CONTROL OF THE SYNTHESIS OF LONG-CHAIN FATTY ACIDS AND TRIACETIC ACID IN E. COLI*

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Received May 9, 1966

The soluble fatty acid synthetase from E. coli produces both saturated and unsaturated long-chain fatty acids from malonyl CoA and acetyl CoA in a process dependent on TPNH and ACP (Lennarz, Light and Bloch, 1962; Goldman and Vagelos, 1962). We have now observed that under certain conditions the synthesis of long-chain acids is suppressed and almost entirely replaced by the production of triacetic acid (TAA, 3,5-diketohexanoic acid). This communication describes the isolation and characterization of TAA as its lactone (TAL), and details the conditions under which it is formed at the expense of long-chain acids.

Methods and Results

The crude fatty acid synthetase was prepared by methods previously described (Lennarz, Light and Bloch, 1962), either from commercial E. coli B (Grain Processing Co., Muscatine, Iowa) or from cells grown in the laboratory. Incubation of the synthetase with malonyl CoA, acetyl CoA, TPNH and ACP,[1] and extraction of the hydrolyzed reaction mixture with either pentane or ether gave a product which was predominantly (80-90%) nonpolar in character as judged by column chromatography on silicic acid. CH_2Cl_2 was used

*Supported by grants-in-aid from the National Science Foundation, the National Institutes of Health, the Life Insurance Medical Research Fund, and Eugene Higgins Trust Fund of Harvard University

[1] Acyl Carrier Protein (Majerus et al., 1964)

to elute the nonpolar material and 10% MeOH in CH_2Cl_2, the polar material. The nonpolar fraction consisted of both saturated and unsaturated fatty acids, cis-vaccenic acid being the major component. These fatty acids were largely in the bound form as judged by the fact that they could be released and extracted only after strong alkaline hydrolysis.

When either mercaptoethanol, glutathione or dithiothreitol was added to the incubation mixture, a striking change in the nature of the product was observed, while total incorporation was not affected significantly (Table I). Product could now be extracted into ether without prior hydrolysis and was almost exclusively polar in nature. No long-chain fatty acids were found under these conditions nor was pentane effective in removing the product from

Table I

Effect of Sulfhydryl Compounds on Product Formation

Additions	% Polar	% Non-Polar	Total Incorporations dpm
- - - -	10	90	24,300
Glutathione (5×10^{-3} M)	98	2	26,600
Mercaptoethanol (2×10^{-2} M)	95	5	22,800
Dithiothreitol (1×10^{-2} M)	97	3	21,900

Reaction mixtures contained: TEA pH 7.5 (50 μmoles), TPNH (1 μmole), ACP (0.020 μmole), malonyl CoA (0.100 μmole,) ^{14}C-acetyl CoA (0.013 μmole, 120,000 dpm) and enzyme (1.5 mg), with additions as above. Final volume 0.5 ml. Incubation at 37° for 1 hour was followed by hydrolysis with 0.1 ml 50% KOH (w/v) at 100° for 20 min. After acidification, product was extracted into ether and separated into polar and nonpolar fractions by chromatography on silicic acid.

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the reaction mixture. Paper chromatography of the polar product in isobutyric acid - NH_3 - H_2O(66:3:30) showed the presence of two radioactive peaks in the approximate ratio of 1:3. These were separated and isolated by column chromatography on Hy-flo Supercel with $CHCl_3$ (equilibrated with 0.5 M H_2SO_4) as eluant. The major component had an identical R_f value to authentic TAL on paper chromatography in the isobutyric - NH_3 - H_2O system and on TLC with

10% MeOH in CH_2Cl_2. The same material when treated with ethereal diazomethane at room temperature for one hour and then injected onto a 6% DEGS column operated at 180°, gave two radioactive peaks, which corresponded exactly, both in retention time and relative radioactivity, to the mass peaks of the two enol methyl ethers of TAL (Herbst et al., 1959). On a SE-30 column at 60° a single radioactive peak was obtained, again corresponding to the unresolved enol methyl ethers of TAL. Characterization of the polar product was completed by co-crystallization from acetonitrile with carrier TAL to constant specific activity; 1st recrystallization 423 dpm/mg; 2nd recrystallization 401 dpm/mg, 3rd recrystallization 428 dpm/mg.

