

Kinetic Analysis of the Interaction between Recombinant Human FcεRIα and Serum IgEs from Allergic Patients

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Binding of IgE to the high-affinity IgE receptor (FcεRI) is the essential event for allergic reaction. Although there are many reports on binding kinetics between myeloma IgE and FcεRI, little is known about the kinetics between heterogeneous polyclonal IgE in the serum and FcεRIα. To elucidate the binding characteristics of heterogeneous serum IgE, we measured kinetic parameters of binding between IgE from allergic patients and a recombinant ectodomain of the human FcεRIα subunit by real-time interaction analysis based on surface plasmon resonance. Purified IgE monomer from the plasma of allergic patients displayed kinetics for the interaction with FcεRIα similar to those of myeloma IgE. In the case of crude IgE samples from allergic patients, one of seven specimens showed significantly higher affinity than highly purified IgE, suggesting that it is possible for IgEs in this specimen to form complexes of higher molecular weight. © 2000 Academic Press

Key Words: IgE; FcεRIα; kinetic analysis.

INTRODUCTION

The high-affinity receptor for IgE (FcεRI) is found mainly on the surface of mast cells and basophils, and the crosslinking of the FcεRI on these cells induces the release of inflammatory mediators, resulting in a type I allergic reaction. FcεRI consists of noncovalently associated subunits, namely one α chain, one β chain, and two disulfide-linked γ chains (1). The α subunit (FcεRIα) has one membrane-spanning region (2, 3) and its extracellular domain can bind IgE molecules (4–6). The β and γ subunits do not participate in the IgE-binding reaction but mediate receptor aggregation signal in the cells, leading to degranulation and the release of inflammatory mediators.

Binding of IgE to FcεRI is a crucial step in the allergic reaction, and therapeutic attempts to shut

down this process using the soluble form of FcεRIα (7–9), the humanized anti-FcεRIα monovalent antibody, the IgE-Fc fragment (10), and the humanized anti-IgE antibody (11–13) have been reported. We also demonstrated that the extracellular domain of the human FcεRIα abolishes the passive cutaneous anaphylaxis response in the rat model and inhibits IgE binding to human peripheral blood basophils, resulting in inhibition of the histamine release from them (7).

The binding kinetics of IgE and FcεRIα has been reported (5, 6, 13–21), and in most of these studies, myeloma IgE molecules were used and little was known about the binding kinetics between serum polyclonal IgE molecules from allergic patients and the FcεRIα molecule. Therefore, we focused on IgE molecules in sera of allergic patients and estimated the binding kinetics between these molecules and the FcεRIα.

For this study, the soluble form of FcεRIα (sFcεRIα), which was produced in secreted form using a baculovirus gene expression system (22), was used. The expressed recombinant α chain was composed of 172 amino acids and could bind to IgE molecules. Serum IgE samples were prepared at high purity from plasma samples that had been obtained from atopic patients by plasmapheresis. The binding kinetics between these IgEs and the immobilized sFcεRIα was evaluated by the BIAcore system, which is a kind of biosensor based on the technology of surface plasmon resonance and enables a specific binding reaction between biomolecules to be monitored in real time (23).

METHODS

The BIAcore system, *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N'*-(3-dimethylaminopropyl) carbodiimide (EDC), 2-(2-pyridinyldithio)ethaneamine hydrochloride (PDEA), and surfactant P20 were purchased from Pharmacia Biosensor AB (now BIAcore AB). Con A-Sepharose, butyl Sepharose 4 Fast Flow, NAP-5, and Protein G-Sepharose were obtained from Pharmacia Biotech Co. POROS

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50HQ was from PerSeptive Co. Anti-IgE monoclonal antibody was from Yamasa Co. (Japan). Myeloma IgEs were from Yamasa Co. and Cosmobio Co. (Japan). An IgE assay kit, "ELISA-IgE," was from International Reagent Co. (Japan). Formyl-celluloffine affinity matrix was from Seikagaku-kogyo Co. (Japan). Dimethylamine borane (DMAB) was from Morton International Ltd. TSKgel 4000SW was from TOSO Co. (Japan). All other reagents were of the highest purity available.

