BIOSYNTHESIS OF TRIACYLGLYCEROLS CONTAINING ISOVALERIC ACID

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Received June 12, 1972

SUMMARY

Melon tissues from the porpoise (Tursiops truncatus) were incubated with L-(U-^{14}C) leucine in Krebs-Henseleit buffer solution fortified with glucose. Rapid decarboxylation of the radioactively labelled L-leucine was accompanied by extensive incorporation of radioactivity into isovaleroyl chains of triacylglycerols. Relatively little radioactivity was incorporated into long-chain acids. The findings show that isovaleryl CoA, a catabolite of L-leucine, is directly incorporated into lipid structure to a significant degree instead of being degraded to acetate and acetoacetate by the pathway characteristic of other mammalian tissues.

It is now well established that isovaleric acid and long-chain iso acids are major constituents of the triacylglycerols from acoustic tissues of porpoises (1-4) and whales (5). Moreover, isovaleric acid was recently detected in alkyldiacylglycerols from the pink portion of the harderian gland of the New Zealand white rabbit (6). Evidence indicating that isovaleroyl glycerides are present in both marine and terrestrial animals elicits an interest in the metabolism of these recently discovered glycerolipids. We report that extensive catabolism of L-(U-^{14}C) leucine in porpoise acoustic tissues is accompanied by the direct incorporation of the isovaleroyl moiety into triacylglycerols.

EXPERIMENTAL

Incubation conditions: Fresh tissues from the interior melon, 5 cm below the apex, were obtained from a porpoise (Tursiops truncatus) immediately after death. Tissue slices weighing 2000 mg $\pm$ 50 mg were incubated in 20 ml of Krebs-Henseleit bicarbonate buffer (7). The solution was fortified with 0.02 M glucose and contained 9 μc of L-(U-^{14}C) leucine (240 mc/mM) (Dhom Products, Ltd., Los Angeles). The incubation medium was equilibrated at 37.5°C with 95% O_2 - 5% CO_2 (pH 7.4) (8). Two experiments were conducted for each time interval

varying from 15 to 300 minutes, one for the collection of evolved CO_2 from L-(U-^{14}C) leucine catabolism and the other for the radioassay of the triacylglycerols.

Carbon dioxide analyses: Carbon dioxide was collected as described previously (8) except that hyamine hydroxide (New England Nuclear Corp., Boston) was used as the trapping agent. The radioactivity associated with CO_2 was analyzed at four time intervals in a Packard Tri-Carb liquid-scintillation spectrophotometer (9).

Lipid analyses: The reaction mixtures were immediately frozen in dry ice at the end of each incubation time. Total lipid, extracted by the method of Hansen and Olley (10), comprised 73% of the interior melon tissues, as determined by gravimetric analysis (11). Triacylglycerols were separated by thin-layer chromatography in hexane:diethyl ether (90:10, v/v) (3) against markers trioleoylglycerol and diisovaleroylmyristoylglycerol. The purity of the triacylglycerols was shown to be greater than 98% by methods previously described (12). The triacylglycerols were transesterified with butanol and catalytic amounts of H_2SO_4 (13). Resulting butyl esters were purified and analyzed by gas-liquid chromatography using the conditions reported elsewhere (1). In addition, triacylglycerols were saponified and resulting acids were subjected to a short-path distillation at atmospheric pressure to separate isovaleric acid (b.p. 176.5°C) from long-chain acids (C_9 - C_{18}). The purity of the distillate (> 99%) as well as the absence of isovaleric acid in the residual long-chain fatty acids was confirmed by gas-liquid chromatography. Moreover, analyses of the primarily saturated residual acids by gas-liquid chromatography revealed that the composition was essentially the same as that of the long-chain acid fraction before distillation. The isovaleric acid and the residual long-chain acids were assayed for radioactivity as previously described (14).

RESULTS

The radioactivity associated with evolved CO_2, a measure of the

Table I.

Conversion of L-(U-^{14}C) leucine to carbon dioxide and incorporation of radioactivity into total lipids from porpoise (Tursiops truncatus) melon tissues.*

Sample	Time (Min)	^{14}C Recovered (total cpm)		^{14}C (% Administered Dose)	
		CO_2	Total Lipid	CO_2	Total Lipid
1.	15	53,300	63,400	0.30	0.30
2.	45	341,000	542,000	1.70	2.70
3.	120	573,000	951,000	2.86	4.80
4.	300	508,000	892,000	2.54	4.50

*Incubation conditions, separation and analytical techniques are described in the experimental section.

decarboxylation of the L-(U-^{14}C) leucine, is given in Table I. After 2 hr the radioactivity present in CO_2 represented 2.9% of the administered dose or 17.4% of the radioactivity of the α-carbon atom of the L-(U-^{14}C) leucine. At the same time interval, 4.8% of the radioactivity of the administered dose was found in total lipids (Table I). Thus, extensive catabolism of L-(U-^{14}C) leucine was accompanied by substantial incorporation of radioactivity from this amino acid into lipids.

