Histamine is a low-molecular-weight biomolecule most commonly recognized as a secretion released in an allergic reaction. Present allergic tests, such as the radioallergosorbent test (RAST) and enzyme-linked immunosorbent assay (ELISA), detect the specific binding of antigens to IgE to identify the antigen incorporated with allergy reactions. However, these tests cannot be used to evaluate whether the antigen-binding IgE activates mast cells and basophils, resulting in secretions such as histamine. Therefore, it is more important to directly monitor the release of mediators such as histamine to evaluate allergic responses.

Over the last few decades, biologically coupled gate field-effect transistors (bio-FETs) have been proposed as bioanalytical tools to monitor biomolecular recognition events. To realize the label-free and real-time detection of allergic responses, we previously reported an approach based on a cell-coupled gate FET (cell-based FET). FET biosensors can directly detect the ionic charges at a gate insulator/electrolyte solution interface on the basis of the field-effect principle. Therefore, small biomolecules such as histamine can be sensitively detected as long as they have molecular charges. In a previous study, rat basophilic leukemia (RBL-2H3) cells were cultured on a sensor surface so that the allergic responses were directly transduced to changes in surface potential. As a result, the cell-based FET biosensor successfully detected the allergy responses as changes in pH based on secretions such as histamine at the sensor/cell interface. Since the sensor was not selective to histamine, however, the effects of histamine secretion on the changes in potential are still unclear. To precisely observe histamine secretion, the detection selectivity of histamine was realized by using an HMIP with higher crosslink density, as shown by comparing the electrical response upon adding L-histidine to an HMIP-FET, which has a similar chemical structure to histamine. The enhancement of selectivity to histamine was clarified on the basis of the binding constant estimated by potentiometric Langmuir isotherm analysis.

**Experimental**

**Chemicals.**—The following chemicals used in the experiments in this study were purchased. Histamine, L-histidine, dimethyl sulfoxide (DMSO), azobisisobutyronitrile (AIBN), 2-(trifluoromethyl)acrylic acid (TFMAA), 1 M hydrochloric acid (HCl), and distilled water were purchased from Wako Pure Chemical Industries Ltd., and N,N'-methylenebisacrylamide (MBAAm) was purchased from Sigma-Aldrich.

**Design of HMIP.**—In this study, two different MIP compositions were designed to investigate the effect of the MIP interface on the detection sensitivity and selectivity of the sensor to histamine. The most important factor in designing an MIP interface is the choice of the functional monomer, because the selective binding sites are created on the basis of the functional monomer/target interaction in the prepolymer solution. In general, it is known that the stronger the intended interaction, the more selective the cavities created in the polymer matrix. In this regard, TFMAA was chosen as a functional monomer because it was previously reported optimized ratio for an HMIP. The choice and amount of the crosslinker also play important roles in strengthening the structure of the polymer matrix and enhancing selectivity. As the sensor is used in an aqueous...
Figure 1. (A) Conceptual illustration of FET biosensor. (B) Source follower circuit. Using the measurement system, the surface potential at the gate of FET can be monitored in real time.

Figure 2. Schematic illustration of molecularly imprinted polymer (MIP). The molecular structure of histamine and L-histidine are also shown in the figure.

solution, a hydrophilic crosslinker, MBAAm, was utilized to control the hydrophilicity of the polymer. Furthermore, a high crosslink density is important in enhancing selectivity. Therefore, we designed two polymer compositions with different monomer/crosslinker ratios, one with a low crosslink density (1/0.85 ratio for HMIP1) and the other with a high crosslink density (1/6.4 ratio for HMIP2). The solubility of MBAAm in an aqueous solution is poor; thus, a mixture of H₂O and DMSO was used as the solvent for HMIP2 to design the highly crosslinked polymer. The compositions of each chemical compound and the solvent for HMIP1 and HMIP2 are listed in Table I.

Fabrication of HMIP-modified extended-gate Au electrode.—On a transparent glass slide (Matsunami Glass), an approximately 100-nm-thick Au thin film was sputtered on an approximately 15-nm-thick Cr layer. A polycarbonate ring (18 mm inner diameter / 20 mm outer diameter) was encapsulated on the Au substrate using an epoxy resin (Pelnox ZC-203T) excluding the sensing surface. Prior to the MIP modification, the prepared Au substrate was immersed in a piranha solution (3/1 v% mixture of H₂O₂/H₂SO₄) for 10 min to clean the surface then thoroughly rinsed with distilled water. The substrate was then kept in a UV/ozone cleaner (Meiwafosis) to prevent the additional adhesion of organic compounds before the copolymerization of the hydrogel.

