

Isolation and Characterization of an Asthma-Inducing Sea-Squirt Antigen¹

Hidenori SUZUKI,*² Satoru OKA,* Seiko SHIGETA,*
Kazuhisa ONO,* Toshihiko JYO,** and
Takashi KATSUTANI***

*Laboratory of Biochemistry, Faculty of Engineering, Hiroshima University,
Higashi-Hiroshima, Hiroshima 724, **Third Department of Internal
Medicine, Hiroshima Prefectural Hospital, Hiroshima 734, and
***Katsutani Clinic, Hatsukaichi-machi, Hiroshima 738

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An antigen capable of inducing asthmatic attack in patients with sea-squirt allergy has been isolated from body fluid of sea-squirt, *Styela plicata*, and designated as *DIIIIa*. The chemical composition and the behavior in anion-exchange chromatography showed that the antigen was a weakly acidic glycoprotein. The weight-average molecular weight of *DIIIIa* was estimated to be 9,880 by the sedimentation equilibrium method. This antigen was apparently discriminated from the previously isolated sea-squirt antigens, Gi-rep and Ei-M, by its activity to induce asthmatic attack and conjunctival congestion, though it gave a strongly positive reaction in skin tests against allergic patients, similarly to the previous antigens. From the cross-reaction of *DIIIIa* to rabbit anti-Gi-rep and anti-Ei-M sera *in vitro*, it was confirmed that the antigen carried essentially the same antigenic determinant as Gi-rep and Ei-M, and this was termed type *a* determinant. Furthermore, the allergenic activities detectable *in vivo* and the reactivity to the rabbit antisera *in vitro* were simultaneously absorbed by immobilized immunoglobulin from anti-Gi-rep serum. Thus, it was suggested that the antigenic determinant responsible for the allergic reactions *in vivo* corresponded to that specified as type *a* *in vitro* on the basis of the reactivity against the rabbit anti-Gi-rep serum.

Previously, it has been shown that two sea-squirt antigen preparations termed Gi-rep and Ei-M are not only active in skin tests on asthmatic patients with sea-squirt allergy but also highly effective in hyposensitization therapy of the patients (1-4). In the therapy, it was also recognized that each of

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² Present address: Central Research Laboratory, Wakunaga Pharmaceutical Co., Ltd., Koda-cho, Hiroshima, Hiroshima 729-64.

Abbreviation: FEV₁, one-second vital capacity in forced expiration.

the antigens rapidly induced a certain blocking antibody which could be neutralized by either of the antigens. Nevertheless, these antigens induced neither conjunctival reaction nor asthmatic attack in the allergic patients (1-4).

On the other hand, radioimmunometric examination of the interaction between the antigens and the antisera prepared in rabbits against the antigens revealed that Gi-rep induced only one type of antibody (type *A*), while Ei-M induced another type of antibody (type *B*) in addition to type *A*. Thus, the induction of type *A* antibody was ascribed to a certain antigenic determinant (type α) carried by the two antigens in common and that of type *B* was ascribed to another determinant (type β) specific to Ei-M (4, 5).

These observations suggested that the blocking antibody (specific IgG) and the reagin (specific IgE) in the patients could be similar to rabbit type *A* antibody in specificity. Thus, it was expected that, though no antigen capable of inducing asthmatic attack in the allergic patients has yet been isolated, the presumed asthma-inducing antigen also carries type α determinant similarly to Gi-rep and Ei-M.

In this connection, in the course of purification of Gi-rep and Ei-M, the antigenic activity was assayed by skin test *in vivo* and by radioimmunoassay *in vitro* (1-5). However, it has been suggested that the activity inducing asthmatic attack correlates closely to the activity inducing conjunctival congestion rather than to that detectable by skin test in the cases of some allergens (6-8). Therefore, in the present study, the asthma-inducing antigen was newly isolated from the body fluid of sea-squirt on the basis of the activity giving conjunctival reaction and compared with the previous antigens in immunological properties as well as in physical and chemical ones.

