LOCALIZATION OF THE CENTRAL AND PERIPHERAL SH-GROUPS ON THE SAME POLYPEPTIDE CHAIN OF YEAST FATTY ACID SYNTHETASE

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SUMMARY: Purified fatty acid synthetase isolated from wild type yeast cells as well as from two different fas-mutant strains was reacted with (1-^{14}C-)iodoacetamide. Tryptic digests of the ^{14}C-carboxamidomethylated enzymes were fractionated on Sephadex G-50. Hereby, essentially only one radioactively labeled peptide was eluted from the column. From this it is concluded that under the experimental conditions employed only the "peripheral" SH-group of yeast fatty acid synthetase becomes alkylated. By sodium dodecylsulfate-polyacrylamide gel electrophoresis of the ^{14}C-carboxamidomethylated fatty acid synthetase it was shown that in all three enzyme preparations studied the inhibitor is bound to the larger one of the two fatty acid synthetase subunits. These findings indicate that the larger fatty acid synthetase subunit accomodates not only the "central" but also the "peripheral" SH-group of the multienzyme complex.

INTRODUCTION

Acetate, the priming substrate of long chain fatty acid biosynthesis, is covalently bound to several chemically distinct sites of the yeast fatty acid synthetase multienzyme complex (1). These sites have been chemically characterized as a serine OH-group and as two different thiol groups, designated as "central" and as "peripheral" SH-group, respectively (2,3). While the "peripheral" SH-group belongs to a cysteine residue of the enzyme protein (4,15, G.-B. Kresze et al., in preparation), the "central" one is attributed to enzyme-bound 4'-phosphopantetheine (5,6). Both thiol groups are assumed to be involved in the condensation of malonate and acetate to form enzymebound acetoacetate according to the following equation, where S_p indicates the "peripheral" and S_c the "central" SH-group:

$$\text{enzyme}S_c\text{—}COCH_2COO^- + \text{enzyme}S_p\text{-}COCH_3 \rightleftharpoons$$

$$\text{enzyme}S_c\text{—}CO\text{-}CH_2\text{-}CO\text{-}CH_3 + CO_2 + \text{enzyme}S_p^-$$

Inhibition studies performed under carefully controlled conditions indicated that the ß-ketoacyl synthetase component enzyme was inactivated concomitantly with the alkylation of three cysteine SH-groups of the fatty acid synthetase complex (15, G.-B. Kresze et al., in preparation). These results

indicated that, under appropriate conditions, iodoacetamide specifically binds to the "peripheral" SH-group but does not react with the "central" pantetheine prosthetic group of the complex. Recently, genetic studies on the biosynthesis of the yeast fatty acid synthetase complex showed that by sodium dodecylsulfate-polyacrylamide gel electrophoresis the multienzyme complex may be separated into two nonidentical protein subunits (7). Subsequently it was found that the larger one of these subunits contained the phosphopantetheine prosthetic group (7). Thus, similar studies with appropriate enzyme-substrate or enzyme-inhibitor compounds should allow the localisation of the corresponding substrate or inhibitor binding sites on either of the two subunits. For instance, by specifically labeling the fatty acid synthetase "peripheral" SH-group with ^{14}C-iodoacetamide it should be possible to identify the ß-ketoacylsynthetase component enzyme among the protein subunits of the complex. Experiments pertinent to this question will be reported in this study.

MATERIALS AND METHODS

The Saccharomyces cerevisiae haploid wild type strain X2180-1B, a-mating type, was originally obtained from Dr. R.K. Mortimer. The fatty acid synthetase deficient mutants fas1-266, fas2-349 and fas2-288 were derived from this strain as described previously (8). For enzyme isolation wild type as well as fas-mutant strains were grown at 30°C in 40 liters of yeast extract-pepton sucrose medium, each. After 36 hours incubation without aeration, the cultures were aerated with compressed air for additional 24 to 36 hours. Under these conditions between 200 and 300 grams of wet yeast cells were obtained. The harvested cells were screened for genetic homogeneity as described earlier (9).

The procedure used for enzyme purification was essentially that described by Lynen (10). After the second 100.000xg sedimentation step the enzyme was reacted with 1-^{14}C-iodoacetamide as described below and subsequently subjected to a final purification by sedimentation in a 5 -30 % sucrose density gradient for 15 hr at 4°C and 100.000xg. After the run, 20 drop-fractions were collected and analyzed for radioactivity and optical density at 280 nm. The enzyme-containing fractions were pooled and, if not used immediately, kept at -15°C. Aliquots of this solution were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis as described below.

