

# An epitope residing in carbohydrate chains of a sea squirt antigen termed Gi-rep

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O-Glycosidically linked oligosaccharides (Gp-1 $\beta$ -b) were liberated from a large size glycopeptide (GP) fraction (Gp-1) in a Pronase digest of a sea squirt antigen termed Gi-rep by the treatment with 0.1 N NaOH per 1 mol/L of NaBH<sub>4</sub>. Gp-1 $\beta$ -b as well as Gp-1 and the intact antigen was capable of inducing a remarkable erythema in the skin of patients with asthma with sea squirt allergy at its concentration of 10  $\mu$ g/ml. N-Glycoside GP fractions (Gp-1 $\beta$ -a and Gp-2 $\beta$ ) having the allergenic activity were also prepared as alkali-resistant GPs from Gp-1 and the other relatively small size GP fraction (Gp-2) of the Pronase digest, respectively, after the alkali treatment. Chemically, the three allergenically active preparations were rich in galactosamine, glucosamine, and fucose in common, although the N-linked carbohydrates were much larger in size than O-linked carbohydrates. Accordingly, it has been expected that the epitope of the sea squirt antigen corresponds to certain oligosaccharide units residing in both of the O- and N-linked carbohydrate chains of the antigen. This suggestion was consistent with the observation that the allergenic activity of Gi-rep was significantly unstable to the periodate oxidation but substantially stable not only to acid, alkali, and heat treatments but also to the enzymatic proteolysis with Pronase E. (J ALLERGY CLIN IMMUNOL 1987;80:57-63.)

The antigen preparations, Gi-rep, Ei-M, and DIIIa, isolated from body fluid of a sea squirt, *Styela plicata*, were all glycoproteins containing significant amounts of hexosamines, and their weight-average molecular weights were estimated to be 106,000, 22,800, and 9,980, respectively.<sup>1-3</sup>

Each of the antigens elicited a remarkably positive reaction on the skin test specific to the patients with asthma with sea squirt allergy at a concentration as low as <10  $\mu$ g/ml.<sup>1-3</sup> Furthermore, every antigen, as well as a crude extract of sea squirt, dose dependently induced a significant release of leukocyte histamine in vitro in the heparin-treated blood of the patients with asthma at concentrations from 10 to 1000  $\mu$ g/ml.<sup>4,5</sup> Thus, the three purified antigens were ex-

## Abbreviations used

RIA: Radioimmunoassay

GP: Glycopeptide

pected to carry certain common epitopes responsible to the in vivo and in vitro allergic reactions mediated by the allergy-specific IgE antibody.

Of the three antigens, Gi-rep and Ei-M were highly effective in hyposensitization therapy; successful cases have amounted to >90%.<sup>6-8</sup> It was also recognized in the therapy by RIA that a certain specific IgG antibody was induced rapidly and remarkably in the patients by injection of the antigens. However, only DIIIa was apparently less effective both in the therapy and in the induction of the specific IgG.<sup>8</sup> Thus, the immunogenicity of DIIIa was considered to be substantially lower than immunogenicity of the other two antigens, although DIIIa was comparable to Gi-rep and Ei-M in the allergenic activity as examined by the skin test in vivo and the histamine-release experiment in vitro.

In contrast, rabbit antisera prepared against Gi-rep and Ei-M were analyzed in antigen specificity by the radioimmunometric method,<sup>2,3,9</sup> and it was revealed that both of the antigens induced a certain antibody (type A) that reacted not only with Gi-rep and Ei-M

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TABLE I. Stability of Gi-rep to physical, chemical, and enzymatic treatments

| Treatment            | Skin reaction |     |    |    |    | Antigenic activity (%) |
|----------------------|---------------|-----|----|----|----|------------------------|
|                      | a             | b   | c  | d  | e  |                        |
| 0. Untreated         | ++            | +++ | +  | ++ | ++ | 100                    |
| 1. 0.1 N HCl         | +++           | ++  | ++ | ++ | ++ | 95                     |
| 2. 0.1 N NaOH        | ++            | ++  | +  | ++ | +  | <2                     |
| 3. 100° C, 1 hr      | +++           | ++  | ++ | ++ | ++ | 82                     |
| 4. NaIO <sub>4</sub> | —             | —   | —  | —  | —  | <2                     |
| 5. Pronase E         | ++            | ++  | +  | ++ | +  | 29                     |

