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Palmityl Coenzyme A Inhibition of Fatty Acid Synthesis

RELIEF BY BOVINE SERUM ALBUMIN AND MYCOBACTERIAL POLYSACCHARIDES*

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SUMMARY

The effects of acyl-CoA derivatives (C_8 to C_{20}) on the activity of the fatty acid synthetases from yeast and *Corynebacterium diphtheriae* have been examined. Both enzyme systems are inhibited by the longer chain acyl thioesters (C_{16} to C_{20}) and protected against this inhibition by bovine serum albumin (BSA). Identical relief from acyl-CoA inhibition is provided by the 6-*O*-methylglucose-containing lipopolysaccharide (MGLP), from *Mycobacterium phlei*. It is shown that MGLP forms a stable complex with palmityl-CoA. This interaction accounts for the BSA-like effects of the polysaccharide. BSA and MGLP have two further effects on the fatty acid synthetases under study, also attributable to complex formation with palmityl-CoA. They stimulate the rate of over-all synthesis from acetyl-CoA and malonyl-CoA, and they cause a shift of the fatty acid pattern towards products of shorter chain length. The observed effects are discussed in terms of the regulation of fatty acid synthesis both with respect to rate and product composition. It is concluded that in the two microbial enzyme systems negative feedback inhibition and its relief are important control mechanisms.

During the isolation and characterization of a multienzyme fatty acid synthetase from *Mycobacterium phlei* we have found the activity of this system to be remarkably enhanced by two types of polysaccharides from the same bacterial source (1). These polysaccharides, which are composed of mannose and 3-*O*-methylmannose and 6-*O*-methylglucose, respectively, have been structurally characterized by Ballou and his colleagues (2–4). The manner in which MMP[1] or MGLP stimulate mycobacterial fatty acid synthesis is not known, except that they markedly lower the K_m values for acetyl-CoA (1). It seemed of interest to ascertain whether MMP or MGLP affect fatty acid synthesis not only in *M. phlei* but also in other microorganisms. Positive, although less marked responses to these polysaccharides have now been obtained with the fatty acid synthetases from yeast and from *Corynebacterium diphtheriae*. The observed effects are qualitatively similar to the way in which BSA enhances fatty acid synthesis.

BSA appears to effect fatty acid synthesis and perhaps also other enzyme systems beneficially by sequestering either added or generated palmityl-CoA, a powerful enzyme inhibitor. Stimulation of fatty acid synthesis by MMP or MGLP can be explained on the same basis. We show that these polysaccharides can counteract inhibition by palmityl-CoA and also that they interact with palmityl-CoA to form stable complexes.

EXPERIMENTAL PROCEDURE

Materials and Methods—Coenzyme A, pantetheine, and BSA (defatted) were obtained from Sigma Chemical Co. and the various acyl-CoA derivatives from P. L. Chemicals. Acetyl-CoA was prepared according to Simon and Shemin (5). New England Nuclear was the source of [2-^{14}C]malonyl-CoA (20.6 mCi per mmole) and [1-^{14}C]palmityl-CoA (52.8 mCi per mmole). TPNH, DPNH, and FMN were supplied by Calbiochem. MMP and MGLP-II, polysaccharides from *Mycobacterium phlei*, were isolated as described (1). MGLP-II is one of a family of methylglucose polysaccharides containing six short chain fatty acid residues and 1 mole of succinate (4).

Enzymes—A culture of *Corynebacterium diphtheriae* was a gift of Dr. A. Pappenheimer, Harvard University. The fatty acid synthetase (6) was isolated and purified to a specific activity of 265 (nmoles of malonyl-CoA incorporated per min per mg of protein).[2] In the presence of BSA the specific activity of this enzyme was 450. The yeast fatty acid synthetase was partially purified through the first ammonium sulfate precipitation according to Lynen (7).

Enzyme Assays—For assay of the *C. diphtheriae* enzyme, incubation mixtures contained 30 μM TPNH, 30 μM DPNH, 2 μM FMN, 100 μM acetyl-CoA, 25 μM [2-^{14}C]malonyl-CoA (2 μCi per μmole), 0.5 M potassium phosphate buffer, pH 7.3, 6 mM dithiothreitol and 0.12 μg of enzyme in a total volume of 0.5 ml. The assay mixture was brought to 37°, enzyme was added, and samples were incubated for 20 min at 37°.

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[1] The abbreviations used are: MMP, methylmannose-containing polysaccharide; MGLP, refers to MGLP-II, the species of methylglucose-polysaccharide that contains 1 succinate residue; BSA, bovine serum albumin.

[2] H. W. Knoche, to be published.

For assay of the yeast fatty acid synthetase, reaction mixtures contained 60 μM TPNH, 2 μM FMN, 100 μM acetyl-CoA, 25 μM [2-^{14}C]malonyl-CoA (2 μCi per μmole), 6 mM DTT, 0.2 M potassium phosphate buffer, pH 6.5, and 2.7 μg of enzyme in a total volume of 0.5 ml. Before addition of enzyme, the solutions were warmed to 37° and then incubated at this temperature for 20 min.

