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ANALYSIS OF SUGAR-CHAIN IN STERSS-COPING HUMORAL LIPOIDS

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Abstract

Stress-coping for keeping adaptation is the nature of animals. Mice given various stresses produces stress-coping substances. Mouse given forced swimming stress produces humoral lipoids preventing death of mouse given repeated immunization stress in the lipoid fractions eluted with 100mM NaCl and with 250 mM NaCl in DE-52 anion-exchange chromatography. The lipoids are not identified, however, cerebroside sulfates fractionated with 100 mM NaCl and with 250 mM NaCl is also reported to prevent mouse death induced by the repeated immunization stress. Cerebroside sulfates have sugar-chains, but analysis of the sugar-chain is difficult because of the sulfation. The stress-coping cerebroside sulfates and the stress-coping humoral lipoids were desulfated using silyl-agents and dehydrated pyridine, and were analysed the sugar-chain reactivity using lectin-ELISA method. Galbeta1-4GlcNAc reactivity was increased in the fraction eluted with 100mM NaCl of cerebroside sulfates and in that of the mouse humoral lipoids. Fucalpha1-3 reactivity was increased in the fraction eluted with 250 mM NaCl of cerebroside sulfates and in that of the humoral lipoids. These suggest stress-coping activity of the cerebroside sulfates and the mouse humoral lipoids would be relating to the sugar-chain Galbeta1-4GlcNAc or Fucalpha1-3.

Key words : stress-coping, humoral lipoid, cerebroside sulfate, desulfation, sugar-chain reactivity

Introduction

Animals produce stress-coping substances for maintaining the life. Mice given various stresses produce stress-coping substances. Mouse given forced swimming stress are indicated to produce humoral lipoids restoring physical strength in the fraction of DE-52 anion-exchange chromatography eluted with 100 mM NaCl, and inducing stress-coping behavior in the fraction eluted with 150 mM NaCl, and keeping stress-recognition in the lipoid fraction eluted with 250 mM NaCl[1,2]. The lipoids fractionated with 100 mM and 250 mM NaCl prevent death of mouse given repeated immunization stress. Cerbroside sulfates fractionated with 100 mM and 250 mM NaCl are reported to have the stress-coping activity[1]. According to PubChemCompound, cerebroside sulfates has a sugar-chain, which is Gal, Galbeta, Fucalpha1-3, or Galbeta-hexose, however, sugar-chains of the stress-coping cerebroside sulfates were not investigated because of the sulfation. In the present study, for identifying the stress-coping cerebroside sulfates and the humoral lipoids, the author confirms the stress-coping activity, performs the desulfation, and analyzes the sugar-chain reactivity.

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Methods

1. Animal

Female 9weeks-old DDY mice were purchased from SLC Co. (Hamamatsu, Japan) for using in the present study. All experiments were conditioned in accordance with animal research regulations at Akita University School of Medicine (the approval number : a-1-2824).

2. Stress-coping lipoid fractions

2-1. Lipoid fractions of mice given forced swimming stress

Ten mice were given forced swimming stress for 5 min in the apparatus previously described[3]. They were sacrificed 10 min after given the stress, and the serum was collected and pooled.

5 ml of chloroform and 10 ml of methanol were added to the 4 ml of pooled serum. The solution was intensively mixed for 3 min and incubated for 10 min at room temperature (RT). Then, 5 ml of chloroform was added to the solution, followed by intensive mixing for 30s. 4 ml of water was added to the solution, followed by intensive mixing for another 30s. The mixture was then centrifuged (150×g) for 10min at RT. The chloroform (lower) layer was collected, and the solvent was evaporated at RT. The extracted lipoids were then suspended in 4ml of water. The solution was applied to 2ml of anion exchanger DE-52 (Whatman Co., Maidstone, UK) column, which had been saturated with 10mM $NaHCO_3$, pH 8.3 and washed with water. These were eluted with 2 ml consecutive washes of 50. 100, 150, 200, 250 and 300 mM NaCl. Fractions eluted with 100 and 250 mM NaCl were then diluted to 4 ml with water as the control humoral lipoid fraction samples.