Preparation of sFc ϵ RI α

sFc ϵ RI α was produced by a baculovirus gene expression system as described previously (22). Briefly, SF-9 cells were infected with the recombinant baculovirus carrying the gene encoding sFc ϵ RI α and cultured for 3 days. The culture supernatants were then collected and applied sequentially to a POROS 50HQ anion-exchange column, a butyl Sepharose 4 Fast Flow hydrophobic interaction column, a Con A-Sepharose column, and an anti-Fc ϵ RI α monoclonal antibody affinity column. Purified sFc ϵ RI α showed heterogeneity due to the number and size of the bound sugar chains and 30–35 kDa of molecular mass.

Purification of IgE

The affinity column coupled with anti-IgE monoclonal antibody was made as follows. The anti-IgE monoclonal antibody solution in 0.2 M potassium phosphate buffer (pH 7.0) was added to a formyl-celluloffine affinity matrix and the reaction mixture was incubated at 4°C for 5 h with gentle stirring. DMAB (reducing reagent) was added to the mixture and reacted at 4°C overnight. The matrix was washed with distilled water and resuspended in 0.5 M ethanolamine buffer (pH 8.3) and incubated with DMAB at 4°C for 4 h. The prepared affinity matrix was packed in a small column and equilibrated with 0.5 M sodium chloride, 0.02 M sodium phosphate buffer (pH 7.5).

Plasma obtained from patients with atopic dermatitis by plasmapheresis was fractionated with 50% ammonium sulfate three times. The precipitates were dissolved in PBS and applied to a Protein G-Sepharose column. The flow-through fraction was collected, concentrated by ultrafiltration, and applied to an anti-IgE monoclonal antibody affinity column. The column was washed with 0.5 M NaCl, 20 mM sodium phosphate buffer (pH 7.5). Bound protein was eluted with 0.05 M glycine buffer (pH 2.8) and the eluate was quickly neutralized by adding 1/5 vol of 0.5 M sodium phosphate buffer (pH 7.5). This fraction was concentrated and applied to a TSKgel 4000SWXL HPLC column. The eluate was fractionated and analyzed by SDS-PAGE followed by silver staining. The fractions con-

taining IgE were pooled and concentrated by ultrafiltration.

The flow-through fractions of Protein G-Sepharose column in the above protocol were used as partially purified IgE samples.

Determination of IgE Concentration

The concentrations of IgE in samples were measured with an ELISA kit (International Reagent Co.) using anti-IgE monoclonal antibody and HRP-labeled goat anti-human IgE polyclonal antibody. The values obtained were adopted for calculation of the association rate constants.

Evaluation for Binding Kinetics of IgE and Fc ϵ RI α

Kinetic parameters were evaluated using the BIAcore system. The reactive disulfide group was introduced to the sFc ϵ RI α molecule in order to immobilize it on the sensor surface by thiol disulfide exchange reaction. The recombinant sFc ϵ RI α (0.2 mg protein) and 1.1 mg of PDEA were dissolved in 1.0 ml of 0.1 M MES buffer (pH 5.0) and cooled on ice. Then 10 μ l of 0.4 M EDC was added and the mixture was reacted for 1 h on ice. Unreacted reagent was removed and the buffer was changed to 10 mM sodium acetate buffer (pH 4.0) using a NAP-5 prepacked gel filtration mini-column.

The PDEA-modified sFc ϵ RI α was immobilized to the sensor chips as follows (23). The sensor chip was activated with 0.05 M NHS, 0.2 M EDC for 2 min. A solution of 0.04 M cystamine, 0.1 M sodium borate buffer (pH 8.5) and a solution of 0.1 M dithioerythritol, 0.1 M sodium borate buffer (pH 8.5) were injected for 3 min each. The PDEA-modified sFc ϵ RI α was injected into the activated sensor for the appropriate time. In order to block excess reactive groups remaining on the sensor surface, 0.02 M PDEA, 1 M sodium chloride, and 0.1 M sodium formate (pH 4.3) was injected for 3 min.

