Molisch-positive Mucopolysaccharides of Gastric Cancers as Compared with the Corresponding Components of Gastric Mucosae

Third Report

On MPS (Mucopolysaccharide) II*

By

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This account deals with "MPSs II" of gastric cancer and of gastric mucosa which have been prepared as described in the First Report.¹⁾ They closely resemble each other regarding biological and physical properties. With respect to the chemical findings, however, it was noticed that glucosamine/galactosamine ratio and contents of glucose and of sialic acid are higher in the cancerous MPS II than in the non-cancerous, while the L-fucose contents of the two mucopolysaccharides are in reversed relationship. The other sugar components show almost no quantitative discrepancy between the two substances.

EXPERIMENTAL

Electrophoretic examination. As solvents were employed 0.1 M veronal-HCl buffer, pH 8.6, I 0.1 + NaCl (0.2 M) and 0.2 M acetate buffer of pH 4.6 (I=0.2). The substances gave single symmetric boundaries with nearly the same mobilities in either of the buffers. The boundary spreading of the cancerous mucopolysaccharide was pretty reversible. See Fig. 1.

Ultraviolet absorption. 50 mg% solutions (pH 6.4) were examined in a photoelectric spectrophotometer (EPB-U type, Hitachi). Cell 1 cm. thick. The mucopolysaccharides gave both a spectrum with a low peak at 270 m μ , suggesting a trace of cyclic amino acids, but there occurred no particular absorption at 260 m μ characteristic of nucleic acids. See Fig. 2.

* 217th report of Masamune and co-workers' "Biochemical Studies on Carbohydrates." Read before the XVIth General Meeting of the Japanese Cancer Association, Osaka, October 30-31, 1957.

Fig. 1. Electrophoretic patterns ($2/3 \times$) of 1% solutions of the cancerous (a) and non-cancerous (b) MPSs II in 0.1 *M* veronal-HCl buffer of pH 8.6 and I 0.1+NaCl (0.2 *M*) (I) and 0.2 *M* acetate buffer of pH 4.6 (I 0.2) (II). Current 8 mA; temperature 15°C; exposed 120 min. after starting current. Mobilities in $\text{cm}^2 \cdot \text{volt}^{-1} \cdot \text{sec}^{-1} \times 10^{-5}$:

$$\text{Cancerous MPS II in veronal-HCl buffer } \frac{(-0.30) \times 0.01278 \times 0.3}{0.008 \times 7200} = -2.0$$

$$\text{in acetate buffer } \frac{(-0.10) \times 0.00588 \times 0.3}{0.008 \times 7200} = -0.3$$

Non-cancerous MPS II in veronal-HCl buffer

$$\frac{(-0.28) \times 0.01278 \times 0.3}{0.008 \times 7200} = -1.9$$

$$\text{in acetate buffer } \frac{(-0.13) \times 0.00588 \times 0.3}{0.008 \times 7200} = -0.4$$

Fig. 2. Ultraviolet absorption spectra of aqueous 50 mg% solutions of the cancerous (—○—○—) and non-cancerous (—●—●—) MPSs II.

Solubility. The two substances behaved similar also in this respect. They dissolve in water of any pH value, 90% (v/v) phenol, saturated $(\text{NH}_4)_2\text{SO}_4$ (room temperature) and saturated Na_2SO_4 (33°C), and are not precipitated from water with lead acetate, cupric chloride, barium acetate, baryta and trichloroacetic acid. They are insoluble in glacial acetic acid, ethanol, acetone and ether.

Test-tube tests. No dissimilarity was found between the substances in the following test-tube tests. Molisch ($\#$), Bial (+), biuret (+), Osaki-Turumi (direct as well as indirect) (+), Neuberg-Saneyoshi (—), Dische (—), tests for phosphorus and hydrolysable sulfur (—).

Paper partition chromatography of components. 1) Sugars. 8 mg. substance was hydrolyzed with 1 N H_2SO_4 , and the hydrolysate chromatographed in a manner similar to the one in the Second Report.²⁾ Glactosamine, glucosamine, galactose, glucose, mannose and L-fucose were detected but no pentose in both of the substances from the cancerous and non-cancerous materials. See Fig. 3.