MATERIALS AND METHODS

Body Fluid of Sea-Squirt—Sea-squirt, *Styela plicata*, grown on oyster shell was harvested and dissected to obtain the body fluid. The body fluid was employed as the source of asthma-inducing antigen after being dialyzed against running water and concentrated under reduced pressure.

Sea-Squirt Antigens and Anti-Sea-Squirt Sera—Two sea-squirt antigen preparations termed Gi-

rep and Ei-M were those prepared and characterized in previous studies (3-5). The rabbit anti-sea-squirt sera, anti-Gi-rep (L 304) and anti-Ei-M (L 305), were those prepared and characterized in the preceding study (5).

Materials for Gel Chromatography and Anion-Exchanger—Ultrogel AcA 44 and AcA 54 (LKB Producter AB, Bromma) were used for gel chromatography. DEAE-Toyopearl 650S (Toyo Soda, Tokyo) was used as an anion-exchanger.

Conjunctival Reaction Test—A lower eyelid of a patient with sea-squirt allergy was turned up and challenged with a drop of an antigen solution to be tested. The other eye of the patient was treated with 0.9% NaCl as a control. Then, 10-15 min after the challenge, the conjunctiva was examined for congestion by comparing it with that of the control eye.

Bronchial Provocation Test—Untreated patients with sea-squirt allergy inhaled 0.3 ml of an antigen solution in 0.9% NaCl in a form of a mist through a nebulizer (Nisshyo Shoji, Tokyo). Then, asthmatic attack induced in the patients was examined within 15 min after the inhalation. For the control test, 0.9% NaCl solution was used instead of the antigen solution. At that time, the initial rate of forced expiration, *i.e.* one-second vital capacity (FEV₁, ml/s), was also measured by using a spirometer (model AS-2300, Minato Ika-gaku, Tokyo).

Skin Test—The procedure for skin test was the same as that used previously (1-4).

Radioimmunoassay—The procedure for radioimmunoassay (RIA) used in the present study was essentially the same as that described in the previous papers (4, 5).

Immobilized Immunoglobulin and Absorption Experiment—Immunoglobulin was obtained from rabbit anti-Gi-rep serum (700 or 250 μ l) by the salting-out technique using Na₂SO₄ (180 mg/ml serum). The precipitate of the globulin was dissolved in a small volume of 0.05 M phosphate buffer, pH 7.6, containing 1% NaCl for 16 h at 4°C with several changes of the buffer. Protein A-Sepharose CL-4B (Pharmacia Fine Chemicals, Uppsala) was swollen and washed with the same buffer. Then, the immunoglobulin solution was added to 250 μ l of the swollen gel in a small plastic vial, the mixture was incubated for 5 h at room temperature under gentle shaking to immo-

bilize the globulin on the gel, and the gel was washed repeatedly with 0.01 M phosphate buffer, pH 7.6, containing 1% NaCl by centrifugation. In absorption experiments, 750 μ l of an antigen dissolved in the same buffer was incubated with the immunoglobulin-trapped gel for 16 h at room temperature under gentle shaking and centrifuged. Then, the supernatant was examined for concentration of the residual antigen by RIA and for allergenic activities *in vivo*.

Methods of Analysis—The methods used for chemical analysis and for sedimentation experiments were the same as those used in the previous studies (3, 4).

RESULTS

Isolation of Antigen Inducing Conjunctival Congestion—To seek an antigen capable of inducing asthmatic attack in patients with sea-squirt allergy, the body fluid of sea-squirt was chromatographed on a column of Ultrogel AcA 44 and the eluate was divided into four fractions (H-G, E, D, and C) as shown in Fig. 1. As asthma-inducing activity has been suggested to correlate closely to the activity inducing conjunctival congestion in the cases of some allergens (6-8), the four fractions were examined for activity towards conjunctiva as well as skin of the allergic patients.