2-2. Fractionation of cerebroside sulfates

40 mg of cerebroside sulfates (CS) isolated from porcine brain (Avanti Polar Lipids Inc., Alabaster, USA) was dissolved in water, applied on the ion exchange chromatography column, and eluted with 100 and 250 mM NaCl as described above. The fractions were collected and diluted to 4 ml with water as the control CS fraction samples.

3. Stress-coping activity of the fraction samples

Stress-coping activity of the samples were examined as previously described[1]. Briefly, 6 mice were intraperitoneally injected with 1 mg/kg of ovalbumin (OVA, Grade V, Sigma-Aldrich Co., St. Louis, MO, USA) dissolved in physiological saline (PS). 10 days after the first immunization, they were given second immunization with the same OVA concentration. Three days after the second immunization, they were injected with OVA 0.5 mg/kg, and 200 μl of the control sample fractions or PS as Control. Death of the mice was assessed at 1 h after the treatment.

Fisher's exact test was used for the statistical analysis. A $p<0.05$ was considered as a significant difference.

4. Sugar-chain reactivity in the samples

4-1. Desulfating method

Silyl-agents are reported to remove acid radicals from substrates in dehydrated pyridine[4]. Elimination of sulfate radical in the samples was performed using TMS-HT kit (Tokyo Chemical Industry Co., Tokyo, Japan). Briefly, stress-coping substances were extracted from 800 μl of the control solution samples using methanol and chloroform as described above. The extracted substances were added 800 μl of the reagent containing silyl-agents and dehydrated pyridine of the kit, and then incubated at 90°C for 3 h. The reacted solutions were added 800 μl of water, and the substances in the solutions were further extracted using methanol and chloroform as described above. The extracted substances were suspended in 800 μl of water as desulfated sample solutions.

4-2. Analysis of sugar-chain reactivity

Enzyme-linked immunosorbent assay (ELISA) was performed as previously described[2]. Briefly, the desulfated sample solutions, the control fraction samples and PS as blank were prepared to 50% ethanol solution. 100 μl of the ethanol solution was poured into a well of a 96 wells plastic plate (Sumitomo Bakelite Co., Tokyo, Japan). ELISA was followed with the use of 300 μl of 5% bovine serum albumin (Sigma-Aldrich Co.) as a blocker, a biotinized lectins of Arachis hypogaea (PNA) recognizing Galbeta1-3GalNAc, Recinus communis (RCA) recognizing Galbeta1-4GlcNAc and Aleuria aurentia (AAL) recogniz-

ing Fucalpha1-3 (Seikagaku Co., Tokyo, Japan), peroxidase-conjugated avidin (Seikagaku Co.) and the coloring kit (Sumitomo Bakelike Co.) Then, the light absorbance was measured at the dual wavelength of 450/655 nm. The ELISA procedure was performed on five different plates.

Mann-Whitney U test was used for the statistical analysis. A $p<0.05$ was considered as a significant difference.

Results

1. Stress-coping activity of the fraction samples

All of 6 mice treated with the fractioned samples, of CS or mice given forced swimming stress eluted with 100 mM NaCl, escaped from death induced by repeated immunization. All of 6mice treated with the fractioned samples of CS or the mice eluted with 250 mM NaCl, also prevented the death. But 3 mice treated with PS were dead (Table 1).