Analysis of Products—Reactions were stopped by the addition of 0.15 ml of 50% KOH and saponified by heating to 100° for 30 min. After acidification with HCl to pH 1, fatty acids were extracted with three 5-ml portions of petroleum-ether, solvent was evaporated at 60°, and the residues were dissolved in 10 ml of 2,5-diphenyloxazole (PPO)-toluene (4 g per liter) for scintillation counting.

For fatty acid analysis the fatty acid samples were methylated according to the method of Schlenk and Gellermann (8) in the presence of carrier fatty acids (10 μg each of C_{14}, C_{16}, and C_{18}). The methyl esters were analyzed by gas-liquid chromatography on a 12-foot column of 10% diethylene glycol succinate with an F & M 400 instrument equipped with a hydrogen flame detector and variable stream splitter. The column temperature was 180°. Effluent was passed directly into a proportional counter (Nuclear Chicago). The values in Table I for the individual fatty acids represent percentages of total radioactivity in the sample.

Complex Formation between Palmityl-CoA and MGLP-II—Solutions (about 50 μl) containing approximately 25 nmoles each of [1-^{14}C]palmityl-CoA and MGLP-II in 0.2 M potassium phosphate buffer, pH 7.0, were chromatographed either separately or as a mixture on a column of Sephadex G-25 (6 mm × 35 cm). The eluting solvent was the same phosphate buffer. Fractions (0.5 ml) were collected and assayed for radioactivity (palmityl-CoA) or for sugar (MGLP-II) with α-naphthol reagent (9) (absorbance at 555 nm). The results are shown in Fig. 8.

Malonyltransacylase—Standard reaction mixtures contained, in a total volume of 0.5 ml, 6.0 μmoles of cysteine, 0.51 μmole of pantetheine, 0.01 μmole of [2-^{14}C]malonyl-CoA (20.6 μCi per μmole, 0.542 μmole per ml), and either 50 or 150 μmoles of potassium phosphate buffer, pH 6.5, for the fatty acid synthetases from yeast and *C. diphtheriae*, respectively. Reactions were initiated by addition of 0.15 μg of the *C. diphtheriae* enzyme or 2.1 μg of the yeast enzyme and were allowed to proceed for 10 min at 37°. Malonyl-pantetheine was isolated and quantitated as described by Lynen (10).

Acetyltransacylase—Standard reaction mixtures contained, in a total volume of 0.5 ml, 6.0 μmoles of cysteine, 0.51 μmole of pantetheine, 0.05 μmole of [1-^{14}C]acetyl-CoA (4 μCi per μmole, 2.5 μmole per ml), and either 50 or 150 μmoles of potassium phosphate buffer, pH 6.5, for the fatty acid synthetases from yeast and *C. diphtheriae*, respectively. Reactions were initiated by addition of 0.4 μg of the *C. diphtheriae* enzyme or 8.3 μg of the yeast enzyme and allowed to proceed for 10 min at 37°. Acetyl-pantetheine was isolated and quantitated as described by Lynen (11).

RESULTS

The fatty acid synthetase from yeast (7) and the recently described enzyme system from *C. diphtheriae* (6) are multienzyme complexes which produce palmityl-CoA and stearyl-CoA as the principal products.

The effects of BSA and polysaccharides on malonyl-CoA incorporation by these two systems are shown in Fig. 1. It should be noted that in the assay system for the yeast enzyme, BSA is routinely included (7). Optimal concentrations of BSA stimulate fatty acid synthetase activity of the *Corynebacterium* enzyme 1.4-fold (Fig. 1*A*) and of the yeast enzyme (Fig. 1*B*) 2-fold. Under the same assay conditions, MGLP and MMP cause activity increases of similar magnitude. MGLP is significantly more effective than either MMP or BSA. The effective concentration ranges for BSA and the polysaccharides are approximately the same but since the molecular weights of the polysaccharides (MMP, 2,000; MGLP-II, 3,500 (2, 4)) are only about 1/30 or 1/20 of that of BSA (69,000), the latter is more effective on a molar basis. Sucrose was without effect on fatty acid synthesis in either system.

The presence of BSA or polysaccharide in the synthetase assay mixtures not only influenced the over-all rate of the elongation process but also caused significant shifts in the composition of the fatty acid end products (Table I). In *C. diphtheriae* and in yeast, both agents raised the relative proportion of palmitate and lowered the percentage of stearate correspondingly. It is well known that the palmitate-stearate ratios are variable and are a function, *inter alia*, of the relative concentrations of acetyl-CoA and malonyl-CoA (12). Such effects are illustrated in Table I, Experiments 1 and 4. They show a shift toward increased proportions of the shorter acids (C_{14} and C_{16}) as the ratio of acetyl-CoA to malonyl-CoA is raised. The ability of BSA and MGLP to raise the proportion of palmitate is evident at both high and low acetyl-CoA to malonyl-CoA ratios. Therefore, these effects appear to be superimposed on the chain length changes which are due to variations in relative substrate concentrations. It is clear from the data in Table I that BSA and MGLP cause major shifts in the fatty acid distribution patterns and, moreover, that both the direction of these effects (toward shorter chain products) and their magnitude are the same for both of these agents. This suggests a similar mode of action.

