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Higher expression of renal sulfoglycolipids in marine mammals

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Abstract Patterns and contents of major acidic glycosphingolipids in the kidney of three marine mammalian species, the Steller sea lion (Pinnipedia), the rough-toothed dolphin and the broad-beaked dolphin (Odontoceti), were examined, and compared with those of terrestrial mesic mammals. The profile of major acidic glycosphingolipids was not significantly different between the terrestrial and marine mammals: predominant gangliosides were GM3 and GD3, and major sulfoglycolipids were SM4s and SM3. On the other hand, the total concentration (nmol/g wet tissue) of sulfoglycolipids was considerably higher in the marine mammals (2.3–3.0 times) than that in the terrestrial mesic mammals with comparable body weights. In contrast, there was no significant difference in the level of renal glycolipids-bound sialic acid between the marine and the terrestrial mammals. These results suggest that higher expression of renal sulfoglycolipids in marine mammals may contribute to the maintenance of osmotic balance of their body fluid against sea water.

Keywords Allometry · Gangliosides · Kidney · Marine mammals · Sulfoglycolipids

Abbreviations

GLC	gas–liquid chromatography
NeuAc	*N*-acetylneuraminic acid
NeuGc	*N*-glycolylneuraminic acid
SM4s	galactosyl sulfatide (GalCer-I^3-sulfate)
SM3	lactosyl sulfatide (LacCer-II^3-sulfate)
GM3(NeuAc)	II$^3\alpha$NeuAcLacCer
GD3(NeuAc-NeuAc)	II$^3\alpha$(NeuAcα2-8NeuAc)-LacCer
GD3(NeuAc-NeuGc)	II$^3\alpha$(NeuAcα2-8NeuGc)-LacCer
GD3(NeuGc-NeuAc)	II$^3\alpha$(NeuGcα2-8NeuAc)-LacCer
GD3(NeuGc-NeuGc)	II$^3\alpha$(NeuGcα2-8NeuGc)-LacCer

Introduction

Acidic glycosphingolipids including gangliosides and sulfoglycolipids are ubiquitous components of the cell membranes of all vertebrates. In terrestrial mammals, acidic glycolipids of the kidney and of the cultured renal cell lines consist of both gangliosides and sulfoglycolipids [3]. The possible functions of gangliosides [4] and sulfoglycolipids [2] for the maintenance of ionic homeostasis have been suggested. It was reported that the expression of sulfogly-

We dedicate this article to the memory of Prof. Ishizuka (deceased).

Abbreviations for lipids follow those of the IUPAC-IUB Joint Commission on Biochemical Nomenclature [1] and the symbols for sulfoglycolipids follow the system of Ishizuka [2].

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colipids of some mammalian renal tubular epithelial cell lines was up- and down-regulated in the hyper- and hypo-osmotic medium, respectively, for adaptation to environmental osmolarity [5–7]. It was also reported that the amount of renal glycolipid-bound sulfate was significantly higher in some marine mammals than that in the terrestrial mammals [8]. This preliminary observation, similar to the case of renal cell lines, could imply that the hyperosmotic environment may cause the increased expression of renal sulfoglycolipids to the marine mammals. In this report, we examined the patterns and concentrations of acidic glycolipids in the kidneys of three marine mammals, the Steller sea lion (Pinnipedia), the rough-toothed dolphin and the broad-beaked dolphin (Odontoceti), and compared the results with those of the terrestrial mesic mammals.

Materials and methods

Kidneys of a Steller sea lion, *Eumetopias jubata*, were purchased from a meat shop at Rausu, Hokkaido Prefecture, Japan. Kidneys of a rough-toothed (*Steno bredanennsis*) and a broad-beaked dolphin (*Peponocephala electra*) were generous gifts from Dr. Shin-ichiro Kawai (Osaka City Institute of Public Health and Environmental Science, Japan).

