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Activities of Hexosaminidases and Hexosidases in the Brain and Spinal Cord of Rats.

YOSHIHO KATAYAMA and HANZABURO SHIDA*

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Abstract

In the present study, activities of 4 kinds of hexosaminidases and 5 kinds of hexosidases in rat brain and spinal cord were examined. All hexosaminidases in the brain were significantly higher in activity than those in the spinal cord at $p < 0.05$. The activity of N-acetyl-hexosaminidases in both organs was as the following order : N-acetyl-β-D-glucosaminidase>>N-acetyl-β-D-galactosaminidase>>N-acetyl-α-D-galactosaminidase. However activity of N-acetyl-α-D-glucosaminidase could be detected in neither brain nor spinal cord. The most active enzyme in N-acetyl-hexosaminidases was N-acetyl-β-D-glucosaminidase. The most active enzyme in hexosidases was β-galactosidase in both brain and spinal cord. And the activity in the latter organ was significantly higher than that of the former. In hexosaminidases, distribution of each enzyme activity in both organs showed the almost similar pattern, although activities themselves of hexosaminidases were higher in brain than those in spinal cord, showing 83~84% for β-GluN-ase, 12% for β-GalN-ase and 4~5% for α-GalN-ase. On hexosidase activity, β-galactosidase occupied 30 to 40% of total hexosidase activity in both organs. Furthermore β-galactosidase activity of the spinal cord was higher in both specific activity itself and distribution ratio than those of the brain.

Key words, N-acetyl-glucosaminidase, N-acetyl-galactosaminidase, glucosidase, galactosidase, mannosidase, brain, spinal cord, rat.

Introduction

N-acetyl-hexosaminidases and hexosidases are well known as the mediators which play a role in the metabolism of glycoprotein, glycosaminoglycan and/or glycolipids. It is evident by many investigations that activities of N-acetyl-hexosaminidases and hexosidases are observed in many tissues and body fluid of the mammals (1~6). The three kinds of endocrine organs of the rats (hypophysis, thyroid glands and adrenal glands), in our previous evidences indicated, showed considerable activities of some hexosaminidases (N-acetyl-β-glucosaminidase and N-

Laboratory of Nutritional-physiology and biochemistry, Department of Food Sciences and Nutrition, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606, Japan.
* Kyoto General Medico-Chemical Laboratory, Yamashina-ku, Kyoto 607, Japan.

acetyl-β-galactosaminidase) and some hexosidases (β-galactosidase, α-galactosidase, α-glucosidase and α-mannosidase) (7). Furthermore a modified technique of histochemical demonstration of N-acetyl-hexosaminidases was also presented previously (8).

Recently it was discovered that metabolic disorders of glycoprotein or glycolipid, such as Fabry's disease, G_{M1} gangliolipidosis and Tay-Sacks disease might relate to genetic defects of these enzymes (9). From these evidences, many investigators focus their interests upon the study of those fields. In the present paper hexosaminidases and hexosidases in the central nervous system have been investigated.

Materials and Methods

Substrates

Substrates used in the experiment are shown in Table 1. For hexosaminidase assay, phenyl-N-acetyl-D-hexosaminide corresponding to each enzyme was used as the substrate. The melting points and $[\alpha]_D$ of these substrates were also indicated in the table. For hexosidases substrates used in the study were phenyl-D-glycosides showing the melting points and $[\alpha]_D$ in the Table 1. These substrates were prepared in our laboratory and were in relatively high purity.

Table 1. Enzymes and their substrates with melting points and $[\alpha]_D$ of the substrates used in the experiment.