Myristate is not produced in appreciable quantities by the *C. diphtheriae* synthetase under any of the conditions tested.

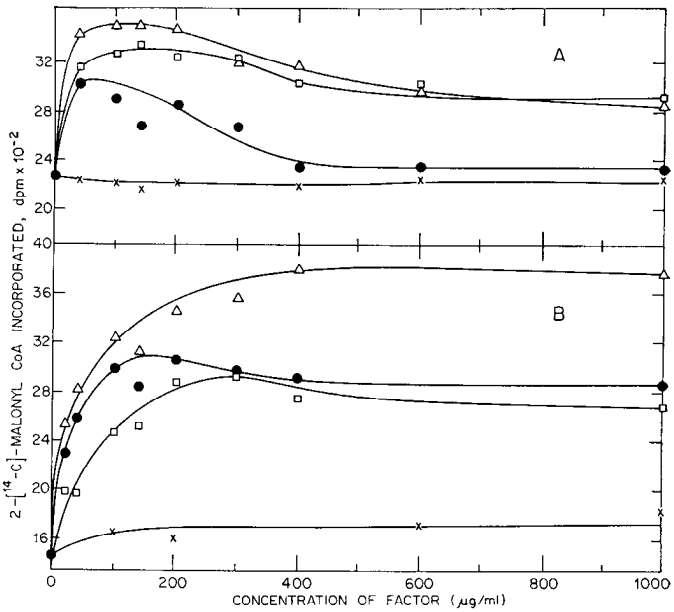

FIG. 1. Stimulation of fatty acid synthetases by various concentrations of BSA (●——●) and by two polysaccharides from *Mycobacterium phlei*. MGLP, △——△; MMP, □——□; sucrose, ×——×. The enzyme assays are described under "Experimental Procedure." *A*, enzyme from *Corynebacterium diphtheriae; B*, synthetase from yeast.

TABLE I

Effect of BSA and MGLP on products of fatty acid synthetases

Assay conditions were those described under "Experimental Procedure" except that in Experiments 1, 2, and 3 acetyl-CoA was 100 μM and [2-^{14}C]malonyl-CoA was 25 μM; and in Experiments 4, 5, and 6 acetyl-CoA was 40 μM and [2-^{14}C]malonyl-CoA was 60 μM.

Additions	*Corynebacterium diphtheriae* fatty acids			Yeast fatty acids			Acetyl-CoA: malonyl-CoA
	Myristic acid	Palmitic acid	Stearic acid	Myristic acid	Palmitic acid	Stearic acid	
	%			%			
1. None	None	85.7	14.2	8.3	45.6	46.1	4.0
2. BSA, 400 μg/ml	None	90.6	9.4	13.8	62.0	24.2	4.0
3. MGLP, 400 μg/ml	None	92.6	7.4	14.2	64.5	24.1	4.0
4. None	None	61.7	38.3	4.4	25.7	69.9	0.67
5. BSA, 400 μg/ml	None	73.7	26.3	6.8	41.6	51.6	0.67
6. MGLP, 400 μg/ml	None	73.8	26.1	5.7	45.7	48.6	0.67

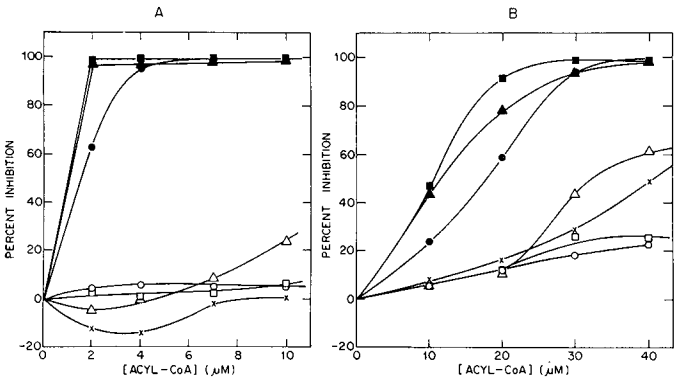

FIG. 2. Inhibitory effect of various acyl-CoA derivatives on *de novo* fatty acid synthesis catalyzed by the enzyme from *Corynebacterium diphtheriae* in the absence (*A*) and presence (*B*) of MGLP (100 μg per ml). C_8, ○——○; C_{10}, □——□; C_{12}, △——△; C_{14}, ×——×; C_{16}, ●——●; C_{18}, ■——■; C_{20}, ▲——▲. The assay conditions were those described under "Experimental Procedure."

