PARTIAL DEGRADATION OF 4.7.10.13.16-DOCOSAPENTAENOIC ACID IN RAT LIVER

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1. Introduction

While the biosynthetic pathway [1–4] as well as the catabolic metabolism via β-oxidation [3, 5] has been firmly established for unsaturated fatty acids, the status of partial degradation is not nearly so clear. A great deal of evidence, both direct and indirect, has indicated that such a reaction might take place. As long as thirty years ago, Nunn and Smedley-MacLean [6] observed an increase of the amount of 5.8.11.14.17-$C_{20:5}$*, in fat deficient rats when 4.7.10.13.16.19-$C_{22:6}$ was added to the diet. During the last ten years other numerous experiments were described, indicating also chain-shortening with or without loss of a double bond [4, 7–12]. For example, in 1964 it was reported that in fat deficient rats, dietary 4.7.10.13.16-$C_{22:5}$ was converted to 5.8.11.14-$C_{20:4}$ [8]. Some of these findings have been confirmed since then by metabolic studies with radioactively labelled acids [13–17]. On the other hand, careful studies [18] with specifically double-labelled 9.12-octadecadienoic and 5.8.11.14-eicosatetraenoic acids revealed that these acids are either incorporated unchanged into complex lipids, or degraded completely. Products of partial degradation could not be observed.

In order to get some insight into the mechanism of partial degradation we have studied the metabolism of 4.7.10.13.16-$C_{22:5}$ in subfractions of rat liver.

* short-hand designation of fatty acids: 5.8.11.14.17-$C_{20:5}$ is all-*cis*-5.8.11.14.17-eicosapentaenoic acid.

2. Experimental

4.7.10.13.16-$C_{22:5}$, 7.10.13.16-$C_{22:4}$, and 5.8.11.14-$C_{20:4}$ labelled with ^3H, were synthesized as described previously [14, 19].

After esterification the crude acids were purified as methyl esters by chromatography on layers of silica gel H, 0.5 and 1.0 mm, impregnated with 20% silver nitrate. The solvent system used was heptane: ethyl acetate:acetone = 50:30:20. The same solvent system was applied when the total methyl esters of one incubation had to be separated according to their degree of unsaturation. The purified methyl esters were hydrolysed with 0.2 N methanolic KOH and subsequently mixed with twice the equivalent of 0.1 N NH_4OH to obtain the ammonium salts. The animals used in these experiments were adult Wistar rats each weighing 200 ± 15 g. They were provided with a normal diet *ad libitum*. The animals were killed by decapitation.

Subcellular fractions of rat liver were prepared essentially according to the procedure of Schneider [20] with the modification of Bygrave [21].

Protein was measured by the biuret method as described by Beisenherz [22].

Glucose-6-phosphatase [23] and succinat-dehydrogenase [24] were used as marker enzymes for the microsomal and the mitochondrial fractions, respectively.

Gas chromatographic analyses were carried out on a Hewlett-Packard Gas Chromatograph, Model 5750. The glass column used was 6 feet long, 1/8 inch I.D., packed with 6% DEGS on Diatoport S, 80/100 mesh. Radioactive methyl esters were trapped after gas chromatography in 10 ml scintillator solution (4 g 2,5-diphenyl-oxazol and 0.5 g 1.4-bis-2(-4-methyl-5-

phenyloxazolyl)-benzene in 1 l toluene) and counted in a liquid scintillation spectrometer, Packard Model 3380. Unless otherwise stated each incubation contained the following: 0.5 μmole radioactive fatty acid as ammonium salt, 1 μmole coenzyme A, 10 μmole ATP, 10 μmole $MgCl_2$, 1 μmole NAD^+, 1 μmole NADH, 1 μmole NADPH, 10 μmole D,L-carnitin, 500 μmole potassium phosphate buffer (pH = 7.4, 0.2 M) and 10 mg protein of the subcellular fractions. The incubations were carried out under nitrogen at 37° and stopped after the appropriate time by adding 2 N NaOH. The total fatty acids of each incubation were separated as methyl esters and subjected to gas chromatography.

3. Results

Incubation of 4.7.10.13.16-$C_{22:5}$ with various subcellular fractions of rat liver showed that the enzyme activity for partial degradation is entirely associated with the mitochondrial fraction. Less than 2% of the acitivity of 4.7.10.13.16-$C_{22:5}$ was found in 5.8.11.14-$C_{20:4}$, or any other fatty acid, if the incubation was carried out with the microsomal fraction or the 110,000 × *g* supernatant. In the mitochondrial fraction, however, two radioactive acids were found in addition to the added 4.7.10.13.16-$C_{22:5}$.