All kinetics experiments were performed with 0.15 M NaCl, 3.4 mM EDTA, 0.005% BIA surfactant P20, and 0.01 M Hepes buffer (pH 7.4) (HBS buffer) at 25°C. IgEs at various concentrations were injected over the sFc ϵ RI α -coupled sensor surface for 15 min and then dissociation was monitored in HBS buffer flow for 30 min. Sensor was regenerated with subsequent injections of 0.01 M sodium acetate buffer (pH 4.0) and 1.5 M sodium thiocyanate for 3 min, respectively. Each IgE sample was evaluated two to five times with varying IgE concentration.

In the case of partially purified IgE fraction, the sensorgram was much more affected by nonspecific adsorption to the sensor chip and bulk refractive index change. To obtain nonspecific binding curve, the sam-

ple, which had been incubated with an excess amount of the sFcεRIα prior to injection, was evaluated. The specific binding curve that was obtained by subtraction of the nonspecific binding curve from the original curve was used for calculation of rate constants.

The kinetic rate constants were calculated using BIAevaluation software version 2.1, which is a nonlinear regression analysis program (23). The averages of the measured association and dissociation rate constant values were calculated and the equilibrium constant was finally calculated based on the values of these rate constants.

RESULTS

Serum IgE Values and Purification of Plasma IgE

Serum IgE values of the patients, from whom plasma or sera were obtained for this study, are listed in Table 1. Plasma or serum samples reacted on broad range of allergens, indicating that the purified IgEs were therefore polyclonal. The fractions of the gel filtration, which was the last step of purification, were analyzed by SDS-PAGE (Fig. 1). Fraction 3, whose retention time corresponded to that of the IgE monomer, showed the highest level of purity of IgE. We concentrated this fraction and used it in the following experiments as purified IgE monomer. SDS-PAGE demonstrated that the fraction of higher molecular weight (Fraction 2) in gel filtration chromatography also contained the bands that corresponded to the molecular weight of IgE, suggesting that IgE would form a complex of larger molecular size in the plasma samples used. The purity of myeloma IgE samples was very high (data not shown).

Sensorgram by SPR

The efficient immobilization of the sFcεRIα to the sensor chip CM5 was followed by the thiol disulfide exchange method. The amine coupling method was most frequently used to immobilize proteins onto the carboxymethyl dextran sensor surface (13, 21, 23), but the efficiency of immobilization of the sFcεRIα by this method was low. In addition, the affinity between the sFcεRIα and IgE on the amine coupling sensor chip was lower than that on the thiol coupling sensor chip described above (data not shown). The immobilization densities of the sFcεRIα on the chips in several experiments were in the range between 540 RU and 850 RU.

Sensorgrams of both association and dissociation phases for each sample were obtained. In the case of monomeric IgE samples purified from plasma of allergic patients and myeloma IgEs, almost smooth sensorgrams were obtained (Figs. 2 and 3). When the sample was preincubated with the sFcεRIα before an injection, an increase of signal in the association phase was per-

fectly blocked (Fig. 2), indicating that the association signal was specific to the sFcεRIα. We applied the nonlinear regression analysis algorithm on these sensorgrams to calculate association and dissociation rate constants.

Affinity Rate Constant of IgE Binding to the FcεRIα

The association rate constant (k_a) and the dissociation rate constant (k_d) were calculated from the average value of the data obtained from three to five independent experiments, and the association equilibrium constant (K_A) was given as fraction of k_a and k_d (Table 2). The association equilibrium constants calculated for myeloma IgEs were on the order of 10^{10} M^{-1} and these values were in good agreement with those previously reported. Between two myeloma IgE samples, the affinity rate constants were different from each other. The calculated k_a values of purified plasma IgE samples from allergic patients were slightly higher than that of myeloma IgE (Cosmobio) and showed no significant difference from that of another myeloma IgE (Yamasa).

