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Active Site Directed Inactivation of Rat Mammary Gland Fatty Acid Synthase by 3-Chloropropionyl Coenzyme A†

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ABSTRACT: 3-Chloropropionyl coenzyme A (CoA) irreversibly inhibits rat mammary gland fatty acid synthase. Enzyme inactivation proceeds with first-order kinetics. NADPH (150 μM) as well as acetyl-CoA (500 μM) affords protection against inactivation, suggesting that the inhibitor is active site directed. In contrast, malonyl-CoA (500 μM) offers little protection. With chloro[1-^{14}C]propionyl-CoA, stoichiometries of modification that approach one per enzyme protomer (240 kilodaltons) have been measured. When chloropropionyl-[3′-^{32}P]CoA is used for inactivation, modification stoichiometries are less than 10% of the value observed in the ^{14}C labeling experiments, suggesting that acylation of the enzyme occurs. Radioactivity remains associated with the ^{14}C-labeled protein after performic acid oxidation, indicating that another linkage, in addition to the thio ester adduct, is formed during inactivation. Recovery of ([^{14}C]carboxyethyl)cysteine from digests of the inactivated enzyme indicates that alkylation of an active site cysteine occurs. The cysteamine sulfhydryl of the acyl carrier peptide is clearly *not* the site of modification. Loss of overall enzyme activity is tightly linked to decreases in the ketoacyl synthase partial reaction. This observation, coupled with the differential protection measured with acetyl-CoA and malonyl-CoA, suggests that the reagent modifies a residue at the active site involved in condensation. While inactivated enzyme shows good ketoacyl reductase activity when *S*-(acetoacetyl)-*N*-acetylcysteamine is used as a substrate, only poor activity for this partial reaction is measured when acetoacetyl-CoA is the substrate. This implies that the function of the acyl carrier peptide (ACP) is impaired during the inactivation process. Since the ACP of one protomer appears to interact with the active site of the condensing enzyme on the adjacent subunit of the dimeric fatty acid synthase [Wakil, S. J., Stoops, J. K., & Joshi, V. C. (1983) *Annu. Rev. Biochem. 52*, 537-579], enzyme inactivated as a result of sequential thio esterification and alkylation events should contain covalently cross-linked protomers. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of inactivated enzyme under conditions designed to preserve labile thio ester linkages demonstrates the formation of cross-linked enzyme. Treatment of a paired sample of inactivated enzyme with hydroxylamine prior to SDS-PAGE eliminates all cross-linking, validating this aspect of our model for the mechanism of chloropropionyl-CoA-dependent inhibition.

A variety of approaches have been used to specifically modify amino acids at the active site of fatty acid synthase. Bloch's group (Helmkamp et al., 1968) utilized 3-decynoyl-*N*-acetylcysteamine to inhibit *Escherichia coli* β-hydroxydecanoyl-thioester dehydrase and subsequently demonstrated that a mechanism-based process accounted for the irreversible inactivation (Endo et al., 1970). The highly reactive analogue

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S-(4-bromo-2,3-dioxobutyl) coenzyme A (CoA) was evaluated as a site-directed inhibitor (Clements et al., 1979, 1982) and found to behave as a simple alkylating reagent rather than as a classical affinity label. Chloroacetyl-CoA was reported to stoichiometrically label fatty acid synthase (Kumar et al., 1980). Subsequently, experiments with this analogue (McCarthy & Hardie, 1982) raised a question concerning whether the target of this reagent was a pantetheine thiol rather than the cysteinyl thiol previously reported.

Recently, we reported that 3-chloropropionyl-CoA irreversibly inhibits 3-hydroxy-3-methylglutaryl-CoA synthase in an active site directed fashion (Miziorko & Behnke, 1985a). Stoichiometric labeling of a cysteinyl sulfhydryl accounts for enzyme inactivation; a peptide containing this active site residue has been isolated and sequenced (Miziorko & Behnke, 1985b). The specificity afforded by this reagent suggested that it might be a useful affinity label of other acyl-CoA utilizing enzymes. Upon screening several potential target enzymes, it was determined that 3-chloropropionyl-CoA is quite effective in inactivating rat mammary fatty acid synthase. This paper presents the results of our experiments designed to test this analogue as an affinity label for fatty acid synthase. In the course of these experiments, we have generated data that provide an independent test of the model that has been advanced (Wakil et al., 1983) to explain why subunit orientation in the dimeric mammalian enzyme is crucial to catalysis. A preliminary account of this work has appeared (Miziorko et al., 1985).

