[Chem. Pharm. Bull. 29(6)1785—1788(1981)]

Studies on Heterogeneous Components of Hog Pancreatic Kallikrein. II. Preliminary Probe of the Varied Structures of Carbohydrate Chain of Hog Pancreatic Kallikrein

Highly purified hog pancreatic kallikreins A and B from the autolysed hog pancreas were digested with pronase and subjected to hydrazinolysis to release asparagine-linked sugar chains. The oligosaccharides released were separated into fractions based on the acidities due to the contents of neuraminic acid residues. Out of these oligosaccharides, fractions having no neuraminic acid obtained from both A and B were further separated according to the molecular size by the 2 times repeated gel filtrations and their carbohydrate contents were analysed. Thus varied primary structures of carbohydrate chains—namely, different number of neuraminic acid residues being bound, straight and branched oligosaccharide chains, and the high mannose containing and the complex carbohydrates containing structures—of these asparagine-linked oligosaccharides being consisted parts of hog pancreatic kallikreins were observed.

Keywords——hog pancreatic kallikrein; heterogeneous components of kallikrein; carbohydrate structures of kallikreins; asparagine-linked sugar chain; neuraminic acid

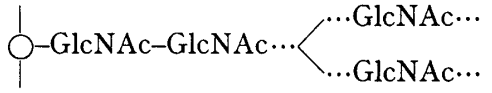
Recently, it has been revealed that the glandular kallikreins, such as human urinary, hog pancreatic, rat submandibular kallikreins and so on, have heterogeneous and further divided micro-heterogeneous forms being separable by the progressed methods in the enzyme chemistry.[1] In our previous papers[2–4] carbohydrate contents in heterogeneous components of hog pancreatic kallikrein were estimated and possible role of the contained neuraminic acid residue was discussed. And differences within these multiple components due to the different carbohydrate compositions were also observed and somewhat elucidated.

In this paper, preliminary probe of the varied primary structures of carbohydrate chains of hog pancreatic kallikrein is tried and discussed.

Highly purified hog pancreatic kallikreins A and B were the starting materials in this study and prepared according to our previously described method[4] with minor modifications from the autolysed hog pancreas.

Hydrazinolysis of the pronase digested hog pancreatic kallikreins A and B were separately performed according to the method of Fukuda *et al.*[5] which had been developed from the original method of Akabori *et al.*[6] The reaction mixture obtained was applied to a column of Sephadex G-25 (Fine) and eluted with distilled water at room temperature. The oligosaccharide fractions were further applied to ion exchange chromatography on a column of DEAE-Sephacel. Collected oligosaccharide fractions were analysed to estimate the contents of neuraminic acid by the periodate-resorcinol method.[7] According to the neuraminic acid content appropriate fractions were pooled and the pooled fractions having the different number of residues of neuraminic acid for kallikreins A and B were obtained, separately. In the present studies the fractions having no neuraminic acid separately obtained from A and B were employed and further separations were carried out according to the molecular size by the 2 times repeated gel permeation chromatography using Bio-Gel P-4 (–400 mesh). Appropriately divided and pooled fractions are shown on Chart 1-A and -B and were given to analyse carbohydrate compositions by gas chromatography[8] on a column (0.3×100 cm) of 0.05% (w/w) ECNSS-M after conversion to alditol acetate derivatives for neutral sugars and by using amino acid analyser for aminosugar. As shown on Chart 1-A and -B, fucose (Fuc), mannose (Man), galactose (Gal) and N-acetylglucosamine (GlcNAc) were also detected and quantitatively measured, and the number of residues of these carbohydrates composed were proposed regarding the main fractions which should be of consisted multiple forms of hog pancreatic kallikreins. In comparison of kallikrein A to B, fairly larger number

of mannose residues in B were observed (Chart 1-B), being indicated so-called the high mannose containing structure in B while the complex carbohydrates containing structures in A.

