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FATTY ACID SYNTHETASE FROM CHLOROPLASTS OF SOYBEAN COTYLEDONS: ACP ACTIVATION AND CoA INHIBITION [1]

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SUMMARY. Fatty acid synthetase from chloroplasts of soybean cotyledons was activated by preincubation with acyl carrier protein and dithiothreitol. The synthetase reaction had a 3-10 min lag which was not eliminated by the preincubation. Acetyl-CoA and malonyl-CoA had no effect on the activation. Fatty acid synthetase from spinach chloroplasts was neither activated by preincubation nor had a lag. The variability of the activity of the soybean enzyme with preincubation suggested that the fatty acid synthetase was present in two forms and that the acyl carrier protein caused conversion to the active form. This fatty acid synthetase and the same synthetase from spinach chloroplasts were inhibited by CoA. The type of inhibition by CoA in soybean was competitive with respect to malonyl-CoA and the Ki was 80μM.

Fatty acid synthetase (FAS)[2] from a large number of animal, plant, and bacterial systems has been studied. The FAS's examined fall into two major classes: Type I FAS which includes the multifunctional enzyme complexes consistently found in animal and yeast systems and Type II FAS which includes the dissociated, ACP-dependent systems found in E. coli and plants (1). Examinations of the mechanisms which could control fatty acid synthesis have demonstrated that fatty acid synthesis in Type I systems is subject to activation and inhibition by citrate and palmityl-CoA respectively (2). In addition, the Type I FAS can be converted from an apoenzyme to a holoenzyme by the ATP-dependent addition of 4′-phosphopantetheine from CoA or ACP (3-5). With respect to Type II FAS, turnover of 4′-phosphopantetheine in E. coli ACP has also been demonstrated (2). In contrast to E. coli and animal systems, very little information is available concerning the control of fatty acid synthesis in plant systems. Plant FAS has been obtained from

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2. Abbreviations: FAS: Fatty Acid Synthetase; ACP: Acyl Carrier Protein; DTT: Dithiotheitol

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leaf chloroplasts, developing and germinating seeds and mesocarp tissues (6). Each is a Type II ACP-dependent synthetase.

We report here the properties of FAS obtained from the chloroplast of soybean cotyledons. This enzyme system differs from previously studied synthetases in that it is activated by preincubation with ACP and, in addition, is inhibited by CoA.

MATERIALS AND METHODS. [2-^{14}C]malonyl-CoA was prepared by the method of Rutkoski and Jaworski (7), and acetyl-CoA was prepared by the method of Simon and Shemin (8). ACP, 80% pure and free to transacylase activity, was a gift of P. K. Stumpf, University of California, Davis. All other reagents are commercially available.

Soybeans (Glycine max L. var Wayne) were grown in constant light and the cotyledons picked 8-10 days after planting. Chloroplasts were isolated as previously described (9), and broken by homogenization in a TenBroeck homogenizer. The homogenate was centrifuged at 30,000xg for 30 min and the supernatant collected. The pellet was rehomogenized with an equal volume of isolation medium, centrifuged and the supernatants combined and used directly. Fatty acid synthetase from spinach was obtained in essentially the same way using spinach purchased locally. Protein concentration was determined by the method of Lowry et al. (10).

Fatty acid synthetase was assayed by a method previously described (11). Each assay contained 0.5mM NADH, 0.5mM NADP$^+$, 4mM glucose-6-phosphate, 0.15 units of Glc-6-P dehydrogenase, 2mM DTT, 38µg of ACP, 0.2mM acetyl CoA, 0.1mM [2-^{14}C]malonyl-CoA (5 Ci/mol), 60mM potassium phosphate, pH 7.4 and 0.5 mg of enzyme in 0.5 ml. Preincubations were carried out at 25° for 10 min in a volume of 0.15 ml, and all additions were present in amounts such that dilution to 0.5 ml yielded the indicated assay concentrations. Reactions were carried out at 25°. Enzyme activity was calculated from the linear portion of the progress curve following the lag.