Experimental Procedures

Materials. Rat mammary gland fatty acid synthase was purified by the method of Ahmad et al. (1982). Activity assays were conducted at 30 °C with the procedure of Smith & Abraham (1975). 3-Chloropropionyl chloride and 3-chloropropionic acid were purchased from Aldrich Chemical Co. The acyl chloride was redistilled before use; the free acid was recrystallized from petroleum ether before use. 3-Chloro[1-^{14}C]propionic acid was obtained from Pathfinder Laboratories, Inc. (St. Louis, MO). S-(Carboxyethyl)cysteine was purchased from Fluka AG. CoASH (lithium salt) and dephospho-CoA were obtained from Pharmacia P-L Biochemicals. Dephospho-CoA kinase was generously provided by Dr. R. Naylor (Pharmacia P-L Biochemicals). All other chemicals were of the highest quality commonly available.

Methods. *S*-(Carboxyethyl)cysteamine was synthesized from 3-mercaptopropionic acid and 2-bromoethylamine hydrobromide with the procedures described by Cavallini et al. (1955) and Hermann et al. (1969). The product was purified on a Dowex 50 column (5 × 22 cm); elution was accomplished with 1 M aqueous pyridine. Purified material was taken to dryness, dissolved in ethanol, and crystallized by addition of acetone. The melting point was 212–214 °C. Identity of the product was verified by proton NMR: δ 3.18 (2 H, t, J = 6.6 Hz), 2.83 (2 H, t, J = 6.6 Hz), 2.74 (2 H, t, J = 7.0 Hz), and 2.44 (2 H, t, J = 7.0 Hz).

Synthesis of unlabeled and radioactively labeled 3-chloropropionyl-CoA has been described in detail by Miziorko & Behnke (1985a). The unlabeled compound was prepared by the method of Simon & Shemin (1953) using a 5-fold molar excess of 3-chloropropionyl chloride. The ^{14}C-labeled material was prepared similarly after conversion of 3-chloro[1-^{14}C]-propionic acid to the corresponding acyl chloride with a 25-fold molar excess of oxalyl chloride (Kass & Brock, 1969). 3-Chloropropionyl-[3′-^{32}P]CoA was synthesized from [3′-^{32}P]-CoA prepared by enzymatic phosphorylation of dephospho-CoA with dephospho-CoA kinase and [γ-^{32}P]ATP.

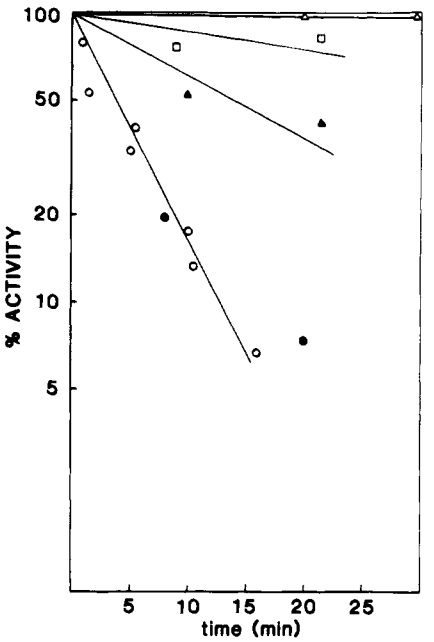

FIGURE 1: 3-Chloropropionyl-CoA inhibition of fatty acid synthase: protective effects of acyl-CoA substrates. Reaction mixtures (100 μL) containing potassium phosphate buffer, pH 7.5 (210 mM), dithiothreitol (0.5 mM), and fatty acid synthase (18.5 μg) were preincubated at 30 °C for 10 min. When acetyl-CoA (0.5 mM, ▲), malonyl-CoA (0.5 mM, ●), or NADPH (150 μM, □) was added, the mixture was incubated for 2 min prior to initiation of the reaction by addition of 3-chloropropionyl-CoA (55 μM, ○, ●, ▲, □; 0 μM, △). Aliquots were withdrawn at the times indicated and assayed for fatty acid synthase activity. In other experiments performed in the absence of protective agents, inactivation rates measured with 10–60 μM chloropropionyl-CoA were essentially the same as those observed at 55 μM, indicating that the enzyme is saturated at these levels of reagent.

