SUBSTRATE SPECIFICITY OF ENZYMES CATALYZING INTERCONVERSIONS OF LONG-CHAIN ACIDS AND ALCOHOLS IN THE RAT

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1. Introduction

It is well established that, in the gastro-intestinal tract of the rat, palmitic acid and hexadecyl alcohol are interconverted [1]. The same is true for other saturated fatty acids and the corresponding alcohols [2].

We have shown recently that in the rat's gut not only saturated but also monounsaturated fatty acids are reduced [2]. Our findings suggested that polyunsaturated fatty acids are not readily reduced to the corresponding alcohols [3, 4]. Other investigators have demonstrated that alcohols, including polyunsaturated alcohols, are oxidized to the corresponding fatty acids [5, 6] during passage through the gastro-intestinal tract.

The following experiment was designed to assess the substrate specificity of the enzymes catalyzing interconversions and interactions of fatty acids and alcohols in the gastro-intestinal tract of the rat.

Groups of rats were fed a basic diet plus supplements of equimolar mixtures of homologous saturated acids (13:0, 15:0, 17:0, 19:0), and of the corresponding alcohols, and equimolar mixtures of vinylogous acids (19:0, 19:1, 19:2), and of the corresponding alcohols. Odd-numbered fatty acids and alcohols are normally not present in the diet of rats, and the lipids of rat tissues contain only traces of such constituents Thus, odd-numbered long-chain compounds can serve as markers in studying interconversions of acids and alcohols, and other metabolic processes.

The acyl and alkyl moieties of the wax esters in the feces of the animals were analyzed to determine whether, and to what extent, dietary fatty acids were reduced to long-chain alcohols and whether, and to what extent, dietary long-chain alcohols were oxidized to fatty acids.

The results of our study confirm that, in the gastro-intestinal tract of the rat, saturated fatty acids and long-chain alcohols are interconverted. Moreover, we could show that acids differing in chain-lengths are reduced at different rates. We could also demonstrate that monounsaturated fatty acids are readily reduced to the corresponding long-chain alcohols whereas polyunsaturated fatty acids are not. Finally, the results presented in this communication suggest that the enzyme system catalyzing the esterification of acids with alcohols is rather non-specific as regards both the chain length and the degree of unsaturation of the long-chain compounds.

2. Materials and methods

2.1. *Lipids*

An equimolar mixture of tridecanoic, pentadecanoic, heptadecanoic and nonadecanoic acids, as well as a mixture of the corresponding alcohols, were prepared from an equimolar mixture of dodecyl, tetradecyl, hexadecyl and octadecyl methanesulfonates [7]. Similarly, an equimolar mixture of nonadecanoic, *cis*-10-nonadecanoic and *cis, cis*-10, 13-nonadecadienoic acids, as well as a mixture of the corresponding alcohols, were prepared from an equimolar mixture of octadecyl, *cis*-9-octadecenyl and *cis, cis*-9, 12-octadecadienyl methanesulfonates [7].

Model mixtures of various lipid classes and reference mixtures of methyl esters as well as alkyl acetates were purchased from Nu-Chek-Prep, Elysian, Minnesota 56028, USA.

2.2. *Animals and their diets*

Groups of 4–5 week old Wistar rats (SW-50), five in each group, were fed *ad libitum*, for 14 days a basic diet consisting of 'Altromin R' (Fa. Altrogge, 4910 Lage, Germany) and water.

Group A: the control group, received the basic diet;
Group B: the basic diet plus 200 mg/day/animal of an equimolar mixture of homologous acids (13:0, 15:0, 17:0, 19:0);
Group C: the basic diet plus 200 mg/day/animal of an equimolar mixture of homologous alcohols (13:0, 15:0, 17:0, 19:0);
Group D: the basic diet plus 200 mg/day/animal of an equimolar mixture of vinylogous acids (19:0, 19:1, 19:2);
Group E: the basic diet plus 200 mg/day/animal of an equimolar mixture of vinylogous alcohols (19:0, 19:1, 19:2).

The supplements of acids and alcohols were each dissolved in 400 mg of olive oil and fed by stomach tube. The feces of the animals were collected daily.

2.3. *Lipid analysis*

Samples of rat feces were stored in hexane at −10°C. The hexane was decanted, evaporated under vacuum, and the residue was taken up in chloroform–methanol (2:1, v/v), which was used later for the extraction of lipids. The feces were homogenized in twenty times their weight of chloroform–methanol (2:1, v/v), the homogenate was filtered, and the crude lipid extract was purified following established procedures [8]; extra volumes of 0.37% aqueous potassium chloride solution were used for washing to make up for the low water content of the feces.