Kinetics for Binding of the Partially Purified IgE to the FcεRIα

On the SPR sensor, refractive index is directly correlated to the mass of substances that bind to the sensor surface, and kinetic parameters were influenced when molecules other than the analyte bind to the sensor. To assess the possibility of other factors binding to the IgE-FcεRIα complex, a similar kinetic analysis was performed with the partially purified IgE samples on the SPR biosensor. In the case of the partially purified IgE fraction, the sensorgram was discontinuous at the beginning and the end of sample injection (Fig. 4). When the partially purified IgE sample was preincubated with the sFcεRIα before an injection, specific binding of IgE to the sFcεRIα on the sensor surface was inhibited. We evaluated the kinetic rate constants from the specific binding curve that was obtained by subtraction of the nonspecific binding curve from the original curve (Fig. 4). The calculated association rate constants of the partially purified IgE appeared higher than those of highly purified IgE and myeloma IgE samples, but the difference was not significant except for sample 3. The dissociation rate constants were not significantly different with all samples (Table 2).

DISCUSSION

Real-Time Biospecific Interaction Analysis

In the present work, we adopted a real-time biospecific interaction analysis sensor to examine the elemen-

TABLE 1
IgE Values of the Patients

No.	Sample status	Total IgE (IU/ml)	Allergen specificity									
			Dermato- phagoides	Japanese cedar	Cat epithelium	Egg white	House dust	Wheat	Rice	Milk	Soybean	<i>Candida</i>
3	Serum	4095	+	+	+	+	ND	ND	ND	ND	ND	ND
9	Serum	4615	+	—	+	+	ND	ND	ND	ND	ND	ND
18	Serum	3005	+	—	+	+	ND	ND	ND	ND	ND	ND
29	Serum	3155	+	+	+	+	ND	ND	ND	ND	ND	ND
127	Plasmapheresis	54774	+	+	ND	+	+	+	+	+	+	—
129	Plasmapheresis	31194	+	+	ND	+	+	+	+	+	+	—
157	Plasmapheresis	24341	+	+	ND	+	+	+	+	+	+	+

Note. The concentration of total IgE was measured by ELISA. The reactivity to each allergen was determined with an allergen-specific IgE detection kit, "AlaSTAT" and "RAST." ND, not determined.

tary reaction between IgE and the FcεRIα. Although there were some works that investigated the reaction between IgE and the FcεRIα using the SPR biosensor (13, 18–21), their experimental conditions were different from each other.