After separating the methyl esters according to the degree of unsaturation, these two radioactive acids were found to be present in the tetraen-fraction. Hydrogenation of this fraction afforded radioactive docosanoic acid as well as eicosanoic acid, indicating that the original acids had been docosa- and eicosatetraenoic acids. Gas chromatography proved these acids to be identical with authentic 7.10.13.16-docosatetraenoic and 5.8.11.14-eicosatetranoic acids.

Between 60 and 90% of the radioactivity added to each incubation mixture was recovered with the methyl ester fraction. When incubation times were increased the absolute activity of 4.7.10.13.16-$C_{22:5}$ decreased whereas the absolute activities of 7.10.13.16-$C_{22:4}$ and 5.8.11.14-$C_{20:4}$ increased (table 1). The specific activities of the acids, however, showed markedly different behaviour. These were of the same order to magnitude and remained constant for 4.7.10.13.16-$C_{22:5}$ and 7.10.13.16-$C_{22:4}$, whereas the specific activity of 5.8.11.14-$C_{20:4}$ were lower and increased with longer incubation times. Incubation of 7.10.13.16-$C_{22:4}$ with the mitochondrial fraction gave rise to 5.8.11.14-$C_{20:4}$ as the only partial degradation product. If other subcellular fractions were used, chain shortening did not take place.

When 5.8.11.14-$C_{20:4}$ was the incubated acid partial degradation did not occur at all.

4. Discussion

The results of these studies not only verify the partial degradation of 4.7.10.13.16-$C_{22:5}$ to 5.8.11.14-$C_{20:4}$ in vitro, but also reveal some details of the mechanism that could not be elucidated by in vivo experiments. From the theoretical point of view one could consider first a chain shortening and subsequently the loss of a double bond or the reverse. This means that either 2.5.8.11.14-$C_{20:5}$ or 7.10.13.16-$C_{22:4}$ should be the intermediate. Schlenk, Gellermann and Sand postulated the existence of the former acid [13].

Table 1

Absolute and specific activities of 4.7.10.13.16-$C_{22:5}$, 7.10.13.16-$C_{22:4}$ and 5.8.11.14-$C_{22:4}$ at different incubation periods.

Acid	30 min		60 min		90 min		120 min		180 min	
	[dpm] [a]	$\left[\frac{dpm}{mm^2}\right]$ [b]	[dpm]	$\left[\frac{dpm}{mm^2}\right]$	[dpm]	$\left[\frac{dpm}{mm^2}\right]$	[dpm]	$\left[\frac{dpm}{mm^2}\right]$	[dpm]	$\left[\frac{dpm}{mm^2}\right]$
4.7.10.13.16-$C_{22:5}$	13,400[c]	48±4	8950	45±6	8450	43±3	7700	44±4	5300	45±6
7.10.13.16-$C_{22:4}$	900	45±5	2085	42±3	2800	40±4	2800	40±5	3250	43±5
5.8.11.14-$C_{20:4}$	750	3.5±0.3	1940	8.6±0.4	2900	10.3±0.3	3500	11.5±0.4	4420	14.5±0.8

[a] Absolute activity of individual peak in one gas chromatogram.

[b] Specific activity of individual methyl ester expressed as absolute activity of one peak divided by the peak area.

[c] Values are the mean of three different incubations.

Our experiments indicate that the retroconversion of 4.7.10.13.16-$C_{22:5}$ takes place in rat liver only in mitochondria, not on the endoplasmic reticulum or in the cytoplasm. Moreover, they show that 7.10.13.16-$C_{22:4}$ not 2.5.8.11.14-$C_{20:5}$ is a second partial degradation product besides 5.8.11.14-$C_{20:4}$. The comparison of the specific activities (table 1) proves that 7.10.13.16-$C_{22:4}$ is the intermediate and 5.8.11.14-$C_{20:4}$ the end product. This reveals that initially the loss of the double bond, and subsequently the chain shortening, should be considered.

Both the observed chain shortening of 7.10.13.16-$C_{22:4}$ to 5.8.11.14-$C_{20:4}$ in mitochondria, and the fact that 5.8.11.14-$C_{20:4}$ is neither partially degraded nor elongated under the same incubation conditions, are in accord with this sequence of events. Similar findings too have recently been reported by Stoffel et al. [17].

How the two reactions proceed is unsolved at the moment. The simplest explanation for the first step would be a direct hydrogenation. But up to now only one mitochondrial enoyl-CoA-reductase has been described [25–27]. This enzyme with a chain length optimum at C_6 reduces a,β-trans-enoyl-CoA esters.

It is not known whether a *cis* double bond at position 4 of an acid with 22 carbon atoms is also hydrogenated by the same enzyme.

The second step is not due to the reversal of the microsomal chain elongation sequence, but might rather be catalysed by the enzymes of β-oxidation. The enzyme activities responsible for chain shortening of 7.10.13.16-$C_{22:4}$ have been shown to be closely associated with the enzyme activities of the β-oxidation cycle [17].

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