Interfacial pH Behavior at a Cell/Gate Insulator Nanogap Induced by Allergic Responses

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Supporting Information

ABSTRACT: In this paper, we clarify the interfacial pH behavior induced by allergic responses at a mast cell/gate insulator nanogap detected by laser scanning confocal fluorescence microscopy. In a previous work, the change in interfacial pH detected on the basis of allergic responses was monitored as a mast cell/gate insulator nanogap interface using a cell-cultured gate ion-sensitive field-effect transistor (ISFET), but the interfacial pH behavior at a mast cell/gate insulator nanogap has not been clarified using other methods. Here, the phospholipid fluorescein is employed as the extracellular pH indicator, which is fixed to the external side of the plasma membrane of mast cells cultured on a substrate. As a result, the interfacial pH at the mast cell/substrate nanogap increases after mast cells with IgE on their membrane are activated by the interaction between IgE and an allergen. This is due to the basicity of histamine molecules released from mast cells. Moreover, the change in the interfacial pH at the mast cell/substrate nanogap is larger than that at the mast cell/bulk solution interface. That is, molecules of substances secreted as a result of allergic responses are assumed to accumulate around the cell/substrate nanogap. The data obtained in this study support the idea that potentiometric ion sensors such as ISFETs can detect a cellular-function-induced change in pH at a cell/electrode nanogap in real time.

1. INTRODUCTION

Allergy is one of the most common diseases at present,1 in particular, allergy in infants often causes death. Therefore, simple and accurate tests for allergy are required for early detection in infants. The specific binding of an allergen to IgE may be detected by various immunological methods, such as enzyme-linked immunosorbent assay, but such methods may be detected by various immunological methods, such as detection in infants. The specificity and accuracy of tests for allergy are required for early detection in infants. Particularly, allergy in infants often causes death. Therefore, the potential of allergen-specific IgE to activate mast cells and basophils, which play important roles in type I allergy or IgE-associated diseases, in patients. That is, the potential of allergen-specific IgE to activate mast cells and basophils from a patient should be evaluated rather than the analysis of simple IgE−allergen binding in the diagnosis of type I allergy.2–4

Nonoptical monitoring of allergic responses has been realized by the detection of the increase in pH induced by basic histamine released from mast cells using a cell-cultured gate ion-sensitive field-effect transistor (cell-cultured gate ISFET).5 Here, rat basophilic leukemia (RBL-2H3) mast cells were utilized as a signal transduction interface to cause allergic reactions on the gate insulator of the ISFET because IgE antibodies, which bind to Fce receptors on the mast cell membrane, are specifically cross-linked by allergens, contributing to allergic responses of RBL-2H3 cells.5–8 Ionic and molecular charges that are related to biological functions are directly detected using an ISFET sensor at the gate surface, which is covered by various functional groups and probe molecules that interact with target substances. Basically, a solution-gate ISFET was developed to detect ions in biological environments.1 Particularly, an electrolyte solution induces the potential at the interface between the solution and the gate insulator. A gate insulator is mostly composed of oxide or nitride membranes such as SiO2, Si3N4, Ta2O5, and Al2O3; that is, hydroxy groups at the oxide or nitride surface in a solution take the equilibrium reaction with hydrogen ions according to protonation (−OH + H+ ⇄ −OH2) and deprotonation (−OH ⇄ −O− + H+) so that a change in the surface charge is measured as a change in pH on the basis of the principle of the field effect.10,11 Thus, the changes in pH based on not only allergic responses5 but also cellular respiration12−18 were measured as the change in the output potential at the cell/gate insulator interface using cell-cultured gate ISFETs.

However, certain issues encountered in a previous work remain to be clarified.5 The release of basic histamine molecules together with other molecules from mast cells is activated following the allergen−IgE interaction at the cell membrane and then causes an increase in pH at the mast cell/gate insulator interface. That is, the pH at the mast cell/gate insulator nanoscale interface, hereafter the interfacial pH, reflects cellular functions in situ. Indeed, previous works showed a cleft of about 50−150 nm at the cell/substrate interface, where focal or nonfocal regions of contact between...
substrates and membrane proteins were observed by total internal reflection fluorescence microscopy. This increase in interfacial pH at the mast cell/gate insulator nanogap induced by histamine released from mast cells was demonstrated using a cell-cultured gate ISFET but has not been confirmed using other methods. On the other hand, cellular respiration was continuously monitored as a decrease in interfacial pH induced by carbon dioxide released from cells at the cell/gate insulator nanogap using a cell-cultured gate ISFET. Such interfacial pH behavior at the nanogap was also clarified by fluorescence imaging. Considering the above, the interfacial pH behavior at the nanogap between the mast cell and the substrate can be analyzed using a lipophilic pH indicator. The results obtained in this study support the detection principle of cell-cultured gate ISFET sensors, to ensure the data obtained using ISFETs.