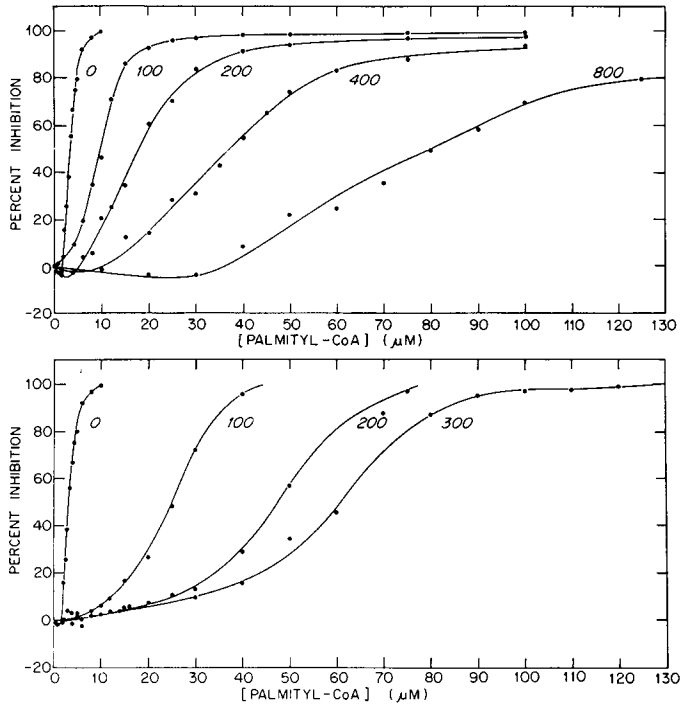

FIG. 3. Protection of *Corynebacterium diphtheriae* fatty acid synthetase against palmityl-CoA inhibition by various amounts of BSA (*upper panel*) and by MGLP (*lower panel*). Numbers indicate concentration of BSA or MGLP in micrograms per ml. The assay conditions were those described under "Experimental Procedure."

The amounts of myristate synthesized by the yeast enzyme, while small, are also elevated significantly in the presence of BSA and MGLP.

Inhibition of Fatty Acid Synthetases by Acyl-CoA Derivatives—Inhibition of fatty acid synthesis by palmityl-CoA is a well known general phenomenon which has been analyzed in detail for the yeast enzyme by Lust and Lynen (13). The synthetase from *Corynebacterium* is similarly sensitive to palmityl-CoA and other long chain acyl-CoA derivatives (Fig. 2*A*). Less than 4 μM palmityl- or stearyl-CoA completely inhibit both synthetases. On the other hand, the *Corynebacterium* system is resistant to myristyl-CoA up to 10 μM, whereas the yeast synthetase is 50% inhibited by this compound at 3 μM (13). In this context it is worth mentioning, as noted before, that under normal assay conditions the *Corynebacterium* synthetase, in contrast to the yeast enzyme, produces essentially no myristate.

BSA effectively protects the yeast fatty acid synthetase against palmityl-CoA inactivation, the degree of relief depending on the relative concentrations of BSA and palmityl-CoA (13). We have confirmed these results and have also noted that even when the BSA concentrations are high (0.5 mg per ml) and those of palmityl-CoA low (5 μM), reversal is incomplete (60 to 70%). In the *C. diphtheriae* system, BSA also relieves palmityl-CoA inhibition (Fig. 3), except that in this case protection is complete even at 30 μM palmityl-CoA provided sufficient BSA is added.

MGLP duplicates the protective effects of BSA both with the synthetase from *Corynebacterium* (Figs. 2*B* and 3) and with that from yeast (Fig. 4). For example, yeast enzyme completely inhibited by 10 μM palmityl-CoA retains 70% of its normal activity in the presence of 100 μg of MGLP or 200 μg of BSA. Similarly, polysaccharide protects the *Corynebacterium* enzyme against palmityl-CoA inhibition and does so at least twice as effectively on a weight basis as BSA.

Another way of assessing the relative protection afforded by BSA and MGLP is to calculate the concentrations of palmityl-CoA required for 50% inhibition of fatty acid synthesis in the presence and absence of these agents. A plot of the data for the enzyme from *Corynebacterium* shows, for example, that in the presence of 200 μg of MGLP, 25 times as much palmityl-CoA is required for 50% inhibition as in its absence (Fig. 5). To achieve the same protection more than twice as much BSA must be included.