Gi-rep (1 mg) was incubated in 200  $\mu$ l of (1) 0.1 N HCl or (2) 0.1 N NaOH at 22° C for 4 hours and neutralized. (3) The antigen (1 mg) in 200  $\mu$ l of 0.9% NaCl was heated at 100° C for 1 hour. (4) Periodate-oxidized Gi-rep at 4 hours in Table II was dialyzed after addition of ethyleneglycol. (5) The Pronase digest of Gi-rep at 180 hours reaction in Table IV was heated at 100° C for 10 minutes and dialyzed. To evaluate the allergenic activity, these samples were diluted with 0.9% NaCl to a concentration of 10  $\mu$ g/ml on weight basis of the original antigen, and direct skin test was carried out against five cases of asthma (a to e) of sea squirt allergy by injection of 20  $\mu$ l of the diluted samples. When erythema, appearing after 15 minutes, was 0 to 10, 10 to 20, 20 to 30, 30 to 40, and >40 mm in diameter, symbols —,  $\pm$ , +, ++, and +++ were assigned, respectively. The antigenic activity in vitro was assayed by RIA and represented with percent activity based on that of Gi-rep.

but also with DIIIa in common, whereas Ei-M also induced the other type antibody (type B) specific to Ei-M and DIIIa in addition to type A antibody. Thus, it was expected that the three antigens carried certain common epitopes (type  $\alpha$ ) capable of inducing and reacting with type A antibody, whereas Ei-M and DIIIa carried, in addition to type  $\alpha$ , the other type of epitope (type  $\beta$ ) specific to the antibody of type B. Furthermore, the allergenic activity detectable by the skin test and the antigenic activity detectable by RIA in each of the three antigens was simultaneously absorbed by an immobilized immunoglobulin of the rabbit anti-Gi-rep serum containing type A antibody alone.<sup>3,9</sup>

From these clinical and in vitro observations, it is expected that type  $\alpha$  epitope specified by the reactivity to the rabbit antibody of type A corresponds to the presumed epitope responsible for the allergic reactions in skin and leukocytes of the allergic patients. However, the correspondence of type  $\beta$  epitope to the allergy-specific one is avoided because Gi-rep lacks the epitope. The purpose of the present work is to elucidate the chemical properties of the presumed epitope specific to sea squirt allergy in relation to chemical properties of type  $\alpha$  epitope.

## MATERIALS AND METHODS

### Materials

**Sea squirt antigen.** An antigen preparation termed Gi-rep, which was isolated from body fluid of a sea squirt, *S. plicata*, was used as a representative sea squirt antigen. Chemical and immunologic properties of the preparation were described in previous articles.<sup>2,3,9</sup>

**Pronase.** A commercial preparation, Pronase E (Kaken-Kagaku, Tokyo, Japan), was used for the proteolysis of the antigen. The specific activity indicated was 1170 tyrosine units per milligram.

**Gel materials.** Ultrogel AcA 44 and 54 (LKB Producter AB, Bromma, Sweden) were used for gel chromatography.

### Allergenic activity

The allergenic activity of a sample was evaluated by the method of skin test in vivo on patients with asthma with sea squirt allergy as previously described.<sup>1-3</sup>

### Antigenic activity

The reactivity of a sample to rabbit anti-Gi-rep serum (L 304) was assayed in vitro by RIA as the antigenic activity,<sup>1-3,9</sup> and the antigenic activity was expressed as Gi-rep equivalents.

### Isotachopheresis

The process of the periodate oxidation reaction of the antigen was monitored by the method of isotachopheresis<sup>10</sup> with an isotachopheretic analyzer, Shimazu (Kyoto, Japan) model IP-2A.