Table I shows that the conjunctival activity was detected only in fraction D, though all fractions gave positive reaction in the skin test. Thus, fraction D was chromatographed again on a column of Ultrogel AcA 54 and most of the con-

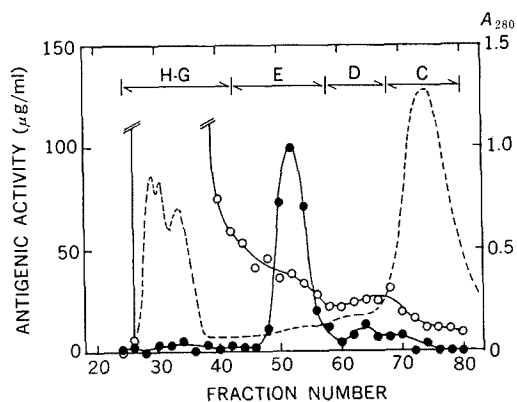


Fig. 1. Chromatographic fractionation of body fluid of sea-squirt. Body fluid from 1.1 kg of sea-squirt was chromatographed on a column of Ultrogel AcA 44 (4.4 $\times$ 90 cm) with 1% NaCl at a flow rate of 120 ml/h (16-ml fractions) after being dialyzed and concentrated under reduced pressure. The indicated fractions were pooled, and subjected to the conjunctival reaction test and skin test. $\circ$, antigenic activity assayed by RIA as Gi-rep; $\bullet$, antigenic activity assayed by RIA as Ei-M; ----, optical absorption at 280 nm.

TABLE I. Antigenic activities in the fractions in Fig. 1 examined by conjunctival reaction and skin test at 1 : 10 and 1 : 81 dilutions, respectively.

Patient	Conjunctival reaction				Skin test			
	H-G	E	D	C	H-G	E	D	C
a	+		+++		+	++	++	++
b	—		+++		$\pm$	$\pm$	+	+
c	—		++		$\pm$	+	$\pm$	$\pm$
d	—	—			+	++	$\pm$	+
e	—	—			+	+	$\pm$	—
f	—	—			+	$\pm$	++	++
g		—	++		++	+	++	+
h		—	+		+	+	++	+
i			++	—	$\pm$	—	$\pm$	—
j			++	—	+	+	+	$\pm$
k			+	—	+	+	+	$\pm$

junctival activity was recovered in fraction DIII, which contained appreciable antigenic activities detected by RIA as Gi-rep and Ei-M, as shown in Fig. 2 and Table II.

After dialysis of fraction DIII against 0.05 M Tris-HCl buffer, pH 8.0, the fraction was applied to a column of DEAE-Toyopearl 650S and eluted with the same buffer, followed by elution with increasing concentrations of NaCl in the same

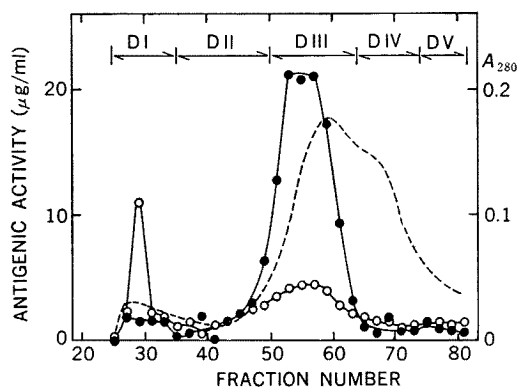


Fig. 2. Rechromatography of the fraction active in the conjunctival reaction test. Fraction D in Fig. 1 (from 3.2 kg of sea-squirt) was chromatographed on a column of Ultrogel AcA 54 (4.4×90 cm) with 1% NaCl at a flow rate of 120 ml/h (16-ml fractions). The indicated fractions were pooled, and examined for the activity in the conjunctival reaction test. Symbols are the same as those used in Fig. 1.

TABLE II. Antigenic activities in the fractions in Fig. 2 examined by conjunctival reaction at 1 : 10 dilution.

Patient	DII	DIII	DIV	DV
l	—	—		
m	—	+		
n	—	+		
o	—	++		
p		—	—	
q		++	—	
r		++	—	
s		+	—	
t		++	—	
u		+++		—
v		+		—

buffer. Two major peaks (a and b) of the antigenic activity were detected by RIA at 0.08 M and 0.2 M NaCl, respectively, as shown in Fig. 3. However, the conjunctival activity was detected only in the fractions of peak a, as shown in Table III. Therefore, fractions No. 15–20 in Fig. 3 were pooled, dialyzed and freeze-dried to obtain an antigen preparation which was designated as *DIIIa*.