HMIP was prepared on the Au electrode via light-induced free-radical co-polymerization. The reagents listed in Table I were mixed in a 1.5 mL Eppendorf tube. After adding AIBN as an initiator, the mixture was deoxygenated with N₂ gas for 20 min. Then, 5 μL of the mixture was placed on the cleaned Au sensing surface, which was then covered with a thin fluorine-coated PET film, and the Au surface was allowed to undergo polymerization for 15 min under UV irradiation. After the polymerization, the PET film was carefully removed, and the template was removed from the polymer by immersing the electrode in 0.1 M HCl in 1/1 v% methanol/water for 24 h.

Real-time measurement of analytes using HMIP-coated FET.—The MIP-coated Au electrode was connected to the gate of a silicon-based n-channel junction-type FET (K246-Y9A, Toshiba), and a gate voltage (V_G) was applied through the Ag/AgCl reference electrode. The gate surface potential was measured in real time using a FET real-time monitoring system (Optogenesys). In this study, V_G, a drain voltage (V_D), and a drain-source current (I_DS) were set to constant values for all the measurements; therefore, the change in the surface potential (∆V_out) at the gate was measured as the change in the gate-source voltage (∆V_GS) using a source follower circuit (Fig. 1B). Thus, ∆V_out is a measure of the change in the threshold voltage (ΔV_T) of FET.

In a measurement, the sensing surface was covered with 1.5 mL of PBS (pH 7.4), and the source–drain current was controlled to 700 μA with a gate voltage of 0 V. After the stabilization of the surface

| Table I. Composition of MIP1 and MIP2. The values are shown as a molar ratio in the solution. |
|---------------------------------------------------------------|
| Histamine | TFMAA | MBAAm | Solvent       |
|---------|-------|-------|---------------|
| HMIP1   | 1     | 4     | 4             | H₂O          |
| HMIP2   | 1     | 2     | 12            | H₂O/DMSO 6/4 |
potential, analytes of various concentrations were added to the solution. The concentration was controlled by exchanging 15 μL of the buffer and the analyte solution to give a 100-fold dilution. A stock solution of the analyte was prepared beforehand and stored at 4 °C. The solution was allowed to warm to room temperature 1 h before the measurement to avoid the effect of a temperature change.

**Results and Discussion**

Detection sensitivity of HMIP-FET biosensor to histamine and L-histidine.—First, the detection sensitivity of the HMIP-FET biosensor to the addition of histamine and L-histidine was investigated. The change in surface potential upon the introduction of the analytes into the HMIP-FET systems was measured in real time. As shown in Fig. 3, the surface potential clearly shifted in the negative direction upon the addition of both histamine and L-histidine. This is because the number of negatively charged carboxyl groups increased in the polymer. As both histamine and L-histidine contain highly basic amino groups, the hydrogen ion on the carboxyl group was removed; thus, the HMIP interface became comparatively negative. As expected, the HMIP-FET biosensor detected histamine from μM order, the same order as the secretion of histamine verified in a previous study. On the other hand, L-histidine was also detected; thus, the detection selectivity appeared to be poor and the effect of the MIP interface was not clear. To discuss the detection selectivity in detail, the result was directly analyzed using the Langmuir isotherm equation in the next step.

Quantitative analysis of HMIP-FET biosensor using langmuir adhesion isotherm.—MIPs are often characterized using adhesion isotherm equations. In this study, the most universally used equation, the Langmuir isotherm equation, was utilized to directly characterize the MIP-FET sensors. For a batch rebinding system, the Langmuir isotherm equation is expressed by

\[
B = \frac{NK[c]}{1 + K[c]},
\]

where \( B \) refers to the signal intensity observed at equilibrium for the MIP-bound template, \([c]\) to the free concentration of the template at equilibrium, \( N \) to the number of available active centers in the MIP per unit volume, and \( K \) to the binding constant. In the MIP-FET system, the surface potential changes upon the adhesion of the analyte to the MIP interface; thus, \( B \) in Equation 1 can be assumed to be proportional to \( \Delta V_{\text{out}} \). Moreover, \( N \) can be assumed to be proportional to \( \Delta V_{\text{out}} \), which is the maximum change in surface potential. Thus, we can express the Langmuir isotherm equation for an MIP-FET system as