Enzymes		Substrates	m.p. $[\alpha]_D$
N-acetyl-α-D-Glucosaminidase (EC 3.2.1.50)	(α-GluN-ase)	Phenyl-N-acetyl-α-D-Glucosaminide	239℃, +210~212° (decomp)
N-acetyl-β-D-Glucosaminidase (EC 3.2.1.30)	(β-GluN-ase)	Phenyl-N-acetyl-β-D-Glucosaminide	239℃, −7 ~ 9° (decomp)
N-acetyl-α-D-Galactosaminidase (EC 3.2.1.49)	(α-GalN-ase)	Phenyl-N-acetyl-α-D-Galactosaminide	243℃, +255~257° (decomp)
N-acetyl-β-D-Galactosaminidase (EC 3.2.1.53)	(β-GalN-ase)	Phenyl-N-acetyl-β-D-Galactosaminide	230℃, +34~37° (decomp)
α-glucosidase (EC 3.2.1.20)	(α-glu-ase)	Phenyl-α-D-glucoside	172~3℃, +181°
β-glucosidase (EC 3.2.1.21)	(β-glu-ase)	Phenyl-β-D-glucoside	174~5℃, −72°
α-galactosidase (EC 3.2.1.22)	(α-gal-ase)	Phenyl-α-D-galactoside	142~3℃, +217°
β-galactosidase (EC 3.2.1.23)	(β-gal-ase)	Phenyl-β-D-galactoside	154~5℃, −43°
α-mannosidase (EC 3.2.1.24)	(α-mann-ase)	Phenyl-α-D-mannoside	131~2℃, +114°

Animals

Wistar strain male rats 2 month age were used in the experiment. Wet weights of brain and spinal cord were measured immediately after autopsy. Each organ was homogenized in buffer solution corresponding to each enzyme with a Potter-Elvehjem glass homogenizer. The homogenates were centrifuged at 4000 r.p.m. for 5 min at 4℃. The supernatants were used for the enzyme assay. If required, supernatants were diluted several times with buffer, and activator was added into incubation medium.

Assay of glycosidase activities (10, 11)

Table 2. Composition of enzyme assay system

	pH	Substrate (ml.)	Buffer (ml.)	2M.NaCl (ml.)	2 % Bovine Serum albumin in 2 M.NaCl (ml.)	dist.water (ml.)	Enzyme (ml.)
α-GluN-ase	4.5	0.5	1.0	—	0	0	0.5
β-GluN-ase	4.3	0.5	1.0	—	0.1	0.3	0.1※
α-GalN-ase	4.3	0.5	1.0	—	0	0.3	0.2
β-GalN-ase	4.3	0.5	1.0	—	0.1	0	0.4※※
	4.2	0.5	1.0	0.1	—	0	0.4
α-glu-ase	6.0	0.5	1.0	0.1	—	0	0.4
	6.6	0.5	1.0	0.1	—	0	0.4
β-glu-ase	6.0	0.5	1.0	0	—	0	0.5
α-gal-ase	4.0	0.5	1.0	0	—	0.3	0.2
β-gal-ase	3.6	0.5	1.0	0.1	—	0.3	0.1
α-mann-ase	4.8	0.5	1.0	0	—	0.1	0.4
	6.6	0.5	1.0	0	—	0.1	0.4

※ Brain 10 times diluted

※※ Spinal cord 4 times diluted

A 0.1M citric acid-citrate buffer and 0.4M phosphate-0.2M citrate buffer at the pH corresponding to each enzyme were used in the assay. Twenty mM substrates were used in the incubation medium. The composition of enzyme assay systems is shown in Table 2.

The reaction mixtures were consisted of each substrate corresponding to each enzyme, enzyme sample, activator (if required) distilled water and were filled up to 2.0 ml of final volume. The reaction mixture was incubated for 1 to 4 h in a water bath at 37℃. The optimal pH of β-galactosidase from rat brain is 5.0 according to the presentation of Gatt (10). In our observation, however, it was at 3.6 as indicated in Fig. 1. Then our enzyme assay of β-galactosidase was carried out at pH 3.6. Furthermore, as indicated in Fig. 1, two values of the optimal pH for α-glucosidase and α-mannosidase were observed at 4.2 & 6.6 and 4.8 & 6.6, respectively. The substrates were hydrolyzed by each enzymatic action and subsequently aglicon, that is, phenol was released. Estimation of released phenol was performed at pH 9.0 by the method of Akamatsu (12).

Estimation of released phenol.

