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Measurement and Analysis of Intracellular Ion Distributions in Single Living Cells with Fluorescent Indicator Dyes

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Summary

The distributions of intracellular pH, Na^+, K^+, Mg^{2+} and Ca^{2+} concentration is measured using various type fluorescent indicator dyes. The measurement of these intracellular ion distributions performed by a ratio imaging method. The equipment constructed with a fluorescence microscope and digital image processer.

Many type indicator dyes had the property of collection in nucleus, but only Na^+ dye had a collection in cytoplasm. The intracellular pH, K^+ and Ca^{2+} distribution was observed homogeneously distribution. However, the intracellular Na^+ and Mg^{2+} shows the concentration in cytoplasm and nucleus were clearly different.

Introduction

Intracellular Ca^+, Mg^{2+}, pH, *etc.* has an important function for control of cellular responses and metabolismes. For example, intracellular free Ca^{2+} has a second messenger, and intracellular Mg^{2+} is a regulation of enzyme activity and polymerization control of cytoskeleton. Already, the measurement of intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) widely uses an experimental technique of life science[1–3].

Recently, various type fluorescent indicator dyes for the measurement of intracellular ions are announced. (Table 1) These indicator dyes had a similar chemical structure that joined by bound of chelator and fluorescent group. The fluorescence changes resulting from metal ion binding in chelator. Many type dyes[4] and measurement system of intracellular ion concentration could be obtained.

The intracellular ion concentration measured two step treatments. First step is the loading of indicator dye into cells. Second step is measurement of fluorescent intensity emitted from the loading dye. In this report, I mentioned the measurement method of intracellular free Ca^{2+}, Mg^{2+}, Na^+, K^+ and pH distributions.

Materials and Methods

Instrument: The measurements of intracellular ion distribution uses two video image microscope model. One model is ARGUS-100 (Hamamatsu Photonics: Hamamatsu, JAPAN) that measured intracellular pH ($[pH]i$), intracellular free K^+ concentration ($[K^+]i$), and intracellular free Na^+ concentration ($[Na^+]i$). Second model is CASALS (Olympus: Tokyo, JAPAN) that measured $[Ca^{2+}]i$ and intracellular free Mg^{2+} concentration ($[Mg^{2+}]i$). Both instruments had almost same construction (Fig. 1) and the same measurement sequence of ratio imaging method. Using CASALS, the two images of living cell, one by a fluorescent microscopy for intracellular ion distribution and the other by microscopic cell image, can be superimposed on an image processer screen.

Materials: The cell used ion distribution measurements is NG108–15 that is hybrid cell of mouse neuroblastoma and rat glioma.

Table 1. Major Type of Fluorescent Indicator Dyes

Item	Indicator dye	Photometry and wavelength*	Characterization
pH	fluorescein	1ex(489)1em(514)	Old type
	carboxy-fluorescein	1ex(490)1em(515)	
	sulfofluorescein	1ex(492)1em(520)	
	naphtho-fluorescein	1ex(594)1em(663)	
	quene-1	1ex(390)1em(530)	
	BCECF	1ex(508)1em(531)	widely use
	resorufin	1ex(571)1em(589)	Acidic pH range
	HPTS(pyranine)	2ex(405,465)1em(514)	Polar pH indicator
	carboxy SNAFL-1	2ex(479–508, 537)1em(543–623)	SNAFL are new
	carboxy SNAFL-2	2ex(485–514,547)1em(546–630)	type dyes
	carboxy SNARF-1	1ex(488–534)2em(579,640)	SNARF are new
	carboxy SNARF-2	1ex(517–577)2em(583,633)	type dyes
	carboxy SNARF-6	1ex(555)2em(600,635) or 2ex(496–524, 556)1em(610)	
	carboxy SNARF-X	1ex(480–587)2em(559,630)	
	C1-NERF	2ex(488,514)1em(540)	NERF are new type
	DM-NERF	2ex(488,514)1em(540)	of acidic pH
Na^+	FCryp-2	1ex(340)2em(395,490)	
	SBFI	2ex(330–345,370–390)1em(530)	
K^+	PBFI	1ex(334–246)1em(525–551)	
Mg^{2+}	Mag-quin-2	1ex(337–353)1em(490)	
	Mag-fura-2	2ex(344,376)1em(500)	
	Mag-indo-1	1ex(340–360)2em(419,475)	
	Mag-fura-5		Low[Mg2+]i range
Ca^{2+}	quin-2	1ex(332–352)1em(492)	Old type
	fura-2	2ex(335,380)1em(510)	Widely use
	indo-1	1ex(331–360)2em(410,485)	
	fluo-3	1ex(506)1em(526)	
	rhod-2	1ex(555)1em(576)	High $[Ca^{2+}]i$ range
	Calcium Green	1ex(505)1em(527)	Widely type
	Calcium Orange	1ex(550)1em(580)	New type
	Calcium Crimson	1ex(590)1em(610)	New type
	Calcium Red	1ex(425–450, 480–500)1em(660)	New type
	fura-5		Low $[Ca^{2+}]i$
Zn^{2+}	TSQ	1ex(335)1em(376)	
Cl^-	SPA	1ex(415)lem(488)	
	SPQ	1ex(344)1em(450)	
cAMP	FICRhR	1ex(495)2em(520,570)	

