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THE ROLE OF COENZYME A IN LIPID SYNTHESIS BY A PARTICULATE FRACTION FROM GERMINATING PEAS

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Abstract—The effect of CoA on fatty acid synthesis by the microsomal fraction from germinating pea (*Pisum sativum*) was examined. Increasing concentrations of CoA progressively decreased total fatty acid synthesis from [^{14}C]malonyl-CoA. However, the synthesis of very long chain fatty acids was relatively unaffected so that their proportion in the reaction products increased. Other CoA-esters also decreased total fatty acid synthesis while increasing the relative accumulation of radioactivity in very long chain fatty acids. The addition of CoA also altered the distribution of newly synthesized fatty acids in different lipid fractions. Complex lipid labelling was relatively increased while that of acyl–acyl carrier proteins was decreased. Very long chain fatty acids accumulated in lipids rather than thioesters. The role of CoA in controlling fatty acid synthesis in the pea microsomal fraction is discussed.

INTRODUCTION

Previous experiments carried out in this laboratory [1–3] have indicated that part of the ability of the microsomal fraction from germinating pea seeds to form very long chain fatty acids depended on the variety of seed used. It was also found that addition of exogenous CoA severely inhibited the total incorporation of radioactivity from [^{14}C]malonyl-CoA into acyl residues [1]. In contrast, addition of exogenous acyl carrier protein (ACP) caused a large increase of total incorporation and this increase was found to affect the incorporation of radioactivity into acyl-ACP's almost exclusively [3]. It was concluded, therefore, that ACP played a more positive role than CoA in the synthesis of fatty acids from [^{14}C]malonyl-CoA in the microsomal fraction.

However, it is generally accepted that the microsomal fraction is the site of acylthiokinase, stearate elongase and acyltransferase activities [4–8] and it is also known that CoA is involved in these activities. Therefore, in order to try to clarify this apparent paradox we have studied in detail the effect of the addition of exogenous CoA on fatty acid and lipid biosynthesis in the pea microsomal fraction.

RESULTS AND DISCUSSION

In previous work [3] it was found that the addition of exogenous ACP to the pea microsomal fraction increased the total synthesis of fatty acids without changing their percentage composition. On the other hand, it was demonstrated that addition of exogenous CoA produced a severe inhibition of fatty acid synthesis [1] and this was assumed to be due to competition between this cofactor and the radiolabelled precursor, [^{14}C]malonyl-CoA, for active sites on the enzymes involved in fatty acid synthesis. The effect of the addition of various concentrations of CoA on fatty acid synthesis from [^{14}C]malonyl-CoA is shown in Table 1. Previous experiments [1] had used a high (0.3 mM) concentration of CoA but it can be seen that a progressive increase in the inhibition of total synthesis was seen with increasing concentrations of CoA. However, when the pattern of [^{14}C]fatty acid products was examined it was found that the proportion of very long chain fatty acids was increased by the presence of CoA. This was because the formation of these acids was relatively unaffected by CoA whereas the synthesis of palmitate and stearate was severely inhibited. In agreement with these results it has been found that mammalian fatty acid synthetase is severely inhibited by low concentrations of CoA [9].

It will also be noticed in Table 1 that the microsomal fraction from germinating peas synthesizes a significant quantity of odd chain-length fatty acids. These products were previously identified as straight-chain saturated acids [3] and it was thought that their synthesis might have been catalysed by α-oxidation enzymes since these had been shown to be present in germinating pea [10]. However, efforts to block the synthesis of odd chain-length fatty acids with imidazole, an effective inhibitor of α-oxidation [10] were unsuccessful [3]. Accordingly, a number of possible precursors of odd chain-length fatty acids were tested and the results of such experiments are shown in Table 2. Propionyl-CoA was considered the

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Table 1. The effect of CoA on fatty acid synthesis by the microsomal fraction from germinating pea

CoA concn (μM)	Total fatty acid synthesis (% control)	No. of expts	Distribution of radioactivity (% total [^{14}C]fatty acids)							
			< 14	14:0	15:0	16:0	17:0	18:0	20:0	22:0
0	100	7	1 ± tr.	4 ± 1	8 ± 1	22 ± 2	25 ± 2	35 ± 1	6 ± 2	tr.
0.5	72	1	1	3	6	25	32	32	tr.	tr.
1.0	48 ± 8	2	2	3	3	17	20	37	8	8
5.0	13 ± 1	3	1 ± 1	3 ± tr.	4 ± 1	12 ± 2	9 ± 2	24 ± 2	39 ± 4	8 ± 7
50.0	6 ± 2	2	ND	ND	ND	ND	ND	ND	ND	ND