Quantitation of radioactivity covalently bound to inactivated enzyme was performed by denaturing the protein with cold 10% trichloroacetic acid, followed by centrifugation to separate precipitated protein from the bulk of the radioactively labeled chloropropionyl-CoA. After the supernatant was discarded, the precipitated protein was suspended in cold 10% trichloroacetic acid, loaded onto glass fiber filters, and washed sequentially with cold 10% trichloroacetic acid, cold 50 mM sodium pyrophosphate in 0.5 M HCl, and cold absolute ethanol. The sample of radioactively labeled protein immobilized on the filter could be used for liquid scintillation counting or for digestion required for identification of the modification site.

Results

3-Chloropropionyl-CoA is a potent irreversible inhibitor of rat mammary gland fatty acid synthase. At elevated concentrations of the reagent, inactivation proceeds with first-order kinetics (Figure 1). Efficacy of substrates in protecting against enzyme modification varies substantially. Upon testing acyl-CoA substrates, it was determined that acetyl-CoA (0.5 mM) afforded substantial protection while an equivalent concentration of malonyl-CoA was ineffective (Figure 1). NADPH (150 μM) protects even more effectively than acetyl-CoA. Thus, while the protection pattern is complex, it seems clear that the reagent is active site directed.

Chloropropionyl-CoA is a considerably more potent inhibitor of fatty acid synthase (50% inactivation upon incubation with 0.7 μM reagent for 10 min) than of HMG-CoA[1] synthase

[1] Abbreviations: ACP, acyl carrier peptide; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

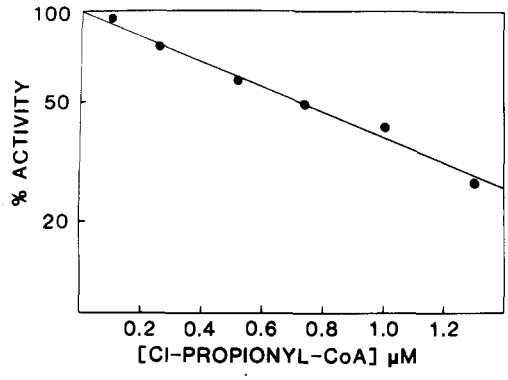

FIGURE 2: Concentration dependence of the chloropropionyl-CoA-dependent inhibition of fatty acid synthase. Enzyme was activated by preincubation in 225 mM potassium phosphate buffer, pH 7.5, containing 0.5 mM dithiothreitol for 30 min at 30 °C. Reaction mixtures containing enzyme (2.9 μg) in preincubation buffer and 3-chloropropionyl-CoA at the concentrations indicated were incubated at 30 °C for 10 min prior to measurement of fatty acid synthase activity.

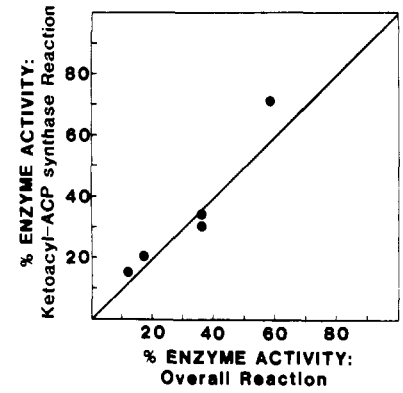

FIGURE 3: 3-Chloropropionyl-CoA inhibition of fatty acid synthase: comparison between effects on the overall reaction and on the ketoacyl ACP synthase reaction. Reaction mixtures (360 μL) containing potassium phosphate (250 mM), pH 6.5, dithiothreitol (0.5 mM), and fatty acid synthase (240 μg) were preincubated for 30 min at 30 °C prior to initiation of inactivation by addition of 3-chloropropionyl-CoA (78 μM). Activity measurements were performed at t = 0, 3, 7, 10, 15, and 26 min. Data points indicating the ratio of activity in the ketoacyl-ACP synthase partial reaction to that measured for the overall reaction are depicted. These data approach the solid line having a slope of 1.0, as expected if loss of overall activity is coupled to a modification at the site where condensation between acetyl-CoA and malonyl-CoA occurs.

