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Fatty-acid chain elongation in rat small intestine

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1. Microsomal fractions from rat small intestine contain a fatty-acid chain-elongation activity. 2. Cofactor requirements are similar to those of the liver microsomal system, but substrate specificity is different. The polyunsaturated arachidonic and timnodonic acids were elongated at very low rates. 3. These results suggest that the relative contents of specific chain-elongation enzymes are different in liver and small intestine.

INTRODUCTION

The small intestine is of central importance in lipid metabolism. A lot of interest has been focused on the uptake of fatty acids into the mucosal cells as well as on their esterification to complex lipids and further transport. Information concerning oxidation and remodelling of fatty acids within this organ is, however, sparse. Previously we have studied peroxisomal β-oxidation (chain-shortening) of fatty acids in rat small intestine (Thomassen *et al.*, 1985). During the course of these studies, which employed an '*in vivo*' lymph-collection technique, we became aware that erucic acid ($C_{22:1}$), as well as being chain-shortened to $C_{20:1}$ and $C_{18:1}$ fatty acids in the small intestine, was chain-elongated to $C_{24:1}$ fatty acid to a considerable extent. This led us to to search for a fatty-acid chain-elongation system in this organ analogous to the systems described in rat liver and brain (Sprecher, 1981; Cook, 1982).

In the present paper we report that a chain-elongation activity is also present in the microsomal fraction isolated from rat small-intestinal mucosa. Furthermore, its cofactor requirements and activity towards different fatty acids have been studied.

EXPERIMENTAL

Male rats of the Wistar strain were used. They were purchased from Møllegaard Breeding Laboratory, Ejby, Denmark. Details about housing conditions were as described by Nilsson *et al.* (1984). The rats were fed a pellet diet obtained from Ewos AB, Södertälje, Sweden; Brood Stock Feed for Rats and Mice-R3, composed according to actual international specifications (National Institutes of Health). Raw materials were cereals, wheat-germ, wheat middlings, fish protein concentrate, soya meal (roasted), fodder yeast, minerals, animal and vegatable fat, vitamin concentrate and trace-element concentrate [crude protein, 21.0%; carbohydrate, 52.0%; crude fat, 6.0% (of wet weight)]. The chemicals were commercially available products of high purity, obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

Rats were killed by a blow on the head and by cutting of the carotid arteries. The microsomal fractions were prepared from liver as described by Norum *et al.* (1979) and from small intestine as described by Thomassen *et al.* (1985), except that the postmitochondrial supernatant obtained was centrifuged at 420000 $g_{av.}$·min. The resulting pellet was resuspended in the homogenization medium and stored frozen for subsequent studies of chain elongation.

Chain elongation of fatty acids was studied by incubating the microsomal fractions for 30 min at 37 °C in a total volume of 2 ml contaiing 100 mM-potassium phosphate buffer, pH 7.4, under standard conditions: 100 μM ^{14}C-labelled fatty acid (0.05 μCi/ml) bound to bovine serum albumin (0.2%), 150 μM-malonyl-CoA, 5 mM-ATP, 0.5 mM-NADPH, 50 μM-CoASH and

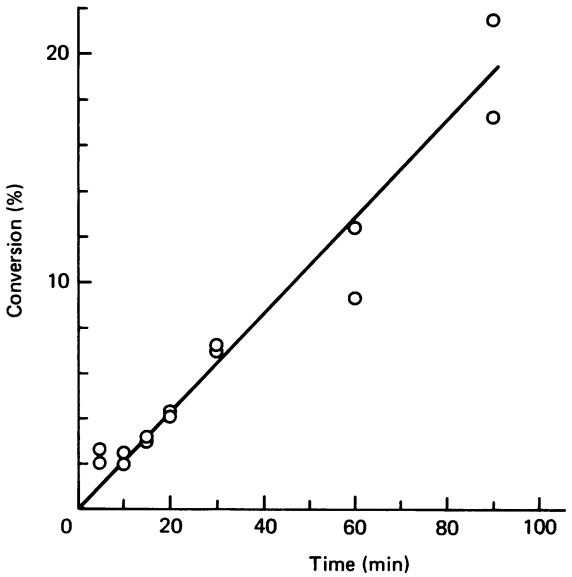

Fig. 1. Effect of incubation period on chain elongation of palmitic acid by microsomal fractions from rat small intestine

Microsomal fractions were isolated from rat small-intestinal scrapings and assayed for chain elongation of ^{14}C-labelled palmitic acid as described in the Experimental section with 1.0 mg of microsomal protein. The elongation rate is given as percentage conversion of added palmitic acid substrate (200 nmol).