2. Sugar-chain reactivity in the samples fractionated with 100 mM NaCl

Galbeta1-3GalNAc reactivity in the desulfated sample solutions of CS and in those of mice given forced swimming stress, was not different from the reactivity in the control fraction samples. Galbeta1-4GlcNAc reactivity was increased in the desulfated sample solutions of CS and in those of the mice. Fucalpha1-3 reactivity was increased in the desulfated sample solution of CS, but not

Table1. Stress-coping activity of the fractioned samples

Dead (total/6)	
Sample fractionated with	
100 mM NaCl	250 mM NaCl
Cerebroside sulfate	
0*	0*
Mice given forced swimming stress	
0*	0*
Control (200 microl PS)	
3	

*$p<0.05$ compared to Control (Fishers exact test)
PS : physiological saline

Table 2. Sugar-chain reactivity in the samples fractionated with 100 mM NaCl

	Light absorbance induced by	
	Desalfated	Control
	PNA	
CS	0.075±0.027	0.052±0.010
SM	0.070±0.008	0.066±0.014
	RCA	
CS	*0.100±0.044	0.042±0.001
SM	*0.117±0.016	0.069±0.002
	AAL	
CS	*0.197±0.015	0.113±0.005
SM	0.234±0.024	0.214±0.014
	Blank (PS)	
	0.050±0.011	

*$p<0.05$ compared to Control (Mann-Whitney U test)
PNA recognizes Galbeta1-3GalNAc, RCA recognizes Galbeta1-4GlcNAc, AAL recognizes Fucalpha1-3
CS : cerebroside sulfates of porcine brain SM : substances of mice given forced swimming stress PS : physiological saline

Table 3. Sugar-chain reactivity in the samples fractionated with 250 mM NaCl

	Light absorbance induced by	
	Desalfated	Control
	PNA	
CS	0.076±0.018	0.040±0.008
SM	0.082±0.018	0.077±0.003
	RCA	
CS	0.076±0.018	0.040±0.003
SM	0.083±0.017	0.069±0.014
	AAL	
CS	*0.179±0.024	0.055±0.003
SM	*0.327±0.012	0.192±0.030
	Blank (PS)	
	0.055±0.017	

*$p<0.05$ compared to Control (Mann-Whitney U test)
PNA recognizes GalNAcbeta1-3GalNAc, RCA recognizes Galbeta1-4GlcNAc, AAL recognizes Fucalpha1-3
CS : cerebroside sulfates of porcine brain SM : substances of mice given forced swimming stress PS : physiological saline

in the desulfated sample solution of the mice (Table 2).

3. Sugar-chain reactivity in the samples fractionated with 250 mM NaCl

Galbeta1-3GalNAc or Galbeta1-4GalNAc reactivity in the desulfated sample solutions of CS and in those of mice given forced swimming stress was not different from the reactivity in the control fraction samples. On the other hand, Fucalpha1-3 reactivity was increased in the desulfated sample solutions of CS and in those of the mice (Table 3).

Discussion

In the present study, the stress-coping CS and the stress-coping mouse humoral lipoids fractionated with 100 mM NaCl, are suggested to have a sugar-chain Galbeta1-4GlcNAc, and the stress-coping CS and the stress-coping mouse humoral lipoids fractionated with 250 mM NaCl, are suggested to have a sugar-chain Fucalpha1-3. These may indicate the stress-coping activities of the CS and the mouse humoral lipoids are relating to the sugar-chains. Stress-coping mouse humoral lipoid fractionated with 100 mM NaCl, is suggested to be induced via serotonergic neuronal activity[1]. Stress-coping mouse humoral lipoid fractionated with 250 mM NaCl, is suggested to protect hippocampal neurons from excessive adrenergic neuronal activity[5]. Serotonergic and noradrenergic neuronal activities are closely related to depressive symptoms. Depression is strongly suggested to be induced by stresses. The stress coping mouse humoral lipoids would be produced for not falling in depression. On the other hand, cerebrosides are primary contained in the brain sensing stresses, furthermore, GalNAcalpha1-3GalNAc reactivity of globopentaosylceramide having a behavioral stress-coping activity, is reported to be detected in lipoid fraction eluted with 150 mM NaCl of mice given forced swimming stress[3]. The presented stress-coping CS may be produced in the mouse fraction eluted with 100 mM or 250 mM NaCl, however, the production mechanism has not been clear.

Competing Interests

The author declares that he has no competing interests.

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