THE EFFECT OF GLUTATHIONE AND GLUCOSE-6-PHOSPHATE ON FATTY ACID SYNTHESIS IN E. COLI*

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The soluble fatty acid synthetase from E. coli catalyses the formation of long-chain saturated and unsaturated acids from malonyl-CoA and acetyl-CoA in a reaction dependent on TPNH and ACP[1] (Lennarz, Light and Bloch, 1962; Goldman and Vagelos, 1962). In the course of an investigation of the detailed products of this reaction, it was discovered that in the presence of glutathione the synthesis of long-chain acids was almost completely suppressed and that a compound identified as the lactone of triacetic acid (TAL) accumulated instead. Addition of glucose-6-phosphate (G-6-P) to the incubation mixture reversed the effect of glutathione and restored the synthesis of long-chain acids (Brock and Bloch, 1966). This communication presents evidence that glutathione and G-6-P exert their opposing effects by acting as substrates for enzymes which accompany the synthetase complex and which consume and produce TPNH.

Methods

Preparation of the fatty acid synthetase has been described (Lennarz, Light and Bloch, 1962). The composition of the synthetase reaction mixture is shown in the legend to the Table. Products of the reaction were separated

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[1] Acyl carrier protein (Majerus et al., 1964).

into polar and non-polar fractions by chromatography on Unisil silicic acid, using solvents already described (Brock and Bloch, 1966). The non-polar fraction consisted of long-chain saturated and unsaturated fatty acids; the polar fraction was made up largely of TAL, but with an unidentified minor component and possibly also small amounts of hydroxy fatty acids. Since only gross changes in the polar/non-polar ratio are discussed, the formation of a predominantly polar product is considered a reasonable measure of TAL synthesis.

For experiments in which the oxidation of TPNH was followed spectrophotometrically, the synthetase preparation was adsorbed onto calcium phosphate gel from 0.01 M KPO_4, pH 6.5 (1 mg gel per 1.19 mg protein) and then eluted by 0.1 M KPO_4, pH 9.0. Enzyme thus treated was largely free of a non-specific TPNH oxidase activity.

Results

A clue to the mode of action of GSH was the finding that both oxidized and reduced forms of the mercaptan were effective in suppressing fatty acid synthesis and stimulating the formation of TAL. The relative ease with which glutathione is oxidized on exposure to air pointed to the oxidized form as the active molecule. Indeed, if glutathione was pre-reduced with sodium borohydride immediately before addition to the incubation mixture, it lost the ability to suppress fatty acid synthesis (Table). The presence of reduced glutathione does, however, have a stimulatory effect on total incorporation, whatever product is formed.[2]

The addition of oxidized glutathione to the synthetase reaction has the same effect on the nature of the products as the removal of TPNH. This suggested the presence in the synthetase preparations of a TPNH-dependent glutathione reductase activity. In Fig. 1 the oxidation of TPNH is followed spectrophotometrically in a basic system consisting of the synthetase preparation and reduced pyridine nucleotide alone (control curve).

[2] The finding that mercaptoethanol and dithiothreitol were able to duplicate the action of glutathione on the products of the synthetase assay (Brock and Bloch, 1966) could not be repeated in later experiments, where TPNH, instead of being added from a stock solution, was dissolved just before addition to the incubation mixture. This observation emphasizes the need to use freshly prepared TPNH solutions when the reduced pyridine nucleotide limits the rate of the reaction under study.

Table

Dependence of Polar/Non-polar Product Ratio

on Components of Assay System

	Polar %	Non-polar %	Total incorporation dpm
Complete	9	91	10,890
+GSH ($5x10^{-3}$M)	98	2	16,988
+GSSG ($5x10^{-3}$M)	99	1	9,680
+pre-reduced GSH ($5x10^{-3}$M)	6	94	21,428
-TPNH	99	1	11,300

Complete system contained: TEA, pH 7.5 (50 μmoles), malonyl-CoA (0.100 μmole), ACP (0.020 umole), TPNH (1 μmole), 1-^{14}C-acetyl-CoA (0.021 μmole, 270,000 dpm) and enzyme (1.5 mg) in a final volume of 0.5 ml. Incubation at 37° for 30 min. was followed by the 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 non-polar fractions as described.

Addition of pre-reduced glutathione has little effect on the oxidation rate, but oxidized glutathione causes a rapid burst of TPNH consumption, which then gives way to a slower rate. The rapid TPNH oxidation is maintained when both oxidized and reduced forms of glutathione are added, or if an "aged" solution of reduced glutathione is used without pre-treatment. These results indicate that TPNH oxidation is dependent on oxidized glutathione as a substrate, but that it is stimulated when the reduced form is also present. Asnis (1955) has shown that E. coli glutathione reductase is a sulfhydryl enzyme, and it seems probable that reduced glutathione serves to maintain the reductase in an active state. In support of this, oxidized glutathione and mercaptoethanol were found to support an oxidation rate as rapid as that shown by oxidized and reduced glutathione.

