Biochemical Studies on Carbohydrates.

CXXVIII. Structural Study of A-specific Carbohydrate from Mucus Mucin of Pig Stomach.

Fourth Paper.

By

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How are molecules of the unit disaccharide (N-acetylglucosamine (1,5)-4- β -D-galactoside(1,5)) are linked mutually in the A group carbohydrate? To dissolve this problem, the writer resorted again to the measurement of periodic acid consumption. The materials adapted were the carbohydrate (I) and a partial-degradation product (II) of it obtained by treatment with 6 N. HCl at a room temperature. Calculated from iodine values and sulfate-rest content, their molecular weights amounted approximately to 3600 and 2500 and further analyses pointed to the presence of 9 and 6 molecules respectively of the disaccharide in one molecule of them. And they reduced 10.99 and 8.10 mols of HIO_4 per mol.

Since the substances gave the Ehrlich aldehyde reaction after short heating with weak alkali (Osaki-Turumi procedure), acetylglucosamine stands at one end of the proper-polysaccharide moiety with carbonyl unbound or as linked in a very unstable way. Accordingly, as assumed as a straight chain, the proper-polysaccharides per se should reduce periodic acid per mol as:

Carbohydrate	Mols of periodic acid, when 1-C of acetylglucosamine in one unit disaccharide combines with galactose of the neighbouring disaccharide at			
	2-C	3-C	4-C	6-C
I	11	3	11	19
II	8	3	8	13

Here the terminal monosaccharides, acetylglucosamine and galactose, in a chain consume 3 mols of periodic acid altogether, as is obvious from what has been cited in the second paper.¹⁾ It is seen that the values actually obtained agree quite accurately with the theoretical when 2- or 4-C

of the intermediate galactose molecules takes part in the union between the adjacent units. Not only the group carbohydrate but also the degradation product possessed an unknown nitrogenous compound and sulfuric acid as further constituents,²⁾ as already mentioned. But, judging from the findings of the periodic acid use, these non-sugar rests apparently attach to the polysaccharide chain at the positions which are indifferent to the reaction concerned. The amount used of periodic acid is decreased as below, varying with the allocation of the above rests:

Sugar	Position of attachment	Decrease of periodic acid reduction (mol)
Acetylglucosamine	Terminal	3-C
		6-C
		3-C, 6-C*
	Inter-mediate	3-C
		6-C
		3-C, 6-C*
Galactose (Terminal and intermediate)	2-C	1
	3-C	2
	4-C	1
	6-C	0
	2-C, 3-C*	2
	2-C, 4-C*	2
	2-C, 6-C*	1
	3-C, 4-C*	2
	3-C, 6-C*	2
	4-C, 6-C*	1

* Cases where the two rests are attached to one and the same sugar.

Namely it looks that the residues are linked at two of the positions 3 of the terminal acetylglucosamine, 3 and 6 of intermediate acetylglucosamine molecules and 6 of galactose molecules, although, if the non-glucosamine nitrogenous component has a reducing force and exactly compensate the decrease of the periodic acid consumption due to its attachment, it must be otherwise allotted.

That 3- or 6-C of intermediate galactose is the position of union between the disaccharide units is least probable, because, on one hand, the reducing force of the non-glucosamine nitrogenous component is hardly estimated so strong as corresponds to 8 (11-3) or 5 (8-3) mols of periodic acid and on the other, wherever the non-sugar residues may be situated, the number of mols of periodic acid reduced can not be lessened to 11 or 8 respectively from 19 (in case of I) or 13 (in case of II). (The maximum decrease by the attachment of the residues is 3).

In consequence, the proper-polysaccharide in the present A group carbohydrate is concluded to have the structure expressed by one of the two diagrams:

That α -form of glucosaminide is assigned is arbitrary. Some of the glucosamine molecules take part in forming the direct-Osaki-Turumi reaction-giving nucleus.⁴⁾

EXPERIMENTAL.

Preparation of the Group Carbohydrate (I).

It was pursued by the method described in the first paper.³⁾

Preparation of Carbohydrate II, a Degradation Product of the Group Carbohydrate.