### Composition analysis

Amino acids and hexosamines were determined with an amino acid analyzer, Hitachi (Hitachi, Japan) model KLA-5, after hydrolysis of a sample in 6 N HCl at 110° C for 24 hours and in 4 N HCl at 100° C for 6 hours, respectively. Neutral sugars and sugar alcohols were determined by gas-liquid chromatography according to the method of Neeser-Schweizer<sup>11</sup> after hydrolysis of a sample in 4 N trifluoroacetic acid at 100° C for 4 hours. Total amount of neutral sugars was determined by the modified phenol-sulfuric acid method.<sup>3</sup>

**TABLE II.** Periodate oxidation of Gi-rep

|                                         | Incubation time (hr) |      |      |      |      |
|-----------------------------------------|----------------------|------|------|------|------|
|                                         | 0                    | 0.5  | 1    | 2    | 4    |
| IO <sub>3</sub> <sup>-</sup> (μmol/ml)  | —                    | 9.0  | 13.0 | 16.4 | 18.2 |
| Formate (μmol/ml)                       | —                    | 0.23 | 0.37 | 0.44 | 0.42 |
| Antigenic activity<br>(mg as Gi-rep/ml) | 5.0                  | 2.48 | 1.11 | 0.35 | 0.09 |

Gi-rep (10 mg) was incubated in 2 ml of 10 mmol/L of NaIO<sub>4</sub> at 25° C, and the process of the reaction was monitored by isotachophoretic analysis of the reaction mixture (5 μl) for the contents of IO<sub>3</sub><sup>-</sup> and formate. The antigenic activity was assayed by RIA in the reaction mixture after reductive inactivation of residual IO<sub>4</sub><sup>-</sup> with ethyleneglycol.

## RESULTS

### Treatment with acid, alkali, and heat

Gi-rep was treated with 0.1 N HCl or 0.1 N NaOH or heated at 100° C, and the treated antigen was examined in vivo and in vitro for the allergenic and antigenic activities, respectively. The allergenic activity detectable by skin test was substantially stable to all of the three treatments. However, the antigenic activity assayed by RIA was significantly unstable to alkali, although the in vitro activity was stable to acid and heat treatments (lines 1, 2, and 3, Table I).

### Periodate oxidation

The carbohydrate moiety of the antigen was examined for its contribution to the in vivo and in vitro activities by the periodate oxidation treatment. Periodate apparently decreased the antigenic activity according to the progress of the oxidation reaction, resulting in a practical loss of the in vitro activity within 4 hours of incubation (Table II). Allergenicity, the oxidation product at 4 hours of incubation was also inactive as examined by skin test (line 4, Table I).

Such concomitant loss of the in vivo and in vitro activities indicates that a certain structure residing in the carbohydrate moiety of Gi-rep is responsible to both of the activities. The oxidized Gi-rep is compared with the intact one in saccharide composition in Table III.

### Proteolysis

The protein moiety of Gi-rep was also examined for its contribution to the antigenic and allergenic activities by treating the antigen with Pronase E. The antigenic activity decreased by the proteolysis in parallel with the liberation of a large amount of ninhydrin-reactive (Katayama, Osaka, Japan) substance (Table IV). However, most of the allergenic activity and about 30% of the antigenic activity of Gi-rep were still conserved in the reaction mixture

**TABLE III.** Saccharide composition of Gi-rep before and after periodate oxidation

| Saccharide    | Before oxidation<br>(μmol/gm) | After oxidation<br>(μmol/gm) |
|---------------|-------------------------------|------------------------------|
| Rhamnose      | 22                            | 8                            |
| Fucose        | 190                           | 48                           |
| Xylose        | 16                            | 8                            |
| Mannose       | 69                            | 27                           |
| Glucose       | 25                            | 16                           |
| Galactose     | 106                           | 33                           |
| Glucosamine   | 426                           | 378                          |
| Galactosamine | 632                           | 411                          |

even after a prolonged incubation (line 5, Table I).

Thus, 2.5 gm of Gi-rep was digested with Pronase E for 180 hours under the same conditions as used in Table IV. Then, the reaction mixture was heated at 100° C for 10 minutes, dialyzed, and freeze-dried to yield 1.5 gm of a Gp preparation, which still conserved most of the allergenic activity and 29% of the antigenic activity of Gi-rep. Subsequently, Gp was chromatographed on a column of Ultrogel AcA 44 into two fractions, Gp-1 and Gp-2 (Fig. 1). The respective fractions were dialyzed and freeze-dried to yield 990 mg of Gp-1 and 240 mg of Gp-2.