(Miziorko & Behnke, 1985). This precluded use of the usual methodology for determining K_i and k_{inact} by replot of the slopes observed in semilog displays of the time course of inactivation measured at different reagent concentrations. A lower limit for k_{inact} was estimated with the approach described by Owens & Barden (1978), which involves measurement of degree of inactivation at a fixed time point using varied concentrations of inhibitor. A plot of log residual activity vs. [chloropropionyl-CoA] produces a straight line (Figure 2), as predicted if the inhibitor concentration used in the incubation is much less than K_i. From a measurement of the slope of the line in Figure 2, it is possible to calculate a lower limit value for the rate of covalent modification [$m = k_{inact}t/(2.3K_i)$]. If K_i = 20 μM is assumed (and, since enzyme is near saturation at this concentration, this certainly represents an upper limit to K_i), then $k_{inact} \geq 2$ min^{-1}.

The specificity of modification afforded by this reagent is indicated not only by the observation of protection by substrates but also by the stoichiometry of labeling, measured with 3-chloro[1-^{14}C]propionyl-CoA. A stoichiometry approaching 0.96 per enzyme active site (i.e., ca. one per 240-kDa protomer) is measured with the ^{14}C-labeled analogue. Selective labeling of the inhibitor with ^{32}P in the CoA moiety resulted in a reagent that eliminated enzyme activity without incorporating radioactivity in the modified enzyme (0.07 mol of ^{32}P/mol of sites). The data suggest that the inhibitor mimics the natural substrates by acylating the enzyme. In contrast, in the case of HMG-CoA synthase, enzyme acylation is not an absolute prerequisite to specific covalent modification (Miziorko & Behnke, 1985a). While fatty acid synthase is acylated by the reagent, the inactivated enzyme does not merely contain a thio ester adduct. Performic acid oxidation of modified enzyme under conditions that labilized >98% of a thio esterified control sample resulted in no appreciable diminuation of radioactivity in a sample of chloro[^{14}C]propionyl-CoA-inactivated fatty acid synthase (untreated, 0.98 mol of ^{14}C/mol of sites; performic acid oxidized, 0.94 mol of ^{14}C/mol of sites).

The data suggest that enzyme is irreversibly alkylated by the inhibitor. Potential alkylation sites are the sulfhydryl groups of the acyl carrier peptide's pantetheine moiety or the cysteinyl sulfhydryl involved in the condensation reaction. The observation of protection by acetyl-CoA but not by malonyl-CoA (Figure 1) supports the latter possibility. Such a protection pattern has been previously observed (Stoops & Wakil, 1981a,b) in studies on agents that modify the β-ketoacyl synthase domain. Upon measuring the activity of this partial reaction, as well as activity for the overall reaction, in chloropropionyl-CoA-containing incubation mixtures, it was established that both activities decrease in a tightly coupled fashion (Figure 3). Thus, a site that is critical to the condensation reaction seems to be a target for the reagent. Furthermore, HCl digestion of enzyme inactivated with ^{14}C-labeled chloropropionyl-CoA followed by thin-layer chromatography of the resulting compounds indicates that the major radioactive component is (carboxyethyl)cysteine (Figure 4). Authentic (carboxyethyl)cysteamine was synthesized and cochromatographed to verify that alkylation of the acyl carrier protein does not account for the data. Similarly, carboxyethyl derivatives of lysine or histidine can be ruled out.

