

CoroNa Red as a potential probe for intracellular sodium imaging

Abstract

We have examined the new sodium indicator fluorescent dye named CoroNa Red using FDSS. Molecular Probe newest Na⁺ indicator is CoroNa Red chloride, which is based on a crown ether that has structural similarity to the Ca²⁺ chelator BAPTA. Unlike SBF1 and Sodium Green, the net positive charge of CoroNa Red targets the indicator to mitochondria and loading of cells does not require use of a permeant ester derivative of the dye. CoroNa Red is only

weakly fluorescent in the absence of Na⁺ and its fluorescence increases ~15-fold upon binding Na⁺. Despite its relatively high K_d for Na⁺ of ~200 mM (Table. 1), CoroNa Red indicator exhibits sensitive responses to cellular Na⁺ influxes through voltage-gated channels and ATP-gated cation pores^{1),2),3)}. Here we present the first data for the use of CoroNa Red with the FDSS "Functional Drug Screening System" (Hamamatsu Photonics K. K., Hamamatsu City, Japan).

Table. 1: Comparison data of each sodium indicator.

	CoroNa Red	Sodium Green	SBFI
MW	773.32	1543	1127
Molecular Probe Cat	C-24430	S-6901	S-1263
K _d (K+free)	~ 200 mM	6 mM	3.8 mM
K _d (K+Sat.)	~ 200 mM	21 mM	11 mM
Selectivity for Na ⁺ than K ⁺		~ 41-fold	~ 18-fold
Fluorescence quantum yield		0.2	0.08
Resting cell			~ 15 mM
Fully depolarized			~ 25 mM
Excitation wavelength	540 nm	480 nm	335 nm
Emission wavelength	590 nm	540 nm	540 nm

Materials and Methods

Human smooth muscle cell were trypsinized and plated at 4×10^4 per well. Cells are typically loaded by adding 5 to 10 μ M CoroNa Red from a 1 mM stock solution in DMSO, incubating for 10 to 30 minutes at 37 °C and finally washing with dye-free medium before FDSS analysis. The light emission was recorded during variable times using the FDSS. Single-wavelength analysis with CoroNa red needs the excitation filters 540 nm, the 560 nm dichroic mirror, and a 590 nm emission filter.

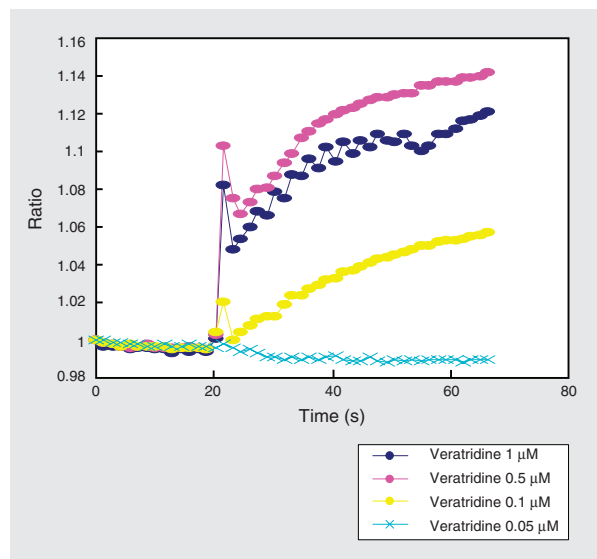
Results and Discussion

To affirm the Na⁺ response of CoroNa red, we used the veratridine/ 3-Veratrolyveracevine (Sigma V5754). Veratridine is one of the neurotoxin recognize the -subunit (site2) of voltage gated Na⁺ channels⁴⁾. As expected, fluorescent ratios were increased immediately after stimulation (Figure. 1). These results are basically consistent with previous data. We present the first data for the use of sodium indicator CoroNa Red with the FDSS. The longer-wavelength absorption of the CoroNa Red indicator results in reduction of the potential for photodamage to the cell because the energy of the excitation light is lower than that of the UV light required for excitation of SBF1. The high K_d for Na⁺ of ~200 mM, approximating physiological ionic strength, CoroNa Red indicator will become the useful dye for imaging the intracellular sodium influxes.

FDSS 7000EX

FDSS/μCELL

CoroNa Red imaging of Human smooth muscle cell



References

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Consumable

CoroNa™ Red chloride

Molecular Formula: C₄₂H₄₉ClN₄O₈

Molecular Weight: 773.32

C-24430 CoroNa™ Red chloride 1 mg

C-24431 CoroNa™ Red chloride *special packaging* 20 × 50 μg

Molecular Probe Catalogue

(<http://www.probes.com/>)

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