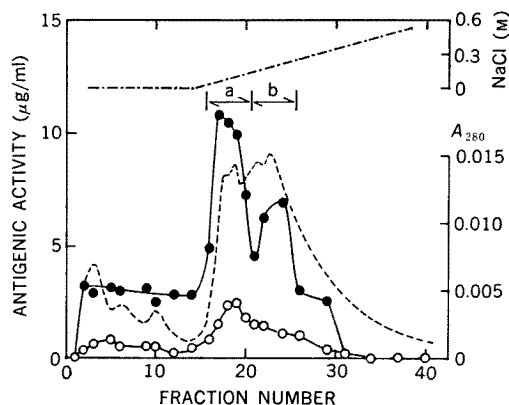


Fig. 3. Purification of antigen active in the conjunctival reaction test on a DEAE-Toyopearl column by salt-gradient elution. Fraction DIII in Fig. 2 was dialyzed against 0.05 M Tris-HCl buffer, pH 8.0, and applied to a column of DEAE-Toyopearl 650S (1.3×11 cm) equilibrated with the same buffer. Then, elution was carried out with the same buffer, followed by elution with increasing concentrations of NaCl in the buffer (8-ml fractions). ○, antigenic activity assayed by RIA as Gi-rep; ●, antigenic activity assayed by RIA as Ei-M; ----, optical absorption at 280 nm; -.-.-, concentration of NaCl.

TABLE III. Antigenic activities in the fractions in Fig. 3 examined by conjunctival reaction at 1 : 10 dilution.

Patient	Fract. No. 3	Fract. No. 10	Fract. No. 18 (peak a)	Fract. No. 23 (peak b)
A	—	—		
B	—		+	
C		—	+	
D		—	+	
E			+	—
F			+	—

The yield of DIIIa was 2.3 mg from 3.2 kg of sea-squirt.

Chemical and Physical Properties—Table IV shows the chemical composition of DIIIa. When compared with Gi-rep and Ei-M, DIIIa contained more uronic acid, but less sulfuric and phosphoric acids. (Contents of uronic, sulfuric, and phosphoric acids in the previous antigens were <40, >150, and >90 $\mu\text{mol/g}$, respectively (4)).

The sedimentation pattern in Fig. 4 shows that DIIIa was apparently homogeneous, giving a sedimentation coefficient of 1.41 $s_{20,w}$. The weight-

average molecular weight of DIIIa was estimated to be 9,880 (specific volume, 0.6710) by the sedimentation equilibrium method.

As was the case with the previous antigens (4), SDS-polyacrylamide gel electrophoresis was not applicable to DIIIa because the antigen was highly soluble even after the usual staining treatment with Coomassie Blue G250. The Ouchterlony test was not applicable because neither sera of the treated patients nor those of rabbits immunized with the previous antigens gave any detectable precipitation at any concentration of DIIIa.

Antigenic Activities In Vivo—Table V shows that DIIIa gave a strongly positive reaction in the

TABLE IV. Chemical composition of DIIIa.

Component	Content ($\mu\text{mol/g}$)
Lysine	310
Histidine	42
Arginine	106
Aspartic acid	502
Threonine	428
Serine	366
Glutamic acid	540
Proline	208
Glycine	466
Alanine	416
Cystine	8
Valine	228
Methionine	72
Isoleucine	150
Leucine	224
Tyrosine	232
Phenylalanine	140
Glucosamine	370
Galactosamine	332
Rhamnose	23
Fucose	34
Xylose	15
Mannose	43
Glucose	6
Galactose	19
Uronic acid as glucuronic acid	199
Sulfuric acid	4
Phosphoric acid	20

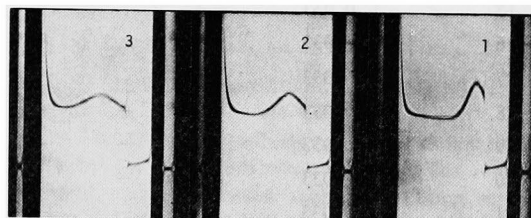


Fig. 4. Sedimentation pattern of DIIIa in 1% NaCl at 60,000 rpm and 20°C. The concentration of the preparation was about 10 mg/ml. Patterns 1, 2, and 3 were photographed at 54, 108, and 162 min, respectively, after reaching 60,000 rpm with a bar angle of 40°. The direction of sedimentation was from right to left.