When chloropropionyl-CoA-containing incubation mixtures were monitored for activity in the ketoacyl reductase partial reaction as well as for overall reaction activity, an interesting contrast was noted. With *S*-(acetoacetyl)-*N*-acetylcysteamine as a substrate, little decrease in reductase activity was observed in comparison to the loss of overall activity. However, if acetoacetyl-CoA is used as the substrate in the ketoacyl reductase assay, a time-dependent loss in activity approaching that measured for the overall reaction is measured (Figure 5). The ability of chloropropionyl-CoA-inactivated enzyme to catalyze the reduction only when the cysteamine derivative is used as substrate suggested that the acyl carrier peptide (ACP) might be affected by the inactivation process (McCarthy & Hardy, 1982). For example, since the inhibitor acylates the enzyme (vide ante), it seemed possible that enzyme inactivation is the result of an initial thio esterification of ACP to form an ACP–S–propionyl chloride species followed by an alkylation event involving the ketoacyl domain (Figure 6). In this case, as long as the thio ester bridge remains intact, ACP may not be able to fulfill all of its normal functions. The model for fatty acid synthase that has emerged as a result of a variety of protein modification and related biochemical studies (Wakil et al., 1983) suggests that the ACP of one fatty acid synthase protomer interacts with the ketoacyl synthase domain located on an adjacent protomer. If this model is correct, it follows that, should chloropropionyl-CoA inactivation result from sequential thio esterification and alkylation events, there should

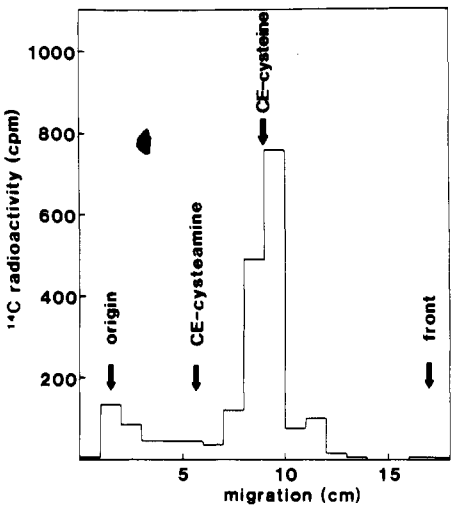

FIGURE 4: Thin-layer chromatographic characterization of the modified residue recovered from hydrolyzed chloro[^{14}C]propionyl-CoA inactivated fatty acid synthase. An aliquot of material recovered after HCl (6 N) digestion of chloro[1-^{14}C]propionyl-CoA inactivated enzyme (precipitated and washed as described under Methods) was dissolved and applied to an activated silica gel thin-layer plate and developed with 1-propanol–H_2O (70:30). Cochromatographed carboxyethylated amino acid standards were detected by spraying the dried chromatogram with 0.25% ninhydrin in acetone. ^{14}C radioactivity was determined by scraping the silica gel from the lane in which the ^{14}C-labeled digest was loaded and counting in liquid scintillator. The major peak of radioactivity coincided with authentic carboxyethylcysteine (R_f 0.48). No radioactivity comigrated with (carboxyethyl)cysteamine (R_f 0.28) or other carboxyethylated standards [N^{ϵ}-(carboxyethyl)lysine, (R_f 0.04; N^{ϵ},N^{ϵ}-bis(carboxyethyl)lysine, R_f 0.09; N^1- and N^3-(carboxyethyl)histidines, R_f 0.24; N^1,N^3-bis(carboxyethyl)histidine, R_f 0.30]. Similar results were obtained upon elution of a cellulose plate with 1-butanol–acetic acid–water (4:1:1). In this sytem, the R_f value for authentic (carboxyethyl)cysteine and the major radioactive component is 0.40, and the R_f for (carboxyethyl)cysteamine is 0.64. The minor radioactive component eluting ahead of (carboxyethyl)cysteine appears to be generated during precipitation and isolation of the inactivated enzyme; it comigrates in both the silica gel (R_f 0.62) and cellulose (R_f 0.74) thin-layer systems with the ethyl ester of (carboxyethyl)cysteine.