At the end of the incubation period, the reaction was stopped by addition of trichloroacetic acid (TCA) (final concentration of TCA was about 2.5%). After centrifuging, 3 ml of the suppernatant was adjusted to pH 9.0 with 2 N KOH solution and about 5 ml of borax solution (pH 9.0) and 0.2ml of each 10% potassium ferricyanide and 0.2% 2, 6-dibromo-4-aminophenol

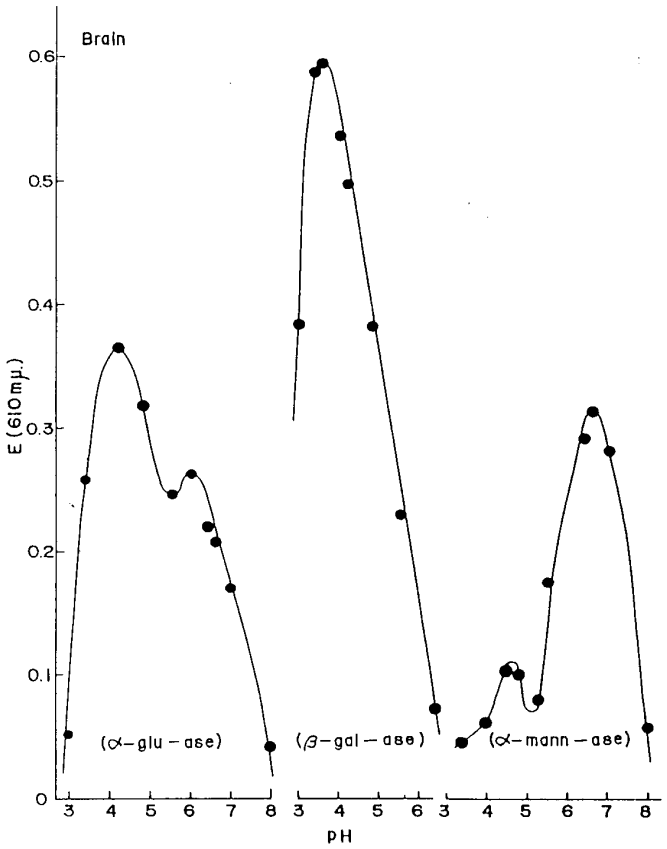


Fig. 1. The optimal pH of α-glucosidase, β-galactosidase and α-mannosidase.

were added to this supernatant. And then final volume was brought to 10 ml with borax solution. Colour was developed at 37℃ for 10 to 15 minutes and the absorption at 610 nm was measured by using a Hitachi Perkin-Elmer UV-VIS spectrophotometer (model 139). Enzyme activity was expressed as μg of phenol released per mg of tissue in 1 h.

Table 3. Body weight (g) and wet weight (mg) of Brain and Spinal cord of Rats.

No.	1	2	3	4	5	Mean ± CI
Body weight	250	290	283	325	305	291 ± 35
Brain	1749	1740	1738	1861	1816	1781 ± 69
Spinal cord	216	178	227	216	207	209 ± 28

CI : half range of confidence interval (confidence limit) at 95% level.

Results and Discussion

1) Body weights and organ weights.

At the autopsy, body weights, the wet weights of brain and spinal cord of each rat are shown in Table 3. Averages were 291± 35 g for the body weight, 1781±69 mg for the wet weight of brain and 209±28 mg for the spinal cord. When compared with data of Kozuma et al. (13), these values were the almost similar in the weights of body and brain. It might be considerd that the rats used in the experiment had nomally grown.

2) Activities of hexosaminidases and hexosidases

Activities of hexosaminidases and hexosidases of the brain and spinal cord in each rat are shown in Table 4 and 5, respectively. And averages±half range of confidence interval (confiden-

Table 4. Hexosaminidase and Hexosidase Activities in the Rat Brain
(μg. of phenol liberated/mg. of tissue in 1 hr.)