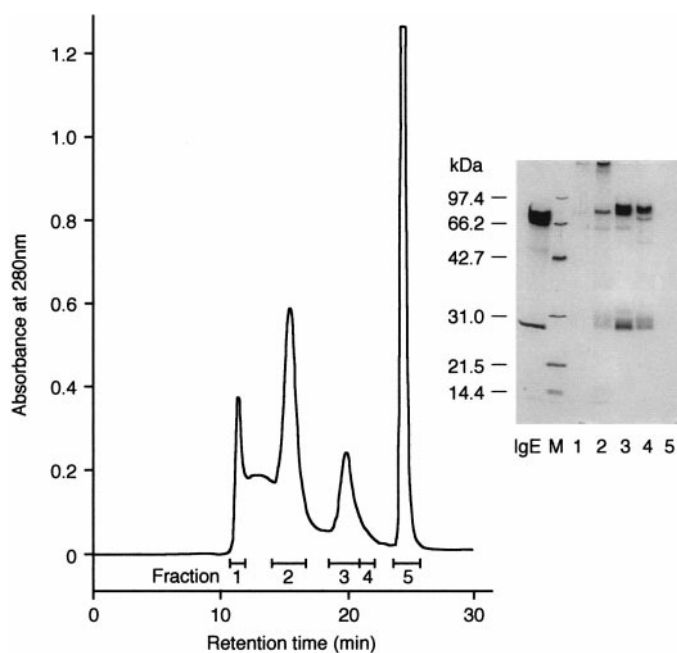


FIG. 1. Purification of plasma IgE by gel filtration chromatography. Eluate from the anti-IgE monoclonal antibody affinity column was fractionated by the TSKgel 4000SWXL column. Each fraction (represented by a bar) was subjected to SDS-PAGE under reducing conditions and visualized by silver staining. Lane IgE, myeloma IgE (Yamasa) as a molecular weight standard; lane M, low-molecular-weight marker; molecular weights are indicated on the left side of the gel. The chromatogram and SDS-PAGE in this figure are a representative sample. The other sample also showed a similar chromatogram and SDS-PAGE pattern including the bands of higher molecular weight in fraction 2.

Immobilization of the sFcεRIα on the Sensor Chip

We immobilized the sFcεRIα on the sensor chip (CM5) by the thiol disulfide exchange reaction, whereas the efficiency of immobilization by the amine coupling method was low. Although the reason for the low efficiency of the amine coupling method was not clear, we thought that the amino groups modified in the coupling reaction could be required for binding between sFcεRIα and IgE or that a steric hindrance effect could occur during the amine coupling immobilization.

One recent study reported that the Lys residue in the FcεRIα molecule was essential for binding to IgE (19). The reason for low-affinity binding to IgE when using the amine coupling method in our system could be modification of the primary amino group of the Lys residue in FcεRIα. That study also documented that two immobilization methods, amine coupling and alde-

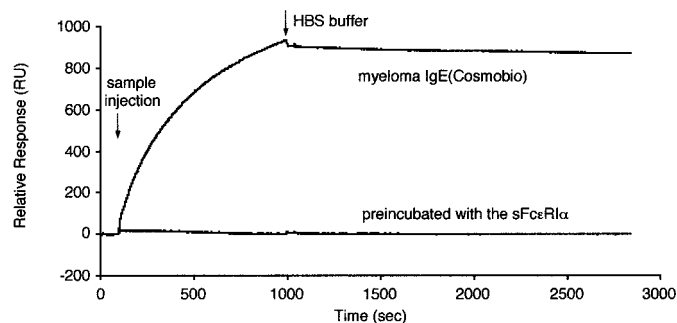


FIG. 2. Sensorgram of myeloma IgE. Myeloma IgE (Cosmobio) was injected onto the sFcεRIα-immobilized sensor chip for 900 s followed by HBS buffer flow for 1800 s. When IgE was preincubated with an excess amount of the sFcεRIα, binding of IgE to the sensor surface was completely blocked.

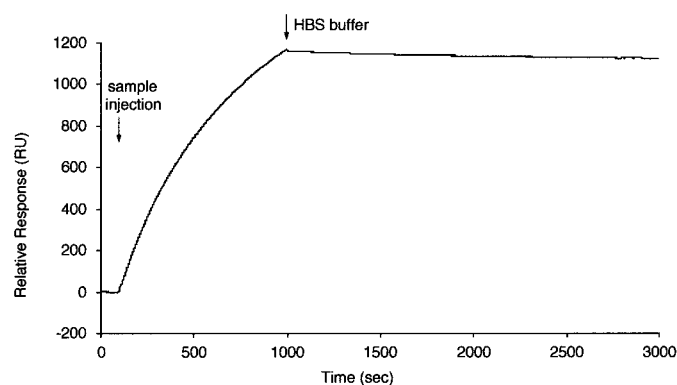


FIG. 3. Sensorgram of purified IgE. IgE purified from the plasma of patient 129 was injected onto the sFcεRIα-immobilized sensor chip for 900 s followed by HBS buffer flow for 1800 s.

hyde coupling, were evaluated for binding kinetics between IgE and the immobilized high-affinity IgE receptor and that there were not significantly different results between the two methods (20). The difference in stability of FcεRIα in the amino-coupling treatment could be due to the gene expression system that produced the soluble FcεRIα molecule. The soluble FcεRIα expressed by a baculovirus gene expression system was 10–15 kDa smaller than the sFcεRIα expressed by mammalian cells (22). This size difference was dependent on the glycosylation state of the product in the expression system used. Though the sugar chains of FcεRIα were believed not to be responsible for binding to IgE (5), they would influence protein stability, chemical resistance, or molecular arrangement on the sensor surface (24).