be transient formation of covalently cross-linked protomers (Figure 6, bottom). This hypothesis seemed verifiable by SDS–PAGE, although it was envisioned that lability of the thio ester linkage might complicate demonstration of the cross-linked species. If chloropropionyl-CoA-inactivated enzyme is briefly denatured in SDS at room temperature (without addition of any thiols) and subjected to SDS–PAGE at 9 °C, it is possible to demonstrate substantial formation of oligomeric material (Figure 7, lane 3). In contrast, untreated enzyme exhibits only a 240-kDa band due to protomer (Figure 7, lane 2). The banding pattern observed with chloropropionyl-CoA-treated enzyme is similar to that observed with a sample of enzyme that had been alkylated with dibromopropane (Figure 7, lane 1), a procedure established to cross-link the dimeric animal fatty acid synthase (Stoops & Wakil, 1981a). Dibromopropanone-treated enzyme produces more than one band corresponding to oligomeric material; similar observations have been reported by Stoops & Wakil (1981a). Chloropropionyl-CoA-inactivated enzyme also produces more then one oligomeric band. It is possible that employing the relatively mild denaturation conditions dictated by the lability of thio ester linkages contributes to the observed heterogeneity. Regardless of the cause of the heterogeneity of the oligomeric material, it is clear that, in the case of chloropropionyl-CoA-inactivated protein, thio ester linkages are involved. In contrast to the irreversible cross-linking produced by using dibromopropanone, our hypothesis predicts

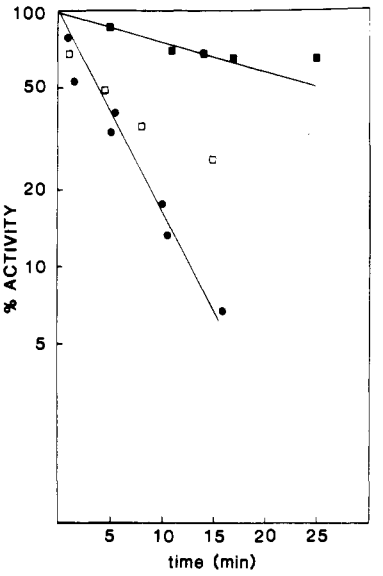

FIGURE 5: Effect of 3-chloropropionyl-CoA on the overall reaction catalyzed by fatty acid synthase and on the ketoacyl reductase partial reaction. Reaction mixtures (100 μL) containing fatty acid synthase (18.6 μg) in potassium phosphate buffer (210 mM), pH 7.5, with dithiothreitol (0.5 mM) were incubated at 30 °C for 30 min prior to initiation of the reaction by addition of 3-chloropropionyl-CoA (55 μM). At the times indicated, aliquots were withdrawn and assayed for activity with a standard assay for the overall reaction (Smith & Abraham (1975) (●) or for ketoacyl reductase activity with either *S*-(acetoacetyl)-*N*-acetylcysteamine (3 mM) as a substrate (■) or acetoacetyl-CoA (0.1 mM) as a substrate (□).

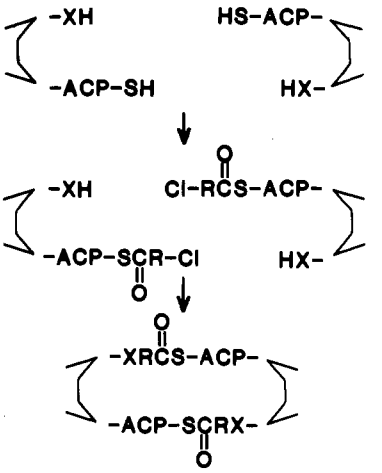

FIGURE 6: Schematic representation of dimeric rat mammary gland fatty acid synthase showing the juxtaposition of the pantetheinyl sulfhydryl group and target residue (×) that is alkylated by chloropropionyl-CoA. One minimal mechanism that could account for inactivation of the enzyme by chloropropionyl-CoA is shown. The results shown in Figure 4 indicate that the target residue (×) is an active site cysteine.

that the oligomers resulting from chloropropionyl-CoA modification can be converted back to protomers. The gel depicted in Figure 7 (lane 4) contains a sample of chloropropionyl-CoA-treated enzyme (similar to that used for lane 3) that was treated with hydroxylamine prior to electrophoresis. The disappearance of all oligomeric material as a result of elimination of thio ester linkages validates our hypothesis and argues for the sequential thio esterification–alkylation process as the mechanism that accounts for enzyme inactivation by chloropropionyl-CoA.

