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Acid Mucopolysaccharide (AMPS) Abnormality in Multiple Sulfatase Deficiency: Chemical Compositions of AMPS in Urine and Liver

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Summary

Extensive chemical analyses of acid mucopolysaccharides (AMPS) were carried out in the urine and tissues (liver and brain) from a Japanese patient and two European patients with multiple sulfatase deficiency (MSD). The Japanese patient with MSD contained excessive quantities of heparan sulfate and moderately increased chondroitin sulfate A/C. Urinary excretion of AMPS in MSD heterozygotes was increased 2-fold compared to our controls. The urinary pattern of AMPS in the mother of the MSD patient showed an increase of 18% heparan sulfate and 36% dermatan sulfate whereas the urinary excretion pattern in the father was increased 21% for heparan sulfate as contrasted to controls (chondroitin sulfate A, 50–52%; chondroitin sulfate C, 38–46%; and heparan sulfate, 3–10%).

Seventy-five % of the AMPS and the MSD liver was heparan sulfate rather than dermatan sulfate. The degree of accumulation of AMPS in the MSD liver was 30–50 times that of the control. Cerebral gray matter from the MSD patient contained 30–40 times that of control (relative increase of heparan and dermatan sulfate) whereas only a 5-fold increase was observed in white matter. It seems that a major site of accumulated AMPS appears to be in the gray matter.

Carbohydrate analysis of the AMPS obtained from MSD urine and tissues was performed by: enzyme digestion with testicular hyaluronidase, heparitinase and chondroitinase ABC, cellulose acetate electrophoresis, Dowex-1 column chromatography and amino sugar analysis by amino acid analyzer. These findings indicate that the major accumulated AMPS in MSD urine and liver is heparan sulfate and thus, the predominant AMPS metabolic defect in MSD is heparan sulfate degradation.

Speculation

The accumulation of negatively charged AMPS, particularly the increased heparan sulfate in the nervous system of patients with MSD, leads to cellular dysfunction resulting in changes in the net charge of neuronal cells.

Multiple sulfatase deficiency (MSD) is considered to be an autosomal recessively inherited disorder characterized by severe neurologic symptoms, gargoyle-like face, mild hepatosplenomegaly, ichthyosis and skeletal abnormalities (1, 20). Biochemically, the accumulations of sulfatide, acid mucopolysaccharides (AMPS) and cholesterol sulfate accompanied with deficiencies of arylsulfatases A, B and C, steroid sulfatase and acid mucopolysaccharide sulfatases (α-iduronosulfatase, heparan N-sulfatase, N-acetylgalactosamine-4-sulfatase, N-acetylgalactosamine-6-sulfatase, N-acetylglucosamine-6-sulfatase) have been described in patient's tissues and cultured skin fibroblasts (1, 2, 10, 20, 21). This disorder is of considerable theoretical interest and distinct from classical MSD, because it appears to be a single mutant gene disorder in which there are deficient activities of at least seven different enzymes involving the genes for enzymes localized in autosomal and X-chromosomes (10).

However, few descriptions provide detailed chemical analysis of AMPS and other complex carbohydrates in MSD tissues and urine. Thus, it is worthwhile to delineate the exact chemical nature of accumulated compounds so as to enhance understanding of the consequences of the multiple sulfatase deficiencies.

This report presents the detailed chemical analysis of complex carbohydrates including AMPS in liver and urine.

MATERIALS AND METHODS

Tissues and urine specimens. Autopsied tissues of two cases with MSD were kindly provided by Rampini (Department of Pediatrics, University of Zurich, Switzerland) and reported elsewhere (22). The chemical and enzymic studies of these cases were reported previously (10–12). The chemical analysis of urine specimens was carried out in the Japanese case of MSD. The clinical and biochemical observations of this case will be reported (13). One or more 24-h urine collections were obtained from patients with Hunter, Sanfilippo. Morquio, G_{M1}-gangliosidosis and compared to specimens from 10 normal children aged from 1-15 years of age.