The extraction and fractionation procedures of renal acidic glycolipids from the sea lion were similar to those described previously [9]. The sugar compositions of isolated glycolipids were determined as trimethylsilyl methylglycosides by gas–liquid chromatography (GLC) [9]. The neuraminic acid species were determined by the method of Yu and Ledeen [10]. Further structural confirmations including methylation analyses and ^{1}H-NMR spectroscopy were performed as described [11]. For quantitative estimation of sulfoglycolipids (galactosyl sulfatide (SM4s) and lactosyl sulfatide (SM3)), their hexose contents were determined by the anthrone-sulfuric acid method [12] using galactose and lactose as the standard, respectively. Glycolipid-bound sialic acids of gangliosides were determined by the periodate-resorcinol method [13] using *N*-acetylneuraminic acid (NeuAc) as the standard. Content of other minor glycolipids was estimated by GLC using mannitol as the internal standard. Their structures were also identified by TLC, one- and two-dimensional ^{1}H-NMR spectroscopy, mild acid hydrolysis, and compositional and methylation analyses.

Renal acidic glycolipids of the two dolphins were extracted, and separated using columns of DEAE-Sephadex A-25 (Pharmacia Japan, Tokyo) [9]. In accordance to their elution profiles, tubes were combined into three groups, monosialosyl fractions, sulfoglycolipid fractions, and disialosyl fractions. For sulfoglycolipid fractions, SM4s and SM3 were separated by silica beads (Iatrobeads (6RS-8060)) (Iatron, Tokyo) column chromatography and the contents were determined as above. Glycolipid-bound sialic acids of mono- and disialosyl fractions were determined as mentioned above. Percentage distributions of GM3 and

Table 1 Composition of the renal predominant acidic glycolipids

	Rough-toothed dolphin	Broad-beaked dolphin	Sea lion
Body weight (kg)	136	228	450
Kidneys wet weight (g)	830	947	2,400
Observed glycolipid-bound sulfate (nmol/g wet tissue)	340.4	237.4	261.2
SM4s (%)	94.7	94.2	96.5
SM3 (%)	5.3	5.8	3.5
Calculated glycolipid-bound sulfate (nmol/g wet tissue)[a]	112.0	105.2	96.9
Observed/calculated ratio	3.0	2.3	2.7
Glycolipid-bound sialic acid (nmol/g wet tissue)	83.5	92.2	62.3
Mono-sialosyl fraction (nmol NeuAc/g wet tissue)	35.5	48.9	46.8
GM3(NeuAc) (%)	41.7	45.7	98.2
GM3(NeuGc) (%)	57.0	31.6	–
Others (%)	3.2	22.7	1.8
Di-sialosyl fraction (nmol NeuAc/g wet tissue)	48.0	43.3	15.5
GD3(NeuAc-NeuAc) (%)	36.3	44.4	91.4
GD3(NeuAc-NeuGc) (%)	9.9	8.2	–
GD3(NeuGc-NeuAc) (%)	ND	ND	–
GD3(NeuGc-NeuGc) (%)	35.2	18.9	–
Others (%)	18.6	28.5	8.6

[a] Calculated by substituting the body weights of marine mammals for x in the equation (y=468 $x^{-0.121}$) (y, nmol/g wet tissue; x, g)
ND Not determined, – not detected

GD3 in mono- and disialosyl fractions, respectively, were analyzed by TLC-densitometry [14]. Briefly, ganglioside mixtures from each fraction were separated on TLC and detected by the resorcinol-HCl reagent [15]. TLC was performed on Silica Gel 60 high performance TLC plates (Merck, Darmstad) using the following two solvent systems: chloroform–methanol–0.2% $CaCl_2$ (55:45:10 *v*/*v*) and chloroform–methanol–3.5 M NH_4OH (60:40:9 *v*/*v*). Densitometric scanning was performed with Chromoscan 3 (Joyce Loeble, Gateshead).

Results and discussion

Compositions of the predominant acidic glycolipids in the kidney of the sea lion, and rough-toothed and broad-beaked dolphins are summarized in Table 1.