The allergenic activities of both preparations were still comparable to that of the original antigen, whereas the antigenic activities of Gp-1 and Gp-2 were assayed to be 32% and 16% of that of Gi-rep, respectively (Table V). Both of the GPs were analyzed for chemical composition (columns 2 and 5, Table VI).

### Treatment with NaOH/NaBH<sub>4</sub>

The large size GP fraction, Gp-1, was first subjected to the mild alkali treatment to examine the presence of *O*-glycosidically linked oligosaccharides. Gp-1 (920 mg) was incubated in 0.1 N NaOH containing

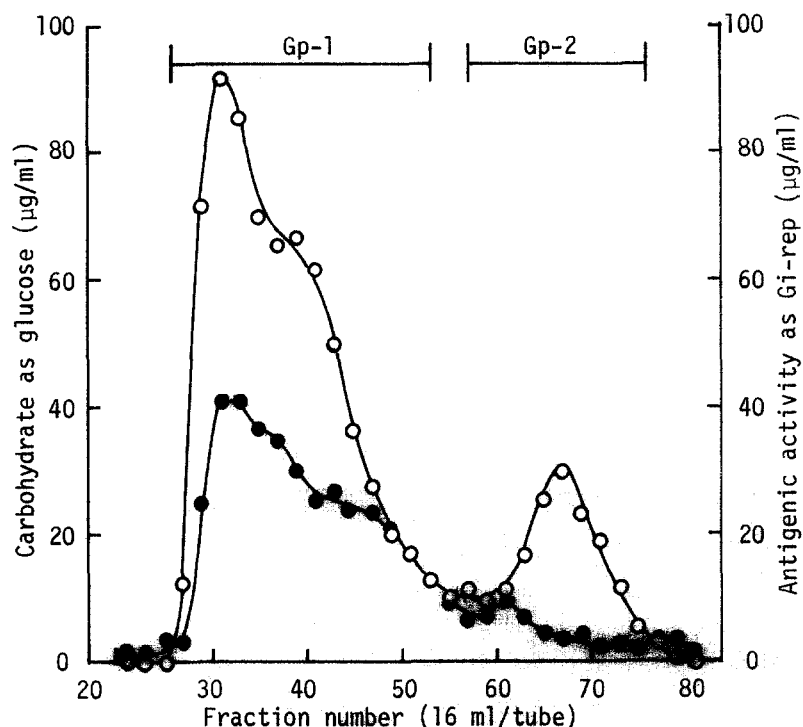


**FIG. 1.** Gel chromatographic fractionation of a Gp substance, derived from Gi-rep by Pronase digestion. Gp (290 mg) was chromatographed on a column of Ultrogel AcA 44 (4.4 by 80 cm) with 1% NaCl at a flow rate of 120 ml/hr. Carbohydrate (○) was determined by the phenol-sulfuric acid method. Antigenic activity (●) was assayed by the method of RIA.

**TABLE IV.** Proteolysis of Gi-rep with Pronase E

|                                         | Incubation time (hr) |      |      |      |      |      |
|-----------------------------------------|----------------------|------|------|------|------|------|
|                                         | 0                    | 25   | 50   | 100  | 150  | 180  |
| Amino acid<br>(μmol as leucine/ml)      | —                    | 0.91 | 1.05 | 1.25 | 1.35 | 1.37 |
| Antigenic activity<br>(mg as Gi-rep/ml) | 2.5                  | 1.03 | 0.83 | 0.75 | 0.72 | 0.72 |

Gi-rep (10 mg) was incubated with 0.4 mg of Pronase E at 30° C in 4 ml of 0.1 mol/L Tris-HCl buffer (pH 8.0) containing 50 mmol/L CaCl<sub>2</sub> under toluene layer. After 50 hours of incubation, 0.23 mg of the enzyme was supplemented, and the incubation was continued. The antigenic activity was assayed by RIA. The amount of amino acids was determined by the Ninhydrin method with leucine as standard.