Discussion

The results presented above indicate the selectivity of chloropropionyl-CoA as a site-directed inhibitor and, together

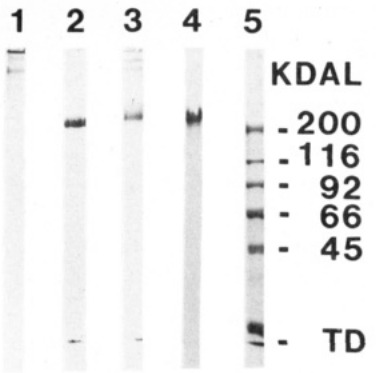

FIGURE 7: Polyacrylamide gel electrophoresis of 3-chloropropionyl-CoA inactivated fatty acid synthase. Reaction mixtures (100 μL) containing fatty acid synthase (37 μg), sodium phosphate (100 mM; pH 6.5 for mixture A, pH 7.5 for mixtures B and C), and dithiothreitol (0.5 mM) were activated by preincubation at 30 °C for 30 min. Mixture 1 (dialkylated enzyme marker) was brought to 100 μM in 1,3-dibromopropanone and incubated at 30 °C for 3 min, at which time enzyme inactivation was complete. Any residual reagent was quenched by addition of dithiothreitol (0.33 mM) prior to denaturation with 1% SDS at room temperature and loading (lane 1; 24 μg of protein applied) on the electrophoresis gel. Mixture 2 (untreated control) was maintained at 30 °C along with mixture 3, which was brought to 55 μM in 3-chloropropionyl-CoA for 30 min. Upon verification of inactivation of enzyme in mixture 3, both mixtures were denatured with 1% SDS at room temperature prior to loading (24 μg of protein) onto gel lanes 2 and 3, respectively. Lane 4 was loaded with chloropropionyl-CoA-inactivated enzyme, prepared as described for mixture 3 except that the inactivated, SDS-denatured sample was brought to 0.5 M in neutralized hydroxylamine for 10 min before loading. Lane 5 contains a mixture of molecular weight markers (myosin, 200 kDa; β-galactosidase, 116 kDa; phosphorylase B, 92 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa). Electrophoresis was performed on a 5% polyacrylamide gel. Sample concentration was achieved by use of a 3% stacking gel. The discontinuous system described by Laemmli (1970) was employed, except that the gel was maintained at 9 °C to maximize preservation of labile thio ester linkages.

with the data reported for HMG-CoA synthase (Miziorko & Behnke, 1985a; 1985b), illustrate the utility of this reagent as a mechanistic probe for enzymes that utilize acyl-CoA substrates. Thus, chloropropionyl-CoA is a valuable addition to the collection of CoA-containing affinity labels, despite the fact that the detailed chemical steps that account for its ability to covalently label the target protein have not been completely elucidated. The simplest mechanism that would account for covalent modification involves a direct attack of an active site nucleophile on C_3 of the acyl group with displacement of chloride. On chemical grounds, this possibility seems unattractive since a chlorine that is situated on a carbon β to the thio ester carbonyl is not a very good leaving group. In the case of chloroacetyl-CoA, such a displacement seems to be more likely since the location of the halogen α to the thio ester carbonyl will strongly enhance its properties as a leaving group. Enzyme inactivation by chloropropionyl-CoA is more likely to be due to a mechanism-based series of events, in which deprotonation of the carbon α to the thio ester carbonyl is followed by elimination of chlorine to form an acrylyl derivative that reacts with an active site nucleophile. Such a hypothesis is quite attractive in explaining the reagent's ability to modify HMG-CoA synthase. This enzyme catalyzes a deprotonation of the α-carbon of acetyl-CoA as a requisite step in formation of the product HMG-CoA. In the case of fatty acid synthase, no such deprotonation step is involved in the condensation event, and a mechanism-based hypothesis might, at first glance, seem unlikely. However, it should be pointed out that formation of an ACP–S–propionyl chloride species affords the possibility of shuttling the acyl group to other loci on this multisite enzyme where a reactive base may extract the proton to produce an ACP–S–acrylyl species. The identity and location of such a base is unclear, and the observation that NADPH affords protection against inactivation does not really narrow the list of possibilities. Binding of this substrate has been shown (Stoops & Wakil, 1982) to induce conformational changes that, inter alia, affect the juxtaposition of the reactive sulfhydryls of ACP and the condensing enzyme, and this behavior could account for protection. In any event, subsequent positioning of the ACP–S–acrylyl adjacent to the condensing enzyme domain would permit attack by a reactive nucleophile and result in stable alkylation of the active site residue. While such a sequence of events is certainly speculative, it is quite compatible with all available experimental data. Future experiments are planned to test the details of this scheme.

