzyme	Sequence
Thioesterase from goose fatty acid synthase	*Ser-Phe-Gly-Ala-Cys-Val-Ala-Phe
Plasmin, human (9)	*Ser-Gly-Gly-Pro-Leu-Val-Cys-Phe
Trypsin, Bovine (9)	*Ser-Gly-Gly-Pro-Val-Val-Cys-Ser
Carboxylesterase, Bovine (9)	*Ser-Ala-Gly-Ala-Glu-Ser

Asteric indicates active serine.

ACKNOWLEDGEMENTS

We thank Dr. A.J. Smith for sequence analysis and Dr. S. Gurusiddaiah for amino acid analysis. This work was supported in part by a grant GM-18278 from National Institues of Health of the U.S. Public Health Service. Scientific Paper 5981, Project 2001, College of Agriculture Research Center, Washington State University, Pullman, Washington 99164.

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