1 mol/L of NaBH<sub>4</sub> at 37° C for 48 hours and acidified with acetic acid. Then, the product (Gp-1β) was chromatographed on a column of Ultrogel AcA 54 into two fractions, Gp-1β-a and Gp-1β-b, with a volatile buffer, 0.1 N of pyridine-acetic acid, pH 5.0 (Fig. 2). After repeated evaporation under vacuum, 205 mg of Gp-1β-a and 215 mg of Gp-1β-b were obtained. Either of the two preparations was still allergenically active, whereas no antigenic activity was detected by RIA in either fractions (Table V).

Chemically, Gp-1β-b contained a significant amount of galactosaminitol but only a relatively small

amount of amino acids (column 4, Table VI). The observation suggested that most of the components in the preparation were the oligosaccharides that were *O*-glycosidically linked to and liberated from Gp-1 by the alkali-mediated β-elimination reaction.<sup>12, 13</sup>

However, no sugar alcohol was detected in Gp-1β-a, suggesting that its carbohydrate moieties still linked to the peptide moieties through a certain alkali-resistant linkage such as *N*-glycosidic one (column 3, Table VI).

The small size GP, Gp-2, was also subjected to the alkali treatment, and the product was chromato-

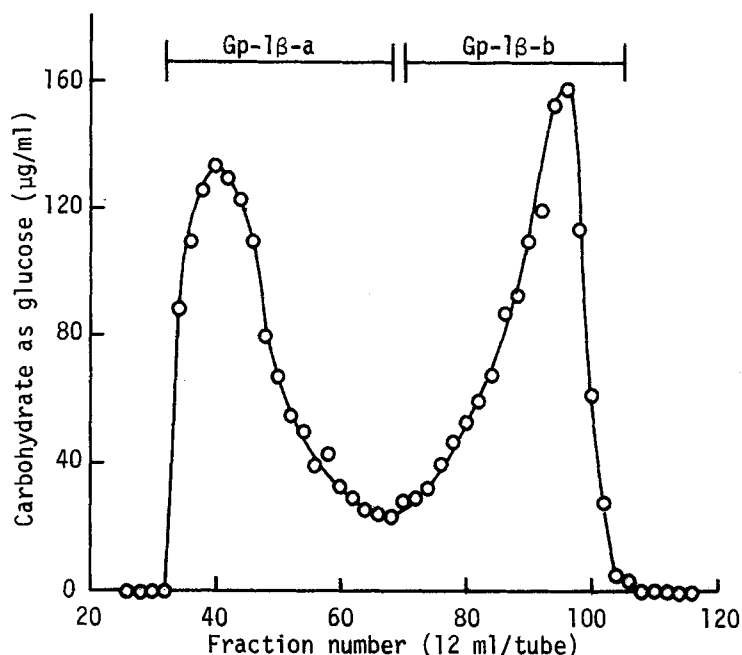


**FIG. 2.** Gel chromatographic fractionation of alkali-treated Gp-1. Gp-1 (920 mg) was incubated in 460 ml of 0.1 N NaOH containing 1 mol/L of NaBH<sub>4</sub> at 37° C for 48 hours and acidified with acetic acid. The product (Gp-1β) was recovered by treating the reaction mixture with Amberlite (Rohm and Haas, Co., Philadelphia, Pa.) CG-120 (H<sup>+</sup> form) and azeotropic distillation with methanol. Then, Gp-1β was chromatographed on a column of Ultrogel AcA 54 (4.4 by 70 cm) with 0.1 mol/L of pyridine-acetic acid buffer (pH 5.0) at a flow rate of 90 ml/hr.

graphed on a column of Ultrogel AcA 54. Because most of the carbohydrate-containing substance was eluted, eliciting a single peak, the peak fractions were pooled and evaporated to recover 69 mg of Gp-2β (Fig. 3). Gp-2β was still allergenically active in skin test but antigenically inactive as examined by RIA (Table V). On chemical analysis, similarly to Gp-1β-a, no sugar alcohol was detected in Gp-2β. Thus, most of the components in the preparation were suggested to be *N*-glycoside GPs.