TABLE V. Antigenic activities of the three antigens examined by skin test.

Patient	Threshold concentration ($\mu\text{g/ml}$)		
	DIIIa	Gi-rep	Ei-M
1	2		10
2	10		10
3	10		10
4	2		2
5	2		10
6	2	10	
7	10	>10	
8	2	10	
9		10	2
10		10	10
11		2	0.4

skin test, similarly to Gi-rep and Ei-M. However, as shown in Table VI, DIIIa induced significant congestion in the conjunctiva of the allergic patients, while the previous antigens were inactive or only slightly active. Furthermore, when DIIIa was used in a bronchial provocation test against

TABLE VI. Antigenic activities of the three antigen preparations examined by conjunctival reaction.

Patient	Concentration of antigen (%)	Conjunctival reaction		
		DIIIa	Gi-rep	Ei-M
12	0.033	++		+
13	0.033	++		++
14	0.033	+++		+
15	0.033	++		—
16	0.033	++		+
17	0.033	+		+
18	0.033		—	—
19	0.033		—	—
20	0.033		—	—
21	0.033		—	++
22	0.1	++		—
23	0.1	+++		—
24	0.1	+++		—
25	0.1	+++	—	
26	0.1	++	—	

TABLE VII. Bronchial provocation test of DIIIa. Two untreated patients (Case 1, female 60 years old; Case 2, female 53 years old) with sea-squirt allergy inhaled 0.3 ml of an antigen solution in 0.9% NaCl in the form of a mist and the induced allergic reactions were examined within 15 min after the inhalation. Both patients were exclusively sensitive to DIIIa in the conjunctival reaction test, though they were highly sensitive not only to DIIIa but also to Gi-rep and Ei-M in the skin test.

	Inhalant	Coughing	Wheezing	FEV ₁ (ml/s)
Case 1	0.9% NaCl	—	—	1,070
	0.0125% DIIIa	++	++	650
Case 2	0.9% NaCl	—	—	2,050
	0.01% Gi-rep	—	—	1,900
	0.01% DIIIa	+	+	1,670

untreated patients, the antigen immediately induced a violent asthmatic attack of coughing and wheezing with a significant reduction in the value of FEV₁, as shown in Table VII.

Antigenic Activity In Vitro—The antigenic activity of DIIIa was also examined *in vitro* by the RIA method as shown in Fig. 5. The binding reaction of radiolabeled Gi-rep to rabbit anti-Gi-