Preparation of AMPS in urine and tissues. Isolation of urinary AMPS was performed by precipitation with 5% cetylpyridinium chloride. AMPS-uronic acid was measured by the carbazol method of Dische (8). The procedure was performed as described by DiFerrante (6). Crude tissue AMPS were prepared by methods reported previously (18, 19).

Dowex-1 column chromatography. CPC-precipitated fractions obtained from urine and tissues were subjected to fractionation by Dowex-1 column chromatography (1-X2; 200-400 mesh, Cl^{1-} form) (1 × 8 cm). The stepwise elution was accomplished by increasing salt concentration up to 3.0–5.0 M NaCl (24). Elution was monitored by measuring uronic acid content.

Electrophoresis. Electrophoresis on Sepharax membrane (Joko Sangyo, Tokyo, Japan) was carried out by Wessler's method (27) in 0.1 M barium acetate for 2 h with a potential gradient of 15 V/cm and in 0.1 M calcium acetate, pH 7.0 at 0.5 mA/cm for 2 h. The membranes were stained with 0.1% toluidine blue. The quantitative measurement of AMPS was carried out according to Saito *et al.* (23).

Enzyme digestions. Testicular hyaluronidase (Seikagaku-Kogyo, Tokyo) digestion was carried out by the method described by Fransson and Roden (16). Heparitinase digestion (a gift from

Seikagaku-Kogyo, Tokyo) was performed as described by Tokunaga *et al.* (26). Chondroitinase AC and ABC (Seikagaku-Kogyo, Tokyo) digestions were carried out by the method of Saito *et al.* (23). The unsaturated sugar mapping after digestion with chondroitinase AB and ABC was accomplished by cellulose thin layer chromatography (DC-Fettigplatten Cellulose-F, Merck, Germany) and the spots equivalent to Δdichondroitin-4S (3-O-beta-Δ-4,5-glucuronosyl-N-acetyl-D-galactosamine-4-O-SO_4) and Δdichondroitin-6S (3-O-beta-Δ-4,5-glucuronosyl-N-acetyl-D-galactosamine-6-O-SO_4) (authentic materials from Seikagaku-Kogyo, Tokyo) were extracted with 2 ml of 0.01 M HCl. Elution was carried out at 50°C for 10 min. After centrifugation, the absorbance of supernatant solution was measured at 232 μ against a reagent blank.

Determination of complexed carbohydrates in tissues. The quantitative analysis of complex carbohydrates in patient tissues was carried out by the method of Brunngraber *et al.* (5). This procedure allows the simultaneous quantitation of complex carbohydrates such as glycoproteins, glycosaminoglycans and glycopeptides.

Other chemical analysis. Hexose was determined by the orcinol method (15). Uronic acid content was determined by the carbazol method of Dische and Shettles (7). Hexosamine was measured after hydrolysis in 4 M HCl at 100°C for 15 h (19) by the Elson-Morgan method (4) and on a Hitachi amino acid analyzer. Total sulfate was measured after hydrolysis with 4 M HCl, using the $BaCl_2$-gelatin turbidity method described by Dodgson and Price (9). Sialic acid was measured by the method of Svennerholm (25). Fucose was determined as described by Dische (8).

RESULTS

Urinary AMPS in MSD patients. Figure 1 shows the quantitative analysis of the total urinary excretion of AMPS in patients with MSD heterozygote carriers of MSD, Hunter, Sanfilippo and Morquio patients and from 10 normal control subjects. Excretion of

