ISOLATION AND PARTIAL CHARACTERIZATION OF A GLYCOPEPTIDE FROM CERUMEN

HIROSHI MASUDA, SHIGEKI SHICHIJO, TOMOYOSHI GOYA
AND MUTSUYA TAKEUCHI

*Institute of Clinical Pathology, Kurume University School
of Medicine, Kurume, 830, Japan*

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A glycopeptide was isolated from cerumen and was assayed by determining the carbohydrate content of material which remained after proteolysis. The component consisted of hexose, peptide, galactosamine, sialic acid, sulfate and glucosamine; hexose and peptide were main components. The separation possessed galactose and glucose as major hexose components.

INTRODUCTION

Glycopeptide and glycosaminoglycans in skin and inner ear have well been studied by many authors (Tanaka, 1967; Saito and Daly, 1970). At present, however, little is known about these substances in external auditory meatus. In this paper it was attempted to isolate and characterize the glycopeptide from the cerumen.

MATERIALS AND METHODS

Materials

Normal cerumen samples were obtained from fifty subjects of both sexes aged between 16 and 55 years old. All materials were stored in a deep freezer until further processed. The samples were put into several changes of acetone for several days, and dried in a desiccator over CaCl_2 .

Isolation of the glycopeptide from the cerumen

The glycopeptide was extracted from the cerumen according to the procedure of Schiller et al. (1961). The samples were digested with papain (EC 3. 4. 4. 10) in the presence of EDTA and cysteine at 65°C for 48 hr. The mixture was adjusted to pH 7.8 and then digested with trypsin (EC 3. 4. 4. 4) at 37°C for 48 hr with simultaneous dialysis against 0.1 M phosphate buffer, pH 7.8. The digested solution was deproteinized with 5% trichloroacetic acid in the cold, and the supernatant was dialyzed against several changes of cold deionized water for 72 hr. Then four volumes of alcohol containing 0.1% acetic acid and 1% potassium acetate were added in order to precipitate the glycopeptide. The precipitate was dried in a desiccator over CaCl_2 and then dissolved in 5 ml of the deionized water for the use of chemical analyses.

Analytical procedures

Hexose was determined by the orcinol-sulfuric acid reaction of Winzler (1955 a) ; peptide by the Lowry method (1951) ; sialic acid by the diphenylamine reaction of Winzler (1955 b) ; fucose by the Gibbons reaction (1958) ; and sulfate by the method of Dodgson and Price (1962) ; respectively. Uronic acid measurement was also tried by the carbazole reaction of Bitter (1962). The separation and quantitative determination of glucosamine and galactosamine were carried out with a Hitachi KLA-3 Amino Acid Analyser, using a 20 cm column in citrate buffer of pH 5.28 (Na : 0.35 N). Neutral sugars were separated and identified by paper chromatography after hydrolysis in 1 N HCl for 3 hr at 100 °C in a sealed ampule ; Whatman filter paper No. 1 was used as a supporting medium ; the solvent system was n - butanol - pyridine - 0.1 N HCl (5 : 3 : 2 v/v/v), Chromatogram obtained by descending paper chromatography, and double development techniques was stained with AgNO₃-NaOH reagent.

RESULTS AND DISCUSSION

The glycopeptide content in the cerumen was 9.1 mg per g of rough weight and 12.5 mg per g of defatted dry weight, respectively. The preparation was subjected to analyses for hexose, peptide, sialic acid, fucose, sulfate, glucosamine, galactosamine and uronic acid ; the results are summarized in Table 1. Hexose and peptide were main components, while uronic acid was scarcely detected. Both galactosamine and glucosamine were also found ; the former was more contained than the latter.

The constitution of the neutral hexose was studied by paper chromatography, as shown in Fig. 1. It was

TABLE 1

Analyses of the glycopeptide from cerumen^a

Hexose	29.3
Peptide	48.1
Sialic acid	7.3
Sulfate	3.5
Fucose	1.5
Galactosamine	12.6
Glucosamine	1.7
Uronic acid	0.1

^a Expressed as percentage of weight.

Fig. 1 Paper chromatogram of hydrolysate of glycopeptide from cerumen.

G : Glycopeptide from cerumen.

M : Mixture of fucose, mannose, glucose and galactose as a reference.

found that the preparation possessed galactose and glucose as main components and only traces of mannose and fucose.

Although several glycopeptides were isolated from several human sources (Odin, 1958; Pusztai and Morgan, 1961; Endo et al., 1973), there are no comparative data concerning the glycopeptide from the human cerumen in the literature. Uronic acid was a little detected in the cerumen of the present study, while it was reported that mucous membrane of human paranasal air sinuses, human skin and rat cochlea contained certain amounts of uronic acid, respectively (Sato, 1966; Shetlar et al., 1972; Saito and Daly, 1970); these may probably show that the glycopeptide isolated by the authors is mainly derived not from skin itself in the external auditory meatus but from the secretion of the glands. Histochemical analysis has indicated that the cerumen is the combined secretion of the sebaceous and the ceruminous glands in the external auditory meatus (Ham, 1974). Further purification of this material will surely be necessary, and accordingly it is in progress by the authors.

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