## DISCUSSION

The three preparations termed Gp-1β-a, Gp-1β-b, and Gp-2β are the fractions of hexosamine-rich fragments derived from a representative sea squirt antigen, Gi-rep (Table VI). These preparations, as well as Gi-rep, are capable of inducing a remarkable erythema in skin of patients with asthma with sea squirt allergy with a concentration as low as 10 µg/ml (Table V). Thus, it is considered that each of the preparations still conserves the epitope that had resided in Gi-rep and reacted with the specific IgE antibody in the skin of the patients.

Chemically, of the three preparations, only Gp-1β-b contained a significant amount of galactosaminitol (Table VI). Thus, the preparation was suggested to be composed of the oligosaccharides that

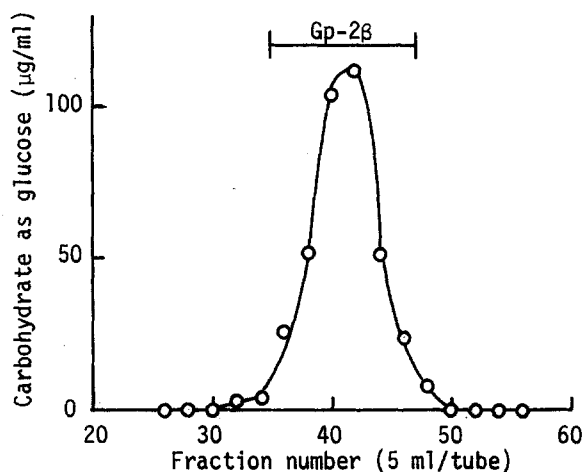


**FIG. 3.** Gel chromatographic fractionation of alkali-treated Gp-2. Similarly to the case of Gp-1, 200 mg of Gp-2 was treated with 0.1 N NaOH in 1 mol/L of NaBH<sub>4</sub>, and the product was chromatographed on a column of Ultrogel AcA 54 (1.6 by 100 cm) with 0.1 mol/L of pyridine-acetic acid buffer (pH 5.0) at a flow rate of 30 ml/hr.

have been *O*-glycosidically linked to and liberated from Gp-1 by the β-elimination reaction.<sup>12, 13</sup> This suggestion is supported by the very low content of amino acids relative to that of galactosaminitol (Table VI) and also by its smaller molecular size observed

**TABLE V.** Allergenic and antigenic activities of GPs and oligosaccharides from Gi-rep

| Preparation     | Allergenic activity detected by skin test |     |     |    |     |       |       |       |
|-----------------|-------------------------------------------|-----|-----|----|-----|-------|-------|-------|
|                 | f                                         | g   | h   | i  | j   | k     | l     | m     |
| Gi-rep          | +                                         | +++ | +   | +  | +++ | +     | ++    | ++    |
| Gp-1            | +                                         | ++  | +++ | ++ | ++  |       |       |       |
| Gp-1 $\beta$ -a |                                           |     |     |    |     | +     | +     | $\pm$ |
| Gp-1 $\beta$ -b |                                           |     |     |    |     | $\pm$ | $\pm$ | $\pm$ |
| Gp-2            | $\pm$                                     | +   | +++ | +  | ++  |       |       |       |
| Gp-2 $\beta$    |                                           |     |     |    |     |       |       |       |

A 20  $\mu$ l portion of 10  $\mu$ g/ml solution of each preparation in 0.9% NaCl was subjected to the skin test against patients with asthma (f to t) of sea squirt allergy. Evaluation and expression of the allergenic activity were the same as those used in Table I. The antigenic activity in vitro was assayed by RIA and represented with percent activity based on that of Gi-rep.

