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$[pH]_o$ imaging in proton releasing cells by an ion image sensor-based chemical microscopy

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Abstract

To examine the functions of proton release from the cells, we measured the extracellular pH ($[pH]_o$) by using an ion image sensor made of a 2 dimensional array of potential sensitive pixels. We recorded the changes in $[pH]_o$ of cells such as osteoclasts, gastric glands and hippocampal slices. After stimulation of cells and/or tissues, $[pH]_o$ was decreased, and the degree of the pH changes was dependent on the doses of stimulants. We established a label free microscopic assessment of 2D-distributions of biologically interesting substances by the present chemical sensing technique. The $[pH]_o$ imaging by chemical microscope promises the usefulness in the medical fields for the analysis of diseases such as osteoporosis, gastric ulcer, and stroke.

Key words: proton, ion image sensor, extracellular pH, osteoclast, gastric mucosa, hippocampus

Development of an ion image sensor for label-free micro-imaging of $[pH]_o$

By using a SiN deposition technique, 32 x 32 pixels of chemical sensor was developed [1]. It produces an electrical output in proportion to the surface potential on each pixel, thus displays the pH of solution with a high spatial resolution (~100 μm). With the chemical microsensor, 2D imaging of not only $[pH]_o$ but also transmitter releases were possible in the living cells without labeling [2, 3]. We used this label-free technique for the detection of proton in the acid secreting cells, osteoclasts and gastric glands.

$[pH]_o$ imaging in osteoclasts and gastric gland

The mature osteoclasts or the excised gastric glands were prepared by the methods described as previously [4, 5]. After the preparation, cells were kept in the recording medium containing 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM D-glucose, and 10 mM HEPES (pH=7.2 with NaOH).

The pH value of solution before placing the cells/tissues on the pH sensor was about 7.2 same as the recording medium. After placing the cells, the pH was slightly decreased (6.5-6.8) in the area where the cells were aligned. When the cells were stimulated by adding a histamine (1 mM)-containing medium, the pH decreased in the 2 min (Fig. 1). The degree of the $[pH]_o$ changes was more than 2 in the osteoclasts, and less than 1 in the gastric glands. Mostly, the pH response in the apical side was larger than that in the basal side of gastric cells. These results indicate that $[pH]_o$ decrease reflects an active release of proton

from osteoclasts for the bone absorption, and from parietal cells for food digestion.

Glutamate-induced $[pH]_o$ decrease in hippocampal slices

We extended the use of the ion image sensor-based $[pH]_o$ imaging to detect the proton release of the brain slices during the excitotoxic neuronal cell death [6]. The hippocampal slice was obtained from the neonatal rat, and organotypically cultured on milli-pore membrane filter for 1 week [7]. Then it was placed on the pH image sensor in a slice-side-down configuration.

The $[pH]_o$ of solution before placing the slice preparation was about 7.2. After placing the hippocampal slice, the pH was slightly decreased in the area where the somata of pyramidal cells were aligned. When the slice was stimulated by adding a glutamate (1 mM)-containing medium over the membrane filter, the pH did not change in the initial 5 min, but then, a clear decrease in pH was observed (Fig. 2). The low pH signal in the region of CA1 sometimes appeared in a shape of a short arc resembling the line of pyramidal cells. Mostly, the pH response in the CA1 region was larger than that in the CA3 region. The selective decrease of $[pH]_o$ in the CA1 region may reflect the glutamate-induced acute neuronal cell death. These results indicate that rapid diagnosis of the neuronal cell death is possible by the 2D-detection of $[pH]_o$ using the chemical microscope system.

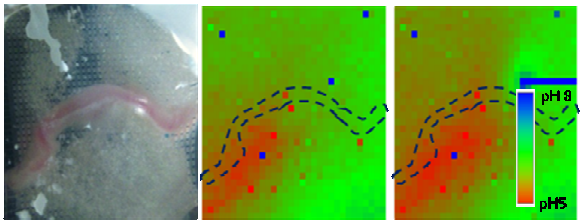

Fig. 1. 2-dimensional $[pH]_o$ imaging of gastric glands. (A) photograph of excised gastric tissue placed on the ion image sensor. $[pH]_o$ image before (B) and after (C) stimulation with 1 mM histamine. Locations of cellular samples were marked by dashed lines in B and C.

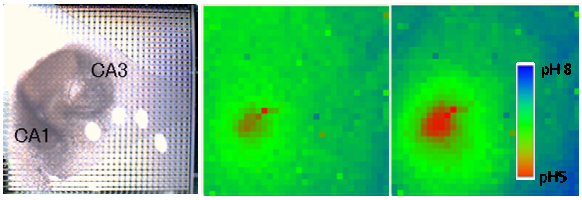

Fig. 2. Glutamate-stimulated neurons of the hippocampal slice. (A) photograph of hippocampal slice placed on the ion image sensor. $[pH]_o$ image before (B) and after (C) stimulation with 1 mM glutamate. Regions of both CA1 and CA3 are indicated in A.

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