ACYLATION OF MONOGLYCERIDES BY LOCUST FAT-BODY MICROSOMES

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Received 9 January 1974

1. Introduction

The synthesis of triglycerides is known to occur by two pathways. The first pathway employs glycerol-3-phosphate as acyl acceptor and triglyceride synthesis occurs via the intermediate formation of phosphatidic acid and diglycerides [1]. The second pathway, known as the monoglyceride pathway, synthesizes triglycerides by the acylation of monoglycerides through a diglyceride intermediate [2, 3]. The enzymes of the first pathway are present in a wide variety of tissues and as suggested by Kennedy (1) this is the major route for the biosynthesis of di- and triglycerides in all tissues except the mucosa of the small intestine which contains high levels of monoglyceride acyltransferase (EC 2:3:1 type) [2, 3].

In a previous communication [4] we described the presence of the enzymes of the glycerol-3-phosphate pathway in the fat-body of the locust, *Locusta migratoria*. In the present communication the occurrence of monoglyceride acyltransferase in the microsomes of the fat body will be demonstrated. The possible role of the monoglyceride pathway in fat mobilization will be discussed.

2. Methods

2.1. *Preparation of C^{14} and H^3 labeled monoglycerides*

Glycerol [1-C^{14}] trioleate or H^3-glyceryltrioleate (Radioactive Center, Amersham, England) were mixed with 20 mg triolein and incubated with pancreatic lipase (EC 3:1:1:3). The incubation conditions and extraction procedure were as described by Chino and Gilbert [3]. The monoglycerides were purified by chromatography on silica gel (0.5 mm thickness) employing benzene: ethyl ether: ethanol: acetone (50:40:2:0.2, by volume) as developing solvent [6]. The ratio of 2 to 1-monoglycerides in each preparation was determined chromatographically [7] and was found to be equal to 1.6–1.7.

2.2. *Preparation of microsomes*

Microsomes were prepared as previously described [4].

2.3. *Assay of monoglyceride acyltransferase activity*

The reaction mixture is described in the legend to the tables. Incubations were done at 30°. Lipids were extracted according to the procedure of Bligh and Dyer [8]. Neutral lipids were separated by thin layer chromatography [9]. The radioactivity associated with the lipid spots was determined and the amount of monoglycerides incorporated into di- and triglycerides calculated.

3. Results

3.1. *Utilization of monoglycerides for the biosynthesis of di and triglycerides*

Monoglycerides were readily metabolized by fat body microsomes. As can be seen from table 1, when monooleate was the only substrate added, it was degraded and ^{14}C-labeled fatty acids accumulated. However when palmityl CoA and/or ATP, CoA and Mg^{2+} were also added, hydrolysis was markedly reduced and di- and triglycerides were formed. ^{3}H-glycerylmono-

Table 1
Metabolism of monoglycerides by fat body microsomes.

Addition	n moles monoglycerides converted to:		
	Diglycerides	Triglycerides	Fatty acids
none	2.8	0	42.5
ATP, CoA, Mg^{2+}	4.1	37.2	5.8
Palmityl CoA	16.8	17.7	13.9
Palmityl CoA + ATP + CoA + Mg^{2+}	7.7	35.3	6.1

The reaction mixture contained: Tris-maleate buffer pH 7.2 250 μmoles, NaF 20 μmoles, glutathione 10 μmoles, Tween 20 2 mg, glyceryl-l-^{14}C-monooleate 57 nmoles, – 10 000 counts/min and fat body microsomes 6.00 mg protein in a total volume 1.7 ml. ATP 20 μmoles, $MgCl_2$ 20 μmoles, CoA 2.5 μmoles and Palmityl CoA 1 μmoles, were added as indicated. Incubation: 1 hr at 30°.

oleate and glyceryl-^{14}C-monooleate were equally available for the transacylation reaction. When the microsomes were incubated, in the presence of palmityl CoA, with a mixture of these monoglycerides the di- and triglycerides which were formed showed the same ^{3}H to ^{14}C ratio (table 2). These results suggest that the monoglyceride molecules were utilized without previous breakdown. Furthermore, ^{14}C-glycerol of high specific activity, was not incorporated into glycerides under these conditions.

Table 2
Acylation of ^{3}H-glycerylmonooleate and glyceryl ^{14}C-monooleate.