The sulfoglycolipids of these marine mammals consisted of SM4s (94–97%) and SM3 (4–6%). The sialic acid species of gangliosides of the two dolphins consisted of both NeuAc and NeuGc, indicating that two GM3 and four GD3 isomers theoretically exist. However, GD3(NeuGc-NeuAc) could not be detected due to low quantity and/or interference by the other GD3 isomers on TLC. The approximate ratios of GM3 isomers, GM3(NeuAc):GM3(NeuGc), of the rough-toothed and the broad-beaked dolphin were 4:6 and 6:4, respectively. The ratios of GD3 isomers, GD3(NeuAc-NeuAc):GD3(NeuAc-NeuGc):GD3(NeuGc-NeuGc), were 4.5:1:4.5 in the rough-toothed dolphin, and 6:1:3 in the broad-beaked dolphin.

In contrast, NeuGc was not detected in the kidney of sea lion. It could be attributed to the phylogenic difference between the sea lion (Pinnipedia) and the dolphins (Odontoceti). It was reported that NeuGc was expressed neither in human kidney (Primates) [16, 17] nor in the cell line derived from a dog (Fissipedia) kidney (MDCK) [18]. The minor gangliosides from the kidney of the sea lion were identified as follows: GM1 ($II^3\alpha NeuAcGg_4Cer$) (1.8 nmol/g wet tissue), GD1a ($II^3\alpha$, $IV^3\alpha(NeuAc)_2Gg_4Cer$) (0.3), and GD1a-GalNAc (II^3,$IV^3\alpha(NeuAc)_2$,$IV^4GalNAcGg_4Cer$) (1.3).

The major species of renal gangliosides (GM3 and GD3) and sulfoglycolipids (SM4s and SM3) in these marine mammals were well coincided with those in all terrestrial mammals investigated except the house musk shrew which contains GM2 as the major ganglioside [9], suggesting that there was no significant difference in the profile of renal acidic glycosphingolipids between the terrestrial and marine mammals.

In our preliminary estimation using five terrestrial mesic mammals, a linear inverse correlation (allometric equation) [19, 20] was observed between the log of the concentration of renal sulfoglycolipids (*Y*, μmol/g wet tissue) and the log of body weight (*X*, kg) as below [8].

$$\log Y = -0.699 - 0.124 \cdot \log X (n = 5, r = -0.995, p < 0.001),$$
$$i.e., y = 471x^{-0.124} (y, \text{nmol/g wet tissue}; x, g). \quad (1)$$

Furthermore, we determined the concentrations of sulfoglycolipids in the kidney of additional seven mesic mammals using ion chromatography, and reconfirmed the