Inhibition of the two fatty acid synthetases as a function of palmityl-CoA concentration both in the presence and in the

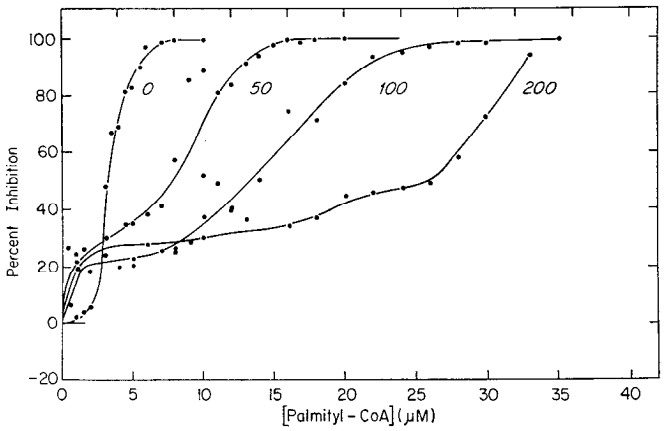

FIG. 4. Protection of yeast fatty acid synthetase against palmityl-CoA inhibition by MGLP. Numbers indicate concentrations of polysaccharide in micrograms per ml. The assay conditions were those described under "Experimental Procedure."

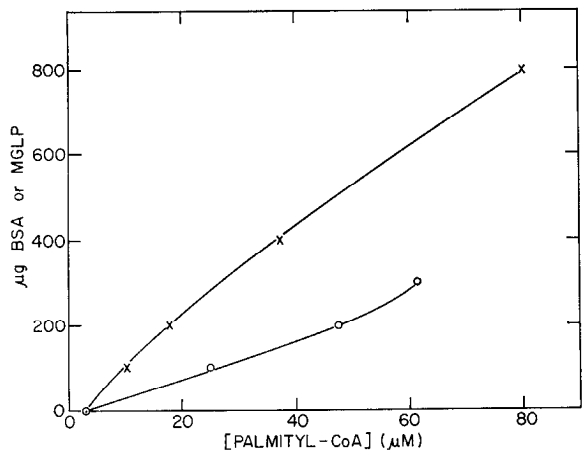

FIG. 5. Concentrations of palmityl-CoA required for 50% inhibition of *Corynebacterium diphtheriae* fatty acid synthetase in the presence of various amounts of BSA (×——×) or MGLP (○——○). The data were recalculated from those in Fig. 3.

absence of BSA or MGLP shows sigmoidal relationships (Figs. 2*B*, 3, and 4). This may be the resultant of two types of inhibition, competitive and noncompetitive.

Nature of Palmityl-CoA Inhibition—When either of the two synthetases was first incubated for 20 min with palmityl-CoA (5 μM) alone, and BSA or MGLP was added at zero time along with substrates and cofactors, enzyme inhibition persisted, whereas a preliminary incubation of enzyme with palmityl-CoA and BSA or MGLP prior to substrate addition left the synthetases largely intact (Table II). These results indicate irreversible inhibition or inactivation by palmityl-CoA, at least at the concentrations used. Whether palmityl-CoA inhibition can be partly or fully reversed at lower concentrations (<5 μM) has not been tested. For the yeast fatty acid synthetase palmityl-CoA inhibition is competitive with malonyl-CoA at 0.5 μM and 2.5 μM palmityl-CoA (13).

Effect of Palmityl-CoA on Partial Reactions of Fatty Acid Synthesis—Acetyl-CoA transacylation and malonyl-CoA transacylation, two of the partial reactions catalyzed by fatty acid synthetase systems, show greatly reduced activity in the presence of palmityl-CoA. These two enzyme activities were assayed by following the transfer of labeled acetyl or malonyl groups from their CoA derivatives to pantetheine (10, 11). The malonyl transacylases from yeast and *C. diphtheriae* are both 50% inhibited by about 10 μM palmityl-CoA (Fig. 6) which is not significantly different from the palmityl-CoA concentration that affords 50% inhibition of over-all fatty acid synthesis. The two acetyl transacylases are considerably more resistant. They require 70 μM (*C. diphtheriae*) and 20 μM (yeast) palmityl-CoA for 50% inhibition (Fig. 7). Malonyl transacylase is, therefore, the more likely step—but not necessarily the only one—which is responsible for the palmityl-CoA sensitivity of over-all fatty acid synthesis.[3]

BSA and MGLP effectively relieve the palmityl-CoA inhibition of the transacylases, *i.e.* they protect individual reactions as well

TABLE II

Irreversible inhibition of fatty acid synthetases by palmityl-CoA

During preincubation solutions contained 6 mM dithiothreitol, potassium phosphate buffer, 0.5 M, pH 7.2, for *Corynebacterium diphtheriae*, and 0.2 M, pH 6.5 for yeast, palmityl-CoA as indicated, and 1.4 μg of enzyme protein in 0.3 ml. After 20 min at 37°, the mixtures were diluted 10-fold and assayed under the conditions described under "Experimental Procedure." In Experiment A, 100 mg of BSA and palmityl-CoA (0.5 μM) was added at zero time along with substrates.