	^{14}C n moles	^{3}H n moles	^{3}H/^{14}C counts/min
Monoglyceride added	57.0	19.0	4.8
Diglyceride formed	20.0	6.0	4.5
Triglyceride formed	13.7	4.1	4.5

The reaction conditions, as described in table 1. 3.9 mg protein were added.

3.2. *Properties of the microsomal monoglycerides acyltransferase*

3.2.1. Dependence on enzyme concentration and time of incubation

In the preliminary experiments described in the previous section, relatively large amounts of microsomal protein were used. To show the dependence of the transacylse reaction on enzyme concentration and on the time of incubation less than 500 μg protein were used and the time of incubation was reduced to 10 min. The results obtained are shown in fig. 1 and 2. Under these conditions, 1,2-diglycerides were the main product (over 90%); less than 10% of the diglycerides formed were further acylated to yield triglycerides.

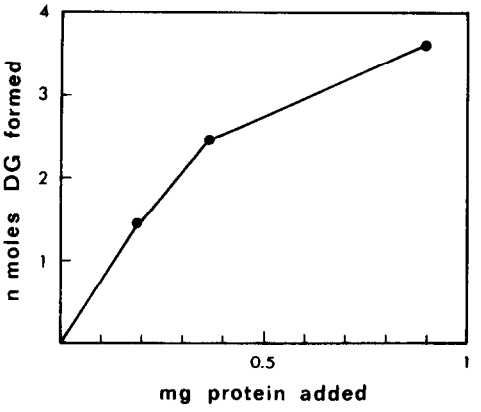

Fig. 1. Monooleate acylation by increasing amounts of microsomes. The reaction mixture contained: Tris-maleate buffer pH 7.2 100 mM, NaF 14 mM, CoA 7 mM, Tween 20 1.4 mg, Palmityl CoA 0.7 μmoles, ^{3}H-glyceryl-monooleate 8.7 nmole, 160.000 counts/min. Total volume 1 ml. Incubation 10 min.

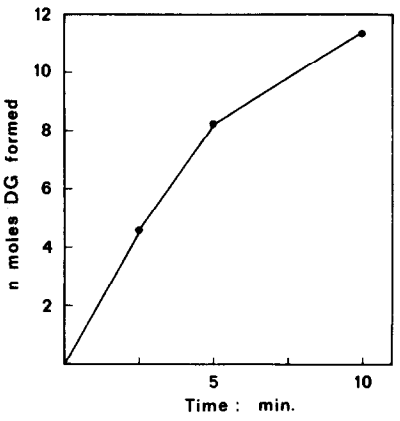

Fig. 2. Dependence of monooleate acylation on time of incubation. Reaction conditions as described in fig. 1. 900 μg of microsomes were added.

3.2.2. Effect of pH

As can be seen in fig. 3, very little activity was detected at pH 5.5. The activity increased markedly over the range of pH 5.5 to 7.0, and only very slightly over the range of 7.0 to 8.5, after which it started to decline. The hydrolytic activity however, markedly increased as the pH was raised.

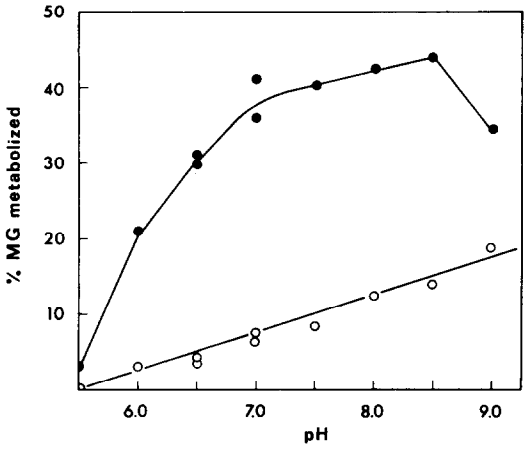

Fig. 3. The effect of pH on monooleate metabolism. Reaction condition as described in fig. 1. 260 μg of microsomes were added. Tris maleate was used throughout. In one experiment (pH values 5.5 to 7.0) 11 nmoles ^{3}H-glycerylmonooleate (20,000 counts/min) were added, in the second experiment (pH 6.5 to 9.0) 7 nmoles (13.000 counts/min) substrate were added. •–•–• Diglycerides formed, o–o–o monoglycerides hydrolysed.

3.2.3. Dependence on monooleate concentration

It can be seen from fig. 4 that the amount of diglycerides formed increased as the amount of monooleate added was increased. Under the experimental conditions used, saturation of the enzyme with substrate was not reached.