Triacylglycerols comprised 85.5% of the lipids from the melon tissues. These glycerides contained 46.5 mole% of isovaleric acid and 22.4 mole% of long-chain iso acids. At each time interval, greater than 90% of the radioactivity of the total lipids was present as triacylglycerols. Moreover, radioassay of the isovaleric acid and long-chain acids in the triacylglycerols revealed that almost all of the radioactivity was associated with isovaleric acid (Table II). In 15 min the radioactivity in isovaleric acid represented 0.28% of the administered dose; however, a maximum value of 4.4% was obtained

Table II.

Incorporation of radioactivity into acids of triacylglycerols from porpoise (Tursiops truncatus) melon tissues.*

Sample	Time (Min)	Specific Activity (cpm/mmole)		^{14}C Recovered (total cpm)		^{14}C (% Administered Dose)	
		Isovaleric Acid	Long-chain Acids	Isovaleric Acid	Long-chain Acids	Isovaleric Acid	Long-chain Acids
1.	15	21,200	1,610	56,000	4,900	0.28	0.03
2.	45	182,000	2,880	480,000	8,800	2.40	0.04
3.	120	330,000	12,800	910,000	39,000	4.40	0.20
4.	300	196,000	28,300	610,000	86,000	2.60	0.43

*Incubation conditions, separation and analytical techniques are described in the experimental section.

after 2 hr. In 5 hr the radioactivity of the long-chain acids reached a level of only 0.43% of the administered dose.

DISCUSSION

The rapid uptake of radioactivity into the triacylglycerols shows that L-leucine is an active precursor of these glycerides. Moreover, the presence of considerable radioactivity in isovaleric acid indicates that isovaleryl CoA was directly incorporated into lipid to a significant extent. Although it was shown previously that L-(U-^{14}C) leucine is readily catabolized in adipose tissues (8), no evidence was found for the direct incorporation of the isovaleroyl moiety into lipids. Thus, a unique feature of the present system is the fact that much of the isovaleryl CoA, which is rapidly degraded to acetate and acetoacetate in other mammalian tissues (8), is used instead for the biosynthesis of isovaleroyl chains of glycerides. This aspect of L-leucine catabolism has not been demonstrated previously. A similar pathway for the utilization of L-leucine may exist in the biosynthesis of the alkyldiacylglycerols containing isovaleric acid in the harderian glands (6).

Because isovaleryl CoA is actively involved in the formation of lipid in this tissue, the possibility existed that the long-chain iso acids are biosynthesized via chain-elongation of isovaleryl CoA with malonyl CoA (15). However, the very low levels of radioactivity in the long-chain acids indicate that neither isovaleryl CoA nor acetyl CoA derived from L-leucine is an effective precursor of the long-chain acids under the experimental conditions. Alternatively, the chain-elongation reaction involving isovaleryl CoA or acetyl CoA may proceed at a very slow rate in comparison to the direct incorporation of isovaleryl CoA into triacylglycerols.

Although we have shown that L-leucine is a major source of isovaleric acid in the triacylglycerols, further work should be directed toward the possibility that the key intermediate, isovaleryl CoA, may be derived under some circumstances from another source, such as β-methylcrotonyl CoA, via a reversal of the pathway of L-leucine catabolism.

Our results show that the isovaleroyl glycerides are actively formed at the site of deposition, although biosynthesis in the liver cannot be excluded at this time. The fact that Lovern (16) was unable to detect isovaleric acid in the liver of Phocoena phocoena, a porpoise containing large amounts of isovaleric acid in acoustic tissues (1), adds strength to the argument that the isovaleroyl glycerides are biosynthesized primarily in the fatty tissues where they are found.

ACKNOWLEDGEMENT

Appreciation is expressed to Dr. T. Bullock, Dr. A. Benson, Dr. R. Phleger, Mrs. E. Baker, Mr. W. Bloodworth, and Mr. W. Reed of the Scripps Institution of Oceanography, La Jolla, California, for their generous help and advice. This work was conducted under Office of Naval Research Contract N00014-72-A-0202-0001.

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