STIMULATION OF TRIACYLGLYCEROL SYNTHESIS BY Z PROTEIN IN RAT LIVER AND INTESTINAL MUCOSA

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1. Introduction

Although the overall pathways of triacylglycerol biosynthesis in intestinal mucosa have been well established [1], little is known concerning the details of fatty acid and acylglycerol specificity in the acyl transfer reactions. Experimental attempts to elucidate this have been frustrated by an apparent discrepancy between in vivo and in vitro results in the stereochemical course of acylation of the 2-monoacylglycerols [2,3] and in the relative yields of the di- and triacylglycerols [4,5]. Furthermore, it has proven difficult to isolate a triacylglycerol synthetase from intestinal mucosa [6,7]. In this connection it was instructive to note that Manley et al. [8] have obtained evidence, that, in the acylation of diacylglycerols to triacylglycerols in rat liver microsomes, there was a requirement for the cytosol fraction, which they showed to contain a soluble protein which was heat sensitive and destroyed by trypsin. Several laboratories [9–11] have reported a binding protein for long chain fatty acids in the cytosol of liver, intestine and other tissues and it has been suggested [10,12] that this binding protein, called Z protein, may be involved in the intracellular esterification of fatty acids.

The present report provides evidence that the Z protein may be involved in the acylation of diacylglycerols to triacylglycerols in microsomes of rat liver and intestinal mucosa.

2. Methods

Microsomes (105 000 *g* particulate fraction) and cytosol (105 000 *g* supernatant) were prepared from rat liver and intestinal mucosa as previously described [13]. The Z protein was prepared from liver and intestinal cytosol as described by Ockner et al. [10]. Diacylglycerol acyltransferase activity was assayed essentially as described by Manley et al. [8]. The standard incubation mixture contained in a final vol of 2 ml: potassium phosphate buffer (0.02 M, pH 7.4) containing 0.1 mM EDTA; 2 mg microsomal protein, 10 nmol 1,2-dioleoyl-*sn*-glycerol, 4 mM $MgCl_2$, 4 mM ATP and 2 nmol [1-^{14}C] palmitoyl CoA (0.1 μCi). Varying amounts of either cytosolic protein (S_{105}) or Z protein were added as described in tables 1 and 2. The diacylglycerol was added in 10 μl of 2:1 dioxane–propylene glycol and the palmitoyl CoA was added in 10 μl of sodium acetate buffer (0.1 M, pH 6.0). All incubations were carried out in a Dubnoff metabolic shaker for 15 min at 37°C.

The incubations were terminated by the addition of 1:2 chloroform–methanol (7.5 ml). Subsequently, chloroform (2.5 ml) and water (2.5 ml) were added as described in the extraction procedure of Bligh and Dyer [14]. Following centrifugation, the chloroform

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layer was removed and dried under a stream of nitrogen. The residue was taken up in toluene and the triacylglycerol fraction was isolated by silicic acid chromatography as described by Manley et al. [8]. To determine net synthesis, triacylglycerol ester was measured quantitatively by the hydroxamate method of Rapport and Alonzo [15]. Radioactivity was determined in a Nuclear Chicago Scintillation counter using ratio method [16]. Protein was determined by the method of Lowry et al. [17] using bovine albumin as standard.

3. Results and discussion

Table 1 shows the effect of liver cytosol and liver cytosol Z protein on the incorporation of [1-^{14}C]-palmitoyl CoA into triacylglycerols by rat liver microsomes. In the absence of either cytosol or Z protein, only a minor amount of synthesis occurred. The addition of increasing amounts of Z protein, which constitutes approx. 2% of the liver cytosolic proteins [18], resulted in a 15-fold stimulation of triacylglycerol biosynthesis. There was no enzymic activity in either the cytosol or the Z protein itself. Table 1 also shows that the addition of albumin, which is present in trace amounts in liver cytosol [19], could not duplicate the action of the Z protein. This suggests that the stimulatory action of the liver cytosol upon triacylglycerol synthesis is due to the Z protein.

