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Measurements of CO_2, lactic acid and sodium bicarbonate secreted by cultured cells using a flow-through type pH/CO_2 sensor system based on ISFET

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Abstract

We developed a pH/CO_2 sensor system using two ion-sensitive field effect transistors (ISFETs) to evaluate the metabolic activity of cultured cells. One ISFET measures total pH changes and the other ISFET, covered with a gas-permeable silicon tube, measures pH changes due to CO_2. These ISFETs were placed in a flow-through chamber with a window to be attached to a glass plate on which cells were cultured. Measurements were performed with a baseline solution of low buffer capacity. In the quantitative estimation of cellular products, we assumed that cells secreted CO_2, lactic acid and sodium bicarbonate ($NaHCO_3$), and then lactic acid and $NaHCO_3$ proceeded to the neutralization reaction to form sodium lactate and additional CO_2. We applied this system to evaluate the metabolic activity of bovine aortic endothelial cells (BAEC) and rabbit aortic smooth muscle cells (RASMC). The production of CO_2 by RASMC was significantly higher than that by BAEC (204 ± 16 and $4.1 \pm 0.3 \times 10^8$ molecules/cell/s, respectively). More $NaHCO_3$ than lactic acid was produced in RASMC, whereas they were not significantly different in BAEC.

Keywords: ISFET; Extracellular acidification; Carbon dioxide; Lactic acid; Sodium bicarbonate

1. Introduction

Measurements of extra cellular acidification (ECA) have been extensively reported, especially since Molecular Devices Corporation developed a system called the Cytosensor Microphysiometer which uses a light-addressable potentiometric sensor [1,2]. For example, Parce et al. [1] measured the ECA of human keratinocytes grown on a glass cover slip, and estimated the net rate of proton production of this cell at about $1 \times 10^8\ s^{-1}\ cell^{-1}$. They also measured the production of lactate by a spectrophotometric enzymatic assay and obtained the rate of $3.3 \times 10^7\ s^{-1}$. Thibodeau et al. [3] measured the ECA of isolated gastric glands, and found that ECA was stimulated by activating H^+-K^+-ATPase with 8-(4-chlorophenylthio) adenosine 3′,5′-cyclic monophosphate, while it was depressed by inhibiting the same enzyme with omeprazole. On the other hand, they found that inhibition of the Na^+/H^+ exchanger did not reduce ECA. Landwojtowicz et al. [4] observed that the ECA of pig kidney cells and mouse embryo fibroblasts was activated or inhibited by selective activators or inhibitors of the ATPase of P-glycoprotein, while treatment of cells with inhibitors of Na^+/H^+ or the Cl^-/HCO_3^- exchanger did not reduce the ECA.

Following these studies, Lehmann et al. [5] first reported the simultaneous measurement of ECA and oxygen consumption using a PO_2 sensor combined with an ion sensitive field effect transistor (ISFET)-based pH sensor. They treated colon adenocarcinoma cells with a glycolysis inhibitor, iodocetate and observed a decline of acidification and an unchanged O_2 consumption. These results showed the possibility of evaluating cellular metabolic production in more detail by using combined sensors.

It has been considered that the compounds causing ECA are mostly lactic acid and CO_2 formed during energy metabolism with glucose and glutamine as carbon sources [6]. However,

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it is reasonable to speculate that cells secrete not only acids but also alkaline components, such as $NaHCO_3$ [8]. In such a case, a conventional microphysiometer which includes a single pH sensor can measure only the overall pH around cells, and therefore it has been impossible to define the amount of CO_2 (p), lactic acid (q) and $NaHCO_3$ (r) secreted. We constructed a flow-through type pH/CO_2 sensor system using two ISFETs that enables the measurements of the overall pH change and the pH change due to CO_2 around the cells. It is impossible to measure CO_2, lactic acid and $NaHCO_3$ separately even by using this combined sensor system, but it was found that the amounts of $p+r$, $p+q$ and $r-q$ can be derived from the output of the two sensors. In the present paper, chemical meanings were given to $p+r$, $p+q$ and $r-q$, and estimations of these values for two kinds of cells will be demonstrated.

2. Experimental

2.1. Software

In the present experiments, two data were obtained as observed values. These are pH values read by the pH sensors placed outside and inside a gas-permeable silicone tube (pH_o and pH_i, respectively). On the other hand, there are three unknowns: the concentrations of secreted CO_2, secreted lactic acid and secreted sodium bicarbonate. Equations correlating observed proton concentrations to the concentrations of secreted acids and base were derived as follows.