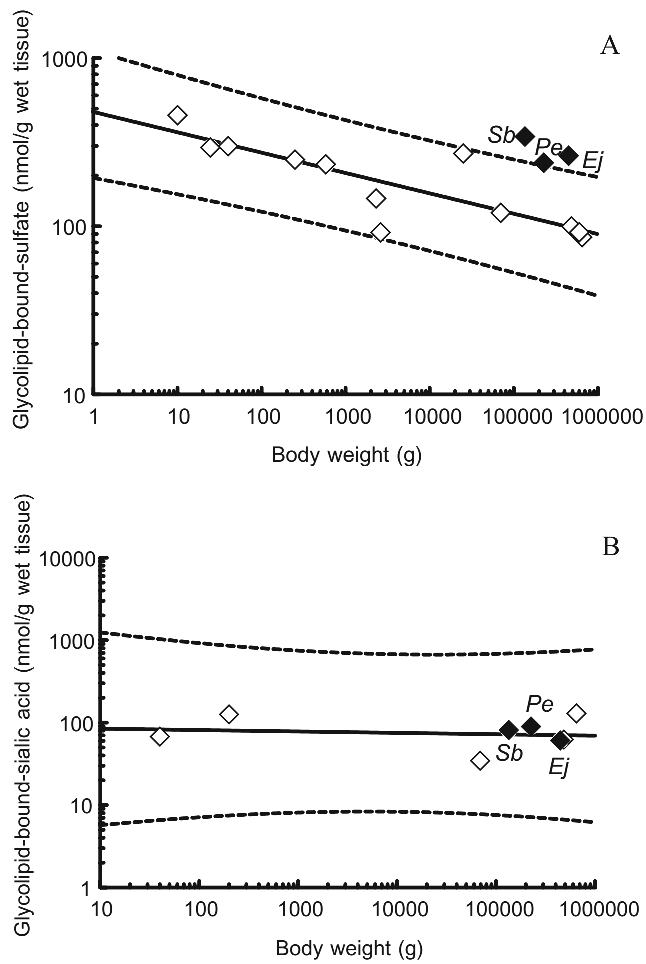

Fig. 1 Relationship between the renal concentration of glycolipid-bound sulfate or sialic acid and the body weight of mammals. The total concentrations (nmol/g wet tissue) of sulfoglycolipid sulfate (**A**) or ganglioside sialic acid (**B**) in the kidney are plotted against the body weights (g). *Open symbols* show slightly modified data on sulfate (**A**) for the terrestrial mesic mammals [21], and data on sialic acid (**B**) for the house musk shrew [9], rat [23], human [16], horse [24] and bovine [25]; *closed symbols* show the present results for marine mammals. *Ej*, *Eumetopias jubata*, Steller sea lion; *Pe*, *Peponocephala electra*, broad-beaked dolphin; *Sb*, *Steno bredanennsis*, rough-toothed dolphin. The data were transformed to logarithms and submitted to linear least squares regression analysis. *Regression lines*: **A**, $y=468\ x^{-0.121}$ (*y*, nmol/g wet tissue; *x*, g) (n=12, r=− 0.845, p<0.001), *i.e.*, log Y=−0.693−0.121•log X (*Y*, μmol/g wet tissue; *X*, kg); **B**, y=87.1 $x^{-0.0171}$ (*y*, nmol/g wet tissue; *x*, g) (n=5, r=−0.140, not significant), *i.e.*, log Y=−1.11−0.0171•log X (*Y*, μmol/g wet tissue; *X*, kg). *Dashed curves* indicate 95% confidence upper and lower limits of the *regression lines*

above allometric equation [21] with a slightly modified equation:

$$\log Y = -0.693 - 0.121 \cdot \log X \quad (2)$$

$$(n = 12, r = -0.845, p < 0.001),$$

$$i.e., y = 468x^{-0.121}.$$

According to the Eq. 2, the total concentrations (observed) of renal sulfoglycolipids (SM4s plus SM3) of the sea lion, rough-toothed and broad-beaked dolphins were found to be considerably higher (2.3–3.0 times) than those (calculated) of the terrestrial mammals with body weights comparable to these marine mammals (Table 1, Fig. 1A).

In contrast to the sulfoglycolipids, we could not obtain a significant correlation between the log concentration of renal glycolipid-bound sialic acid of five mesic mammals and their log body weight (n=5, r=−0.140, not significant) (Fig. 1B). In addition, there was no significant difference in the concentrations of glycolipid-bound sialic acids between the marine and the terrestrial mammals (Fig. 1B).

It was reported for MDCK cells that the concentration of sulfoglycolipids (SM4s plus SM3) was positively correlated with the osmolarity of culture medium (100–500 mosmol/l) made by reduction or addition of NaCl, while no correlation was observed between the concentration of GM3 and the osmolarity [5]. These data on the cultured cells are well coincided with the present results of marine mammals. Further, we observed recently that biosynthesis of the renal sulfoglycolipids was significantly elevated in rats deprived of water for 24 h [22]. Taken collectively, it is possible to speculate that the higher expression of renal sulfoglycolipids in marine mammals contributes to the maintenance of osmotic balance of their body fluid against sea water, yet the molecular mechanism remains to be elucidated.

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