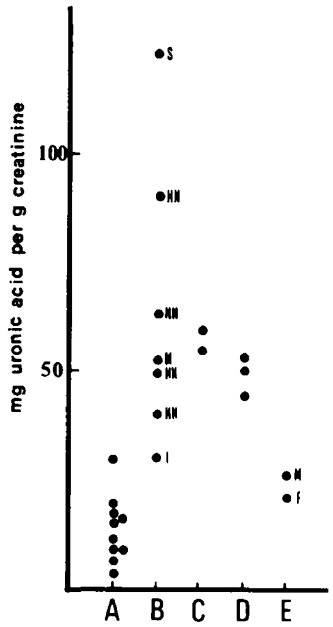

Fig. 1. Urinary acid mucopolysaccharide excretion in patients with MSD and its heterozygots, mucopolysaccharidoses, mucolipidosis and lipidosis. *Lane A*; control subjects, *lane B*, mucopolysaccharidoses and mucolipidosis (abbreviations used: S, Sanfilippo syndrome; HN, Hunter syndrome; M, Morquio syndrome; and I, I-cell disease), *lane C*; G_{M1}-gangliosidosis, *lane D*; MSD patient, *lane E*; MSD obligate heterozygote (M, patient's mother and F, patient's father). High values of uronic acid in G_{M1}-gangliosidosis are due to high hexose content by carbazol reaction. AMPS excreted in urines were expressed as μg uronic acid per g creatinine.

MSD in the MSD patients' urine was 4–5 times higher than the controls, whereas the urinary AMPS excreted by MSD obligate heterozygotes was about twice that of control subjects. Urinary AMPS excretion by the MSD patient was quantitatively equivalent to that seen in patients with mucopolysaccharidosis. Crude urinary AMPS from all these subjects were subjected to cellulose acetate electrophoresis and determined quantitatively by the spectrophotometric method. The AMPS from the MSD patients contained major components with mobilities similar to authentic heparan sulfate (50%) and chondroitin sulfate A/C (50%). On the other hand, the mother of the MSD patient excreted dermatan sulfate, heparan sulfate and chondroitin sulfate A/C, 36, 18, and 46%, respectively, whereas the patient's father excreted only heparan sulfate and chondroitin sulfate A/C, 21 and 79%, respectively. Control subjects excreted heparan sulfate and chondroitin sulfate A/C, 3–10 and 90–97% of the total AMPS, respectively.

The AMPS from control, MSD, Hunter and Sanfilippo patients were subjected for the fractionation of AMPS on a Dowex-1 column. Each AMPS fraction isolated from the Dowex-1 column chromatography was subjected for carbohydrate and analysis, performed by the orcinol, carbazol, Elson-Morgan methods, and hexosamine ratio by Hitachi amino acid analyzer (Table 1). It was also analyzed by cellulose acetate electrophoresis. The analytical data and findings with cellulose acetate electrophoresis of the AMPS indicated that excreted AMPS in MSD urine were heparan sulfate and chondroitin sulfate A/C. The identified heparan sulfate was completely digested by heparitinase and was resistant to testicular hyaluronidase and chondroitinase ABC. The chondroitin sulfate A/C fraction was completely digested by testicular hyaluronidase. Digestion by chondroitinase AB and ABC produced disaccharide 4-S and 6-S unsaturated sugar (57 and 43%, respectively).

AMPS analysis in MSD patient's tissues. The carbohydrate composition of liver and brain from MSD and G_{M1}-gangliosidosis is shown in Table 2. In "the CPC-precipitable AMPS" fraction of MSD liver, AMPS measured by the carbazol reagent was 30–40 times higher than those of controls. In the "dialyzable oligosaccharides" fraction, the MSD patient liver contained approximately twice the amount of small oligosaccharide fragments probably derived from AMPS. "The non-dialyzable glycopeptides" fraction, obtained from MSD liver, was increased about 6 times when compared with the control valve. The G_{M1}-gangliosidosis tissue accumulated about 25 times the concentration of glycopeptides in controls. Accumulated AMPS in the MSD patient's cortical gray matter was 30–40 times that of controls as uronic acid content, whereas in the white matter it was only 5 times higher than controls. Hexosamine and uronic acid contents were increased about 2–6 times higher than those of controls in the "dialyzable oligosaccharides" fractions obtained from MSD gray matters. Fucose and sialic acid concentrations were not significantly altered in each fraction of brain and liver with MSD (data is not shown). The cellulose acetate electrophoretic patterns of crude AMPS isolated from the MSD patient's liver consisted predominantly of heparan sulfate with some increase of dermatan sulfate (75, 20% of total AMPS, respectively). The relative concentrations in normal liver of dermatan sulfate, chondroitin sulfate A/C and heparan sulfate comprised 30, 45 and 25%, respectively. The heparan sulfate band was similar to the authentic standard in that it was not digested by testicular hyaluronidase and chondroitinase ABC but digested completely by heparitinase. Further characterization of MSD liver AMPS was performed by fractionation with Dowex-1 column chromatography. Major fraction of MSD liver AMPS was eluted from 1.25–1.5 M NaCl gradient. Each fraction was subjected to cellulose acetate electrophoresis and chemical analysis. The major peak eluted with 1.5 M NaCl was heparan sulfate with slightly increased dermatan sulfate and a trace of chondroitin sulfate A/C. The peak fraction eluted with 0.25 M NaCl was not AMPS because this fraction contained high amounts of hexose and hexosamine. Table 3 depicts the data of the chemical analysis of AMPS fractions isolated from Dowex-1 column chromatogra-

