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. 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

<table>
<thead>
<tr>
<th>Acronym</th>
<th>MW</th>
<th>Abs (mm)</th>
<th>e (cm⁻¹M⁻¹)</th>
<th>Em (nm)</th>
<th>Ksv(M⁻¹)</th>
<th>Molecular Probe Cat</th>
</tr>
</thead>
<tbody>
<tr>
<td>MQAE</td>
<td>326</td>
<td>350</td>
<td>2800</td>
<td>460</td>
<td>200</td>
<td>E-3101</td>
</tr>
<tr>
<td>MEQ</td>
<td>315</td>
<td>344</td>
<td>3900</td>
<td>442</td>
<td>145</td>
<td>M6868</td>
</tr>
<tr>
<td>SPQ</td>
<td>281</td>
<td>344</td>
<td>3700</td>
<td>443</td>
<td>118</td>
<td>M440</td>
</tr>
<tr>
<td>Incigenin</td>
<td>511</td>
<td>455</td>
<td>7400</td>
<td>505</td>
<td>390</td>
<td>L6868</td>
</tr>
</tbody>
</table>

Table 2: Stern-Volmer constant of MQAE and SPQ

<table>
<thead>
<tr>
<th></th>
<th>Cl⁻</th>
<th>Br⁻</th>
<th>I⁻</th>
<th>SCN⁻</th>
<th>citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>MQAE</td>
<td>200</td>
<td>293</td>
<td>456</td>
<td>410</td>
<td>41</td>
</tr>
<tr>
<td>SPQ</td>
<td>118</td>
<td>175</td>
<td>276</td>
<td>211</td>
<td>15</td>
</tr>
</tbody>
</table>

Materials and Methods
T84 and the cystic fibrosis transmembrane conductance regulator (CFTR) expressing cells were trypsinated and plated at 5 x 10⁴ 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 dichroic mirror 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 acetooethyl 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 excitation². 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 prepared³. With the exception of diH-MEO, 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⁻] 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, fluorescence-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 cell membrane of living cells.

![Fig. 1: cAMP-dependent, increased Cl⁻ loss in CFTR expressing cells](image1)

![Fig. 2: Cl⁻ loss of T84 cells by osmotic change.](image2)

**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**


© 2013 Hamamatsu Photonics K.K.

**FDSS** is registered trademark of Hamamatsu Photonics K.K. (China, France, Germany, Italy, Japan, U.K., U.S.A.)

**Product and software package names noted in this documentation are trademarks or registered trademarks of their respective manufacturers.**

- Subject to local technical requirements and regulations, availability of products included in this promotional material may vary. Please consult your local sales representative.

- Information furnished by HAMAMATSU is believed to be reliable. However, no responsibility is assumed for possible inaccuracies or omissions. Specifications and external appearance are subject to change without notice.

© 2013 Hamamatsu Photonics K.K.