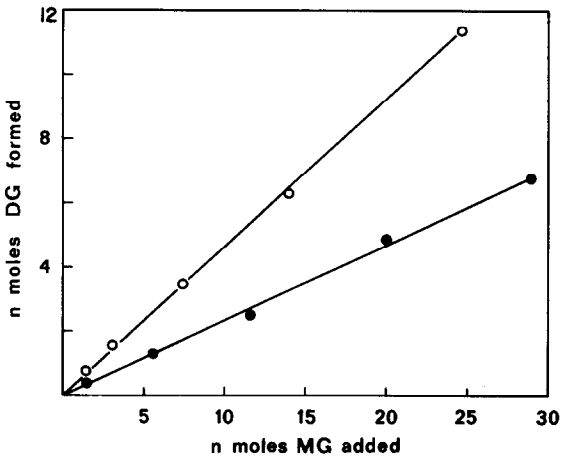

Fig. 4. Dependence on increasing concentrations of monooleate. Reaction conditions as described in fig. 1. ^{3}H-glyceryl monooleate containing 1820 counts/min per nmole was used throughout. •–•–• 900 μg protein, o–o–o 180 μg protein.

4. Discussion

In a previous publication [4] we demonstrated that the locust fat-body microsomes contained glycerophosphate and diglyceride acyltransferase activities (EC 2:3:1:15 and EC 2:3:1:2). In the present communication it was shown that these microsomes also contained monoglyceride acyltransferase activity. Monoglyceride acyltransferase was previously shown to be present in the microsomal fraction of the intestinal mucosa of several laboratory animals [2, 10–13], cats [14] and probably also in pig kidney, rabbit pancreas, rat liver [15] and mammary gland [16]. However, whereas in the liver and mammary gland the enzymes of the glycerophosphate pathway predominated in the intestinal mucosa the monoglyceride pathway was more active.

The enzymes of the intestinal mucosa were extensively studied by Hubscher, Johnston and their

associates. The intestinal monoglyceride acyltransferase was shown to utilize the following monoglyceride isomers: D and L-1-monoglyceride and 2-monoglyceride. Only the primary hydroxyl group was acylated. Thus, 1-isomers yielded 1, 3-diglycerides while the 2-isomer gave a 1, 2-diglyceride. The enzyme preferentially acylated 2-monoglycerides [17]. The specificity of the fat body enzyme for the 3 isomers is at present under investigation. The observation that the incubation of the fat body microsomes with a mixture of 2 and 1-monoglycerides (molar ratio 1.6) yielded primarily 1, 2-diglycerides suggests that our enzyme, like the enzymes of the intestinal mucosa, preferentially acylates 2-monoglycerides.

Brindley and Hubscher [14] calculated that under optimal conditions (pH 8.0, 3.3. mM monoolein) 2980 ± 1030 nmoles of monoglyceride were acylated by 1 mg of cat intestinal mucosa enzyme per hr; the specific activity of the glycerol-3-phosphate acyltransferase was only 1150. Under our experimental conditions (pH 7.2 and 30 nM monoolein) approximately 250 nmoles of monoolein were acylated by 1 mg of locust fat body microsomes per 1 hr. The concentration of monoolein employed in these experiments is 100 times lower than that used by Hubscher, and as shown in fig. 3 the enzyme was not saturated with monoolein. To demonstrate the acylation of glycerol-3-phosphate, relatively high concentrations of this substrate are required; negligible activity was observed when glycerol-3-phosphate was added at 30 nM.

Monoglyceride acyl transferase plays an important role in intestinal fat absorbtion; the glycerol-3-phosphate pathway seems to be involved in the biosynthesis of phospholipids only [3]. Preliminary evidence suggests that the monoglyceride pathway plays a central role in fat mobilization during flight: storage triglycerides are degraded by a lipase to 2-monoglycerides, these are reacylated to 1, 2-diglycerides which are then released from the fat-body into the surrounding hemolymph. This reaction sequence requires the expenditure of one mole of ATP (for the activation of one mole of fatty acid). The formation of diglycerides from glycerol and 2 fatty acids however requires 3 moles of ATP. Furthermore, monoglycerides have a much higher affinity for the acyltransferase system than glycerol-3-phosphate. It seems unlikely that high enough concentration of the latter substrate are available during flight. In feeding animals, however, glycerol-3-phosphate is continously formed from glucose. Thus it seems likely that the glycerol-3-phosphate pathway is operative during build-up periods while the monoglyceride pathway predominates during fat mobilization.

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