Sulfated Glycopeptides from Middle Ear Effusions of Secretory Otitis Media

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AIKAWA, J., MUNAKATA, H., ISEMURA, M., YOSIZAWA, Z., TADA, K., SUZUKI, M. and SAKURAI, T. Sulfated Glycopeptides from Middle Ear Effusions of Secretory Otitis Media. Tohoku J. exp Med., 1985, 146 (4), 461-467 — The middle ear effusion specimens were obtained by myringotomy and aspiration from 4 children of 4-7 years old, who had been diagnosed as patients with secretory otitis media on the basis of conductive hearing loss and tympanogram. In cases 1 and 2, their ear fluids were macroscopically serous, while those of cases 3 and 4 were mucous. These ear fluids were digested with pronase and the digests were analyzed by cellulose acetate membrane electrophoresis with alcian blue and high-iron-diamine stainings. All samples were found to contain glycopeptides possibly derived from sulfated mucin-type glycoproteins with small amounts of glycosaminoglycans. The glycoconjugates from cases 3 and 4 were further examined after hyaluronidase and chondroitinase ABC treatments, followed by heparitinase digestion. The resultant glycopeptide fractions appeared to be electrophoretically homogenous and their chemical compositions suggested that they were typical mucin-type glycopeptides. Furthermore, they contained sulfates. The data suggest that in secretory otitis media, one of the major components of middle ear effusions is sulfated mucin-type glycoprotein. —— sulfated glycopeptides; middle ear effusion; secretory otitis media

By histological and histochemical studies of secretory otitis media, many investigators have reported that there is goblet cell hyperplasia of middle ear mucosa (Hussl and Lim 1969; Hentzer 1972). However, there were only a few chemical examinations of viscosogenic substance from middle ear effusion (Juhn et al. 1971; Vered et al. 1972; Palva et al. 1975). In the present paper, the data are described to suggest that middle ear effusions of secretory otitis media contain sulfated mucin-type glycoproteins.

**Materials and Methods**

*Middle ear effusion.* Middle ear effusions were collected by myringotomy and aspiration.
tion from four patients: Case 1, a 4-year-old girl; case 2, a 5-year-old girl; case 3, a 7-year-old girl; case 4, a 7-year-old boy. Middle ear fluids, which were aspirated (final volume: 5ml with added physiological saline), were collected and stored at −20°C until use.

Preparation of a glycoconjugate fraction from middle ear effusions. To a major portion (4.5 ml) of each middle ear fluid was added 0.5 M Tris-HCl buffer (0.5 ml) containing 0.05 M calcium acetate and 0.05% benzoic acid (pH 8.0). Pronase (500 μg) was added to each solution, and the mixture was incubated at 45°C with shaking for 24 hr. The digestion was continued with additional pronase (500 μg) for 24 hr. To the solution was added 50% trichloroacetic acid (final 7%) in an ice bath and centrifuged. To the resultant supernatant were added 4 volumes of ethanol containing 1% potassium acetate. After centrifugation, the precipitate was separated and dialyzed exhaustively against distilled water. The non-dialyzable fraction yielded a glycoconjugate fraction in each case.

With the remaining portion (0.5 ml) of the middle ear fluid, protein contents were determined by Bio-Rad Protein Assay (Nippon Bio-Rad Laboratories, Tokyo) using bovine serum albumin as a standard.

Mucopolysaccharidase treatment. Hyaluronidase and chondroitinase ABC were immobilized on Sepharose 4B as follows. Streptomyces hyaluronidase (100 TRU) with umbilical hyaluronic acid (5 mg) and chondroitinase ABC (5 U) with alkali-treated chondroitin sulfate C (5 mg) were separately dissolved in 1 ml of 0.1 M sodium bicarbonate and each solution was mixed with 2 ml of Sepharose 4B which had been activated with CNBr (Höök et al. 1976). Heparitinase was obtained from Seikagaku Kogyo Co., Tokyo. The conditions of digestion were similar to those described previously (Aikawa et al. 1984a).

Electrophoresis. Electrophoresis was performed as described previously (Aikawa et al. 1984a). The staining was performed with alcian blue and high-iron-diamine (Munakata et al. 1985a).

Chemical composition. Neutral sugars, sialic acid, hexosamines, sulfate and amino acids were determined as described previously (Aikawa et al. 1984b).

Ultrafiltration. UK-200 ultrafilter membranes (filtration limit, Mr=200,000) were obtained from Toyo Kagaku Sangyo Co., Tokyo. Glycopeptide fractions from cases 3 and 4 were separately subjected to ultrafiltration using Toyo ultrafilter (UK-200). The contents of neutral sugars in the residual solution and the filtrate were determined by the phenol-H₂SO₄ reaction.