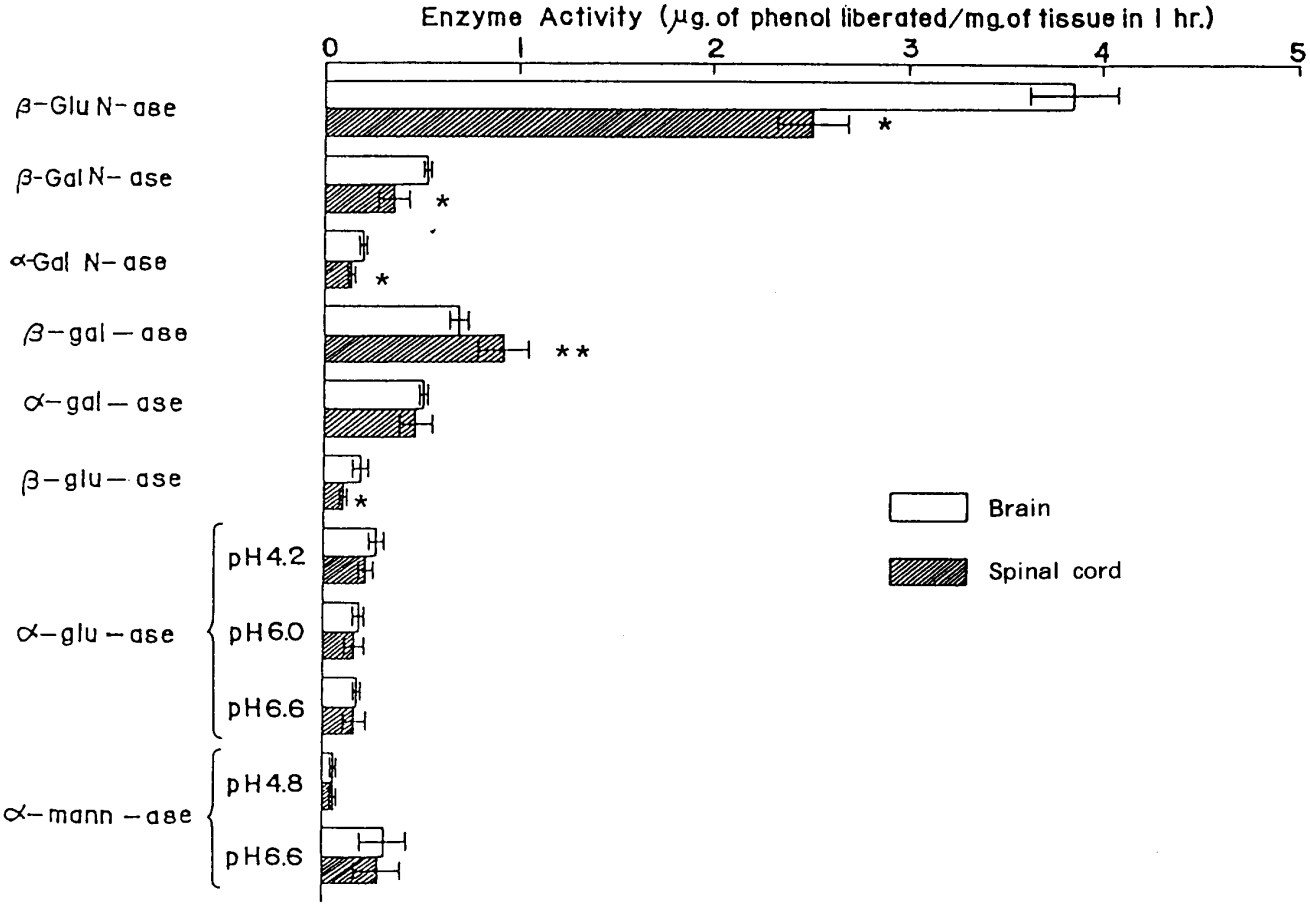
No.	β-GluN-ase	β-GalN-ase	α-GluN-ase	α-GalN-ase
1	3.63	0.51	trace	0.18
2	4.08	0.54	trace	0.19
3	3.71	0.54	—	0.22
4	3.84	0.54	—	0.20
5	3.97	0.54	—	0.20

No.	β-gal-ase	α-gal-ase	β-glu-ase	α-glu-ase			α-mann-ase	
				pH4.2	pH6.0	pH6.6	pH4.8	pH6.6
1	0.66	0.49	0.20	0.25	0.18	0.16	0.05	0.25
2	0.64	0.54	0.22	0.26	0.16	0.16	0.06	0.41
3	0.73	0.52	0.19	0.30	0.21	0.20	0.04	0.33
4	0.71	0.51	0.16	0.25	0.17	0.16	0.04	0.36
5	0.69	0.51	—	0.31	0.20	0.17	0.04	0.17

Table 5. Hexosaminidase and Hexosidase Activities in the Rat Spinal Cord
(μg. of phenol liberated/mg. of tissue in 1 hr.)