Table 1. *Chemical analysis of fractions eluted from Dowex-1 column chromatography in Hunter, Sanfilippo and MSD patients' urines.*[1]

Fraction	Hunter				Sanfilippo				MSD			
	Uronic acid[2]	Hexose[2]	C/O ratio	$GalNH_2/glcNH_2$	Uronic acid	Hexose	C/O ratio[4]	$GalNH_2/glcNH_2$	Uronic acid	Hexose	C/O ratio	$GalNH_2/glcNH_2$
1 (0.5 M NaCl)	0.89	1.93	0.46	9.9/90.1	0.84	0.82	1.02	0.1/99.1	1.66	1.17	1.42	13.1/86.9
2 (0.75 M NaCl)	3.60	1.30	2.76	7.2/92.8	2.65	0.61	4.34	n.d.[3]	2.04	0.83	2.46	18.8/81.2
3 (1.0 M NaCl)	3.82	1.16	3.29	21.4/78.6	1.39	0.37	3.76	n.d.	1.62	0.70	2.31	30.7/69.3
4 (1.25 M NaCl)	4.10	1.48	2.77	63.6/38.4	1.05	0.29	3.62	n.d.	1.36	0.91	1.49	72.7/27.3
5 (1.5 M NaCl)	3.82	3.67	1.04	70.4/29.6	0.34	0.15	2.26	n.d.	0.94	0.56	1.67	78.9/21.1
6 (1.75 M NaCl)	1.16	1.27	0.91	76.5/23.5	0.10	0.11	0.91	n.d.	0.04	0.02	2.0	82.3/17.7
7 (2.0 M NaCl)	0.54	0.79	0.68	80.1/19.9	0.08	0.16	0.50	n.d.	0.08	0.05	1.6	81.7/18.3

[1] Values obtained from 150 ml urine.
[2] Values for uronic acid and hexose are expressed as μmoles per fraction.
[3] Not determined.
[4] C/O ratio, carbazol to orcinol value ratio.
[5] $galNH_2/glcNH_2$, galactosamine to glucosamine ratio.

Table 2. *Carbohydrate compositions in gray matter, white matter and liver of patients with MSD, according to the method of Brunngraber (5)*[1]