In regard to the amount of GlcNAc residues, the complex structure containing 2 GlcNAc residues might be straight chain, while that containing 4 would be branched, because 2 GlcNAc residues might be located as –GlcNAc–GlcNAc·····in the straight chain and in the case of 4 GlcNAc residues as  in the branched chain.

hog pancreatic kallikrein A
↓ hydrazinolysis
glycopeptides
Sephadex G-25
DEAE-Sephacel chromatographies

		Fractions	Number of residues estimated			
			Fuc	Man	Gal	GlcNAc
A-0 neuraminic acid negative	A-0-1 molecular size large	[A-0-1-1]				
		[A-0-1-2]				
		[A-0-1-3]				
		[A-0-1-4]**	2	4	6	4
		[A-0-1-5]				
A-1 neuraminic acid 1	A-0-2 molecular size medium	[A-0-2-1]***	1—2	4	2	4
		[A-0-2-2]				
		[A-0-2-3]				
A-2 neuraminic acid 2	A-0-3 molecular size smaller	[A-0-3-1]				
		[A-0-3-2]*	0—1	4	5	2
		[A-0-3-3]				
		[A-0-3-4]**	1	5	2	2
		[A-0-3-5]				
		[A-0-3-6]*	0—1	4—5	0—1	2

Chart 1-A. Varied Carbohydrate Compositions in Hog Pancreatic Kallikrein A

***, main fraction. **, next main. *, next main over.

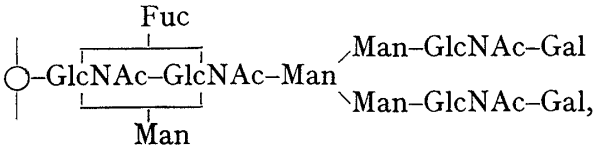
hog pancreatic kallikrein B
↓ hydrazinolysis
glycopeptides
Sephadex G-25
DEAE-Sephacel chromatographies

		Fractions	Number of residues estimated			
			Fuc	Man	Gal	GlcNAc
B-0 neuraminic acid negative	B-0-1 molecular size large	[B-0-1-1]				
		[B-0-1-2]*	1	9	3	2
		[B-0-1-3]				
B-1 neuraminic acid 1	B-0-2 molecular size medium	[B-0-2-1]**	0	9—10	1—2	2
		[B-0-2-2]				
B-2 neuraminic acid 2	B-0-3 molecular size smaller	[B-0-3-1]				
		[B-0-3-2]*	1	7	1	2
B-3 neuraminic acid 3—4						

Chart 1-B. Varied Carbohydrate Compositions in Hog Pancreatic Kallikrein B

**, main fraction. *, next main.

As our probe of the primary structure of carbohydrate chain in one of main fractions (see ***1 sign on Chart 1-A) derived from kallikrein A would be now proposed as,



being based on the calculated number of the composed carbohydrate residues.

Thus the varied and complicated primary structures of carbohydrate chains of hog pancreatic kallikreins (derived from the autolysed hog pancreas) could have been proposed as shown on the schematic Figure 1. There would be multiple forms of kallikrein molecules, in which at least being found high mannose and complex structures of carbohydrates, straight and branched chains, and varied number of neuraminic acid residues located in the terminal of the proposed primary structure of carbohydrate chains.

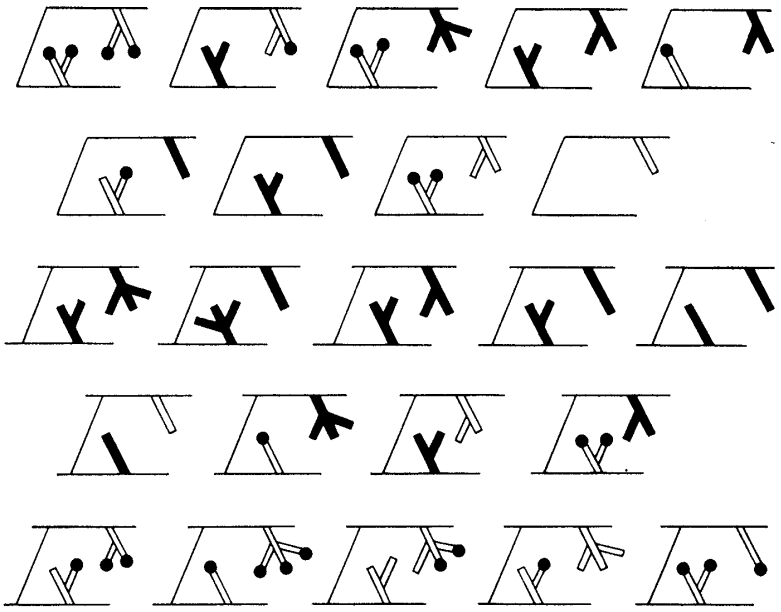
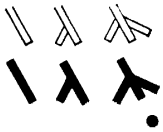


Fig. 1. Expected Heterogeneous Components bound Varied Structures of Carbohydrate Chains of Hog Pancreatic Kallikrein

Figure illustrates the schema which there would be 2 different kinds of structure of carbohydrate chains having the straight and the branched, and varied number of neuraminic acid residues located in the terminal of the carbohydrate chains.