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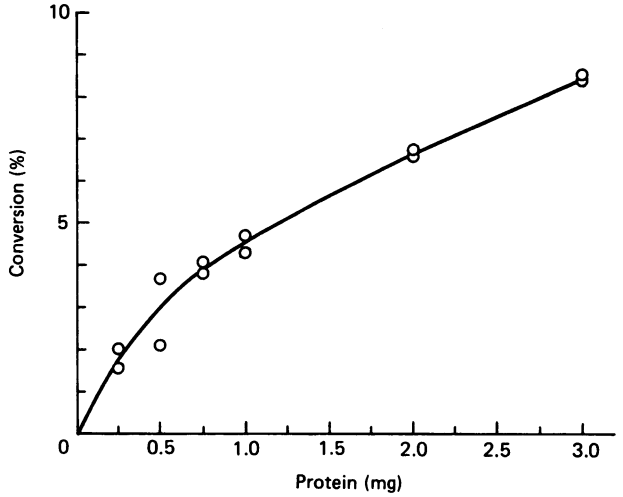

Fig. 2. Effect of microsomal protein concentration on chain elongation of palmitic acid in rat small intestine

Microsomal fractions were isolated from rat small-intestinal scrapings and assayed for chain elongation of ^{14}C-labelled palmitic acid for 30 min as described in the Experimental section. The elongation rate is given as percentage conversion of added palmitic acid substrate (200 nmol).

1.0 mg of microsomal protein, essentially as described by Bernert & Sprecher (1975). The reaction was terminated by the addition of 2 ml of 15% (w/v) KOH in methanol. The mixture was then heated for 45 min at 65 °C, cooled on ice, neutralized by adding 6 M-HCl, and non-esterified fatty acids were then extracted into hexane and prepared for radio-g.l.c. as described by Thomassen *et al.* (1979). Fatty acid methyl esters were separated by using 10% SP-2340 on Supelcoport 100/120 (Supelco, Bellefonte, PA, U.S.A.) in a Varian 2100 g.l.c. apparatus connected to an ESI nuclear radiodetector (Hagve & Christophersen, 1983).

RESULTS

With incubation conditions similar to those used in studies on liver microsomal chain elongation, microsomal fractions from mucosal-cell scrapings reveal a considerable elongation of palmitic acid (Fig. 1). The reaction was found to be linear with time up to an incubation period of at least 100 min. The elongation rate also increased with increasing microsomal protein consentration (Fig. 2).

The elongation process showed an absolute requirement for malonyl-CoA and ATP (Table 1). ATP and CoASH are routinely added as cofactors for the activation of non-esterified fatty acids to acyl-CoA. We found, however, that the addition of CoASH in our system resulted in an inhibition of the chain elongation of palmitic acid. Omission of NADPH, or substituting NADH for NADPH, decreased the elongation rate by more than 30%.

The activity of the intestinal chain-elongation system towards different fatty acids was studied and compared with that of the liver system (Table 2). The rate of palmitic acid ($C_{16:0}$) elongation in the intestine was very similar to the rate obtained with the liver system. Oleic acid ($C_{18:1,\ n-9}$) was not elongated in detectable amounts

Table 1. Cofactor requirements for the microsomal chain-elongation system in rat small intestine

Microsomal fractions were isolated from rat small-intestinal scrapings and assayed for chain elongation of ^{14}C-labelled palmitic acid as described in the Experimental section. With the complete system the elongation rate was 7.2 nmol/30 min per mg (100%)

System	Elongation rate (% of control)
Complete (control)	100
−ATP	0
−Malonyl-CoA	0
−NADPH	69
−NADPH+0.5 mM-NADH	59
−CoA	172

Table 2. Chain elongation of different fatty acids by microsomal fractions from rat small intestine and liver

Microsomal fractions were isolated from rat liver and from small-intestinal scrapings, and assayed for fatty-acid chain elongation as described in the Experimental section. The values given are means ± S.D. obtained with four individual preparations (each with six animals). Abbreviation used: n.d., not determined.