Results

Yields of glycoconjugate fractions

The pronase digestion of middle ear effusions afforded glycoconjugate fractions, the yields of which are shown in Table 1. The protein concentrations of

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Type</th>
<th>Glycoconjugate fraction (mg)*</th>
<th>Glycoconjugate fraction (mg)* per g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>F</td>
<td>Serous</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>F</td>
<td>Serous</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>F</td>
<td>Mucous</td>
<td>1.1</td>
<td>3.2</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>M</td>
<td>Mucous</td>
<td>1.9</td>
<td>5.3</td>
</tr>
</tbody>
</table>

* Expressed as dry weight.
collected middle ear effusions were determined by Bio-Rad Protein Assay. There was a difference in yield between serous group and mucous group.

**Constituents of glycoconjugate fractions**

Electrophoretic analyses revealed that all the glycoconjugate fractions derived from four specimens of middle ear effusions contained alcian blue-stainable

Fig. 1. An electrophoretogram of glycoconjugate fractions from otitis media effusions. Electrophoresis was carried out in formic acid-pyridine buffer (pH 3.0) at 1 mA/cm for 25 min. The anode is at the top and the substances were stained with alcian blue. 1, An authentic mixture of chondroitin sulfate C, dermatan sulfate and hyaluronic acid (from top to bottom); 2, 3, 4 and 5, glycoconjugate fractions from cases 1, 2, 3, and 4, respectively.

Fig. 2. An electrophoretogram of glycoconjugate fractions from middle ear effusions of otitis media. Electrophoresis was carried out in 0.06 M sodium barbiturate buffer (pH 8.6) at 1 mA/cm for 25 min. The anode is at the top and the substances are visualized by the high-iron diamine staining. 1, An authentic sample of chondroitin sulfate C; 2, 3, 4, and 5, glycoconjugate fractions from cases 1, 2, 3, and 4, respectively.
glycopeptide materials moving slightly slower than hyaluronic acid together with glycosaminoglycans (Fig. 1). These bands were also positively stained with high-iron-diamine (Fig. 2) under the condition where sulfated glycoconjugates are specifically stained (Munakata et al. 1985a).

**Characterization of glycopeptides derived from effusions of cases 3 and 4**

In order to characterize glycopeptide materials, the glycoconjugate fractions from cases 3 and 4 were digested with hyaluronidase, chondroitinase ABC and heparitinase. The glycopeptide fractions thus obtained were almost homogenous as judged by electrophoresis on cellulose acetate membranes (Fig. 3). The yields of glycoconjugate fractions of cases 1 and 2 were small (Table 1), and they were not examined further.

Chemical compositions of the glycopeptide fractions from cases 3 and 4 are listed in Table 2. The carbohydrate compositions of both glycopeptides were consistent with a concept that they were derived from mucin-type glycoproteins (Pigman 1977b). It was noteworthy that both fractions contained substantial amounts of sulfates.

Their amino acid compositions are shown in Table 3. Aspartic acid, threonine, serine, glutamic acid, glycine, alanine and proline were the major amino acids, representing 85–87% of the total, which is again a feature characteristic to mucin-type glycoproteins (Isemura et al. 1983).
Molecular size

When the glycopeptide fractions from cases 3 and 4 were ultrafiltered with a membrane, UK-200, the samples were quantitatively retained as judged by the
neutral sugar analysis. These data indicated that both glycopeptides had a molecular size larger than 200,000. A large molecular size of glycopeptides obtained after exhaustive pronase digestion was also compatible with a concept that it was derived from mucin-type glycoprotein (Munakata et al. 1985b).

**DISCUSSION**

Many investigators have studied secretory otitis media by histological and histochemical procedures (Henzer 1972; Hussl and Lim 1969). There have been, however, only a few biochemical studies of secretory otitis media (Juhn et al. 1971; Vered et al. 1972). In the present work, trials were made to characterize the viscogenic substances from the middle ear effusions of secretory otitis media. After pronase digestion, 4 specimens from different cases all yielded sulfated glycopeptide fractions together with small amounts of glycosaminoglycans. After mucopolysaccharidase treatments of glycoconjugates of cases 3 and 4, each fraction gave an electrophoretically homogenous band. Their carbohydrate and amino acid compositions suggested that the glycopeptides obtained were derived from sulfated mucin-type glycoproteins.

As to the content of these glycopeptides, there were obvious differences between serous type and mucous type of secretory otitis media. The effusions from mucous-type otitis media contained larger amounts of glycopeptides than those from serous-type ones.

In general, mucin-type glycoproteins are secreted by goblet cells and from exocrine glands (Pigman 1977a). In the middle ear lining, there is goblet cell hyperplasia in the secretory otitis media (Henzer 1972). The present results suggest that there are sulfated mucin-type glycoproteins in middle ear effusions of secretory otitis media. These glycoproteins are possibly secreted by goblet cells.

The etiology of secretory otitis media is still unknown. It seems that the goblet cells of middle ear lining secrete sulfated mucins which are responsible for the high viscosity of middle ear effusions.

**Acknowledgments**

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

**References**