No.	β-GluN-ase	β-GalN-ase	α-GluN-ase	α-GalN-ase
1	2.50	0.36	trace	0.13
2	2.56	0.33	trace	0.13
3	2.60	0.43	—	0.13
4	2.63	0.42	—	0.16
5	2.26	0.28	—	0.17

No.	β-gal-ase	α-gal-ase	β-glu-ase	α-glu-ase			α-mann-ase	
				pH4.2	pH6.0	pH6.6	pH4.8	pH6.6
1	0.91	0.48	0.08	0.18	0.15	0.16	0.04	0.18
2	1.08	0.37	0.11	0.20	0.12	0.11	0.05	0.33
3	0.93	0.48	0.09	0.26	0.21	0.16	0.05	0.39
4	0.88	0.56	0.11	0.26	0.20	0.23	0.07	0.32
5	0.80	0.46	—	0.21	0.14	0.12	0.05	0.17



* indicates significant difference from the brain at $p<0.05$.
**indicates significantly higher activity from brain at $p<0.05$.

Fig. 2. Comparison of activities of hexosaminidases and hexosidases in the central nervous system.

ce limit) at $p<0.05$ of each enzyme activity are indicated in Fig. 2. As seen in these tables and the figure, hexosaminidases in the brain were significantly higher active than those of the spinal cord $p<0.05$. In hexosaminidases, however, activity of N-acetyl-α-D-glucosaminidase could be detected in neither brain nor spinal cord. In the both brain and spinal cord, the most active enzyme was N-acetyl-β-D-glucosaminidase, and activity of N-acetyl-hexosaminidase was as the following order: N-acetyl-β-D-glucosaminidase≫N-acetyl-β-D-galactosaminidase≫N-acetyl-α-D-galactosaminidase (symbol ≫ shows significant difference between values of both sides of symbol). The latter two enzyme activities were about one-seventh activity of the former enzyme for N-acetyl-β-D-galactosaminidase and 1/18～1/20 of that of the former for N-acetyl-α-D-galactosaminidase, respectively. The most active enzyme in hexosidases was β-galactosidase in both brain and spinal cord. Furthermore this enzyme in spinal cord was significantly higher than that of the brain at $p<0.05$. On the other hand, β-glucosidase activity was observed inverse intensity against β-galactosidase between two organs at $p<0.05$. There were, however, no detectable differences in the activities of the other enzymes. From these activities of hexosaminidases and hexosidases described above, distribution (ratios of each enzyme per total enzyme activity) of hexosaminidases and hexosidases in each organ was calculated here. As indicated in Table 6, distribution showed the almost similar pattern between brain and spinal cord, although activities of hexosaminidase were different in intensity between both organs. Namely 83～84%

of total activity was occupied with β- N-acetyl-D-glucosaminidase, 12% with β-N-acetyl-D-galactosaminidase and 4 ~ 5 % with α-N-acetyl-D-galactosaminidase. In both organs, large parts of hexosaminidase activity were occupied with glucosaminidase, especially β-type of the enzyme. There was, however, no activity of α-type enzyme. There were no different distributions of some hexosidase activities between brain and spinal cord except for the β-galactosidase. For β-galactosidase, distribution of activity was 32% in the brain and 42% in the spinal cord, respectively. The β-galactosidase showed higher values in both intensity of specific activity and distribution ratio in the spinal cord rather than those in the brain.

Table 6. Distribution of the enzyme activities in the brain and spinal cord.

Enzymes		Brain (%)	Splnal Cord (%)
Hexosaminidase	β-GluN-ase	84	83
	β-GalN-ase	12	12
	α-GluN-ase	0	0
	α-GalN-ase	4	5
Hexosidase	β-gal-ase	32	42
	α-gal-ase	23	21
	β-glu-ase	9	5
	α-glu-ase	12, 8 } 20	10, 7 } 17
	α-mann-ase	2, 14 } 16	2, 13 } 15

When compared with those activities in endocrine organs (7), the hexosaminidases and hexosidases in the brain and spinal cord were much less active than those of the endocrine organs. It was coincident evidence that N-acetyl-β-D-glucosaminidase was the most active in hexosaminidases and the most active enzyme in hexosidases was β-galactosidase, although N-acetyl-α-D-glucosaminidase activity was not detected.

With regard to localization of these enzymes in the central nervous system, the moter nerve cells in the ventral horn of the spinal cord showed a number of granules stained by histochemical determination in perikaryon of the cytoplasma of nerve cells without nucleus. Furthermore, in the central nervous system, Purkinje cells and nerve cells of hippocampal regions were moderately stained by histochemical methods. However granular layer cells in the cerebellum and neuroglia cells remained unstained (8).

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