Figures represent the means (± s.e.m. where appropriate) for independent experiments carried out in triplicate. tr. = < 0.5; ND = not determined.

most likely 'primer' which could give rise to odd chain-length fatty acids but its addition did not alter the latter's synthesis. Succinyl-CoA could have given rise to odd chain-length products if it had substituted for malonyl-CoA in the condensation reaction of fatty acid synthetase. However, both reagents decreased the synthesis of odd chain-length fatty acids (Table 2). Furthermore, the addition of increasing concentrations of two other CoA-containing compounds, acetyl-CoA and malonyl-CoA, caused identical effects to propionyl-CoA and succinyl-CoA. That is, all the reagents caused an inhibition of total fatty acid synthesis and, at the same time, increased the relative incorporation of radioactivity into very long chain fatty acids. During the incubation period, all of these compounds were subjected to thioesterase action which resulted in a progressive release of free CoA [unpublished data]. We conclude, therefore, that the similarity of effect of the addition of CoA-esters (Table 2) to that of CoA (Table 1) is probably due to the release of the latter by thioesterase activity.

Since the odd chain-length fatty acid products did not seem to be the result of the use of odd chain-length primers or elongation units by fatty acid synthetase, we re-examined the possible presence of α-oxidation. Although it proved impossible to inhibit the formation of odd chain-length fatty acids more than 50% with imidazole, [U-^{14}C]palmitate was chain-shortened by one-carbon units into a whole series of saturated fatty acids by the microsomal fraction [Khor, H. T. and Harwood, J. L., unpublished results]. We conclude, therefore, that α-oxidation of newly-formed palmitate and stearate is responsible for the formation of pentadecanoate and heptadecanoate, respectively.

Having shown that CoA would severely inhibit palmitate and stearate synthesis, we tested its effect on the distribution of radiolabel incorporated from [^{14}C]malonyl-CoA into different acyl fractions. It was found that the presence of CoA in the incubation medium doubled the proportion of radiolabelled acyl residues in the lipid fraction while decreasing the proportion accumulating as acyl-ACP's (Table 3). So, interestingly, while the addition of exogenous ACP increased the incorporation of radioactivity into acyl-ACP's almost exclusively [3], the addition of CoA favoured the transfer of radiolabelled fatty acids in acyl lipids without producing an accumulation in acyl-

Table 2. The effect of CoA esters on the incorporation of radioactivity from [^{14}C]malonyl-CoA into fatty acids by the microsomal fraction from pea

Addition	Conc (μM)	Total fatty acid synthesis (% control)	Distribution of radioactivity (% total [^{14}C]fatty acids)							
			< 14	14:0	15:0	16:0	17:0	18:0	20:0	22:0
None	—	100	1	4	7	22	23	32	8	3
Acetyl-CoA	5	63	1	2	4	12	15	26	27	13
	20	54	—	tr.	tr.	13	17	25	37	9
	40	47	tr.	tr.	2	8	10	19	41	20
	200	32	—	—	1	8	4	24	48	14
Propionyl-CoA	5	56	—	tr.	4	13	13	25	45	tr.
	20	43	—	tr.	tr.	15	13	27	36	9
	40	37	—	—	2	7	8	23	46	15
	200	20	—	—	1	6	7	26	49	11
Succinyl-CoA	1	91	1	4	4	10	16	22	31	12
	5	83	tr.	3	3	7	10	20	38	19
	50	38	—	2	3	2	7	17	54	15
Malonyl-CoA	1	61	1	4	4	16	10	22	30	14
	5	21	ND	ND	ND	ND	ND	ND	ND	ND

For details of incubations see Experimental. Malonyl-CoA was added at the same specific radioactivity as the normal substrate. ND = not determined.

Table 3. The influence of CoA on the distribution of fatty acids synthesized by the pea microsomal fraction between different lipid classes

Addition	Fraction	% total counts	Distribution of radioactivity (% total [^{14}C]fatty acids)							
			<14	14:0	15:0	16:0	17:0	18:0	20:0	22:0
None (control)	Acyl lipids	35	1	2	4	16	21	39	17	ND
	Acyl-ACP's	53	3	10	6	22	22	38	ND	ND
	Acyl-CoA's	12	ND	4	5	32	32	27	ND	ND
5μM CoA	Acyl lipids	74	1	1	1	5	3	13	45	32
	Acyl-ACP's	12	6	4	4	22	21	44	ND	ND
	Acyl-CoA's	14	ND	2	6	34	18	33	9	ND

ND = not determined.