Fractions	Hexose			Hexosamine			Uronic acid		
	Gray matter	White matter	Liver	Gray matter	White matter	Liver	Gray matter	White matter	Liver
"Papain-resistant materials"									
control, 1.5 y	1.32	2.61	1.34	0.83	0.87	0.39	0.58	0.47	0.38
control, 5 y	1.58	n.d.[2]	1.20	0.81	n.d.	0.51	0.41	n.d.	0.34
control, 7 y	n.d.	3.21	1.16	n.d.	1.20	0.56	n.d.	1.14	0.32
MSD, M.C.	3.21	2.50	2.97	0.51	1.04	0.48	0.51	0.54	0.69
S.A.	n.d.	2.15	n.d.	n.d.	0.81	n.d.	n.d.	0.36	n.d.
GM1-gangliosidosis	1.02	2.53	1.79	0.442	1.32	1.02	0.20	0.41	0.47
"Dialyzable oligosaccharides"									
control, 1.5 y	2.27	2.44	3.34	0.97	1.24	0.66	0.53	0.44	0.69
control, 5 y	2.61	n.d.	3.18	1.13	n.d.	0.82	0.57	n.d.	0.72
control, 7 y	n.d.	2.52	6.80	n.d.	0.95	1.16	n.d.	0.58	1.27
MSD, M.C.	4.10	6.50	13.79	2.53	2.66	2.36	2.97	1.24	3.27
S.A.	n.d.	6.36	n.d.	n.d.	2.31	n.d.	n.d.	1.18	n.d.
GM1-gangliosidosis	4.06	8.97	16.71	2.20	4.50	10.22	0.62	1.05	1.70
"CPC-precipitable AMPS"									
control, 1.5 y	0.06	0.17	1.37	0.03	0.20	0.21	0.02	0.10	0.21
control, 5 y	0.02	n.d.	1.07	0.01	n.d.	0.17	0.03	n.d.	0.21
control, 7 y	n.d.	0.18	0.64	n.d.	0.23	0.17	n.d.	0.08	0.06
MSD, M.C.	0.90	0.53	41.80	0.29	1.01	1.73	0.60	0.74	6.57
S.A.	n.d.	0.76	n.d.	n.d.	0.60	n.d.	n.d.	0.40	n.d.
GM1-gangliosidosis	0.05	0.12	3.89	0.03	0.32	2.32	0.08	0.05	0.53
"Nondialyzable glycopeptides"									
control, 1.5 y	2.94	1.42	1.63	2.03	1.33	1.03	0.33	0.18	0.22
control, 5 y	2.03	n.d.	1.62	1.70	n.d.	0.96	0.23	n.d.	0.21
control, 7 y	n.d.	1.23	0.85	n.d.	1.12	0.71	n.d.	0.14	0.12
MSD, M.C.	4.58	2.61	3.65	2.73	0.94	3.18	0.56	0.39	1.24
S.A.	n.d.	2.73	n.d.	n.d.	1.67	n.d.	n.d.	0.39	n.d.
GM1-gangliosidosis	2.85	2.44	23.87	1.06	2.18	25.64	0.18	0.22	2.67

[1] Values are expressed as μmoles per 100 mg dry tissues.
[2] Not determined.
[3] 1.5 y, 5 y and 7 y indicate the age (years) of patients.

phy. The data indicate that major AMPS in MSD liver was heparan sulfate with a slight increase of dermatan sulfate.

DISCUSSION

AMPS metabolism in MSD patients has not been previously studied in detail. Bischel *et al.* (3) reported that a major compound excreted in MSD patient's urine and in the kidney might be heparan sulfate, whereas Rampini *et al.* (22) reported higher excretion of dermatan sulfate and heparan sulfate in two patients. Our case excreted heparan sulfate as the major AMPS with some increase in chondroitin sulfate A/C in urine. Isolation and characterization of isolated AMPS by Dowex-1 column chromatography, enzyme digestion and chemical analysis confirmed that the major AMPS excreted were heparan sulfate and chondroitin sulfate A/C. The proportion of heparan sulfate and chondroitin sulfate A/C was about 50/50. The ratio of chondroitin sulfate A to C was 57/43. The total excreted AMPS in MSD urine was equivalent to that seen in the other mucopolysaccharidoses such as Hunter, Morquio and Sanfilippo syndromes. In the heterozygotes, the mother of the patient excreted a higher proportion of dermatan sulfate with a relative increase of heparan sulfate, while

Table 3. *Chemical analysis of fractions eluted from Dowex-1 column chromatography in control and MSD liver*[1]