**TABLE VI.** Chemical compositions of allergenically active fragments prepared from Gi-rep

| Component                      | Gp-1<br>( $\mu$ mol/gm) | Gp-1 $\beta$ -a<br>( $\mu$ mol/gm) | Gp-1 $\beta$ -b<br>( $\mu$ mol/gm) | Gp-2<br>( $\mu$ mol/gm) | Gp-2 $\beta$<br>( $\mu$ mol/gm) |
|--------------------------------|-------------------------|------------------------------------|------------------------------------|-------------------------|---------------------------------|
| Lysine                         | 24                      | —                                  | —                                  | 32                      | —                               |
| Histidine                      | 18                      | —                                  | —                                  | 14                      | —                               |
| Arginine                       | —                       | —                                  | —                                  | 22                      | —                               |
| Aspartic acid                  | 82                      | 7                                  | 22                                 | 200                     | 84                              |
| Threonine                      | 159                     | 16                                 | 24                                 | 178                     | 22                              |
| Serine                         | 114                     | 15                                 | 61                                 | 104                     | 10                              |
| Glutamic acid                  | 85                      | 9                                  | 17                                 | 119                     | 24                              |
| Proline                        | 40                      | —                                  | —                                  | —                       | —                               |
| Glycine                        | 87                      | 19                                 | 48                                 | 107                     | 27                              |
| Alanine                        | 76                      | 10                                 | 48                                 | 68                      | 27                              |
| Cystine                        | 10                      | —                                  | —                                  | 27                      | 14                              |
| Valine                         | 58                      | —                                  | 23                                 | 56                      | 16                              |
| Methionine                     | 14                      | —                                  | 20                                 | 19                      | —                               |
| Isoleucine                     | 37                      | —                                  | 7                                  | 40                      | 10                              |
| Leucine                        | 31                      | —                                  | 8                                  | 32                      | —                               |
| Tyrosine                       | 34                      | —                                  | 11                                 | 16                      | —                               |
| Phenylalanine                  | —                       | —                                  | —                                  | 19                      | —                               |
| Rhamnose                       | 44                      | —                                  | —                                  | —                       | —                               |
| Fucose                         | 344                     | 204                                | 212                                | 130                     | 342                             |
| Xylose                         | 103                     | 113                                | 11                                 | —                       | —                               |
| Mannose                        | 27                      | —                                  | —                                  | 28                      | 180                             |
| Glucose                        | 17                      | 44                                 | 16                                 | —                       | —                               |
| Galactose                      | 277                     | 440                                | 136                                | 112                     | 135                             |
| Glucosamine                    | 628                     | 280                                | 499                                | 760                     | 920                             |
| Galactosamine                  | 861                     | 463                                | 333                                | 795                     | 1,330                           |
| Galactosaminitol               | —                       | —                                  | 271                                | —                       | —                               |
| Uronic acid as glucuronic acid | 216                     | 572                                | 72                                 | 507                     | —                               |

in gel chromatography (Fig. 2). Therefore, it is considered that the epitope in Gp-1 $\beta$ -b should correspond to a certain structural unit contained in such oligosaccharides.

In contrast, Gp-1 $\beta$ -a and Gp-2 $\beta$  contained no sugar alcohol, suggesting that the carbohydrates have still been linked to the oligopeptide moieties through an

alkali-resistant *N*-glycosidic linkage (Table VI). Although amino acid contents of Gp-1 $\beta$ -a and Gp-2 $\beta$  are much lower than amino acid contents of the corresponding GPs before the alkali treatment, the loss of amino acids is ascribed to a certain spontaneous degradation of the peptide moiety in the mother GPs under the alkaline conditions but not to the

| Allergenic activity detected by skin test |   |   |    |   |   |   | Antigenic activity (%) |
|-------------------------------------------|---|---|----|---|---|---|------------------------|
| n                                         | o | p | q  | r | s | t |                        |
| ++                                        | + | + | ++ | + | + | + | 100                    |
| +                                         | ± |   |    |   |   |   | 32                     |
| ++                                        | + |   |    |   |   |   | <1                     |
|                                           |   | + | ++ | + | ± | + | <1                     |
|                                           |   | + | +  | + | ± | + | 16                     |
|                                           |   |   |    |   |   |   | <1                     |

$\beta$ -elimination reaction. Furthermore, the carbohydrates in the presumed *N*-linked GPs are considered to be substantially large size polysaccharides from the elution behavior of these GPs (Figs. 2 and 3).

However, similarly to Gp-1 $\beta$ -b, the two large size GP fractions are capable of inducing the skin reaction. Thus, they should also contain the oligosaccharide epitope that is equivalent to the epitope residing in Gp-1 $\beta$ -b. This suggestion is supported by the high contents of hexosamines and fucose in common with the case of Gp-1 $\beta$ -b, whereas the contribution of the peptide moiety to the epitopic function is rejected by their very low contents of amino acids (Table VI).