CoA's. However, because of the general inhibition of fatty acid synthesis, total acyl lipid synthesis was decreased. When the fatty acid compositions of the individual lipid fractions were analysed, it was found that [^{14}C] very long-chain fatty acids were almost exclusively located in the acyl lipid fraction. The absence of these acids as acyl-ACP's even when this fraction contained the majority of radiolabelled products (i.e. in the absence of exogenous CoA), fits in well with the generally accepted view that acyl-ACP's are not suitable substrates for elongation to very long chain fatty acids [1–7].

Altogether these results are in agreement with the idea that fatty acids are synthesized, from [^{14}C]malonyl-CoA, by fatty acid synthetase and then palmitate elongase with the mediation of ACP up to stearoyl-ACP [5–6] and then they are transferred to stearoyl-CoA's for acylation into complex lipids [11]. Although it has been suggested [7] that acyl-CoA's are the substrates for very long chain fatty acid formation, the microsomal fraction from germinating pea would not utilize exogenous stearoyl-CoA for arachidate synthesis [unpublished results; 1]. In contrast, the accumulation of very long chain fatty acids in the complex lipids (Table 3) and the use of stearoyl-phosphatidylcholine for elongation [1] suggests that complex lipids are the substrates for very long chain fatty acid synthesis in peas [2, 8, 12]. However, it is possible that the difficulty of demonstrating the elongation of stearoyl-CoA may reflect a problem in the access of a water-soluble substrate to a membrane-located enzyme (cf ref. [5]). In this connection it is worth emphasizing that some mammalian brain systems also fail to elongate exogenous acyl-CoA's [Mead, J. F., personal communication] even though these compounds have been widely assumed to be the actual substrates [13]. The situation in germinating peas (and other plant systems) can only be unequivocally resolved when stearate elongase enzymes are purified.

It is interesting to see how the results shown in Table 3 agree with those previously obtained in this laboratory [1] in relation to the distribution of radioactivity among different acyl fractions (lipids 77%, acyl-ACP's 16%, acyl-CoA's 7%). Bearing in mind that the concentration of [^{14}C]malonyl-CoA used in the earlier experiments [1] was much higher, it is clear that the differences noticed in the incorporation of radiolabelled precursor into different acyl fractions and in the proportion of very long chain fatty acids synthesized, are determined to a large extent by the concentration of CoA in the incubation medium. All these results demonstrate the central role of CoA in the biosynthesis of fatty acids and in their transfer into complex lipids. They also illustrate the importance that has to be placed in carefully considering the concentrations of cofactors added to the incubation medium especially if these additions are reagents containing CoA.

EXPERIMENTAL

Materials. Pea (*Pisum sativum* cv Feltham First) seeds were purchased from Asmer Seeds, Leicester, U.K., and were surface-sterilized and germinated as previously described [1]. After 24 hr germination pea seeds were homogenized and the microsomal fraction was isolated as previously [1, 3]. [2-^{14}C]Malonyl-CoA (sp. radioactivity 53 Ci/mol) was purchased from The Radiochemical Centre, Amersham and acyl carrier protein was isolated from *Escherichia coli* [14].

Incubations. Reaction mixtures contained NADH (0.67 μmol), NADPH (0.67 μmol), ATP (3 μmol), *E. coli* acyl carrier protein (1 mg), 0.02 μCi [^{14}C]malonyl-CoA and 0.4 ml microsomal fraction made up to 1.0 ml with KPi buffer, pH 7.0 (0.03 M final concn). Incubations were carried out at 25° in triplicate with continuous shaking for 4 hr.

Analysis of products. Analysis of reaction products was carried out by the method of Sanchez and Mancha [15]. In these cases the incubations were terminated by the addition of 1 ml of 2.5% (w/v) HOAc in propan-2-ol. For total fatty acid analysis, the reaction was stopped and extraction was carried out as previously [1]. Fatty acid esters were prepared by trans-methylation with 2.5% (v/v) H_2SO_4 in MeOH and separated by GC in 15% (w/w) EGSS-X on Chromosorb W AW (80–100 mesh). Quantification of radioactivity was with a Panax gas flow proportional counter. Radioactivity estimation for total fatty acid methyl esters and acyl lipids was as previously described [1].

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