Communications to the Editor

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THE STRUCTURE OF NEPHRITOGENOSIDE [1)]

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We have isolated a glycopeptide named nephritogenoside, which was purified from the glomerular basement membrane of rats. Nephritogenoside contains a new type of carbohydrate-peptide linkage having the α-D-configuration. After the cleavage of the amido-linkage of nephritogenoside between carbohydrate and peptide by ion-exchange resin Amberlite(IRA 410), 1N-hydrochloric acid eluate was chromatographed on a column of Sephadex G-100 to give a single peptide peak (Fr. 10). The amino acid sequence of Fr. 10 has been determined with an amino acid sequencer.

KEYWORDS —— nephritogenoside; ion-exchange resin; amido linkage; amino acid sequencer; HPLC; dansylation; carboxypeptidase

We have isolated a glycopeptide named nephritogenoside, which was purified from the glomerular basement membrane of rats, using several steps of experimental procedures.[2)] From 1200 normal rats, only 5-6 mg of nephritogenoside were isolated. S. Shibata and T. Nagasawa have established a new experimental model for adult human glomerulonephritis which was induced in homologous animals by a single footpad injection of nephritogenoside and Freund's incomplete adjuvant.[3)] Six to eight months later the injected animals became afflicted with chronic glomerulonephritis, contracted kidney. Typical histological changes of contracted kidney was induced in 96-98% of the injected animals.[4)]

From methylation analysis,[5)] the concanavalin A test,[6)] and ^{13}C-NMR data compared with those of related synthetic glycosylamine derivatives,[7)] Shibata et al.[8)] proposed structure 1 for the nephritogenoside. The sugar moiety of 1 has the Glcα-(1→6)Glcβ-(1→6)Glc structure shown in Fig.1. The number of amino acids is 18±1, and the amino acid profile is characterized by high contents of glycine, glutamic acid, and aspartic acid, and by a low content of basic amino acid. It is of particular interest that no tyrosine, half cystine or hydroxylysine were found in this compound. This is the first example among natural compounds of a new type of carbohydrate-peptide linkage having a direct, α-N-glycosyl linkage between a glycosyl and an amino acid residue.

We wished to isolate the peptide from the nephritogenoside to analyze the structure of the peptide moiety. In our previous paper,[9)] we reported the cleavage of the amido linkage of various glycosylamine derivatives, N-(L-γ-glutamyl)-α- and -β-D-glucopyranosylamine, N-(L-β-aspartyl)-α- and -β-D-glucopyranosyl-

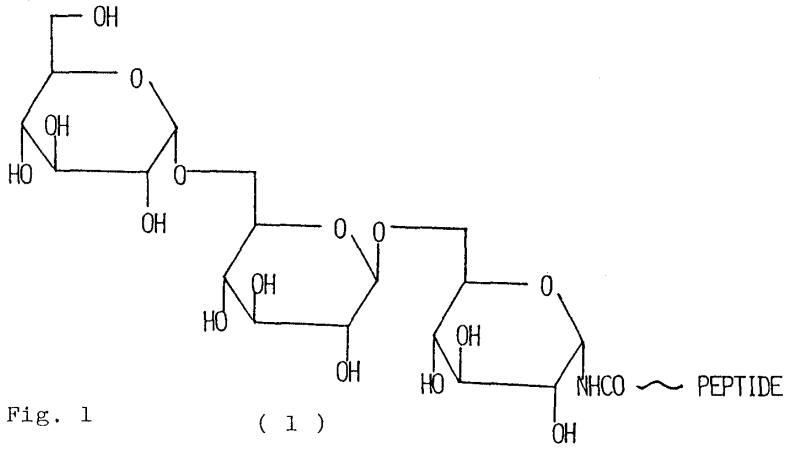

Fig. 1 (1)

amine, and N-glycyl-α-D-glucopyranosylamine, by Amberlite IRA-410(OH^-), as evidenced by the appearance of the aglycone amino acids and the disappearance of the starting amide. This resin treatment was successfully applied to nephritogenoside.

After the cleavage by resin of the amido-linkage between the carbohydrate and peptide of nephritogenoside, the part of the aqueous eluate which shows the colour-reaction with anthrone-sulfuric acid reagent was discarded. The 1N-hydrochloric acid eluate, which does not show the colour-reaction, was lyophilized and chromatographed on a column of Sephadex G-100 (23 cm x 2.6 cm i.d.). The effluent was collected in 11 ml fractions. Fraction 10 (Fr. 10) was shown by high-performance liquid chromatography (HPLC) with a fluorescence detector to be homogeneous. The method, which involves derivatization of the amino acid with o-phthalaldehyde (OPA), is extremely sensitive.[10] Fr. 10 was hydrolyzed with 6N-HCl for 24 h at 110°C and the acid hydrolysate gave Asp_2, Thr_1, Ser_1, Glu_2, Pro_3, Gly_6, Leu_1, Ile_2, Ala_1, Val_1, and Phe_1. There were 21 amino acid residues in all. The NH_2-terminal sequence analysis of Fr. 10 was performed by sequential Edman degradation in the presence of polybrene in an Applied Biosystems sequenator, model 470A.[11] The use of a sequenator makes it possible to unambiguously identify all residues normally encountered in the automatic degradation of Fr. 10, except those at positions 4, 7, 13, 16, and 19. As the basis for the increased background of amino acids observed during the course of a sequencing experiment, positions 4 and 7 show both glycine and phenylalanine, and glycine and alanine, respectively. At position 19 valine occurs in addition to glycine; this position was also slightly contaminated but not sufficiently to obscure the true residue, valine, which was confirmed in the C-terminal analysis as follows. The C-terminal amino acid of Fr. 10 has been determined by carboxypeptidase A and/or Y[12] to be one mol of glutamine. The C-terminal sequence, -Ile.Val.Gly.Gln, was postulated on the basis of the rates of release of the amino acids by CPase. At position 13 and 16, the threonine and serine, in addition to glycine, could be due to a slight under correction, considering the amino acid composition of Fr. 10. By the method of K. Yagi et al.,[13] Dns-Asp was clearly detected as an N-terminal amino acid by reverse-phase HPLC on Develosil C-18, using a linear gradient made from Tris-HCl buffer (pH 7.75) and

methanol. Fluorescence was monitored by a Shimadzu RF-500 LC spectrophotometer. Exitation and emission wave lengths were 340 and 530 nm, respectively.

The sequence deduced after considering all these factors is as follows:

^{1}Asp-Pro-Leu-$\frac{\text{Gly}}{\text{Phe}}$-^{5}Gly-Ile-$\frac{\text{Gly}}{\text{Ala}}$-Gly-Glu-^{10}Asp-Gly-Pro-$\frac{\text{Gly}}{\text{X}}$-Gly-^{15}Pro-$\frac{\text{Gly}}{\text{X}}$-Gly-Ile-$\frac{\text{Val}}{\text{Gly}}$-^{20}Gly-^{21}Gln.

An additional symbol, X, is used for serine or threonine.

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