2 gm. of the group carbohydrate (I) were dissolved in 50 cc. of 6 n. HCl and stood in air (room temperature, 20°C). The solution changed brown gradually. After 40 hours, 10 volumes of 94% alcohol and 5 volumes of ether were added and the appearing flocculent precipitates centrifuged, washed twice with 10 cc. of alcohol and dried in a vacuum desiccator (H_2SO_4). A white powder given amounted to 1.4 gm. In the next, its brownish solution in 70 cc. of water was submitted to electrodialysis in a Pauli apparatus (160–200 volts) until renewed water in the outer compartments showed no shift of pH, which took 3 days. Some deposit that came out was filtered off and the filtrate was distilled in vacuo to a small volume at first, then three times to dryness with 20 cc. of absolute alcohol and finally placed in a vacuum desiccator. Yield, 1.2 gm.

TABLE I.

Analysis	Carbohydrate I %	Carbohydrate II %
Nitrogen*	3.67	3.78
Glucosamine	43.27	41.90
Galactose	43.61	42.16
Indole r.	44.31	42.43
Orinol r.	42.91	41.88
Hydrolysable sulfur	0.95	1.14
Acetyl	10.50	10.15
Ash†	0.32	0.20
Iodine value‡	5.87	8.46

* Micro Kjeldahl. † Micro analysis. ‡ cc. of 0.1 n. iodine solution used by 1 gm. of the carbohydrates. Calculated out from the iodine use by Macleod and Robison method.

The Properties and Composition of the Carbohydrates I and II.

The both carbohydrates resembled each other in following reactions:— Molisch positive; direct Ehrlich aldehyde faintly positive, the indirect distinctly positive (Osaka-Turumi procedure); biuret and ninhydrin negative.

Quantitative-analytical figures obtained are embodied in Table I.

Optical rotation in water.

$$\text{Carbohydrate I: } [\alpha]_{\text{D}}^{20} = \frac{-0.508 \times 100}{1 \times 1.92} = -26.46^{\circ}$$

$$\text{Carbohydrate II: } [\alpha]_{\text{D}}^{20} = \frac{0.094 \times 100}{1 \times 1.28} = +7.34^{\circ}$$

Molecular weight.

Carbohydrate I: From the sulfur content, $32 \times \frac{100}{0.95} = 3370$. From the iodine value of it and that of the unit acetylglucosamine galactoside (m. w. 383, iodine value 55.26⁵⁾), $\frac{55.26 \times 383}{5.87} = 3606$.

Carbohydrate II: From the iodine value, $\frac{55.26 \times 383}{8.46} = 2502$.

Serological activity.

By our usual procedure, I was found to specifically inhibit the isoagglutination of A-erythrocytes at a dilution of 1:10⁶, but II only at a dilution of 1:10³.

Measurement of Periodic Acid Reduction by the Carbohydrates.

The measurement was carried out in a like manner as described in the second paper¹⁾:— To 5 cc. each of 3.84% solutions of the carbohydrates I and II were added respectively 5 cc. of 0.4364 mol. solution and 10 cc. of 0.1402 mol. solution of periodic acid. Temperature, 20°C. In the both mixtures, change of rotation ceased after 90 minutes from the commencement of reaction (Fig. 1), when 2 cc. of the former mixture and 3 cc. of the latter were pipetted out for determining the acid remaining unreduced. The quantities of the acid reduced reached $0.1172 \times \frac{5}{1000}$ and $0.1244 \times \frac{5}{1000}$ mol, and accordingly those reduced per mol by the carbohydrates are calculated at 10.99 and 8.10 mols.

Fig. 1.

Change of rotation during the oxidation of Carbohydrates
I and II by periodic acid.

CONCLUSION.

The extents of reducing periodic acid of the A group carbohydrate and a partial degradation product of it suggested that, in the carbohydrate chain, neighbouring two disaccharide units link together through 2- or 4-C of galactose of one molecule and 1-C of accetylglucosamine of the other.

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Bibliography and Notes.

- 1) Yosizawa, Tohoku J. Exp. Med., this volume, p. 111.
- 2) The readers are referred also to Sinokawa (Tohoku J. Exp. Med., 1949, **50**, 167) and others in this series of investigation.
- 3) Yosizawa, Tohoku J. Exp. Med., 1949, **51**, 51.
- 4) It is because the carbohydrate gives the direct Ehrlich reaction. Regarding the structure of this nucleus cf. Hiyama, Tohoku J. Exp. Med., 1949, **51**, 319.
- 5) Cf. 3).