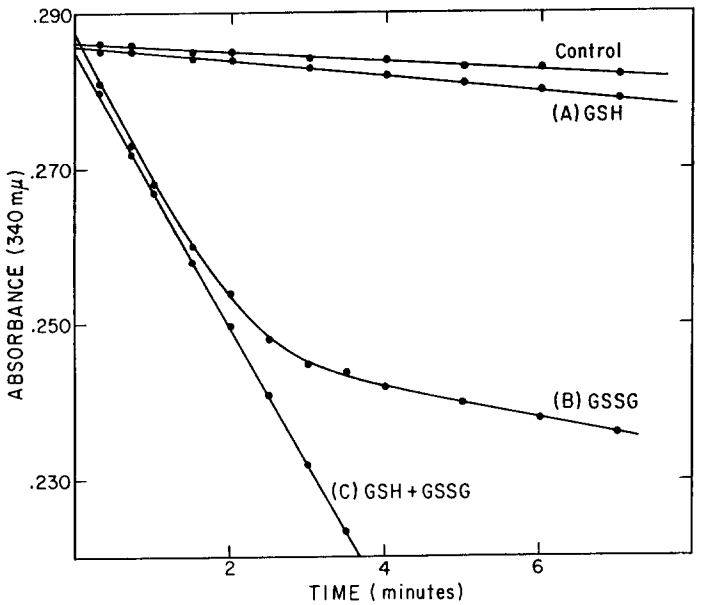

Figure 1. Effect of oxidized and reduced glutathione on TPNH oxidation Cuvettes contained in a total volume of 1.0 ml: 10 μmoles potassium phosphate buffer, pH 7.0, and 0.050 μmoles TPNH: in (A) 1 μmole GSH prereduced by sodium borohydride, in (B) 1 μmole GSSG, in (C) 1 μmole prereduced GSH + 1 μmole GSSG. Reactions were started by the addition of 0.04 mg calcium phosphate purified synthetase preparation and the decrease in 340 mμ absorbance followed at 30°.

In the synthetase reaction a high level of G-6-P was able to overcome the effect of added glutathione and allowed long-chain acid synthesis to proceed (Brock and Bloch, 1966). The influence of G-6-P on the glutathione-dependent oxidation of TPNH is shown in Fig. 2. The initial rate of oxidation is the same in the presence or absence of G-6-P; however, with G-6-P, TPNH consumption is slowed before the base-line is reached and is then slightly reversed. It appears that two competing reactions are taking place; a glutathione-dependent TPNH oxidation and the regeneration of TPNH through a TPN-linked G-6-P dehydrogenase. The presence of the latter enzyme in the synthetase preparation is demonstrated in Fig. 3. The net effect of the two competing reactions is that when G-6-P is added to the synthetase

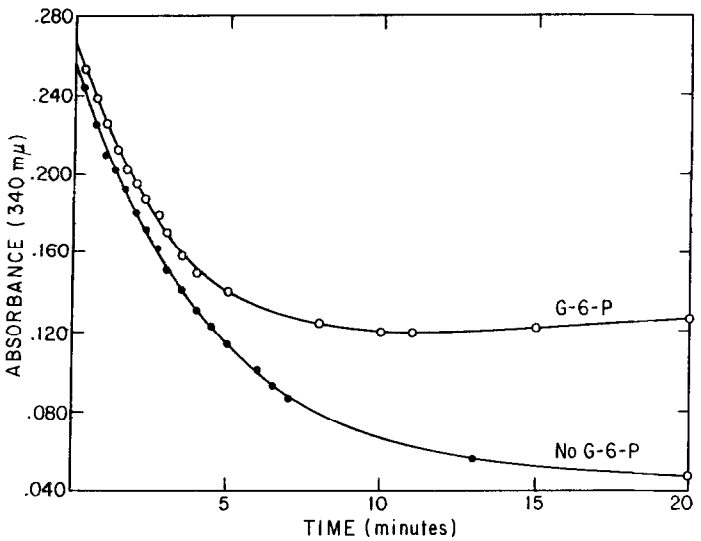

Figure 2. Effect of G-6-P on glutathione-dependent TPNH oxidation. Cuvettes contained in a total volume of 1.0 ml: 10 μmoles potassium phosphate buffer, pH 7.0, 0.050 μmole TPNH, 2.5 μmoles GSH (not pre-reduced) and where indicated, 5 μmoles G-6-P. Reactions were started by the addition of 0.1 mg calcium phosphate treated synthetase preparation.