Protein samples were denatured by incubation with 1 % sodium dodecyl sulfate in the presence of 0.5 % mercaptoethanol for 3 hr at 25°C. After the addition of 30 % (w/v) solid sucrose the samples were placed on top of urea-sodium dodecylsulfate-polyacrylamide gels as described previously (7) Small cylindrical gels (15 x 0.5 cm) were run for 12 hr at 20 volts and 22°C, slab gels were run in a DESAGA slab electrophoresis chamber for about 60 hr at 23 volts and 22°C. Subsequently, the gels were stained with 0.05 % Coomassie Blue G 250 (7). For radioactivity determination the destained slab gels were frozen in a dry ice/methanol bath and cut into slices of 0.5 -1 mm width. Each of the slices was soaked overnight with 1 ml of Soluene® (Packard) at 60°C under slight agitation. For subsequent counting, 16 ml of a 1:1 mixture of PPO/POPOP-scintillation fluid (7) and Triton X 100 (Serva) were added together with 0.1 ml of glacial acetic acid to each sample.

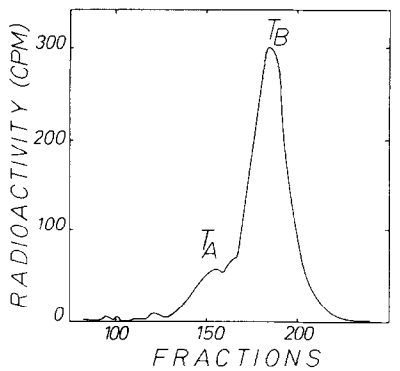

Fig. 1: Sephadex G-50 chromatography of the tryptic hydrolysate of ^{14}C-carboxamidomethyl-synthetase. For conditions of digestion, see Materials and Methods. The hydrolysate of the ^{14}C-carboxymethylated fas2-349 fatty acid synthetase was dissolved in 1 ml 50 % (v/v) acetic acid and subjected to chromatography on Sephadex G-50. Column: 1.65 x 87 cm; fraction volume: 0.72 ml; eluant: 50 % (v/v) acetic acid. 0.2 ml of every second fraction were tested for radioactivity.

About 20 mg of the enzyme were reacted with 1 mM (1-^{14}C) iodoacetamide (18.4 mCi/mmole, Commissariat à l'Energie Atomique, Gif-sur-Yvette, France) at 0°C in 0.1 M potassium phosphate buffer pH 6.5 for 120 min. Unreacted (1-^{14}C) iodoacetamide was removed by filtration of the mixture over a Sephadex G-25 column (6 x 0.5 cm) at 0°C. Tryptic digestion of ^{14}C-carboxamidomethyl synthetase was conducted at 25°C for 20 hours (0.5 mg of N-tosyl-L-phenylalanyl-chloromethan-treated trypsin per ml 0.1 M tris.HCl, pH 8.1). Then, the mixture was dried on a rotatory evaporator, the residue dissolved in 1 ml of 50 % (v/v) acetic acid and subjected to chromatography on Sephadex G-50 (column 87 x 1.6 cm) in the same solvent.

RESULTS

1. Chromatography of the tryptic hydrolysate of ^{14}C-carboxamidomethyl synthetase. As will be reported in detail elsewhere (G.-B. Kresze et al., in preparation) tryptic digestion of ^{14}C-carboxamidomethyl-labeled fatty acid synthetase produces two radioactive peptides, T_A and T_B separable by Sephadex G-50 chromatography. Depending on the digestion conditions used the proportion of the larger peptide T_A varies. From this it was concluded that T_B originates from T_A by further digestion and that there is only one iodoacetamide-sensitive site in the yeast fatty acid synthetase. To confirm these findings also for the mutant fatty acid synthetase used in this study the tryptic digests of the ^{14}C-carboxamidomethylated mutant enzymes were analyzed by Sephadex G-50 chromatography. In Fig. 1, the radioactivity pattern obtained with one representative enzyme isolated from mutant fas1-349 is shown. Though the amount of peptide T_A was very small in this experiment