*1ex 1em: One excitation and one emission, 1ex 2em: One excitation and two emissions, 2ex 1em: Two excitations and one emission. The number in parenthesis shows major wavelength for excitation and emission.

Fluorescent indicator dyes of BCECF, BCECF/AM, PBFI/AM, SBFI, SBFI/AM, Mag-fura-2 and Mag-fura-2/AM were obtained from Molecular Probes (Oregon, USA). Fura-2 and fura-2/AM were purchased from Dojindo Laboratories (Kumamoto, JAPAN).

Methods: Preparation of culture cells and loading of fluorescent indicator dyes performed by the steps shows in Fig. 2[5]. The loading conditions are same dye concentration and same incubation time with any type indicator dyes for the comparison of loading characterise of each dye.

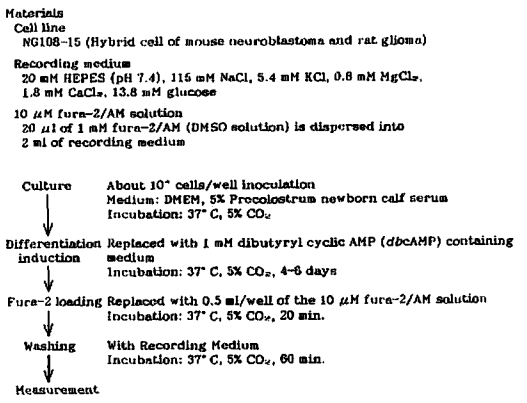

Fig. 1. Loading condition of fluorescent indicator dyes.
The condition wrote the case of fura-2 loading into NG108-15 cells. Loading of another indicator dyes also was the same condition as fura-2 in these experiments.

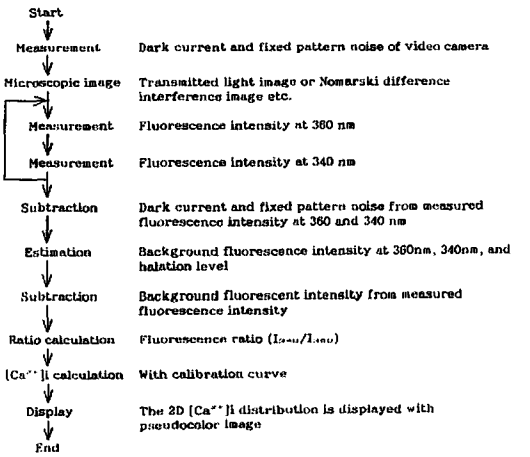

Fig. 2. Image processing sequence.
This sequence shows fura-2 case. But, another indicator dye case, measurement and image processing were same sequence only exchange the excitation and emission filter of microscope to fitted wavelength. Input of microscopic image for the superimposition of ion distribution and cellular structure performed only CASALS.

The method of quantitative measurement sequence shows in Fig. 3[5]. This measurement sequence of both instruments of ARGUS-100 and CASALS is a dual wavelength fluorometry method.

The excitation filters exchanged with each indicator dye to fit excitation and emission light wavelength. The measurement of [pH]i with BCECF excited at 450 nm and 490 nm, and measured of fluorescent intensity at 520 nm. The measurement of $[K^+]i$ with PBFI, $[Na^+]i$ with SBFI, and $[Ca^{2+}]i$ with fura-2 excited at 340 nm and 360 nm, and fluorescence measured at 510 nm. In the case of $[Mg^{2+}]i$ with Mag-fura-2, excitation was 430 nm and 480 nm, and fluorescence measured at 510 nm.