Regeneration of the Sensor Surface

To regenerate the sensor surface for repeated use, noncovalently associated analyte should be washed out under regeneration conditions and the immobilized ligand should withstand this step. An acidic buffer such as lactate buffer (pH 3.0–4.0) was used to dissociate IgE from the FcεRIα on the cell surface in previous reports (14). We had tried applying the same conditions to our system, but 10–20% of the total amount of associated IgE still remained on the sensor surface and was not removed by subsequent injection of the same buffer (data not shown). This finding suggests that IgE might not be thoroughly removed from the cell surface in the previous works or that the recombinant sFcεRIα in our system could have different characteristics from the native receptors on the cell surface in the previous works. Some groups used 10 mM or even higher concentrations of HCl for dissociation of IgE (13, 21). Though IgE molecules were completely removed from the sFcεRIα by this method, the sFcεRIα molecule became damaged gradually by repeated regeneration. Our regeneration method, which was performed with a solution of 0.01 M sodium acetate buffer (pH 4.0) and a solution of 1.5 M sodium thiocyanate, was appropriate for complete dissociation of IgE without inactivation of the sFcεRIα on the sensor surface.

Kinetic Parameters and Clinical Implication

The binding reaction between IgE-Fc and FcεRIα was well analyzed with a biphasic kinetics model (19). The previous study used immobilized sFcεRIα or a FcεRIα-IgGFc chimera molecule as a probe and IgE-Fc

TABLE 2
Kinetic Rate Constants between IgE and the sFcεRIα

IgE		Association rate constant k_a ($10^5/\text{ms}$)	Dissociation rate constant k_d ($10^{-5}/\text{s}$)	Association equilibrium constant K_A ($10^{10}/\text{M}$)	n	K_A t test/ myeloma (Yamasa)	K_A t test/ myeloma (Cosmobio)
Myeloma (Yamasa)	Purified	2.02 ± 0.12	1.73 ± 0.31	1.17	3		$P < 0.01$
Myeloma (Cosmobio)	Purified	1.48 ± 0.12	1.28 ± 0.30	1.15	3	$P < 0.01$	
No. 129	Purified	2.05 ± 0.31	1.23 ± 0.17	1.67	5	—	$P < 0.05$
No. 157	Purified	2.07 ± 0.18	1.08 ± 0.21	1.92	5	—	$P < 0.01$
No. 3	Partially purified	3.67 ± 0.04	1.53 ± 0.74	2.40	2	$P < 0.01$	$P < 0.01$
No. 9	Partially purified	2.86 ± 0.53	1.42 ± 0.45	2.01	2	—	—
No. 18	Partially purified	3.69 ± 0.69	0.99 ± 0.57	3.73	2	—	—
No. 29	Partially purified	2.65 ± 0.31	1.17 ± 0.53	2.26	2	—	—
No. 127	Partially purified	2.92 ± 0.30	1.18 ± 0.21	2.47	2	—	—
No. 129	Partially purified	3.03 ± 0.53	1.37 ± 0.18	2.21	2	—	—
No. 157	Partially purified	2.33 ± 0.34	1.46 ± 0.63	1.60	2	—	—

Note. Rate constants were average values of two to five independent experiments (the value n is the number of experiments). Each rate constant is expressed as mean $\pm$ SD. The difference in k_a values against those of two myeloma IgEs was examined by t test. The association equilibrium constant, K_A , was calculated as a fraction of the association rate constant, k_a , and the dissociation rate constant, k_d .

