Synopsis of Original Research Paper

Development of a red fluorescence probe for monitoring dynamics of cytoplasmic calcium ion to elucidate physiological mechanism in skin

Kenjiro Hanaoka

Graduate School of Pharmaceutical Sciences, The University of Tokyo

The development of sophisticated fluorescence sensor probes has contributed to elucidation of the molecular mechanisms of many complex biological phenomena. In particular, calcium ion (Ca^{2+}) is a pivotal second messenger inside cells, and fluctuation of intracellular Ca^{2+} works together with various biomolecules in biological systems. So, we expect that simultaneous visualization of Ca^{2+} and other biomolecules, i.e., multicolor imaging, brings us many biological findings. However, color choices are not sufficient at present, that is, reported long wavelength fluorescence probes for Ca^{2+} have some disadvantages. For example, AM ester form of Rhod-2, one of the most widely used red fluorescence probe for Ca^{2+}, often localizes into mitochondria and monitors mitochondrial Ca^{2+} concentration change, although cytoplasmic Ca^{2+} is much more important for the research of Ca^{2+} signaling. Thus, we set out to develop a red fluorescence probe for Ca^{2+} with excellent properties including the cytoplasmic distribution to elucidate cytoplasmic Ca^{2+}-related biological phenomena such as hydraulic pressure stimulation in skin and epithelial wound-healing.

So far, we have developed a novel fluorescein analogue, TokyoMagenta (TM), in which the O atom at the 10 position of the xanthene chromophore of fluorescein is replaced with a Si atom. The absorption and emission wavelengths of TM were about 90 nm longer than those of TokyoGreen (a fluorescein derivative). In this study, we introduced chlorine into the fluorophore and developed dichloro TokyoMagenta (DCTM). Then, by utilizing DCTM, we developed a red fluorescence probe for Ca^{2+}, CaTM-2, and its activation ratio of the fluorescence intensity reaching 16-fold was practically useful. The chlorination of the fluorophore was also advantageous, and the pK_a value of CaTM-2 was greatly shifted to the acidic region compared with that of TM, and was sufficiently low (pK_a = 5.1) for practical use. For cellular application, we synthesized CaTM-2 AM, an AM ester form of CaTM-2. CaTM-2 AM diffused into cytosol uniformly in living cells, and showed the change in its fluorescence intensity by the histamine stimulus, monitoring the change of the cytoplasmic Ca^{2+} concentration. As a further demonstration of the usefulness of CaTM-2 AM, we confirmed that it could be applied to rat hippocampal slice cultures for monitoring activities of neurons. Thus, CaTM-2 and CaTM-2 AM would provide an innovative approach for researchers to work on many challenges related to Ca^{2+}.