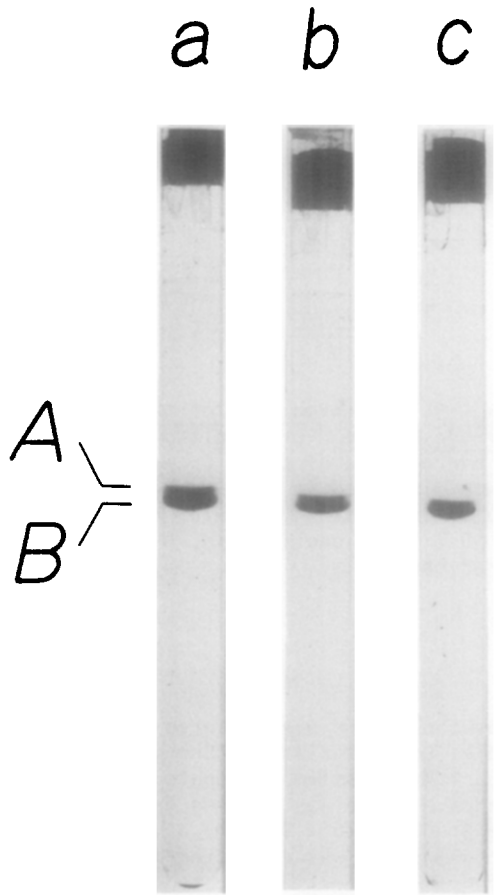

Fig. 2: Sodium dodecylsulfate-polyacrylamide gel electrophoresis of purified wild type (a), fas2-349 (b) and fas1-266 (c) fatty acid synthetase. Experimental conditions were as described earlier (7).

it is evident that essentially the same carboxamidomethylated peptides are obtained with wild type and fas-mutant fatty acid synthetases.

2. The iodoacetamide binding protein component of yeast fatty acid synthetase. The purified and ^{14}C-carboxamidomethylated synthetase preparations of the mutants fas1-266 and fas2-349 as well as from wildtype yeast cells studied were subject to analytical as well as preparative sodium dodecylsulfate-polyacrylamide gel electrophoresis. As shown in Fig. 2 the analytical gels revealed the presence of exclusively the two fatty acid synthetase subunits A and B described earlier (7). After slicing the gels the radioactivity associated with the individual bands was determined. As

