Chloride ion sensitive fluorescent dye for Drug Screening

Abstract

The chloride-sensitive fluorescent indicator MQAE (N-[ethoxycarbonylmethyl]-6-methoxy-quinolinium bromide) has been used for determination of the intracellular free chloride concentration in mammalian cells. The MQAE had a high Cl^- sensitivity (Stern-Volmer constant 200 M^{-1}), peak excitation and emission wavelengths of 355 and 460 nm and a molar absorbance of 4850 M^{-1} cm^{-1} (320 nm). MQAE fluorescence was not altered by the physiological anions HCO3, SO4, and PO4, by cations, or by pH.

The MQAE leaked out of cells less than 20 % in 60 min at 37 ˚C. Recently, the MQAE has been used in a fluorescence microplate assay that has potential for screening compounds that modify Cl-ion-channel activity[1]. Here we present the first data for the use of MQAE with the FDSS "Functional Drug Screening System" (Hamamatsu Photonics K. K., Hamamatsu City, Japan). The results show that the chloride-sensitive fluorescent indicator MQAE is a useful tool when determining intracellular chloride activity, and in quantitative determination of chloride fluxes in living cells.

Table. 1: Properties of chloride indicator

Acronym	MW	Abs (mm)	e ($cm^{-1}M^{-1}$)	Em (nm)	Ksv(M^{-1})	Molecular Probe Cat
MQAE	326	350	2800	460	200	E-3101
MEQ	315	344	3900	442	145	M6886
SPQ	281	344	3700	443	118	M440
Incigenin	511	455	7400	505	390	L6868

Table. 2: Stern-Volmer constant of MQAE and SPQ

	Ksv (M^{-1})				
	Cl^-	Br^-	I^-	SCN^-	citrate
MQAE	200	293	456	410	41
SPQ	118	175	276	211	15

Materials and Methods

T84 and the cystic fibrosis transmembrane conductance regulator (CFTR) expressing cells were tripsinized and plated at 5×10^4 per well. Cells are typically loaded by adding 4-10 mM MQAE, incubating for 4-24 hours at 37 ˚C and finally washing 2 times with dye-free medium before FDSS analysis. The light emission was recorded during variable times using the FDSS. Single-wavelength analysis with MEAQ the excitation filters 360 nm, the UV dichroicmirror for Fura-2, and a 460 nm emission filter.

Results and Discussion

Fluorescence-optical measurements of the intracellular chloride concentration facilitate identification of chloride movements across the cell membrane of living cells. The two main dyes used for this purpose are 6-methoxy-N-(3-sulfopropyl) quinolinium (SPQ) and 6-methoxy-quinolyl acetoethyl ester (MQAE) (Table. 2). But there are some reports that the use of both substances is impaired by their poor membrane permeability and therefore the loading of the cells to be studied is limited. In some case, MQAE demonstrated some toxicity with fluorescence excitation[2]. Some researcher improve the use of 6-methoxy-N-ethyl-1,2-dihydroquinoline (diH-MEQ), a chloride-sensitive dye for which a membrane-permeable form is easily prepared[3),4)]. With the exception of diH-MEQ, Cl-indicators must be loaded into cells by long-term incubation (up to eight hours) in the presence of a large excess of dye or by brief hypotonic permeabilization.

A microplate chloride ion channel assay, using N-(6-methoxyquinolyl) acetoethyl ester (MQAE) fluorescence changes has been developed (Fig. 1, Fig. 2). In Anal Biochem 1996, the Stern-Volmer constant for MQAE fluorescence in T84 cells was calculated as 28.3 ± 0.9 M^{-1} and the [Cl^-]i in untreated T84 cells was determined as 52.4 ± 0.6 mM[1]. We tested the two cell line. Forskolin stimulation of CFTR expressing cells caused cAMP-dependent, increased Cl^- loss in CFTR expressing cells (Fig. 1). In conclusion, fluroscence-optical measurements using MQAE as the chloride-sensitive dye provide a reliable and easy-to-use method for measuring changes of the chloride flux across the cellmembrane of living cells.

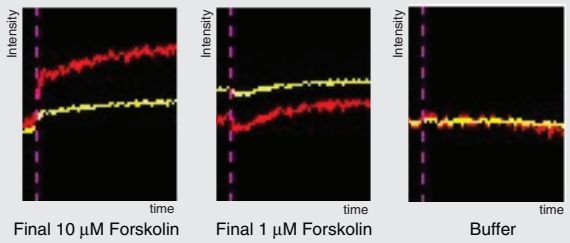

Fig. 1: cAMP-dependent, increased Cl^- loss in CFTR expressing cells

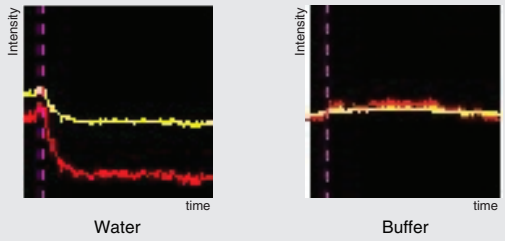

Fig. 2: Cl^- loss of T84 cells by osmotic cange.

Consumable

6-Methoxy-N-(3-sulfopropyl) quinolinium (SPQ, M-440)
N-(Ethoxycarbonylmethyl)-6-methoxyquinolinium bromide (MQAE, E-3101)
6-Methoxy-N-ethylquinolinium iodide (MEQ, M-6886)

References

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