	Control				MSD			
Fraction	Hexosamine ($glcNH_2/galNH_2$)[2]	Uronic acid	Hexose	C/O	Hexosamine ($glcNH_2/galNH_2$)	Uronic acid	Hexose	C/O
1 (0.25M NaCl)	0.50 (1.00, 96/4)[2]	0.32 (0.64)	2.90 (5.80)	0.11	0.09 (1.00, 71/29)	4.07 (45.2)	19.98 (222)	0.20
2 (0.5M NaCl)	1.28 (1.00, 89/11)	0.24 (0.19)	1.00 (0.78)	0.24	2.27 (1.00, 90/10)	1.66 (0.73)	2.24 (0.99)	0.74
3 (0.75M NaCl)	0.41 (1.00, 57/43)	0.34 (0.83)	0.41 (1.00)	0.83	2.45 (1.00, 88/22)	2.19 (0.89)	1.61 (0.66)	1.36
4 (1.0M NaCl)	0.50 (1.00, 53/47)	0.45 (0.90)	0.28 (0.56)	1.60	3.23 (1.00, 93/7)	3.19 (0.99)	1.33 (0.41)	2.39
5 (1.25M NaCl)	0.31 (1.00, 58/42)	0.26 (0.84)	0.28 (0.90)	0.93	6.38 (1.00, 98/2)	6.18 (0.99)	1.82 (0.29)	3.40
6 (1.5M NaCl)	0.26 (1.00, 61/39)	0.16 (0.62)	0.31 (1.19)	0.52	5.77 (1.00, 86/14)	5.86 (1.02)	1.61 (0.29)	3.64
7 (1.75M NaCl)	0.11 (1.00, 80/20)	0.10 (0.91)	0.17 (1.55)	0.50	3.15 (1.00, 78/22)	2.55 (0.81)	1.16 (0.37)	2.20
8 (2.0M NaCl)	—	—	—	—	2.12 (1.00, 48/52)	1.40 (0.66)	1.05 (0.50)	1.33

[1] Values are expressed as μmole/10 g wet tissues.
[2] $glcNH_2/galNH_2$, glucosamine to galactosamine ratio.
[3] The parenthesis indicates values when hexosamine ratio was 1.00.
[4] C/O ratio carbazole to orcinol ratio.

the father excreted a relatively increased heparan sulfate. The increase of dermatan and heparan sulfate in the MSD mother suggests that the gene for sulfoiduronate sulfatase is located on the X-chromosome.

The chemical analysis of AMPS in MSD patients' tissues reported here demonstrated that MSD liver accumulated predominantly heparan sulfate, while brain tissue contained increased heparan and dermatan sulfate. Bischel et al. (3) reported the accumulation of glycosaminoglycans in kidney, possibly heparan sulfate and Murphy *et al.* (20), using ethanol precipitation methods, reported the accumulation of heparan and dermatan sulfate in the liver of two cases of MSD. However, neither detailed chemical analysis of AMPS in this disorder described in this study nor quantitation of the degree of accumulation of AMPS in MSD tissues have been reported previously. As shown in Table 2, a 30–50-fold increase in AMPS is observed in liver, a 30-fold increase in gray matter and a 5-fold increase in white matter.

Structural analysis of hepatic AMPS identified the major accumulated compound as heparan sulfate and a fraction consisting of chondroitin sulfate A/C.

In conclusion, the chemical analysis of AMPS in MSD urine and in tissues from two cases indicates that the major accumulated compound in this disorder is heparan sulfate rather than dermatan sulfate. In MSD, heparan sulfate N-sulfatase, glucosamine-6-sulfatase and alpha-iduronosulfatase for the degradation of heparan sulfate have been described to be deficient (2, 10). It seems that pronounced accumulation of heparan sulfate rather than dermatan sulfate in MSD liver and urine arises due to the triple enzyme deficiencies or simply due to tissue-specific AMPS metabolic defects by several sulfatase deficiencies. Further exact explanation in this regard could be brought by how much degree of the enzyme deficiencies *de novo* in MSD tissues.

The accumulation of heparan sulfate in MSD tissues, probably in lysosomes, is the primary cause of organomegaly such as hepatosplenomegaly and of mental retardation (17).

Recently, Fiddler *et al.* (14) reported that this disorder is probably caused by reduced enzyme activity of normal enzymes and not by quantitatively abnormal enzymes. The nature of multiple enzyme deficiencies for the degradation of ASPS is still puzzling.

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Erratum

In the article by Stanley *et al.* entitled "Intragastric Feeding in Type I Glycogen Storage Disease: Factors Affecting the Control of Lactic Acidemia" in Pediatric Research *15:* 1504 (1981) the formula on page 1505 was incorrectly printed. The formula, appearing on line 11, should read:

$$Y\ (mg/min) = 0.0014X^3 - 0.214X^2 + 10.411X - 9.084$$

We regret the error.