RESULTS AND DISCUSSION

The FAS from chloroplasts of soybean cotyledons after germination had cofactor and pH requirements similar to the FAS from the developing cotyledons (12). The concentrations of all substrates and cofactors in the assay were at or above the concentration required for maximum activity. [2-^{14}C] Malonyl-CoA was used as the substrate for the FAS instead of [^{14}C]malonic acid. This increased incorporation 15-20 fold, and under optimal conditions, the reaction was 0.9 nmole/min/mg of protein. This activity was sufficiently high to allow careful examination of the initial progress of reaction. We consistently observed a 3-10 min lag before a linear increase in fatty acid synthesis occurred. To determine if this lag could be due to rate-limiting activity of one of the transacylase reactions, the

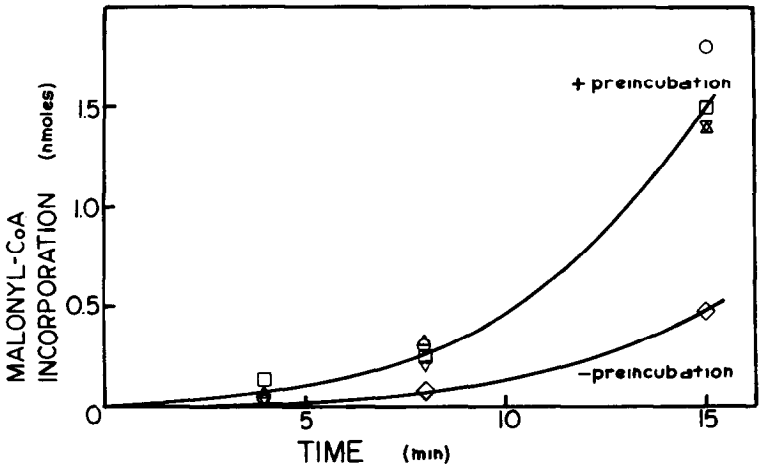

Figure 1. The effect of preincubation on the progress of the fatty acid synthetase reaction. Preincubations were carried out for 10 min at 25° in the presence of ACP (○), ACP and acetyl-CoA (□), ACP and malonyl-CoA (▽), and ACP, acetyl-CoA, and malonyl-CoA (△). Progress of the reaction with no preincubation is shown by the lower curve (◇). Final concentration of all substrates and cofactors in the assay were as indicated in Materials and Methods.

FAS was preincubated at 10 min in the presence of acetyl-CoA, malonyl-CoA, ACP and DTT (Fig. 1). Preincubation did not eliminate the lag, but did increase the post lag activity. However, this effect was dependent only on ACP and DTT. The DTT was required not only to reduce the ACP, but also affected the enzyme during the preincubation (Table I). While the enzyme was active in the absence of DTT if the ACP was reduced immediately before the reaction, the activity was increased during the initial 10 min if DTT was present along with reduced ACP in the preincubation. The effect of the preincubation could not be attributed simply to reduction of ACP by DTT since reactions carried out without preincubation but using reduced ACP exhibited the same lag and activity as reactions using ACP that was not reduced prior to the reaction.

While it initially appeared that preincubation with ACP and DTT was simply activating the FAS, numerous preparations of the enzyme

Table 1. Effect of ACP on fatty acid synthetase activity

Additions		[^{14}C]malonyl-CoA incorporation (CPM X10^3/ml of Assay)		
To Preincubation[1]	To assay Mixture[2]	10 min	20 min	20-10 min
none	ACP,DTT	9.5	105.0	95.4
ACP,DTT	none	89.6	177.0	87.4
ACP(reduced)[3],DTT		78.8	142.0	63.4
ACP(reduced)	DTT	47.0	106.0	58.9
ACP(reduced)		48.0	114.0	66.3
ACP	DTT	12.1	113.0	101.0
DTT	ACP	13.0	112.0	99.0
ACP	none	0.8	1.3	0.5

1. Enzyme was preincubated for 10 min at 25° in the presence of the indicated cofactors.
2. Additions listed were added to the other cofactors used which include NADH, $NADP^+$, Glc-6-P, Glc-6-P dehydrogenase, acetyl-CoA and [2-^{14}C]malonyl-CoA at concentrations listed in Materials and Methods.
3. ACP (75μg) was reduced with 1mM DTT for 10 min at 25°, precipitated with 0.1N H_2SO_4, and collected by centrifugation. The ACP pellet was rinsed with 0.1N H_2SO_4, dissolved in buffer and used immediately. Lower activity in these samples is due to loss of ACP during precipitation.

indicated that without preincubation, enzyme activity varied from very low to equal to preincubated enzyme (e.g. compare post-lag activity in Fig. 1 and Table I for preincubated vs. non-preincubated). Similarly, the length of the lag was variable. On the other hand, the activity of FAS after preincubation varied very little from one enzyme to another, and this activity was consistently high. Thus, it appeared that the FAS was being converted from an inactive form to an active form in the presence of ACP and DTT and that the ratio of active to inactive enzyme varied from one preparation to another. A similar study of FAS from spinach chloroplast was also carried out. Neither an activation nor an initial lag in the reaction was observed. Thus,