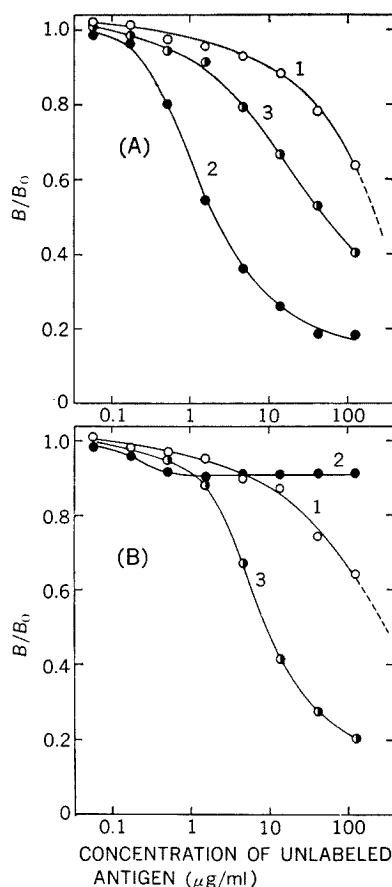


Fig. 5. Inhibition of antibody-binding of radiolabeled Gi-rep and Ei-M by the three unlabeled antigens. The RIA method was used (4): (A) the immunological reaction between rabbit anti-Gi-rep serum (1 : 1,200) and ¹²⁵I-labeled Gi-rep, and (B) that between rabbit anti-Ei-M serum (1 : 1,200) and ¹²⁵I-labeled Ei-M were inhibited by each of the unlabeled antigens. Curves 1, 2, and 3 show the inhibitions by DIIIa, Gi-rep, and Ei-M, respectively. B/B_0 is the ratio of antibody-bound radioactivity in the presence of an unlabeled antigen (B) to that of a control experiment with labeled antigen alone (B_0).

rep serum (containing type *A* antibody alone (5)) was apparently inhibited by increasing concentrations of unlabeled DIIIa as well as by unlabeled Gi-rep (containing antigenic determinant of type *a* alone (5)) and Ei-M (containing determinants of types *a* and *β* (5)) (Fig. 5A). DIIIa also inhibited the reaction of radiolabeled Ei-M with rabbit anti-Ei-M serum (containing antibodies of types *A* and *B* (5)) similarly to unlabeled Ei-M. However, Gi-rep was different from the other two antigens in the mode of inhibition (Fig. 5B). In connection with the limited inhibition by Gi-rep, it has been suggested that type *a* determinant of Gi-rep could not replace type *β* determinant of labeled Ei-M, which bound to type *B* antibody in anti-Ei-M serum, though it could replace type *a* determinant of the labeled antigen, which bound to type *A* antibody in the same serum (4, 5). Therefore, the results in Fig. 5 suggest that the type *a* determinant is a common antigenic determinant for the three sea-squirt antigens, though DIIIa carries type *β* determinant in addition to type *a*, similarly to Ei-M.

Thus, the expected type *a* determinant of DIIIa was further examined for specificity to type *A* antibody in anti-Gi-rep serum. As shown in Fig. 6, radiolabeled DIIIa apparently reacted to anti-Gi-rep serum and the immunological reaction was inhibited not only by unlabeled DIIIa but also by either unlabeled Gi-rep or Ei-M. This result

indicates that the anti-Gi-rep-reacting determinant of DIIIa is essentially the same in specificity as the determinant of type *a* carried by Gi-rep and Ei-M in common.

Furthermore, according to the procedure described in the preceding paper (5), the affinity con-

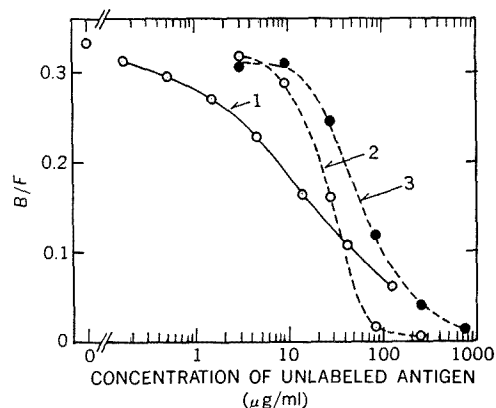


Fig. 6. Inhibition of antibody-binding of radiolabeled DIIIa by the three unlabeled antigens. The immunological reaction between rabbit anti-Gi-rep serum (1 : 20) and ^{125}I -labeled DIIIa (ca. 150,000 cpm/ml) was inhibited by unlabeled DIIIa (curve 1), Gi-rep (curve 2), and Ei-M (curve 3); the RIA method was used (5). In this case, the B/F value was employed in place of B/B_0 to analyze the interaction between DIIIa and the anti-serum. B/F is the ratio of antibody-bound radioactivity (B) to the free one (F).