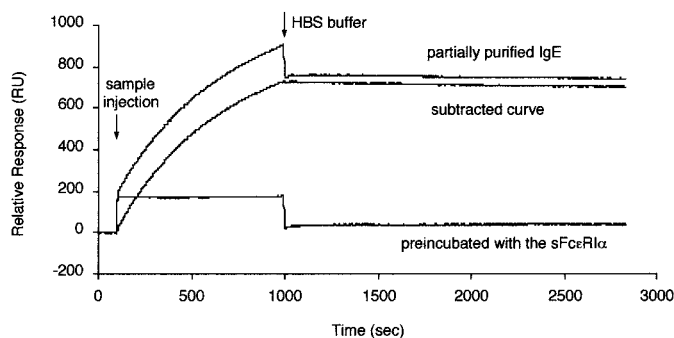


FIG. 4. Sensorgram of the partially purified IgE sample. The partially purified sample 29 serum was injected onto the sFcεRIα-immobilized sensor chip for 900 s followed by HBS buffer flow for 1800 s. The resonance signal of the partially purified IgE preincubated with an excess amount of the sFcεRIα revealed the nonspecific bulk refractive index change. Specific binding signal was obtained by subtraction of nonspecific signal from the original curve.

fragment as a ligand in their system and there was no difference between sFcεRIα and the FcεRIα-IgGFc chimera.

We employed a monophasic kinetics model in analysis of the binding kinetics between IgE and immobilized FcεRIα and obtained good results. As IgE molecules from patients' samples used in our experiments were heterogeneous and each IgE molecule might show different binding kinetics, the binding kinetics between native serum IgEs and FcεRIα would be more complicated and like that of a multidimensional reaction.

Our data demonstrated that IgE purified from allergic patients appeared to have an affinity to FcεRIα that was almost equal to or slightly higher than that with myeloma IgE. In a past report, polyclonal IgE that was partially purified from high IgE serum had lower affinity than myeloma IgE (6). Our results in this work indicated that the polyclonal IgE from allergic patients was fully functional to bind to FcεRIα, suggesting that the polyclonal IgE in the previous report was inactivated for some reason.

To assess some other substances that could bind to the IgE-FcεRIα complex, we compared the rate constants between the purified IgE sample and the partially purified IgE sample from the same origin: samples 129 and 157. Though the association rate constant of the partially purified IgE with the sFcεRIα appeared higher than that of the purified IgE, we could not conclude whether the difference was significant because the calculated standard deviation of the k_a value of the partially purified IgE was large. Since SDS-PAGE of the gel filtration chromatography fraction with higher molecular weight than the IgE monomer also contained the bands that corresponded to the molecular weight of IgE (Fig. 1), the apparent higher

affinity of this crude sample could be due to complex formation of IgE of larger molecular size in the plasma.

We demonstrated that IgE monomer from allergic patients was fully functional so far as binding ability to the FcεRIα was concerned. Allergy does not always occur even in individuals who have high serum IgE levels, whereas it is certain that IgE with appropriate antigen triggers the allergic reaction. In the present study we did not identify other factors bound to IgE-FcεRIα complex such as histamine-releasing anti-IgE antibody (25) and/or anti-FcεRI antibody (26) that had been shown to exist in patients with chronic urticaria. It would be interesting to analyze these molecules in detail by SPR as well as a direct binding between IgE and FcεRIα.

As we mentioned above, partially purified IgE samples (Nos. 129 and 157) demonstrated a higher association rate constant than the purified IgE and appeared to form a complex with some other factors in the serum. This kind of IgE complex may more efficiently trigger mast cells in the allergic state. In this respect, evaluating the binding kinetics for this type of IgE complex can be of importance to obtain further insights into the allergic state, although further investigation at the molecular level is required to identify serum factors that form the IgE complex.

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