Fig. 3. Sugar paper chromatograms of MPSs II developed by descending irrigation with solvent butanol-pyridine-water (5:3:2 by volume) (A) or with solvent *n*-butylacetate-acetic acid-ethanol-water (3:2:1:1 by volume) (B) at $25^\circ \pm 1^\circ\text{C}$ for 18 hrs. Paper slivers: Toyo Roshi filter paper No. 2, 10×60 cm. Spray reagent: 2% aniline phthalate in moist butanol. Reference runs were carried out using 0.02 cc. of 1% solutions with respect to individual sugars.

a: reference run. *b*: the hydrolysate of the cancerous MPS II. *c*: the hydrolysate of the non-cancerous MPS II. 1 galactosamine, 2 glucosamine, 3 galactose, 4 glucose, 5 mannose, 6 xylose, 7 L-fucose.

2) Amino acids. 6 mg. substance was subjected to 6 N HCl-hydrolysis and following operations described in the Second Report.²⁾ Fig. 4 is a reproduction of the chromatogram of the cancerous MPS II after hydrolysis. The hydrolysate of the non-cancerous MPS II gave a similar chromatogram. Namely, either of the two MPSs II proved to contain

aspartic and glutamic acids, serine, glycine, hexosamine, lysine, histidine, arginine, threonine, alanine, tyrosine (weak spot), proline, valine and/or methionine and leucine and/or isoleucine and/or phenylalanine.

Fig. 4. Amino acid paper chromatogram of the cancerous MPS II. Paper square (Toyo Roshi filter paper No. 50) 30×30 cm. (A) Primary irrigation with solvent butanol-acetic acid-water (4:1:2 by volume) (13 hrs., $25^\circ \pm 1^\circ\text{C}$). (B) Secondary irrigation with solvent 80% phenol in ammonia-containing atmosphere (13 hrs., $25^\circ \pm 1^\circ\text{C}$). Spray reagent 0.2% ninhydrin in moist butanol.

1 aspartic acid, 2 glutamic acid, 3 serine, 4 glycine, 5 hexosamine, 6 lysine, 7 histidine, 8 arginine, 9 threonine, 10 alanine, 11 tyrosine, 12 proline, 13 valine and/or methionine, 14 leucine and/or isoleucine and/or phenylalanine.

Quantitative analysis. The analytical figures obtained are embodied in Table I. Nitrogen-, total-hexosamine-, galactose- and mannose-contents and iodine uses don't noticeably differ between the cancerous and non-cancerous MPSs II. On the contrary, glucosamine/galactosamine ratios and glucose-, sialic acid- and L-fucose-contents are discrepant from substance to substance, the cancerous MPS surpassing in the first three points and falling behind the non-cancerous in the last one point.

Optical rotation. The watery solutions examined were of pH 6.8. The two substances were found levorotatory to resembling extents.

$$\text{Cancerous MPS II} \quad [\alpha]_D^{25} = \frac{-0.195 \times 1}{0.0100 \times 1} = -19.5^\circ$$

$$\text{Non-cancerous MPS II} \quad [\alpha]_D^{25} = \frac{-0.228 \times 1}{0.0120 \times 1} = -19.0^\circ$$

Blood group potency. The glass plate method of Masamune *et al.*⁸⁾ was applied. The results are shown in Table II. The cancerous substance

TABLE I
Composition of MPSs II of Stomach Cancer and of
Stomach Mucosa

Analysis	In per cent		In equivalents per equivalent weight	
	Stomach cancer	Stomach mucosa	Stomach cancer	Stomach mucosa
N*	5.3	5.6	3.63	3.68
Total hexosamine†	27.0	28.9	1.44	1.48
$\frac{\text{Glucosamine}}{\text{Galactosamine}}$ ratio†	4.4	3.3		
Galactose‡	18.8	19.6	1.0	1.0
Glucose‡	7.3	2.9	0.39	0.15
Mannose‡	6.3	6.7	0.34	0.34
L-Fucose§	6.9	10.8	0.40	0.61
Sialic acid//	11.0	6.7	0.34	0.20
Ash¶	2.1	1.6		
Iodine use as glucose**	5.2	5.2		