Experiment	Preincubation at 37° for 20 min in presence of			Inhibition compared to control (A)	
	Palmityl-CoA	BSA	MGLP	*C. diphtheriae* synthetase	Yeast synthetase
	μM	μg/ml	μg/ml	%	
A	0	0	0	0	0
B	5	0	0	76	52
C	5	200	0	34	33
D	5	0	100	22	15
E[a]	5	200	0	88	56

[a] Same as Experiment B except that after the preliminary 20 min incubation, 200 μg of BSA were added and the sample was kept at 0° for 20 min before enzyme assay.

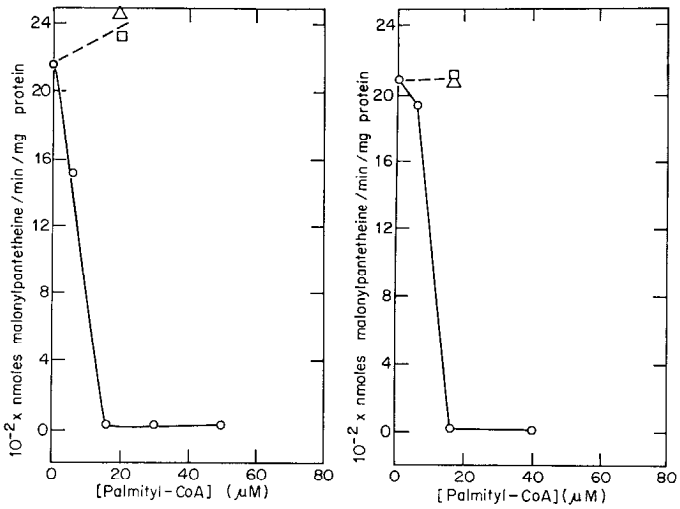

FIG. 6. Inhibition of malonyltransacylase activity by palmityl-CoA. *Left*, *Corynebacterium diphtheriae* synthetase; *right*, yeast synthetase. Additions to the standard incubation are: △- - -△, 180 μg of MGLP-II; □- - -□, 200 μg of BSA.

[3] Taketa and Pogell (14), describing the inhibition of a variety of enzymes by palmityl-CoA, cite a private communication of P. R. Vagelos stating that in adipose tissue all of the individual enzyme activities of fatty acid synthesis are sensitive to palmityl-CoA.

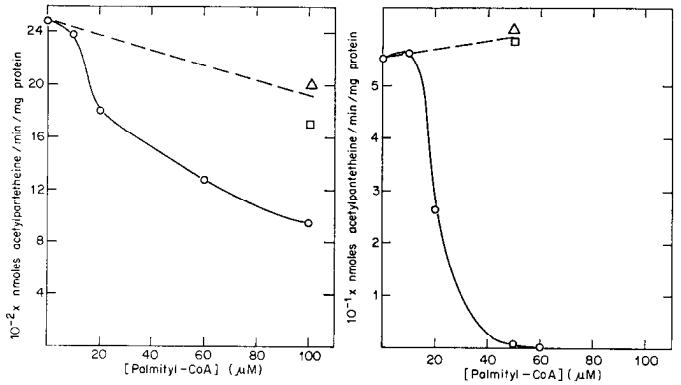

FIG. 7. Inhibition of acetyltransacylase activity by palmityl-CoA. *Left, Corynebacterium diphtheriae* synthetase; *right*, yeast synthetase. Additions to the standard incubation are: △- - -△, 180 μg of MGLP-II; □- - -□, 200 μg of BSA.