Identification of the major polar component as the lactone of triacetic acid did not establish whether the product of the enzymatic reaction was the the acid or the lactone, since lactonizing conditions were used in isolation and characterization. The unidentified minor component had an R_f value on paper closely similar to that of TAL, which suggests that it might be the acid[2]. If this is the case, it seems probable that the acid is the direct product of the enzymatic reaction, since TAL, one formed, is extremely resistant to hydrolysis.

Dependence of TAL formation on the various components of the incubation mixture is shown in Table II. With ^{14}C-acetyl CoA as source of radioactivity, the dependence on both malonyl CoA and ACP is nearly absolute. In the presence of glutathione or mercaptoethanol, the omission of TPNH is without effect.

When TPNH was replaced by a TPNH generating system (TPN, glucose-6-phosphate, glucose-6-phosphate dehydrogenase), the addition of glutathione or mercaptoethanol to the incubation mixture was no longer able to divert synthesis to TAL. This suggested that one of the components of the generating system counteracted the effect of added sulfhydryl compounds. This component was found to be glucose-6-phosphate, which at sufficiently high concentration allowed the formation of the normal long-chain fatty acids even in the presence of mercaptans. Figure I shows the effect of increasing levels of G-6-P in a TAL-synthesizing system containing malonyl CoA, acetyl CoA, ACP, TPNH, and GSH. G-6-P appears to stimulate total product formation by super-

[2] Dr. R. Light, personal communication

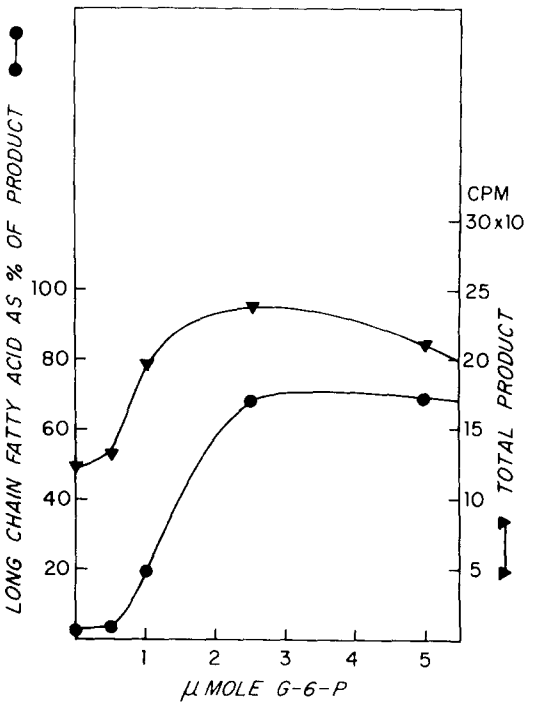

Figure 1. Effect of G-6-P on long-chain fatty acid and TAL synthesis. Conditions were the same as in the legend to Table I, except that glutathione (5×10^{-3} M) was added.

Table II

Dependence of TAL Synthesis of Components of Assay System

	dpm	mμmoles TAL*
Complete System	23,400	21.6
-malonyl CoA	394	0.4
-ACP	2,050	1.9
-TPNH	20,000	18.5
-TPNH, -GSH	11,300	10.5
-enzyme	225	0.2

*Based on the assumption that only one molecule of acetate is incorporated into TAL.

Conditions were the same as in the legend to Table I, except that glutathione (5×10^{-3} M) was added.

imposing long-chain fatty acid synthesis on a basal level of TAL formation. This property is unique to G-6-P; addition of glucose-1-phosphate, fructose 1, 6-diphosphate, 6-phosphogluconic acid, DL-α-glycerophosphate, 3', 5'-cyclic AMP or inorganic phosphate (all at a concentration of 10^{-2}M) was without effect.