Site-directed modification of acyl-CoA utilizing enzymes has been attempted with the α-haloacyl-CoA analogues bromoacetyl-CoA and chloroacetyl-CoA. Interpretation of the data generated with these relatively reactive compounds has not always been straightforward. Clements et al. (1976) demonstrated that the methodology previously reported (Chase & Tubbs, 1969) for bromoacetyl-CoA synthesis yielded a mixture of components, including thiophenylcarboxymethyl-SCoA as the major product, and explained how this material could account for most of the modifications attributed to bromoacetyl-CoA. Similar methodology, involving thiol exchange, was used by Kumar et al. (1980) to synthesize chloroacetyl-CoA, which was used to inactivate fatty acid synthase. Cysteine was reported to be the target of this reagent. Using a different synthetic procedure, McCarthy & Hardie (1982) prepared chloroacetyl-CoA and, in experiments on fatty acid synthase, generated data that argued for a cysteamine sulfhydryl as the site of modification. Our preparation of chloropropionyl-CoA is more straightforward due to the inherently lower reactivity of the β-haloacyl group. While this reagent clearly modifies a cysteinyl sulfhydryl, this observation should not be used to argue for the validity of either of the chloroacetyl-CoA reports, since a substantially different mechanism is likely to account for the protein modification; i.e., instead of direct attack on the reagent by a protein-derived nucleophile, conversion of the chloropropionyl group to a more reactive species is likely to precede alkylation. Another novel aspect of this β-haloacyl-CoA involves the fact that chloropropionyl-CoA is the first site-directed reagent that has been clearly shown to mimic the normal substrates by acylating fatty acid synthase. With a chloroacetyl-CoA preparation, Kumar et al. (1980) showed a "nonspecific" association of the ^{3}H-labeled CoA moiety with acid-precipitated protein. Unlike the results obtained when the acyl group was ^{14}C-labeled, the level of protein-bound [^{3}H]CoA decreased with time. While inactivation was accounted for by invoking transfer of the chloroacetyl group to the enzyme, no data were provided to suggest that any thio ester linkage between enzyme and the chloroacetyl group forms. In experiments using 3-decynoyl-*N*-acetylcysteamine to modify β-hydroxydecanoyl-thioester dehydrase, a component of the bacterial fatty acid synthase system, Helmkamp et al. (1968) reported that the same degree of labeling was observed regardless of whether the reagent was labeled in the acyl group or in the acetylcysteamine moiety. Thus, those studies provide an additional contrasting example in which no thio esterification of a fatty acid synthase system by the site-directed reagent could be documented.

The selectivity that makes chloropropionyl-CoA valuable as an affinity label also adds significance to its ability to

cross-link fatty acid synthase. Undoubtedly, the development of dibromopropanone as a cross-linking reagent (Stoops & Wakil, 1981a, 1982) has been instrumental in leading to the model that predicts subunit arrangement as well as the orientation of various domains of fatty acid synthase. However, dibromopropanone is a relatively reactive reagent without the structural features that might be expected to strongly steer the molecule to a specific site on the protein. The utility and specificity that have been observed with dibromopropanone apparently rely on the unusual reactivity of the fatty acid synthase sulfhydryls that it modifies. Thus, the use of the more specific CoA-containing affinity label to achieve cross-linking between ACP and the condensing enzyme domain provides strong support for the validity of the structural model. This aspect of the experimental findings underscores the versatility of chloropropionyl-CoA as a mechanistic probe.

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