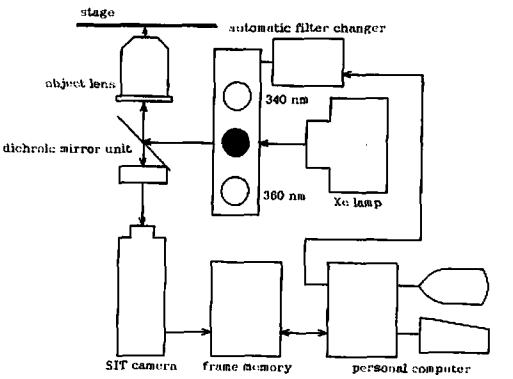

Fig. 3. Block diagram of the measurement system.
This system is composed of an inverted vertical light fluorescent microscope attached to a xenon lamp and automatic changer of excitation filter. Image data, recorded by SIT camera, are processed by frame memory and computer and displayed on TV monitor. Basically, ARGUS-100 and CASALS had same construction.

Results and Discussion

By the loading condition showed in Fig. 2, NG108-15 cells had high intracellular BCECF concentration to measurement [pH]i. The NG108-15 nucleus collects BCECF dye and observed strong fluorescence intensity. (Fig.4A) However, [pH]i distribution shows in Fig. 4B indicated the uniform intracellular distribution. Nevertheless, pH value differed cell by cell and had 6.7 to 7.7 over the cells.

Fluorescence from PBFI loaded into cell slightly week intensity comparison with BCECF. PBFI dye also has the trend of collection in nucleus. The measurement of $[K^+]i$ distribution could be done easily. $[K^+]i$ shows the homogeneous distribution, and similar concentration of all cells. (Fig. 5)

However, SBFI fluorescent from cells shows week intensity. While BCECF and PBFI dyes case, SBFI dye loaded into NG108-15 cells mainly exists in cytoplasm comparison with nucleus. The measurement

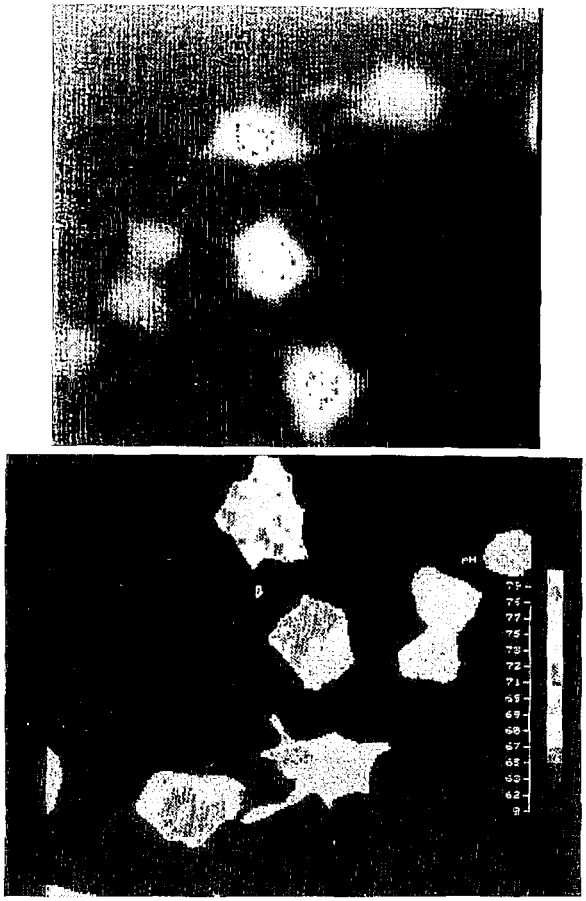

Fig. 4. Fluorescent image of BCECF loaded NG108–15 cells and [pH]i distribution.

A. Fluorescent microscope image.

BCECF, PBFI, fura-2 and Mag-fura-2 had the property of cellected in nucleus, while SBFI exists in cytoplasm.

B. [pH]i distribution shows pseudocolor.

[pH]i indicated the uniform intracellular distribution, but pH value differed cell by cell.

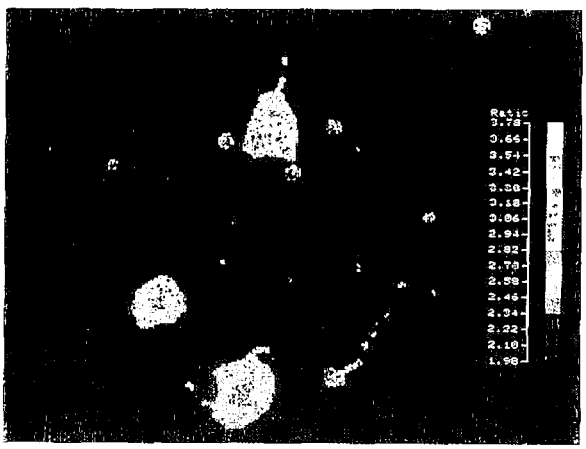

Fig. 5. $[K^+]i$ distribution image with PBFI.
The pseudocolor image shows ratio image of fluorescent intensity.