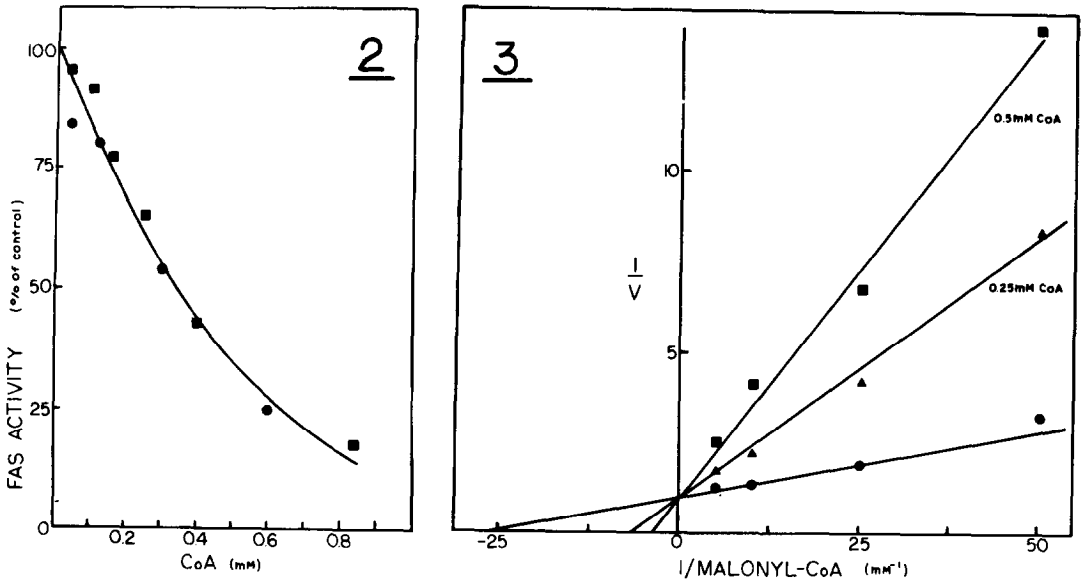

Figure 2. CoA inhibition of fatty acid synthetase from soybean cotyledons (●) and spinach (■). In the presence of no CoA, synthetase activities in soybean and spinach were 0.51 and 1.13 nmole per min per mg protein, respectively.

Figure 3. CoA inhibition of fatty acid synthetase from soybean cotyledons in the presence of increasing concentrations of malonyl-CoA. Assays were carried out with a 10 min preincubation with ACP and under conditions described in Materials and Methods except that the indicated levels of malonyl-CoA and CoA were used. Km for malonyl-CoA was 0.037mM (S.E. - 0.006); Ki for CoA was 0.08mM (S.E. = 0.01); and Vmax was 1.01 nmole per min per mg protein (S.E. = 0.05).

either the activation by ACP is not a general property of FAS from all plants, or the active form of FAS from spinach was more stable.

In light of the 4′-phosphopantetheine donation by either ACP or CoA to Type I FAS, to form the holoenzyme (4), the effect of CoA on the soybean FAS was examined. In our system, CoA exhibited only inhibition (Fig. 2). FAS from chloroplasts of either soybean cotyledons or spinach were similarly inhibited. While CoA inhibition of a microsomal FAS has been recently reported (13), inhibition by CoA of a Type II synthetase has not been previously reported. Palmityl-CoA inhibition of Type I FAS has been reported for a number of systems (2).

The type of inhibition observed in our work was competitive with respect to malonyl-CoA (Fig. 3), and the Ki = 80μM (S.E. = 12). Data was analyzed using a non-linear regression (14) comparison of the fit to equations for competitive, uncompetitive and noncompetitive inhibition (computer programs provided by C. C. Griffin, Miami U.). While reasonable overall fits were obtained for competitive or noncompetitive types of inhibition, the Ki (intercept) for noncompetitive inhibition had a standard error greater than itself, thus indicating that the fit to noncompetitive inhibition was meaningless.

The CoA inhibition of FAS argues against a transfer of 4′-phosphopantetheine during activation. In addition, we observed that the activation by ACP and DTT occurred equally well at 0° and 25° and did not have an ATP requirement. The activation at 0° would indicate a reaction with a low activation energy was taking place. One possible reaction might be a simple protein-protein association.

In summary, we report that FAS from chloroplasts of soybean cotyledons was activated by ACP and DTT and competitively inhibited by CoA. Both ACP and CoA are substrates or products of FAS and thus these observations may implicate a role for these two molecules in the control of FAS in plants.

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