TABLE VIII. Interaction between DIIIa and rabbit anti-Gi-rep antibody. K is the affinity constant of an antigen to the antibody in anti-Gi-rep serum. B_m is the maximum amount (μg) of the antigen which can be bound by the antiserum. K was calculated from B_0/F_0 (B/F value for the control experiment with the radiolabeled antigen alone) and $T_{0.5}$ (the concentration of the unlabeled antigen giving 50% inhibition of the binding of the labeled antigen) by Eq. 1 (5),

$$K = [1 + 0.5 (B_0/F_0)] / (T_{0.5} + t^*) \quad (1)$$

where t^* is the concentration of radiolabeled antigen (negligibly small for the case of DIIIa (ca. 15 ng/ml)). Then, B_m was calculated from K and B_0/F_0 by Eq. 2 (5),

$$B_m = (B_0/F_0) / K \quad (2)$$

Data for DIIIa were obtained by using curve 1 in Fig. 5 and those for Gi-rep and Ei-M were taken from the preceding paper (5).

Antigen	Concentration of anti-Gi-rep ($\mu\text{l/ml}$)	B_0/F_0	$T_{0.5}$ ($\mu\text{g/ml}$)	K ($\text{ml}/\mu\text{g}$)	B_m ($\mu\text{g}/\mu\text{l}$)
DIIIa	50	0.332	13.9	0.084	0.079
Gi-rep	1.25	5.13	0.540	6.54	0.627
Ei-M	1.25	0.29	0.395	2.87	0.081

TABLE IX. Absorption of the three sea-squirt antigens by immobilized immunoglobulin from anti-Gi-rep serum. For the control experiments, the antigens were treated with immobilized immunoglobulin from normal rabbit serum instead of the antiserum.

Antigen	Amount of antigen applied (μ g)	Amount of anti-Gi-rep serum used for immunosorbent (μ l)	Concentration of residual antigen assayed by RIA	
			Control (μ g/ml)	Treated (μ g/ml)
DIIIa	50	700	53.6	12.0
Gi-rep	50	250	49.6	0.6
Ei-M	10	250	12.6	1.3

TABLE X. Residual allergenic activities in DIIIa after treatment with immobilized immunoglobulin from anti-Gi-rep serum. The treated DIIIa sample in Table IX was diluted to 1/20 and 1/2 with 0.9% NaCl for testing the reactivities to skin and conjunctiva, respectively, in the allergic patients.

Patient	Allergenic reaction	
	Control	Treated DIIIa
<i>vs. skin</i>		
27	+++	$\pm$
28	+++	—
29	+++	—
30	+++	$\pm$
31	++	—
<i>vs. conjunctival</i>		
32	+	—
33	++	—
34	++	—
35	++	—
36	++	—
37	++	+

stant, K , of DIIIa to anti-Gi-rep serum and the maximum amount, B_m , of the antigen which could be bound by 1 μ l of the antiserum were calculated from the concentration of unlabeled DIIIa giving 50% inhibition, $T_{0.5}$, in curve 1 of Fig. 6. Table VIII shows that B_m for DIIIa is comparable to that for Ei-M, though K of DIIIa is significantly lower than those of Gi-rep and Ei-M.

Allergenic Activity and Type α Determinant—As DIIIa was confirmed to carry type α determi-

nant, which is expected to react also with the IgE specific to sea-squirt allergy (3–5), the correlation between the allergenic activity of DIIIa *in vivo* and its reactivity to type α antibody *in vitro* was examined. DIIIa (50 μ g) was reacted with immobilized immunoglobulin from 700 μ l of anti-Gi-rep serum and the residual antigenic activity depending on type α determinant was assayed by RIA. As shown in Table IX, a large portion of DIIIa applied was absorbed by the immobilized immunoglobulin, though the efficiency of absorption was somewhat lower than in the cases of Gi-rep and Ei-M.

The antigen sample thus treated was also examined for activity on skin and conjunctiva of the allergic patients. As shown in Table X, most of the allergenic activities was absorbed by the immobilized immunoglobulin in parallel with the absorption of the *in vitro* reactivity to type α antibody.