In the present experiments, a solution composed of 15 mM D-glucose, 140 mM NaCl, 5 mM KCl, 0.9 mM $MgCl_2$, 0.34 mM $NaHCO_3$ and 0.028 mM HEPES (*N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethane-sulfonic acid) was used as the baseline solution. $NaHCO_3$ was added to this baseline solution to adjust the pH of the final solution to 7.4. Because all components other than $NaHCO_3$ and HEPES had a neutral pH, it was assumed that the pH of the baseline solution was determined by the initial concentrations of $NaHCO_3$ (α) and HEPES (β), and the concentrations of CO_2 (p), lactic acid (q) and $NaHCO_3$ (r) secreted by cells. The effects of other neutral components, such as D-glucose, NaCl, KCl and $MgCl_2$ on the acid–base equilibrium were neglected in the following discussions.

Thus, the chemical equations responsible for determining the pH of the baseline solution are the following three equations. The dissociation of bicarbonate ion to carbonate ion was assumed to be negligible.

$$CO_2 + H_2O = H^+ + HCO_3^- \tag{1}$$

$$RCOOH = H^+ + RCOO^- \tag{2}$$

$$H_2O = H^+ + OH^- \tag{3}$$

Here, RCOOH and $RCOO^-$ mean lactic acid and lactate anion, respectively. The equilibrium constant for each equation is defined by the following equations:

$$K_1 = \frac{(H^+)(HCO_3^-)}{CO_2} = 10^{-6.3525} \tag{4}$$

$$K_2 = \frac{(H^+)(RCOO^-)}{RCOOH} = 10^{-3.858} \tag{5}$$

$$K_w = (H^+)(OH^-) = 10^{-13.997} \tag{6}$$

HEPES is assumed to be dissociated completely to proton and HEPES anion ($HEPES^-$) because it is a strong acid.

In the baseline solution surrounding the pH_o, the positive ions are the Na^+ of the original and secreted sodium bicarbonate and proton, and the anions are bicarbonate ion, hydroxide ion, lactate ion and HEPES anion. Due to the neutral charge condition, one can obtain the following equation:

$$(Na^+)_o + (H^+)_o = (HCO_3^-)_o + (OH^-)_o + (RCOO^-)_o + (HEPES^-) \tag{7}$$

Here, subscript 'o' in each term means that these values are for the solution outside the silicone tube. Because the concentrations of HEPES anion outside and inside the silicone tube are identical, ($HEPES^-$) has no suffix. The sum of CO_2 and bicarbonate ion should be equal to $\alpha + p + r$, giving Eq. (8).

$$(CO_2)_o + (HCO_3^-)_o = \alpha + p + r \tag{8}$$

For lactic acid,

$$(RCOOH)_o + (RCOO^-)_o = q \tag{9}$$

and for HEPES ion,

$$(HEPES^-) = \beta \tag{10}$$

From (4) and (8), Eq. (11) can be obtained.

$$(HCO_3^-)_o = \frac{\alpha + p + r}{1 + h_o/K_1} \tag{11}$$

Here, h_o is $(H^+)_o$. Eq. (12) is also obtained.

$$(RCOO^-)_o = \frac{q}{1 + h_o/K_2} \tag{12}$$

Because $(Na^+)_o = \alpha + r$, one can obtain Eq. (13) from Eqs. (7)–(12).

$$\alpha + r + h_o = \frac{\alpha + p + r}{1 + h_o/K_1} + \frac{K_w}{h_o} + \frac{q}{1 + h_o/K_2} + \beta \tag{13}$$

Because lactic acid and lactate ion cannot permeate the silicone tube, the neutral charge condition for the baseline solution inside the silicone tube is Eq. (14).

$$(Na^+)_i + (H^+)_i = (HCO_3^-)_i + (OH^-)_i + (HEPES^-) \tag{14}$$

Here, subscript 'i' means that the value is for the baseline solution inside the silicone tube. Because $(Na^+)_i$ is α, Eq. (15) can be derived from Eq. (14), using Eqs. (4)–(6).

$$\alpha + h_i = \frac{K_1(CO_2)_i}{h_i} + \frac{K_w}{h_i} + \beta \tag{15}$$

Here, h_i is $(H^+)_i$. It is assumed that the concentration of molecular CO_2 is identical on both sides of the silicone tube in the