Structure and Function of Sphingolipids

1. Differences in Sphingolipid Long-chain Base Pattern between Kidney Cortex, Medulla, and Papillae*

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Kidney is a suitable tissue for attempts to correlate sphingolipid structure with function, as almost all known animal sphingolipids have been detected in this organ,[1-4] and as there is a complexity of functions, most of which are transport processes associated with membranes. In the present work, which is the first in a series of investigations on kidney sphingolipid topography, the long-chain bases, specific constituents of the sphingolipids, were prepared from kidney cortex, medulla, and papillae.

One adult human kidney (autopsy material) and one bovine kidney were macroscopically dissected into cortex and medulla, the latter including papillae. Papillae were separately dissected from several bovine kidneys. Homogenized and lyophilized tissues were extracted with hot chloroform-methanol (2:1, v/v), and to avoid losses of polar lipids, no partition against water was made. The evaporated extracts were refluxed for 6 h in 2 M HCl in water,

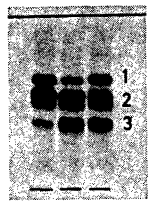

Fig. 1. Thin layer chromatogram of purified DNP derivatives of bovine kidney long-chain bases. From left to right: cortex, medulla, and papillae. Derivatives of the following bases are seen: (1) saturated dihydroxy bases, (2) unsaturated dihydroxy bases with *erythro* (above) and *threo* isomers, and (3) trihydroxy bases. Silica gel G (Fluka) containing 2 % sodium borate was used as adsorbent and hexane-chloroform-methanol 50:50:20, v/v/v, as solvent.

partitioned [4] and separated into fatty acids and long-chain base hydrochlorides on silicic acid.[4] The base hydrochlorides were converted to their corresponding dinitrophenyl (DNP) derivatives [4] and these were fractionated on silicic acid to remove reagent products and

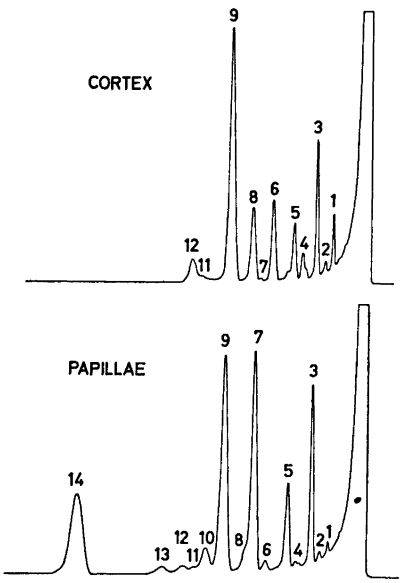

Fig. 2. Gas chromatograms of aldehydes derived from long-chain bases of bovine kidney cortex and papillae. For peak identity, see Table 1.

* *Abbreviations used in the present paper:* For convenience, all long-chain bases with two hydroxy groups, *e.g.* sphingosine (1,3-dihydroxy-2-amino-4-*trans*-octadecene), dihydrosphingosine (1,3-dihydroxy-2-amino-octadecane) and related bases are designated dihydroxy bases. Analogously, all long-chain bases with three hydroxy groups, *e.g.* phytosphingosine (1,3,4-trihydroxy-2-amino-octadecane) and related bases, are designated trihydroxy bases. In the shorthand formulas, d indicates dihydroxy and t trihydroxy; the figure before the colon expresses chain length and the figure after the colon number of double bonds. Thus, d18:1 means sphingosine, d18:0 dihydrosphingosine, t18:0 phytosphingosine, and iso d18:1 an iso branched-chain dihydroxy compound with 18 carbon atoms and one double bond.

Table 1. Results from the long-chain base analysis of kidney cortex, medulla and papillae. The following amounts of purified DNP derivatives were prepared from the different sources (mg/g dry weight): human cortex 1.8; human medulla 2.0; bovine cortex 2.9; bovine medulla 2.3; bovine papillae 1.2.

Peak No.	Aldehyde	Relative amounts					Probable parent base
		Human		Bovine			
		Cortex	Medulla	Cortex	Medulla	Papillae	
1	14:0	trace	1	3	3	trace	t17:0 (trace of d16:0)
2	iso 15:0	—	—	1	3	trace	iso t18:0
3	15:0	2	15	11	27	12	t18:0 (trace of d17:0)
4	iso 16:0 + 14:1	2	2	3	2	trace	iso t19:0 + d16:1
5	16:0	3	5	6	3	7	d18:0
6	15:1	2	2	11	3	1	d17:1
7	17:0	—	2	trace	7	23	t20:0
8	iso 16:1	1	trace	11	4	2	iso d18:1
9	16:1	86	72	46	39	26	d18:1
10	18:0	—	—	—	trace	4	d20:0
11	16:2	3	1	1	trace	1	d18:2
12	iso 17:1	trace	—	5	3	1	iso d19:1
13	17:1	—	—	trace	trace	1	d19:1
14	18:1	—	trace	—	5	20	d20:1
	Dihydroxy bases	97	83	83	60	65	
	Trihydroxy bases	3	17	17	40	35	