\[
\Delta V_{\text{out}} = \frac{\Delta V_{\text{out}}^{\max} K[c]}{1 + K[c]},
\]

where \([c]\) is the concentration of the target biomolecule at equilibrium, which is obtained from the saturated electrical signal in a real-time measurement. In the analysis, \( \Delta V_{\text{out}} \) was first calculated for each concentration of histamine by subtracting \( V_{\text{out}} \) at \( t = 0 \) (offset). Then, the resultant data were plotted against the histamine concentration, as shown in Fig. 4. \( \Delta V_{\text{out}} \) is the average of 10 data points taken 5 min after the addition of histamine. The best-fit adsorption isotherm equations were determined by optimizing \( K \) and \( \Delta V_{\text{out}}^{\max} \) using application software to minimize \( R^2 \) (in Microsoft Excel).

Equation 2 was applied to the result to further characterize the MIP film interface. Firstly, the changes in surface potential upon the addition of histamine and L-histidine to HMIP1- and HMIP2-based FET sensors were plotted against the corresponding concentration. Then, to draw the calibration curves, the maximum change in surface potential (\( \Delta V_{\text{out}}^{\max} \)) and the binding constant (\( K, \text{M}^{-1} \)) in Equation 2 were optimized (Fig. 4 and Table II). As shown in Fig. 4, the calibration figures show the calibration curves for the HMIP-FET biosensors.
curves of $\Delta V_{out}$ for the HMIP1 and HMIP2 films upon adding histamine were similar, whereas for L-histidine addition, the detection sensitivity (linear slope) of HMIP2-FET biosensor at low concentrations appeared to be smaller than that of the HMIP1-based FET biosensor. Therefore, the HMIP-FET biosensor with higher crosslink density exhibited the more selective detection of histamine at low concentrations, which verified the effect of the HMIP film interface. In fact, the result can be discussed quantitatively using the data listed in Table II. For L-histidine addition to HMIP1 and HMIP2, the binding constant $K$ decreased from 1300 M$^{-1}$ to 490 M$^{-1}$. On the other hand, as the binding constant for histamine addition to HMIP2 was 1200 M$^{-1}$, the HMIP2-based FET biosensor was more selective to histamine than the HMIP1-based FET biosensor. In addition, the relative selectivity $S$, which is defined as the ratio of the binding constant (in this study, $S = K_{\text{histamine}}/K_{\text{histidine}}$), can be used to quantify the selectivity. From the results, $S_{\text{HMIP2}}/S_{\text{HMIP1}}$ was approximately 5, which showed that the HMIP2-based sensor was five times more selective to histamine than the HMIP1-based sensor. As can also be seen from the calibration curves in Fig. 4B, the difference in selectivity appeared at low concentrations ($\mu$M to mM order), making the HMIP2-based sensor advantageous for the detection of histamine secretion.17

The detection selectivity of the HMIP-FET biosensor was improved due to the difference in the functional monomer/crosslinker ratio in the polymer composition (1/0.85 and 1/6.4 for HMIP1 and HMIP2, respectively). As explained in the previous section, the monomer/crosslinker ratio is one of the important factors in enhancing selectivity. In general, higher selectivity can be obtained by increasing the ratio of the crosslinker because a high crosslink density results in the target-specific cavities having a rigid structure. Since HMIP2 has a higher crosslink density than HMIP1, it showed higher selectivity to histamine.

Conclusions

In this study, we demonstrated the label-free and selective detection of histamine using an HMIP-coated gate FET. The induction of molecular charges based on the histamine/monomer complex in the HMIP film interface contributed to the direct detection of histamine by the HMIP-FET biosensor. To enhance the selectivity of HMIP to histamine, it was important to control the crosslink density in the HMIP film. The reason for the more selective detection of histamine using the HMIP-FET biosensor was clarified on the basis of potentiometric Langmuir isotherm analysis. The potentiometric analysis based on intrinsic molecular charges enabled the direct evaluation of the detection selectivity of the HMIP film interface. Thus, a platform based on the MIP-FET system is suitable for use as an analytical tool to evaluate the selectivity of an MIP film to a target molecule as well as to selectively detect a target molecule as a label-free biosensor.

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**Table II. Binding constant ($K$, M$^{-1}$) estimated from potentiometric Langmuir isotherm equation.**

|        | MIP1 | MIP2 |
|--------|------|------|
| Histamine | 580  | 1200 |
| Histidine | 1300 | 490  |