Table 2 demonstrates that the Z protein of the intestinal mucosa is effective in the promotion of triacylglycerol biosynthesis in mucosal microsomes. The degree of stimulation achieved is comparable to that noted for the Z protein of the rat liver cytosol, for which it can be exchanged in the incubation medium. These results indicate that the Z protein can stimulate triacylglycerol formation in both liver and intestinal microsomes.

In an earlier study, Manley et al. [8] showed that triacylglycerol synthesis in rat liver microsomes can be stimulated by the addition of cytosol and suggested that the cytosol may contain one or more proteins which operate as carriers of acyl CoAs in the synthesis of triacylglycerols. Suzue and Marcel [20] have recently reported the isolation of a new fatty acid binding protein from the cytosol of rat liver and have made a preliminary examination of its effect upon the acylation of 1-palmitoyl lyso phosphatidylcholine by rat liver microsomes. No stimulation could be demonstrated for the new cytosolic protein or albumin and the Z protein which were clearly resolved from it. Mishkin and Turcotte [21], however, had earlier found a strong stimulatory effect of Z protein in the formation of monoacylglycerolphosphate in liver microsomes. The present study supports this earlier claimed stimulation of acyl transferase activity by the Z protein and extends it to the active promotion of the activity of diacylglycerol acyltransferase in microsomes of rat liver and intestinal mucosa.

Table 1
Stimulation of incorporation of [1-^{14}C] palmitoyl CoA into triacylglycerols in liver microsomes by cytosol and liver cytosol Z protein

Incubation	Additions	Incorporation of [1-^{14}C] palmitoyl CoA
		nmoles/2 mg microsomal protein
1	None	0.05 ± 0.1
2	S_{105} (10 mg protein)	0.16 ± 0.2
3	S_{105} (20 mg protein)	0.35 ± 0.2
4	Z protein (10 nmol)	0.43 ± 0.2
5	Z protein (20 nmol)	0.59 ± 0.3
6	Z protein (30 nmol)	0.80 ± 0.3
7	Albumin (20 nmol)	0.07 ± 0.1
8	Albumin (40 nmol)	0.09 ± 0.1

Standard incubation medium contained 10 nmol of 1,2-dioleoyl-*sn*-glycerol as the acceptor, as described in Methods. S_{105} = 105 000 *g* cytosol. The results represent the mean ± S.E.M. of four experiments.

Table 2
Stimulation of incorporation of [1-^{14}C] palmitoyl CoA into triacylglycerols in intestinal microsomes by cytosol and intestinal cytosol Z protein

Incubation	Additions	Incorporation of [1-^{14}C] palmitoyl CoA
		nmoles/2 mg microsomal protein
1	None	0.04 ± 0.1
2	S_{105} (10 mg protein)	0.15 ± 0.1
3	S_{105} (20 mg protein)	0.33 ± 0.3
4	Z protein (10 nmol)	0.38 ± 0.2
5	Z protein (20 nmol)	0.56 ± 0.2
6	Z protein (30 nmol)	0.74 ± 0.3
7	Albumin (20 nmol)	0.08 ± 0.1
8	Albumin (40 nmol)	0.09 ± 0.1

The enzyme was assayed as described in table 1, except that intestinal cytosol and intestinal cytosol Z protein were used. Results are the mean ± S.E.M. of four separate experiments.

Since the Z protein of liver cytosol is known to have different affinities for various fatty acyl CoAs [10,11], it is possible that it may play a role in fatty acid specificity in triacylglycerol biosynthesis. Furthermore, the loss of the Z protein in the supernatant may account at least for part of the deficiency in diacylglycerol acyltransferase activity observed with conventional preparations of mucosal microsomes [4,5], and for the difficulty in isolating the mucosal triacylglycerol synthetase [6,7]. It remains to be demonstrated whether or not the cytosolic proteins of rat liver and intestinal mucosa also play a role in the positional and stereochemical specificity of acylation of acylglycerols.

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