acid induced by-products of long-chain bases.[4] Although some bases, especially those containing allylic groups, are lost more than others in this way, values obtained from identically treated fractions are useful for comparative purposes. The purified DNP derivatives (see Fig. 1) were oxidized and the derived aldehydes analyzed by gas chromatography.[4] For differentiation of dihydroxy and trihydroxy bases that may produce the same aldehydes,[5] dihydroxy and trihydroxy DNP derivatives were prepared by thin layer chromatography and analyzed separately. For further identification of probable parent bases of the aldehydes, comparison was made with earlier described DNP derivatives of long-chain bases, *e.g.* d16:1, d17:1, d19:1, d20:1, d18:2, t17:0, t18:0, t19:0, and t20:0 (see Refs. 5–10).

Peak 8 in Fig. 2 was analyzed by a combination of gas chromatography and mass spectrometry (this analysis was made by S. Ställberg-Stenhagen). The molecular weight was found to be 238, identical to that of peak 9, and the presence of the fragment M−41 (41 is the mass of isopropylene), not obtained from peak 9, was taken as evidence for an iso structure (suggestion by E. Stenhagen). The identification of peaks 2, 4, and 12 was based on retention times before and after catalytic hydrogenation of the bases. Evidence for the existence of branched-chain bases in other tissues was recently presented.[10-12]

As shown in Figs. 1 and 2 and Table 1, about fifteen long-chain base species are present and regional differences exist in their pattern. The relative content of trihydroxy bases is much higher in medulla than in cortex. C_{17} bases (peaks 1 and 6 in Fig. 2) are relatively more concentrated in cortex, while C_{20} bases (peaks 7, 10, and 14) are dominating in papillae and apparently absent from cortex. The branched-chain compounds (peaks 2, 4, 8, and 12) are relatively more abundant in cortex than in papillae. Finally, dihydroxy bases show the same chain length distribution as trihydroxy bases (*cf.* peaks 5, 9, and 14 with 1, 3, and 7).

Although comparison between bovine (fresh) and human (aged, and from autopsy) kidney must be done with caution, some species differences seem to exist: bovine kidneys contain more of trihydroxy bases, and more of the branched-chain compounds.

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An Extracellular Glucan Produced by the Rot Fungus *Stereum sanguinolentum*

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The enzymes produced by the rot fungus *Stereum sanguinolentum* (Basidiomycetes) have been studied by Eriksson *et al.*[1-3] When the fungus was cultivated in a medium containing cellulose as the carbon source, an extracellular, slimy polysaccharide was formed. The same polysaccharide was produced in a higher yield when cellobiose was used as the carbon source, and the present paper reports structural studies on this polysaccharide.

The polysaccharide, which caused the culture solution to become viscous, was isolated by precipitation as a fibrous material which was insoluble in water. It gave $[\alpha]_{578}$ + 10° (1 M KOH) and on acid hydrolysis yielded D-glucose only, showing that it was a β-glucan. Two methylations with dimethylsulphinyl sodium-methyl iodide, following the procedure devised by Sandford and Conrad,[4] yielded the fully methylated polysaccharide. The mixture of methylated sugars obtained on acid hydrolysis of this material was converted into the alditol acetates and analyzed by GLC [5] — mass spectrometry.[6] A unique mass spectrum is obtained for each substitution pattern in the partially methylated alditol acetates. As all the methyl ethers are derived from D-glucose, the components have been fully identified by their mass spectra. The identifications were further confirmed by the *T*-values, which were the same as those given by the corresponding authentic substances.

The methylation analysis, summarized in Table 1, shows that the polysaccharide consists of a backbone of β-(1→3)-linked D-glucose residues, with a branch in the 6-position on approximately every third residue. The insolubility of the polysaccharide prevented the application of periodate oxidation studies, enzymic hydrolysis or other methods, which might have given information about the length

Table 1. Methyl ethers from the hydrolysate of the methylated glucan.

Sugars	T [a]	mole %
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.00	27.5
2,4,6-Tri-*O*-methyl-D-glucose	1.95	46.0
2,4-Di-*O*-methyl-D-glucose	5.10	26.5

[a] Retention times of the corresponding alditol acetates on the ECNSS-M column relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

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