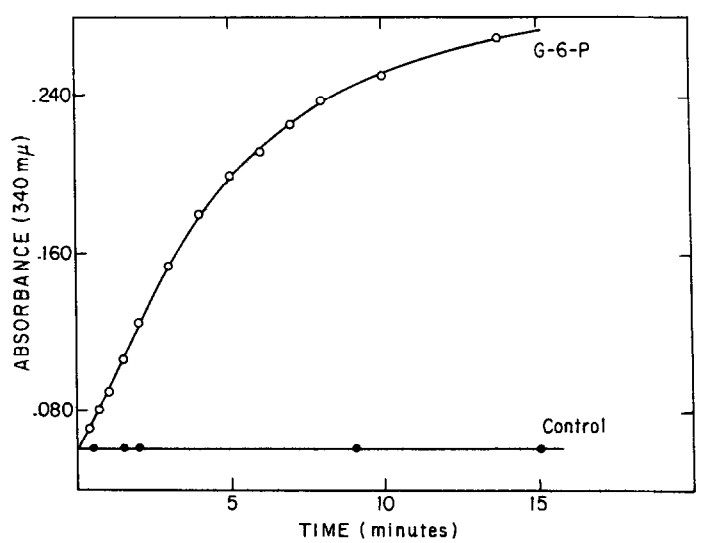

Figure 3. Effect of G-6-P on TPN reduction. Cuvettes contained in a total volume of 1.0 ml: 10 μmoles potassium phosphate buffer, pH 7.0, 0.060 umoles TPN, and where indicated 5 μmoles G-6-P. Reactions were started by the addition of 0.1 mg calcium phosphate treated synthetase preparation.

reaction mixture, the TPNH concentration is maintained at a level where long-chain acid synthesis can proceed.

Discussion

The biosynthesis of long-chain fatty acids in E. coli has been extensively explored. In the first step, condensation of acetyl-ACP and malonyl-ACP yields acetoacetyl-ACP; this is then followed by reduction of the ketoacyl intermediate to β-hydroxy butyryl-ACP in a TPNH-dependent reaction (Goldman et al., 1962, Alberts et al., 1964). Though the enzymatic formation of triacetic acid (or its lactone) has received little detailed attention, it is reasonable to assume that it arises by the condensation of a malonyl unit with acetoacetyl-ACP. Two different reactions can therefore be presumed to operate on acetoacetyl-ACP, and depending on their relative rates varying amounts of long-chain acids and TAL will ultimately arise.

In this communication, evidence is presented that the products of the synthetase-catalyzed reaction are determined by factors influencing the level of TPNH. When TPNH is available in sufficient supply, long-chain acids are the major product. Addition of glutathione, either as the oxidized form or as a mixture of oxidized and reduced forms, to the reaction mixture, promotes glutathione reductase activity, thereby causing a rapid consumption of TPNH and impairment of fatty acid synthesis.[3] Acetoacetyl-ACP is then elongated to triacetic acid. The addition of G-6-P to the reaction mixture allows G-6-P dehydrogenase to restore the level of TPNH to a point where most of the acetoacetyl-ACP can be reduced to β-hydroxybutyryl-ACP and then elongated to long-chain acids. This phenomenon may explain in part the stimulatory effects of G-6-P on fatty acid synthesis observed by Wakil et al. (1966).

The physiological significance of an enzyme system in E. coli capable of synthesizing triacetic acid or its lactone is not clear. The relationship

[3] Though both oxidized and reduced glutathione are necessary to maintain a maximal rate of TPNH oxidation in the spectrophotometric assay (Fig. 1), the ability of oxidized glutathione alone to prevent fatty acid synthesis may be explained in terms of the very much greater amount of enzyme used in the synthetase assay (Table).

of TAL to other complex "polyacetate" structures found in fungi has been discussed (Richards and Hendrickson, 1964).[4] Though the metabolic fate of TAL in E. coli is unknown, it is of interest that when fatty acid synthesis is blocked because of the absence of TPNH, chain buildup does not stop at acetoacetyl-ACP, but proceeds to a six carbon length. It may also be of significance that control of the TPNH level can be effected through the ubiquitous cellular constitutent, glutathione.

The E. coli fatty acid synthetase as obtained after breakage of the bacterial cells is a mixture of several enzymes readily susceptible to resolution (Majerus, Alberts and Vagelos, 1964; Wakil, Pugh and Sauer, 1964). Extensive purification of the whole complex to the extent achieved for the yeast (Lynen, 1961) and pigeon-liver systems (Bressler and Wakil, 1961; Hsu, Wasson and Porter, 1965) is not possible with the bacterial extracts. The crude preparations of E. coli fatty acid synthetase will therefore contain necessarily a number of adventitious enzymes. As the present experiments show, G-6-P dehydrogenase and GSSG reductase are two such enzymes. Whether these activities are merely contaminating or whether they play a more fundamental role in bacterial fatty acid synthesis will have to be established by reconstitution of the synthetase system from the isolated and purified components.

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[4] Reports that both triacetic acid and its lactone can be cleaved to acetoacetic acid and acetic acid in liver homogenates (Breusch and Ulusoy, 1947; Witter and Stotz, 1948) raise the possibility of TAL synthesis providing an alternative pathway to ketone bodies in animal tissues.

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