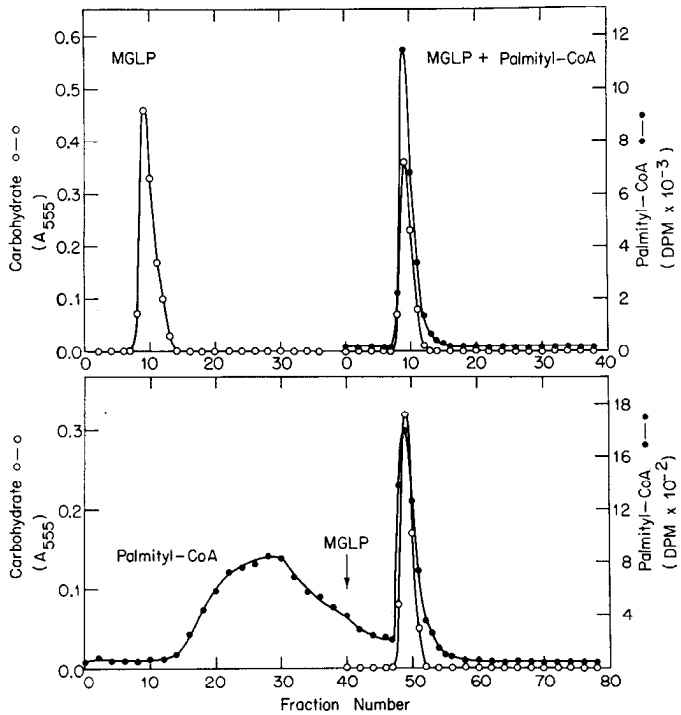

FIG. 8. Chromatography of MGLP and palmityl-CoA on a column of Sephadex G-25. The *upper panel* shows the elution pattern of MGLP alone and of a mixture of palmityl-CoA and MGLP. In the experiment depicted in the *lower panel*, palmityl-CoA was applied to the column and 40 fractions were collected. At this point a solution of MGLP was added to the column and elution continued as indicated. Fractions were assayed for palmityl-CoA by scintillation counting and for carbohydrate (MGLP) by measuring absorbance at 555 nm after addition of α-naphthol reagent.

as over-all synthesis (Figs. 6 and 7). However, whereas total synthesis is significantly stimulated above base-line levels by these agents in the absence of palmityl-CoA (Fig. 1), this is not the case for acetyl or malonyl transacylation.

Complex Formation between Palmityl-CoA and MGLP—Samples of palmityl-CoA and MGLP were chromatographed on Sephadex G-25 separately and as a mixture. The elution patterns are shown in Fig. 8. Palmityl-CoA alone emerges broadly with an indistinct peak at Fraction No. 30, but in the presence of MGLP, palmityl-CoA is eluted as a sharp peak which completely coincides with the fractions containing MGLP (Fraction No. 10). The position of the MGLP peak is not shifted by the presence of palmityl-CoA. In preliminary experiments the composition of this peak fraction has been found to correspond to a ratio of 1 mole of palmityl-CoA per mole of MGLP.[4] Thus, these two components interact to form a stoichiometric complex which is stable, at least under the conditions employed.

DISCUSSION

A number of enzymes both related and unrelated to lipid metabolism are inhibited by the CoA derivatives of long chain fatty acids in the micromolar range and the point has been made that these effects may be those of a potent nonspecific detergent (14). BSA protects many of the affected enzymes, either partially or completely, presumably by complexing acyl-CoA and thereby abolishing its detergent properties. The capacity of BSA for tight binding of long chain fatty acids is well known (15), and it may be assumed that this hydrophobic binding occurs also with the CoA derivatives of fatty acids.

As for the inhibition of a multienzyme fatty acid complex by long chain acyl CoA derivatives and the relief of this inhibition by BSA, our findings confirm those obtained by Lust and Lynen with the fatty acid synthetase of yeast (13). A very similar but not identical pattern of inhibition and relief is also observed for the fatty acid synthetase from *C. diphtheriae*. One of the major differences between the two systems is the sensitivity of the yeast enzyme to myristyl-CoA (50% inhibition at 5 μM), whereas the bacterial synthetase is not affected even at 10 μM myristyl-CoA. Secondly, BSA provides complete protection against palmityl-CoA in the *C. diphtheriae* system, whereas in yeast this inhibition is only partially reversed even at high BSA concentrations.

We report here the novel finding that mycobacterial polysaccharides have effects on fatty acid synthesis in yeast and in *C. diphtheriae* identical to those shown by BSA. Like BSA, MGLP stimulates total fatty acid synthesis from acetyl-CoA and malonyl-CoA and it relieves the palmityl-CoA inhibition of this process. On a weight basis, but not on a molar basis, MGLP is more effective. BSA can be assumed to protect by binding and, therefore, sequestering palmityl-CoA. We have now shown that palmityl-CoA can also be complexed by a polysaccharide (Fig. 8) and we assume that it is thereby rendered ineffective as an enzyme inhibitor. MGLP and MMP, which were first shown to stimulate fatty acid synthesis in *M. phlei* (1), characteristically have a high content of methoxy groups. Being relatively nonpolar polysaccharides, they probably interact with palmityl-CoA by hydrophobic binding.

The effects of BSA and MGLP on palmityl-CoA-inhibited fatty acid synthesis can be readily explained in terms of removal or lowering of the effective concentration of an agent which inactivates fatty acid synthesis enzymes whatever the specific mechanism of inhibition. It is not immediately apparent, however, why in some cases BSA and MGLP enhance fatty acid synthesis under normal assay conditions, *i.e.* in the absence of added palmityl-CoA. With the *C. diphtheriae* system these effects are relatively slight (30 to 50%); they are more pronounced (2- to 2.5-fold) with the yeast synthetase, and they range from 10- to 50-fold for the *M. phlei* enzyme system (1). Moreover, when BSA and MGLP stimulate fatty acid synthesis (malonyl-CoA incorporation) the fatty acid pattern also changes (Table I), and this may provide a clue to the mode of action of these agents. In the stimulated systems there is a shift toward

[4] Y. Machida, unpublished experiments.

