TRIHYDROXY LONG-CHAIN BASES IN BOVINE MILK SPHINGOMYELIN

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1. Introduction

Trihydroxy long-chain bases, which are the principal bases in plant sphingolipids, are also found in protozoa, yeasts and fungi [1]. Saturated trihydroxy (t:0) bases have not been reported in mammalian tissues other than kidney where they are sometimes present in significant amounts [1–6]. In a previous paper [7] from this laboratory it was reported that there were no t:0 bases in bovine milk sphingomyelin. Recent work with other milk samples suggested that there were traces of t:o bases present, and prompted re-examination of the original sample. This communication describes the composition of the t:0 bases and their apparent relationship to the dihydroxy long-chain bases in bovine milk.

2. Materials and methods

Bovine milk sphingomyelin [7] was isolated and converted to ceramides. Thin-layer chromatography (TLC) on borate-impregnated silica gel layers gave four ceramides (A–D) [7]. It was necessary to grossly overload the plates (for separation of ceramides A and B) to obtain adequate amounts of ceramide D from the milk sample. Ceramide D from milk sphingomyelin (< 1% of total ceramides), consisting of t:0 bases and normal fatty acids, was isolated for further examination. Aldehydes prepared from the intact ceramide by oxidation of the t:0 base moieties with sodium metaperiodate [8] were analysed by gas-liquid chromatography on a polar (EGSS-X) liquid phase (conditions as in [7], but column 10 ft long).

Ceramides A and B, containing saturated dihydroxy (d:0) and *trans*-4 unsaturated dihydroxy (d:1) bases, respectively, were analysed for comparison. The ceramides were hydrolysed with 2 M KOH in methanol as before [7], the hydrolysate adjusted to pH < 2 with HCl and extracted with chloroform–methanol–water [9]. The free fatty acids and long-chain bases were separated by preparative TLC on silica gel G with chloroform–methanol–acetic acid (90:10:1) as solvent. The plates were neutralised with ammonia and the bases detected with 2′,7′-dichlorofluorescein. The bases were eluted from the silica gel with methanol and oxidised immediately with sodium metaperiodate [8]. This revised procedure was found to give better results than the method [7] based on oxidation of base-dinitrophenyl derivatives.

3. Results and discussion

The t:0 bases (table 1) are similar to the d:1 bases or total (hydrogenated) bases. The bases contain a substantial proportion of branched structures (notably *i* d18:0, *i* d18:1 and *i* t18:0), and it has been suggested that these may be derived from human micro-organisms [1, 7]. Since t:0 bases are not characteristic of mammalian sphingolipids they too may be of exogenous origins, but this seems unlikely for two reasons. Firstly, their composition in milk sphingomyelin is quite different from t:0 bases from any plant or other exogenous source; and secondly, their composition is very similar to the dihydroxy bases which are largely synthesised by the cow. This similarity in composition between d:1 and t:0 bases is also seen in bovine kidney spingolipids [10], and suggests that the cow can synthesise t:0 bases [1] from the same fatty acids as are used for synthesis of dihydroxy bases. This possibility is now being investigated.

Table 1
Composition[a] of long-chain bases in bovine milk sphingomyelin.

Chain length[b]	Long-chain base type			
	d:0	d:1	t:0	total, hydrog.
14	0.9 ± 0.2	0.5 ± 0.0		
16	54.0 ± 1.5	26.4 ± 1.6	22.0 ± 1.5	27.4 ± 0.6
i 17	0.9 ± 0.0	1.4 ± 0.1	2.0 ± 0.3	1.3 ± 0.1
17	6.6 ± 0.2	8.5 ± 0.3	10.7 ± 0.5	8.9 ± 0.2
i 18	4.3 ± 0.2	11.7 ± 0.3	9.2 ± 0.3	11.2 ± 0.1
18	29.6 ± 1.3	45.8 ± 0.7	49.0 ± 1.2	45.5 ± 0.5
i 19	0.8 ± 0.1	1.1 ± 0.2	1.9 ± 0.5	1.3 ± 0.1
ai 19	1.3 ± 0.2	3.3 ± 0.4	3.8 ± 0.6	2.9 ± 0.2
19	0.5 ± 0.0	1.0 ± 0.1	1.4 ± 0.4	1.1 ± 0.1
20	1.1 ± 0.1	0.3 ± 0.0		0.4 ± 0.0
Percentage of total bases	17	82	< 1	100

[a] Values are given as uncorrected weight percentages ± 1 standard deviation. Sizes of samples were d:0 = 6, d:1 = 9, t:0 = 10.
[b] Prefix *i* = iso, *ai* = anteiso.

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