Accordingly, it is expected that the allergy-specific epitope of Gi-rep corresponds to a certain structural unit residing in the carbohydrate chains of the above mentioned allergenically active fragments in common. The chemical structure of the oligosaccharide epitope is being studied as the next problem to be solved.

In this connection, these allergenically active carbohydrate-rich preparations demonstrated no antigenic activity as examined by RIA (Table V). This observation appears to be inconsistent with the previous suggestion<sup>1-3, 9</sup> that the allergy-specific epitope corresponds to type  $\alpha$  epitope specified by the reactivity with the rabbit polyclonal antibody of type A. However, type  $\alpha$  epitope as well as the allergy-specific epitope is considered to be contained in the three sea squirt antigens in common.<sup>1-3, 9</sup> Furthermore, as observed in the periodate-oxidation of Gi-rep (Tables I and II), the *in vitro* activity as well as the *in vivo* activity appeared to depend mostly on the carbohydrate structure in the antigen. Therefore, at least partial overlapping of the carbohydrate structures could still be expected in type  $\alpha$  and the allergy-specific epitopes. The possibility of the structural overlapping should be further examined by the method with monoclonal antibodies.

## REFERENCES

- Oka S, Suzuki H, Jyo T, Tsuji M. Purification of two types of sea squirt antigens. *Int Arch Allergy Appl Immunol* 1979;59:408.
- Oka S, Suzuki H, Hashimoto H, Jyo T. The properties of two sea squirt antigens. *J Biochem* 1982;91:247.
- Suzuki H, Oka S, Shigeta S, Ono K, Jyo T, Katsutani T. Isolation and characterization of an asthma-inducing sea squirt antigen. *J Biochem* 1984;96:849.
- Tanifuji K, Daibo A, Sudo M, Tamura M, Jyo T, Katsutani T, Oka S, Suzuki H. The correlation between sea squirt crude and purified antigens and histamine release in sea squirt asthma. *Jpn J Allergol* 1984;33:78.
- Daibo A, Tanifuji K, Kitazawa S, Tamura M, Jyo T, Katsutani T, Aono M, Oka S. Influence of blocking antibody on *in vitro* histamine release in sea squirt asthma. *Jpn J Allergol* 1986;35:747.
- Jyo T, Kohmoto K, Katsutani T, Otsuka T, Tsuboi S, Oka S. Measurement of specific IgG antibody using protein A-Sepharose CL-6B in patients treated with hyposensitization therapy. *Jpn J Allergol* 1978;27:842.
- Jyo T, Oka S. Purification of sea squirt antigens and hyposensitization therapy with purified antigens. *Allegria* 1985;14:26.
- Jyo T, Kodomari Y, Kodomari N, Katsutani T, Otsuka T, Tsuboi S, Oka S, Shigeta S, Ono K. Therapeutic effect and titers of the specific IgE and IgG antibodies in cases of sea squirt allergy (Hoya asthma) under a long-term hyposensitization with three sea squirt antigens [submitted]. *J ALLERGY CLIN IMMUNOL*.
- Oka S, Suzuki H, Tsuji M, Jyo T. Immunogenic properties of two types of sea squirt antigens. *J Biochem* 1982;91:1403.
- Oka S, Hirotsune M, Shigeta S. Isotachopheresis for periodate oxidation analysis of a small amount of carbohydrate. *Anal Biochem* 1979;98:417.
- Neeser JR, Schweizer TF. A quantitative determination by gas-liquid chromatography of neutral and amino sugars (as *O*-methyloxime acetates), and a study on hydrolytic conditions for glycoproteins and polysaccharides in order to increase sugar recoveries. *Anal Biochem* 1984;142:58.
- Anderson B, Seno N, Sampson P, Riley GR, Hoffman P, Meyer K. Threonine and serine linkages in mucopolysaccharides and glycoproteins. *J Biol Chem* 1964;239:PC2716.
- Tanaka K, Pigman W. Improvements in hydrogenation procedure for demonstration of *O*-therone glycosidic linkages in bovine submaxillary mucin. *J Biol Chem* 1965;249:PC1487.