Total lipid extracts were applied as a band to layers of Silica Gel H, 0.5 mm in thickness. The plates were developed in jars lined with filter paper using hexane–diethyl ether–acetic acid (60:40:1, v/v/v) as solvent; reference compounds were run alongside the samples to be analyzed [9]. The various fractions were detected in U.V. light after spraying the layers with 2′,7′-dichlorofluorescein solution [9]. The fraction of wax esters plus steryl esters was eluted from the adsorbent with chloroform–methanol–water–acetic acid (50:39:10:1, v/v/v/v) [10] and purified by thin-layer chromatography on Silica Gel H using hexane–diethyl ether (90:10, v/v) as developing solvent [4]. Wax esters and steryl esters, which are not resolved by chromatography on silica gel, were separated from each other by thin-layer chromatography on magnesium oxide with hexane–diethyl ether–ethyl acetate (60:40:1, v/v/v) as developing solvent [11].

The wax esters isolated were subjected to methanolysis [12], and the resulting methyl esters and alcohols were resolved by thin-layer chromatography; the methyl esters derived from the acyl moieties of wax esters were analyzed by gas chromatography whereas the alcohols derived from the alkyl moieties were acetylated [13] and also analyzed by gas chromatography.

A Hewlett–Packard instrument, Model 5750 G (Hewlett–Packard, Palo Alto, California 94303, USA) equipped with a hydrogen flame ionization detector was used for gas chromatographic analyses. All samples were analyzed on a "non-polar column", 6 ft by 1/8 inch, packed with 10% UCC-W-982 on acid-washed chromosorb W, 80–100 mesh which had been treated with dimethyl-chlorosilane (Hewlett–Packard). Some of the samples were also analyzed on a "polar column", 6 ft by 1/8 inch, packed with 20% DEGS on Anachrom A, 100–110 mesh (Analabs, Inc., North Haven, Conn. 06473, USA) Helium served as carrier gas; its flow rate as well as those of air and hydrogen was adjusted to obtain optimum separations. All analyses were carried out at 170°C. In some cases, fractions of methyl esters as well as alkyl acetates of uniform chain length were isolated by gas chromatography on the non-polar column and then analyzed on the polar column.

3. Results and discussion

Small amounts of "free" fatty acids and long-chain alcohols were found in the feces of rats of the control group as well as in the feces of rats that had received supplements of acids or alcohols. Wax esters were present in much larger proportions.

Table 1 shows the composition of the acyl and alkyl moieties of wax esters isolated from the feces of rats.

The results presented in this table prove that, in the gastro-intestinal tract of the rat, saturated fatty acids are reduced to the corresponding alcohols and saturated alcohols are oxidized to the corresponding fatty acids. Monounsaturated acids and alcohols are also interconverted, though at lower rates. Polyunsaturated

Table 1
Acyl and alkyl moieties of wax esters in the feces of rats fed equimolar mixtures of homologous or vinylogous acids or alcohols.

Chain length: (number of double bonds)	Acyl moieties* (%)					Alkyl moieties* (%)				
	Control animals	Animals fed mixtures of homologous		vinylogous		Control animals	Animals fed mixtures of homologous		vinylogous	
		acids	alcohols	acids	alcohols		acids	alcohols	acids	alcohols
13:0	tr.	0.3	–			3.6	0.1	6.5		
14:0	0.8					–				
15:0	0.6	2.0	–			28.6	24.0	16.5		
16:0	13.9					11.1				
16:1	1.4					1.5				
17:0	–	3.5	14.0			3.7	14.6	38.6		
18:0	7.8					11.3				
18:1	48.6					7.5				
18:2	12.0					tr.				
18:3	6.0					–				
19:0	–	4.9	1.8	1.5	0.4	0.8	2.4	35.5	4.8	32.8
19:1	–			1.8	0.1	tr.			4.6	33.7
19:2	–			0.9	0.1	–			0.5	22.9
20	7.2					6.5				
20	–					25.1				

* Only acyl and alkyl moieties corresponding to the dietary acids and alcohols are listed for the experimental groups.

acids, however, are not readily reduced to alcohols. As some of the unsaturated fatty acids are oxidized to hydroxy fatty acids while passing through the gastro-intestinal tract [14, 15] it is understandable why polyunsaturated acyl and alkyl moieties occur only in small proportions in the wax esters found in the feces of rats fed polyunsaturated fatty acids.

It is evident that the formation of wax esters proceeds in a rather non-specific manner. If supplied with the diet, saturated, monounsaturated and polyunsaturated long-chain alcohols are esterified at comparable rates.

In many animal tissues long-chain alcohols serve as precursors of alkoxylipids [16]. As polyunsaturated alcohols are not found in mammalian tissues where both saturated and monounsaturated alcohols occur [17, 18], it is obvious why both alkyl and alk-1-enyl moieties in the alkoxylipids of mammalian tissues are exclusively saturated and monounsaturated [19].

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