* Micro Kjeldahl. † Masamune and Yosizawa.³⁾ ‡ Masamune and Sakamoto.⁴⁾ § A modification of Dische and Shettles.⁵⁾ // Direct Ehrlich reaction of Werner and Odin.⁶⁾ Calcd. as $C_{11}H_{19}NO_9$. ¶ Pregl method without use of conc. H_2SO_4 . ** Macleod and Robison.⁷⁾

TABLE II
Anti-hemagglutinative Potency of MPSs II

Goat: normal anti-O goat serum. Anti-E: eel serum. Anti-OS: anti-OS saliva chicken serum. — no hemagglutination, $\pm$ faint hemaggl., + slight hemaggl., ++ marked hemaggl., ### strong hemaggl.

MPS II	Red cells	Serum	Dilution of the substances (1:10 ³ ×)										
			1	2	4	8	16	32	64	128	256	512	∞
Cancerous	A	α	+++	++	++	++	++	++	++	++	++	++	++
	B	β	+++	++	++	++	++	++	++	++	++	++	++
	O	Goat	+	++	++	++	++	++	++	++	++	++	++
	O	Anti-E	+++	++	++	++	++	++	++	++	++	++	++
	O	Anti-OS	+++	++	++	++	++	++	++	++	++	++	++
Non-canc.	A	α	—	+	++	++	++	++	++	++	++	++	++
	B	β	+	++	++	++	++	++	++	++	++	++	++
	O	Goat	—	++	++	++	++	++	++	++	++	++	++
	O	Anti-E	±	++	++	++	++	++	++	++	++	++	++
	O	Anti-OS	—	—	++	++	++	++	++	++	++	++	++

TABLE III
Anemia-inducing Potency of MPSs II

No. of assay	Rabbit			Material intravenously injected	Dose per kg. body wt. (mg.)	Number of red cells ($10^4 \times$; per cmm.)						
	No.	Body wt. (kg.)	Sex			Before injection	Time after injection (hrs.)					Maximum decrease (%)
							1	2	3	4	5	
1	7	2.2	♂	Cancerous MPS II	1	746	745	725	723	751	712	5
2	8	2.7	♀		„	585	607	592	608	613	596	
3	9	2.7	♂		3	662	668	662	658	633	670	4
4	10	2.7	♂		„	679	694	651	645	678	653	5
5	11	2.3	♂		„	652	664	674	647	621	625	5
6	12	2.5	♂	Non-cancerous MPS II	3	639	654	643	638	632	622	3
7	13	2.7	♀		„	602	595	570	571	576	580	5
8	14	2.3	♀		„	600	578	600	583	593	605	4

TABLE IV
Liver-catalase Depression *in vivo* of MPSs II

In each test-set, the average volume of O_2 evolved for each dose is expressed in per cent of the corresponding volume of O_2 in the blank control.

No. of test-set	No. of mice used	Amount of the substances intraperitoneally injected (mg.)					
		Cancerous MPS II			Non-cancerous MPS II		
		5	3	1	5	3	1
1	9			91			96
2	15	90		97	98		97
3	15	96	96		103	100	
4	15	90	97		92	105	

exhibited no group potency. That the non-cancerous was slightly inhibitory to the agglutination of A and O erythrocytes is most probably due to faint contamination with the very strong group mucopolysaccharide or mucopolysaccharides.

Anemia-inducing potency (KIK activity). It was examined as by Masamune and Kawasaki.⁹⁾ As shown in Table III, the substances caused no more decrease than 5% of number of erythrocytes, even when injected into a rabbit in dosage of 3 mg. per kg. body weight.

Toxohormone activity. It was determined according to Masamune and others¹⁰⁾ (gasometry of catalase activity). Injection of even 5 mg. amounts of MPSs II did not entail depression of liver catalase in mice. See Table IV.

SUMMARY

1. MPSs II, cancerous and non-cancerous, in the First Report were examined physically, chemically and biologically.

2. They proved homogeneous electrophoretically and absorbed no ultraviolet light near 260 m μ .

3. They are made up of same components.

4. However, glucosamine/galactosamine ratio and contents of glucose, of sialic acid and of L-fucose vary from substance to substance.

5. Their optical rotations resemble each other.

6. They don't possess group activity within ABO classification. They are also devoid of KIK- and toxohormone-activity.

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