2. RESULTS AND DISCUSSION

2.1. Interfacial pH Behavior at Cell/Substrate Induced by Type 1 Allergic Response. RBL-2H3 cells with or without IgE on their membrane were precultured on a glass substrate under appropriate conditions (see Section 4.2) and then labeled with fluorescein DHPE. Here, the phospholipid fluorescein [N-(fluorescein-5-thiocarbamyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, DHPE] can be fixed as an extracellular pH indicator to the external side of the plasma membrane of a cell by inserting its lipophilic alkyl chain into the membrane and utilized to detect the change in interfacial pH.

In this study, we investigated the interfacial pH behavior based on allergic responses at a mast cell/substrate nanogap by laser scanning confocal fluorescence microscopy. The phospholipid fluorescein DHPE was utilized as the extracellular pH indicator. The results obtained in this study support the detection principle of cell-cultured gate ISFET sensors, which are expected to be widely applied as an analytical tool in the fields of cell biology and medicine.
The data shown are the average of six cells in the MT buffer medium (pH 8.0). The fluorescence image was observed in a circular manner (along the cell membrane), that is, the x−y cross-sectional image was observed at an optical slice for z > 0.38 μm. (b) z-Stack image of IgE-bound RBL-2H3 cells stained with fluorescein DHPE in MT buffer. The fluorescence image was observed in plane (on the cell membrane), that is, the x−y cross-sectional image was observed at an optical slice for z = 0−0.38 μm. The fluorescent backgrounds found around the RBL-2H3 cells also seemed to be due to the debris with a lipophilic double layer derived from these cells. (c) Calibration curve of fluorescence peak intensity for interfacial pH measured by ratiometric analysis (488/458 nm). The data shown are the average of six cells in the MT buffer at different pH values.

Figure 2. (a) z-Stack image of IgE-bound RBL-2H3 cells stained with fluorescein DHPE in MT buffer medium (pH 8.0). (b) z-Stack image of IgE-bound RBL-2H3 cells stained with fluorescein DHPE in MT buffer. (c) Calibration curve of fluorescence peak intensity for interfacial pH measured by ratiometric analysis (488/458 nm). The data shown are the average of six cells in the MT buffer at different pH values.

Figure 3. Change in interfacial pH at the interface between the mast cell and substrate for incubation time. IgE-bound (IgE(+)) or unmodified (IgE(−)) RBL-2H3 cells were used for reaction with the antigen (50 ng/mL). Interfacial pH was analyzed on the basis of the ratio of fluorescence intensities (Figure S1 in the Supporting Information) and the calibration curve (Figure 2c). The data presented are the average of five cells with IgE and seven cells without IgE.

2.2. Comparison of Interfacial pH at the Cell/Substrate Interface with Interfacial pH at the Cell/Bulk Solution Interface. Figure 5 shows the change in pH (ΔpH) along the z-stack position for the IgE-bound RBL-2H3 cells upon adding DNP-HSA. The allergic reactions were allowed to proceed for 2, 5, and 8 min. ΔpH at the cell/substrate interface (z = 0−0.38 μm) was clearly different from that at the cell/bulk solution interface (z > 0.38 μm). The interfacial pH at the mast cell/substrate interface markedly increased after the addition of DNP-HSA and reached a peak (ΔpH ≈ 0.5) after 5 min, followed by a decrease in ΔpH to about 0.2 after 8 min. On the other hand, ΔpH at the mast cell/bulk solution interface did not significantly change and remained constant at about ±0.15 after the addition of DNP-HSA. This may be due to the diffusion rate of histamine released from the IgE-bound RBL-2H3 cells stimulated by the antigen. That is, the concentration of histamine near each interface should depend on the balance of the inflow of histamine from intracellular granules and the outflow of histamine diffusing to the bulk solution. At the mast cell/substrate interface, the diffusion of histamine molecules released from the mast cells was restricted to the horizontal direction because this interface was sandwiched by the cellular membrane and the substrate. On the other hand, at the mast cell/bulk solution interface, the released histamine easily diffused in every direction in the bulk solution. Therefore, the outflow of histamine at the mast cell/substrate interface would have been smaller than that at the mast cell/bulk solution interface, resulting in the local accumulation of histamine at the mast cell/substrate nanogap interface. Moreover, the morphological change of RBL-2H3 from a spherical shape to a flat shape may have contributed to the accumulation of histamine molecules at the mast cell/substrate nanogap interface. The morphological change to the flat shape increased the area of adhesion of RBL-2H3 cells to the substrate (Figure 4) and may have prevented the released histamine from diffusing to the bulk solution from the mast cell/substrate nanogap. In a previous work, the cellular morphology was found to likely affect the accumulation of ions at the cell/substrate nanogap. The contribution of the cellular morphology to the accumulation of histamine should be considered in the future including the distance between the mast cell and the substrate, although the nanogap distance before and after the allergic response has not yet been correctly measured.