, complex type carbohydrate chain.

, high mannose type carbohydrate chain.

, neuraminic acid

The present schematic Figure 1 is our probe of the structure of carbohydrate chain and actual structures will be determined in further experimental analysis.

Acknowledgement The authors wish to thank Professor Dr. T. Osawa and Dr. T. Tsuji, Faculty of Pharmaceutical Sciences, University of Tokyo, for their grateful instructions in the carbohydrate analysis. This work has been supported in part by grants from the Ministry of Education, Science and Culture, of Japan.

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Received April 10, 1981

[Chem. Pharm. Bull. 29(6)1788—1790(1981)]

Identification of the Aglycon Part of Vineomycin A_1 with Aquayamycin

Vineomycin A_1 (formerly OS-4742 A_1) produced by *Streptomyces matensis* subsp. *vineus*, is an antibacterial and antitumor antibiotic. The aglycon part obtained by mild hydrolysis turned out to be identical with aquayamycin, and its ^{13}C-NMR assignment was also determined.

Keywords——vineomycin A_1; OS-4742 A_1; *Streptomyces matensis* subsp. *vineus*; antibiotic; aquayamycin; P-1894B; ^{13}C-NMR

Vineomycin A_1 (formerly OS-4742 A_1, **1**) is a component of new antibiotics, vineomycins (A_1, A_2, B_1 and B_2), which are produced by *Streptomyces matensis* subsp. *vineus*.[1] Ōmura *et al.* have reported that it is active against Gram-positive bacteria and Sarcoma 180 solid tumor on mice and has a quinone-type chromophore and sugar moieties.[1] During the investigation of the structure and biosynthesis of the antibiotic, we realized that a chromophoric aglycon obtained by mild acid hydrolysis turned out to be identical with aquayamycin.[2] Described in the present paper is the identification together with the ^{13}C-nuclear magnetic resonance (^{13}C-NMR) assignment of the aglycon of **1**.

In order to obtain a chromophoric aglycon, **1** was treated under mild conditions (0.6 N HCl, room temperature) providing a reddish-orange product. After purification by preparative thin-layer chromatography (TLC) ($CHCl_3$–MeOH, 5: 1), recrystallization from a benzene–hexane mixture gave red plates (**2**): $C_{25}H_{26}O_{10}$, *Anal.* Found C; 61.88, H; 5.58%. Calcd C; 61.72, H; 5.39%, mp 175—180° (dec.), MS (FD); *m/z* 486 (M^+) 478 ($M^+ - H_2O$) 450 ($M^+ - 2H_2O$), $[\alpha]_D^{20}$ +130° (c=1, dioxane).

The infrared absorption (ν_{max}^{KBr} cm^{-1}; 1635, 1615) and the ultraviolet spectrum [λ_{max}^{EtOH} nm (log ε); 219 (4.30), 318 (3.61), 419 (3.65)] of **2** indicated the presence of a quinone group, which was supported by signals at 184.3 ppm (C-12) and 190.4 ppm (C-7) in the ^{13}C-NMR spectrum, the latter carbonyl group is hydrogen-bonded to the phenolic hydroxyl group.[3] A non-protonated carbon signal at 116.0 ppm (C-7a) was characteristic of the C-2 carbon of an enol form of 1,3-diketone system[4] and a phenolic carbon signal at 159.5 ppm (C-8) was also observed. In addition, an aromatic AB spin system [δ_H 7.44 ppm (H-11) and 7.73 ppm (H-10), J=8 Hz] was observed in the ^{1}H-NMR spectrum of **2**. These data suggest a 2,3,6- or 2,3,8-trisubstituted