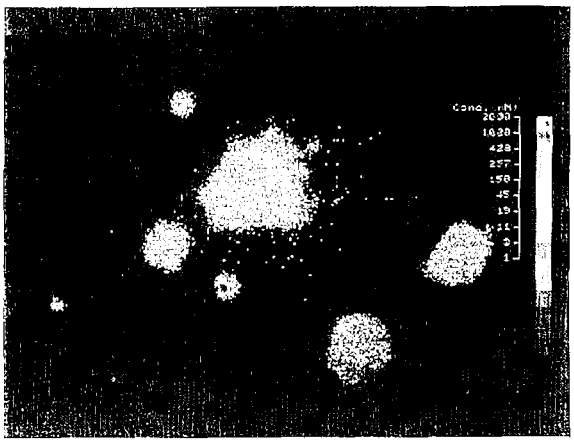

Fig. 6. $[Na^+]i$ distribution image with SBFI.
SBFI fluorescence from loading cells week intensity, and the ratio image has a large noise.

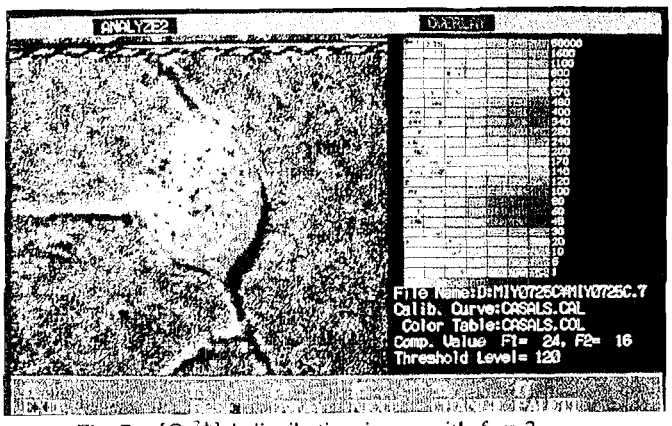

Fig. 7. $[Ca^{2+}]$ i distribution image with fura-2.
This $[Ca^{2+}]i$ distribution superimposed on Nomarski differential interference image of NG108-15 cell. $[Ca^{2+}]i$ shows the homogeneous distribution.

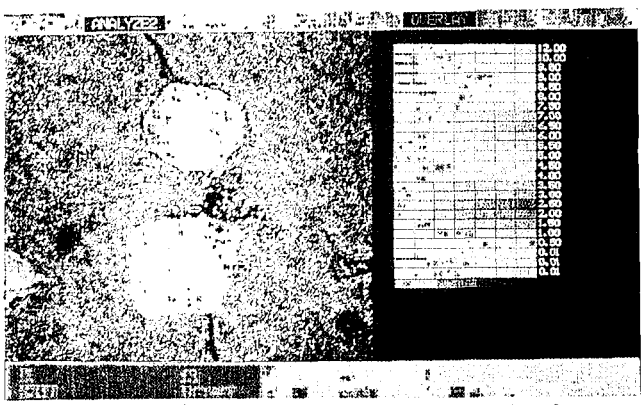

Fig. 8. $[Mg^{2+}]i$ distribution image with Mag-fura-2.
This $[Mg^{2+}]i$ distribution superimposed on transmission light microscopic cell image. $[Mg^{2+}]i$ shows the low concentration in nucleus and high in cytoplasm.

of $[Na^+]i$ distribution performed maximum sensitivity of SIT camera. By the case of SBFI loading into NG108–15 cells, long incubation time and/or high SBFI/AM concentration is necessary to obtain the good signal-to-noise ratio images. The $[Na^+]i$ distribution indicated slightly low concentration of nucleus comparison with cytoplasm. (Fig. 6)

In the case of Mag-fura-2 and fura-2, sufficient concentration of dyes was loaded in NG108–15 cell by the condition shows in Fig. 2. These fura-2 and Mag-fura-2 dyes also had the trend of collection in nucleus comparison with cytoplasm. The superimpose of $[Ca^{2+}]i$ distribution and Nomarski differential interference image shows in Fig. 7. The $[Ca^{2+}]i$ distribution was measured after stimulation with high concentration KCl, and observed homogeneously distribution.

Figure 8 shows a superimpose image of $[Mg^{2+}]i$ distribution and transmission light microscopy. The $[Mg^{2+}]i$ distributed low nucleus concentration (below 1mM) and high cytoplasm concentration (2–3mM) same as the case of $[Na^+]i$.

Reference

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