Considering the local increase in the interfacial pH detected on the basis of the accumulation of basic histamine molecules at the mast cell/substrate nanogap, our results suggest that a dish-type sensor with cultured cells such as cell-cultured gate ISFETs has a structural merit in terms of enhancing the detection sensitivity to substances released from cells, compared with other measurement methods (e.g., the use of...
In this study, we have demonstrated the interfacial pH behavior based on the allergic response at a RBL-2H3 cell/substrate nanogap by the laser scanning confocal fluorescence microscopy. In particular, the induction of the type I allergy reaction resulted in extracellular alkalization at the mast cell/substrate nanogap interface, which was induced by basic secreted molecules accumulated at the mast cell/substrate nanogap interface, resulting in the increase in the interfacial pH, whereas minute changes in pH were monitored at the mast cell/bulk solution interface. Thus, the results of this study have proved the detection principle for allergic responses using the mast cell-coupled gate ISFET sensor and further support the idea that potentiometric ion sensors such as ISFETs can detect a cellular-function-induced change in pH at a cell/electrode nanogap in real time.

4. EXPERIMENTAL SECTION

4.1. Chemicals. The chemicals used were obtained from the following sources: dinitrophenyl-conjugated human serum albumin (DNP-HSA; A6661), mouse monoclonal anti-DNP IgE (D8406), fetal bovine serum (FBS), bovine serum albumin (BSA), and p-nitrophenyl-2-acetoamido-2-deoxy-β-D-glucopyranoside (pNPG) from Sigma-Aldrich Japan (Tokyo, Japan). Ringer buffer was composed of 126 mM NaCl, 4 mM KCl, 3 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM N-(2-hydroxyethyl)-piperazine-N’-ethanesulfonic acid (HEPES), and 15 mM glucose. MT buffer was composed of 137 mM NaCl, 2.7 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, 5.6 mM glucose, 20 mM HEPES, and 0.1% (v/v) BSA. The pH of each buffer was adjusted by adding 1 M NaOH and measured using a conventional pH meter (HORIBA).

4.2. Cell Culture. RBL-2H3 cells were seeded in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% FBS, 50 U/mL penicillin, and 50 μg/mL streptomycin on a cell culture dish (φ = 10 cm) at 37 °C in 5% CO$_2$ in an incubator system at a density of 2.5 × 10$^5$ cells/dish. The cells were cultured for 5 days including the replacement of medium on day 3. Cells were collected by trypsin treatment and used for each experiment.

4.3. Confirmation of Release of β-Hexosaminidase. β-Hexosaminidase released from RBL-2H3 cells was used as the index of type I allergy reaction. RBL-2H3 cells were transferred to 24-well plates at a density of 2.5 × 10$^5$ cells/well. RBL-2H3 cells were cultured in RPMI culture medium containing the 50 ng/mL IgE antibody, as described in Section 4.2. After cultivation overnight, cells were washed twice with MT buffer solution. MT buffer solution (500 μL) containing 50 ng/mL DNP-HSA as the antigen was added to each well, and the plates were incubated for 30 min in an incubator (5% CO$_2$, 37 °C). The sample plates were cooled with ice for 10 min to stop allergic reactions. The supernatant (50 μL) from each well was transferred to 96-well plates. Then, 100 μL of substrate solution containing 0.1, 1, 3.3, or 10 mM pNPG in 100 mM citrate buffer (pH 4.5) was added to each sample and the sample was incubated for 25 min in an incubator (5% CO$_2$, 37 °C). One hundred microliter of 2 M glycine buffer (pH 10.4) was added to each well to stop the enzyme reaction, and the

Figure 4. Change in morphology of IgE-bound RBL cell upon adding antigen (50 ng/mL). (a) Before addition of antigen. (b) After addition of antigen.