DISCUSSION

As described above, an antigen capable of inducing asthmatic attack in patients with sea-squirt allergy was isolated from body fluid of sea-squirt based on assay of activity to cause a conjunctival reaction and was designated as DIIIa. DIIIa, like Gi-rep and Ei-M (4), was fairly homogeneous on gel chromatography, anion-exchange chromatography, and sedimentation analysis (Figs. 2, 3, and 4). The weight-average molecular weight of the antigen was estimated to be 9,880. As the molecular weights of Gi-rep and Ei-M are 106,000 and 22,800, respectively (4), DIIIa has the lowest molecular weight among the three sea-squirt anti-

gens, in accordance with the elution behavior observed in gel chromatography (Figs. 1 and 2).

Chemically, DIIIIa was considered to be a glycoprotein containing more uronic acid and less sulfuric and phosphoric acids (Table IV) than the previous antigens. In the chromatography with DEAE-Toyopearl 650S, DIIIIa was eluted with 0.08 M NaCl (Fig. 3), while Gi-rep and Ei-M were eluted with 0.3–0.6 M NaCl from QAE-Sephadex A-25 (3, 4). The lower acidity of DIIIIa relative to the previous antigens may be ascribed to the lower contents of the inorganic acids.

DIIIIa was also discriminated from Gi-rep and Ei-M by the *in vivo* activity to induce asthmatic attack and conjunctival congestion in the allergic patients (Tables VI and VII). However, the antigen gave a strongly positive reaction in the skin test specific to the allergy, similarly to the previous antigens (Table V). This observation suggests that the three antigens carry a common antigenic determinant which is capable of reacting with the specific IgE distributed in the skin of the allergic patients. Nevertheless, Gi-rep and Ei-M hardly reacted with the IgE located in bronchial and conjunctival mucosa; only DIIIIa apparently reacted. Thus, it is considered that, among the three antigens, only DIIIIa can diffuse into mucosa to react with the IgE. The lower molecular weight and the lower acidity of DIIIIa relative to those of Gi-rep and Ei-M might contribute to its capability to diffuse into mucosa.

On the other hand, in the radioimmunometric examination using antisera prepared in rabbits against the previous antigens, it was observed that DIIIIa apparently inhibited the reaction between radiolabeled Gi-rep and anti-Gi-rep as well as that between labeled Ei-M and anti-Ei-M (Fig. 5). Furthermore, the reaction of labeled DIIIIa with anti-Gi-rep serum was inhibited not only by unlabeled DIIIIa but also by either unlabeled Gi-rep or unlabeled Ei-M (Fig. 6). These results indicate that the anti-Gi-rep-reacting determinant of DIIIIa is essentially the same in specificity as type α determinant carried by Gi-rep and Ei-M in common (4, 5). Thus, it was confirmed that the type α determinant specific to rabbit type A antibody (4, 5) is a common antigenic determinant for the three sea-squirt antigens.

Furthermore, the allergenic activity of DIIIIa

in vivo and its antigenic activity *in vitro* were simultaneously absorbed by the immobilized immunoglobulin containing type A antibody (Tables IX and X). Thus, it is suggested that the determinant of DIIIIa responsible for the allergic reaction *in vivo* corresponds to type α determinant specified *in vitro* by the reactivity to type A antibody. In this connection, it has been observed that the *in vivo* and *in vitro* reactivities of Gi-rep and DIIIIa were simultaneously eliminated by periodate oxidation treatment, suggesting that a certain carbohydrate moiety behaves as the determinant responsible for both activities (Shigeta, S., Ono, K., & Oka, S., unpublished results).

However, rabbit type A antibody in anti-Gi-rep serum must be polyclonal, though it appears to be monospecific. The affinity constant, K , of DIIIIa for the antibody was apparently lower than those of the other antigens, resulting in a relatively low efficiency in absorption of DIIIIa by immobilized type A antibody (Tables VIII and IX). Such a weak cross-reactivity of DIIIIa to the antibody might be ascribed to a minor structural difference between type α determinant of DIIIIa and those of the other antigens. Therefore, various monoclonal antibodies capable of recognizing different portions of the determinant are desirable for further characterization of type α determinant as the antigenic determinant specific to sea-squirt allergy.

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