Discussion

The biosynthesis of long-chain fatty acids from acetyl CoA and malonyl CoA is a cyclic process, involving in each cycle a condensation, two reductions and a dehydration (Lynen et al., 1961), (Fig. II). In the case of E. coli, the individual enzymes catalyzing the steps in the first cycle have been isolated and purified (Alberts et al., 1964). It is clear that acetoacetyl-ACP can occupy a pivotal position in the sequence; reduction of the keto group gives β-hydroxybutyryl-ACP and then leads to long-chain acids, whereas direct condensation of acetoacetyl-ACP with another malonyl unit could lead to TAA. This hypothesis is supported by the finding of Brodie et al. (1964) that a purified fatty acid synthetase complex from pigeon liver produces small amounts of a product having the paper-chromatographic properties of TAA when TPNH is omitted from the system. Our data show that in E. coli various mercaptans will cause production of TAL even in the presence of TPNH, and that under these conditions, TAL appears to be the sole product of the reaction. The ease with which synthesis may be directed from TAL into long-chain fatty acids or vice versa, merely by the addition or omission of glucose-6-phosphate, indicates that one or more of the enzymes acting on acetoacetyl-ACP may be the site of control. A likely possibility is that β-ketoacyl ACP reductase, the

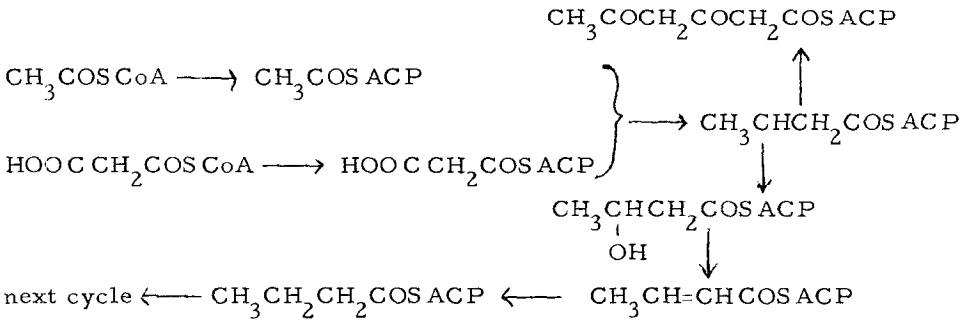

Figure 2

enzyme responsible for the reduction of acetoacetyl-ACP to β-hydroxybutyryl-ACP (Alberts et al., 1964) is blocked by sulfhydryl compounds and that this inhibition is released by G-6-P. Alternatively the enzyme responsible for adding a malonyl unit to acetoacetyl-ACP (which may or may not be the same as β-ketoacyl ACP synthetase described by Alberts et al. 1965) could be activated by sulfhydryl compounds or inhibited by G-6-P. These and other possibilities are currently under investigation.

References

1. Alberts, A. Majerus, P., Talamo, B., and Vagelos, P., Biochemistry, 3, 1563 (1964).
2. Alberts, A., Majerus, P., and Vagelos, P., Biochemistry, 4, 2256 (1965).
3. Brodie, J. D., Wasson, G., and Porter, J. W., J. Biol. Chem., 239, 1346 (1964).
4. Goldman, P., and Vagelos, P. R., Biochem. Biophys. Res. Com., 7, 414 (1962).
5. Herbst, D., Mors, W., Gottlieb, O., and Djerrassi, C., J. Am. Chem. Soc., 81, 2327 (1959).
6. Lennarz, W., Light, R., and Bloch, K., Proc. Nat'l Acad. Sci. U. S., 48, 840 (1962).
7. Lynen, F., Federation Proc., 20, 941 (1961).
8. Majerus, P., Alberts, A., and Vagelos, P., Proc. Nat'l Acad. Sci. U. S., 51, 1231 (1964).