Figure 5. Change in interfacial pH (ΔpH) along the z-stack position upon adding antigen (50 ng/mL). The allergic reactions were allowed to proceed for 2, 5, and 8 min. ΔpH at the cell/substrate interface (slice number 0; z = 0−0.38 μm) and ΔpH at the cell/bulk solution interface (slice number 1; z = 0.38−0.76 μm, slice number 2; z = 0.76−1.14 μm, slice number 3; z = 1.14−1.52 μm, and slice number 4; z = 1.52−1.90 μm) were evaluated. The data shown are the average of five cells.
4.4. Preparation of RBL Cells Stained with Fluorescein DHPE. RBL-2H3 cells were transferred to a glass bottom dish (φ = 35 mm) at a cell density of 2 × 10^5 cells/dish. The IgE antibody (50 ng/mL) was added to the culture medium for sample measurement. Samples to which no IgE antibody was added were used as the control. After cultivating overnight, the culture medium was removed and washed with Ringer buffer (pH 7.4). RBL-2H3 cells were stained by incubating them in Ringer buffer containing 30 μM fluorescein DHPE for 30 min at room temperature. Stained cells were washed twice in Ringer buffer.

4.5. Fluorescence Imaging by Laser Scanning Confocal Microscopy. An LSM510 laser scanning confocal microscope (Carl Zeiss Co., Ltd.) was used for fluorescence imaging of stained RBL-2H3 cells. An Ar laser (458 and 488 nm) was used as the excitation laser. A long-pass filter (～505 nm) was used as the emission filter for each excitation wavelength. The ratio of emission intensity at 488 nm to that at 458 nm was calculated for the ratiometric analysis of fluorescence intensity. An oil immersion objective (40×, numerical aperture = 1.4) was utilized, and the pinhole size was set at one Airy disk unit. The image size was set to 256 × 256 pixels, and the pixel size was 0.56 × 0.56 μm. An optical slice was set at an interval of 0.38 μm in the normal direction to the glass substrate, and z-stack images of stained RBL cells were captured. The entire process of fluorescence imaging was conducted at 37 °C in air.

The pH behavior around RBL-2H3 cells caused by allergic responses was evaluated using fluorescence images. The IgE-bound RBL-2H3 cells or unmodified RBL-2H3 cells cultured on the glass bottom dish were stained with fluorescein DHPE, as described in Section 4.4. MT buffer solution (pH 7.3) was used as a fluorescence measurement buffer. Z-Stack images were obtained every 2 or 3 min for the RBL-2H3 cells with or without IgE. At 10 min after the start of measurement, the antigen was added to the cultured cells at the final concentration of 50 ng/mL. Fluorescence images were evaluated using ZEN lite software (Carl Zeiss Co., Ltd.), and the plugin “Ratio Plus” in Image J was utilized to estimate the ratio of fluorescence intensities (488/458 nm).

4.6. Calibration of Fluorescence Intensity Ratio for Interfacial pH. To construct the calibration curve between the ratio of fluorescence intensity and the pH of measurement solution (Figure 2c), the pH of MT buffer solution was adjusted from 6.8 to 8.0 upon adding 1 M NaOH and analyzed using a conventional pH meter (HORIBA). The z-stack images of RBL-2H3 cells stained with fluorescein DHPE in MT buffer were obtained using the same protocol as in the above-mentioned microscopy measurement at different pH values. An optical slice was moved around the substrate surface (under the cell/substrate interface) to the top of adhering cells to find the region with the peak fluorescence intensity, where the mast cell/substrate interface was found. From the peak intensity, the fluorescence ratio at the cell/substrate interface around the center of a cell was calculated. pH calibration was conducted for the IgE-bound RBL cells or unmodified RBL-2H3 cells.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b01872.

Fluorescence intensities along the z-axis; evaluation of β-hexosaminidase; and change in pH along the z-stack position for the IgE-bound RBL-2H3 cells (PDF)

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Notes

The authors declare no competing financial interest.

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