	Chain elongation (nmol/30 min per mg)	
Fatty acid	Intestine	Liver
$C_{16:0}$	5.9±1.6	6.1±1.8
$C_{18:1,\ n-9}$	n.d.	n.d.
$C_{18:2,\ n-6}$	n.d.	3.4±3.4
$C_{18:3,\ n-3}$	1.3±0.3	1.8±0.7
$C_{20:4,\ n-6}$	3.0±0.3	55.1±3.0
$C_{20:5,\ n-3}$	3.2±0.9	82.1±14.5
$C_{22:1,\ n-9}$	6.6±5.4	2.4±0.5

in either of the two organs, whereas linoleic acid ($C_{18:2,\ n-6}$) and linolenic acid ($C_{18:3,\ n-3}$) were chain-elongated to a small extent in the liver, and linolenic acid to a small extent in the intestine. The long-chain polyunsaturated acids arachidonic ($C_{20:4,\ n-6}$) and timnodonic ($C_{20:5,\ n-3}$) were elongated at a considerable rate in the liver, but only to a small extent in the intestine. Erucic acid ($C_{22:1,\ n-9}$) was elongated at a rate comparable with that for palmitic acid in the intestine, and possibly at a somewhat lower rate in the liver.

DISCUSSION

Chain elongation of fatty acids has been detected in microsomal fractions from several mammalian organs. This reaction has been shown to require malonyl-CoA, acyl-CoA and NADPH, and has been extensively studied in liver and brain (Sprecher, 1981; Cook, 1982). However, to the best of our knowledge, elongation of fatty acids has not previously been demonstrated in rat small intestine. The cofactor requirements of this system seem to be analogous to those observed previously in

liver and brain. The lack of requirement for added CoASH has also been noted before in both plant and animal systems (Cook, 1982; Agrawal & Stumpf, 1985) and is probably due to the presence of endogenous CoASH as well as to free CoASH generated from added malonyl-CoA. However, the fatty acid specificity of the intestinal system seems to differ greatly from the systems in rat liver and brain. Although the activity towards saturated and monounsaturated fatty acids may seem to be similar in liver and small intestine, highly polyunsaturated fatty acids are markedly poorer substrates for the intestinal system. In brain, microsomal chain elongation of fatty acids seem to be catalysed by several elongation systems depending on chain length rather than the degree of unsaturation (Yoshida & Takeshita, 1984). Sprecher (1974) has suggested that, in liver, the chain-elongation system for polyunsaturated fatty acids is distinct from the one for palmitic acid, and recently Kawashima & Kozuka (1985) presented evidence supporting this suggestion. On the basis of these findings, the difference observed in fatty acid specificity in the present study may suggest that intestine lacks the specific 'polyunsaturated' elongase that is present in liver.

The active chain elongation of erucic acid observed in the present study is in good agreement with the results from studies *in vivo* by Thomassen *et al.* (1985), where a significant amount of radioactive labelled $C_{24:1}$ fatty acid was detected in lymph lipids after intraluminal injection of [^{14}C]erucic acid. When the same '*in vivo*' technique was used, no metabolic conversion into other fatty acids of intraluminally injected [^{14}C]linoleic acid could be detected (E. N. Christiansen, P. Helgerud & M. S. Thomassen, unpublished work). This finding is also confirmed by the lack of elongation of linoleic acid in the intestine *in vitro* (Table 2).

In conclusion, the present results show that there is an active microsomal chain-elongation system in rat small-intestinal mucosa that is able to chain-elongate both saturated, monounsaturated as well as polyunsaturated fatty acids. The fatty acid specificity seems, however, to be different from that of other mammalian organs studied.

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