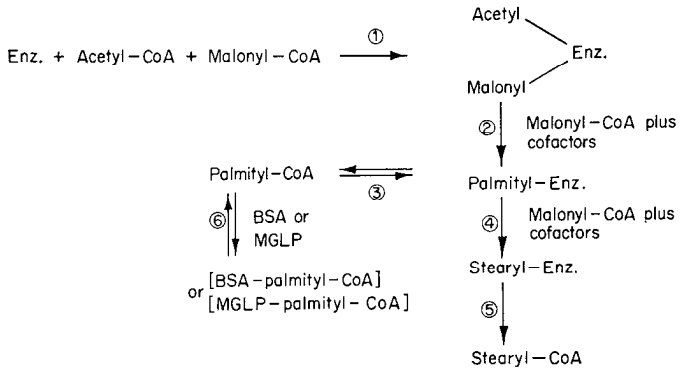

FIG. 9. Proposed mode of action of BSA and MGLP in counteracting palmityl-CoA inhibition of fatty acid synthesis.

a higher proportion of palmitate at the expense of stearate in both yeast and *C. diphtheriae*, and in yeast toward increased amounts of myristate as well. BSA and MGLP, therefore, favor earlier chain termination. A simplified speculative scheme accounting for these results is shown in Fig. 9. It seems reasonable to assume, on the basis of the evidence of Sumper *et al.* (12), that chain termination is a function of two competing processes, these being transacylation of a long acyl chain from enzyme to CoA (*Reaction 3*) and further elongation by condensing enzyme (*Reaction 4*). If palmityl-enzyme is the substrate for both Reactions 3 and 4, their relative rates will determine the C_{16} to C_{18} ratio. Furthermore if Reaction 3 is reversible, then this ratio will be raised by any process that removes or lowers the concentrations of the product of Reaction 3. BSA and MGLP, by complexing palmityl-CoA will, therefore, accelerate Reaction 3, at the expense of Reaction 4, causing palmitate to accumulate. The differences in the response of the yeast enzyme and the enzyme from *C. diphtheriae* to BSA and MGLP and the differences in the C_{16}:C_{18} ratios can be explained on the same basis. In yeast, Reaction 3 is slower relative to Reaction 4 than it is in *C. diphtheriae*, and hence removal of palmityl-CoA will have a greater effect both on over-all rate and on the C_{16}:C_{18} ratio. A necessary assumption in the above argument is that BSA and MGLP will interact strongly with CoA derivatives only when the acyl chain is C_{16} or longer. It will, therefore, be of interest to ascertain the binding constants of these molecules for acyl-CoA as a function of chain length.

The arguments given above lead to the postulate that BSA and MGLP influence fatty acid synthesis by sequestering not only externally added palmityl-CoA but also palmityl-CoA that is internally generated. According to this view, the complexing agents prevent palmityl-CoA from reaching inhibitory levels, *i.e.* they counteract negative feedback inhibition by the major or principal end product of the synthetase process. It would then follow that end product inhibition is indeed an important mechanism for regulating fatty acid synthesis, a conclusion reached tentatively by Lust and Lynen on the basis of their studies on the inhibition of the yeast fatty acid synthetase by added acyl-CoA derivatives (13). Until now, acetyl-CoA carboxylase has been thought to be the principal site for the regulation of fatty acid synthesis, but evidence that the carboxylase is the control enzyme exists only for animal systems (16, 17).

The ability of BSA and MGLP to influeuce not only the rate of synthesis but also fatty acid patterns in favor of products of shorter chain length may be an important mechanism for determining the composition of cellular lipids. This mechanism could operate independently of the regulation of chain length which is achieved by variations in substrate concentration (acetyl-CoA-malonyl-CoA), or it might be superimposed on it. Depending on the chain length specificity of the complexing agent, wide variations in the fatty acid pattern produced by a given synthetase are conceivable.

Pertinent to this discussion is the observation by Barnes and Wakil (18) that the low molecular weight palmityl thioesterase from *Escherichia coli*, when added to the fatty acid synthetase system from the same organism, caused a significant increase in the production of palmitate, relative to stearate. These authors suggest that thioesterase, by hydrolyzing palmityl-acyl carrier protein, will prevent further elongation and in this manner influence the length of the fatty acid chain. They considered that interpretation tentative since in control experiments the production of stearate was variable. Nevertheless, these experiments and earlier observations by Porter and Long (19) support the contention that removal of palmityl-CoA, whether by sequestration or by hydrolysis, may be an important control mechanism in fatty acid synthesis. Related aspects are discussed in accompanying papers (20, 21).

Finally, it should be pointed out that the phenomena described here are not necessarily significant physiologically. BSA and MGLP are foreign to yeast and *C. diphtheriae* and there is no evidence so far for the presence of such complexing agents native to these microorganisms. This qualification does not apply to *M. phlei*, an organism in which the fatty acid synthetase activity is profoundly modulated by polysaccharides from the same source (1). The properties of the mycobacterial system and its regulation are described separately (20, 21).

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