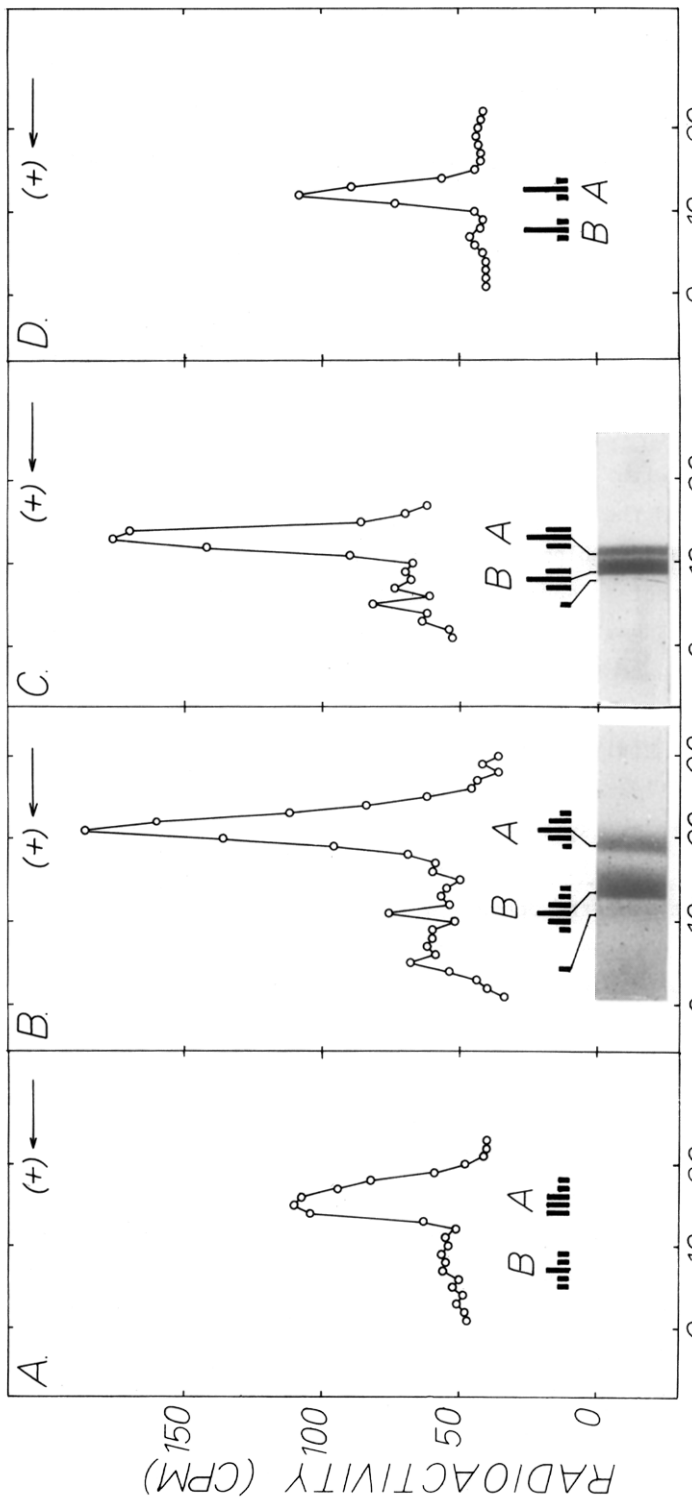

Fig. 3: Protein and radioactivity banding patterns of ^{14}C-carboxamidomethylated (A-C) and ^{14}C-pantothenate-labeled (D) fatty acid synthetases upon sodium dodecylsulfate-polyacrylamide gel electrophoresis. The enzymes studied were isolated from wild type (A), fas1-266 (B) and fas2-349 (C) cells. The pattern of ^{14}C-pantothenate-labeled fas2-288 synthetase (D) was adapted from Schweizer et al. (7) 0.9 ml containing 1.500 - 2.000 cpm radioactively labeled sodium dodecylsulfate-denatured fatty acid synthetase (0.2 - 0.4 mg protein) were applied to each gel. Radioactivity determinations were averaged from 5-6 10-min-countings. The black bars below the tracings indicate relative staining intensities of the gel slices at the positions of the fatty acid synthetase subunits A and B. For other experimental details see Materials and Methods.

it is shown in Fig. 3 A-C, the radioactive inhibitor is exclusively bound to the larger subunit A of all three enzymes studied. For comparison, the banding pattern observed with a ^{14}C-pantothenic acid-labeled fatty acid synthetase is shown in Fig. 3 D. Obviously, both the pantetheine and carboxamidomethyl residues are bound to the same polypeptide chain of the yeast fatty acid synthetase complex.

DISCUSSION

Genetic evidence obtained earlier in this laboratory suggested that in yeast fatty acid synthetase the ß-ketoacyl synthetase activity is associated with the larger one of the two known protein subunits of the complex (7). This view has now been further confirmed chemically by specific labeling of the "peripheral" SH-group with ^{14}C-iodoacetamide. According to the results reported in this study both the "central" pantetheine SH-group as well as the"peripheral" SH-group are combined within a single polypeptide chain, i.e. the larger fatty acid synthetase subunit A. Furthermore, genetic data to be reported elsewhere (E. Schweizer, in preparation) suggest that this fatty acid synthetase subunit accomodates even a third function, namely the ß-ketoacyl reductase activity. Thus, the fas2 encoded fatty acid synthetase subunit A appears to be a multifunctional polypeptide chain comprising at least three different functions of the multienzyme complex, i.e. the acyl carrier protein, the ß-ketoacyl synthetase and the ß-ketoacyl reductase. Correspondingly, the four known fas1-encoded fatty acid synthetase activities, i.e. the enoyl reductase, the dehydratase, the malonyl and the palmityl transferase component enzymes are assumed to be associated with component B, the second fatty acid synthetase protein subunit, as it is indicated by various lines of genetic evidence obtained by Tauro et al. (11) as well as by Knobling and Schweizer (in press). These findings together with the known FMN (12) and pantetheine (13) content of yeast fatty acid synthetase suggest an A_6B_6 stoichiometry of the intact yeast fatty acid synthetase complex.

On the other hand, this conclusion seems to be somewhat at variance with results obtained by Kresze et al. (in preparation) indicating that not six but only three carboxymethyl residues are bound to one molecule of the multienzyme complex concomitant with complete inhibition of fatty acid synthetase activity. This apparent discrepancy, however, may be resolved by the assumption that yeast fatty acid synthetase is endowed with "half-site-reactivity" characteristics as they are observed with numerous other multimeric enzymes (for a review see 14). Although a reaction mechanism

of the fatty acid synthetase complex involving half-site-reactivity of the "peripheral" SH-groups may easily by envisaged it must remain speculative until more is known about the molecular interaction and the spatial arrangement of the various active centers within the yeast multienzyme complex.

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