A Selective, Cell-Permeable Optical Probe for Hydrogen Peroxide in Living Cells

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Hydrogen peroxide is a major reactive oxygen species (ROS) in living organisms, and its homeostasis can have diverse physiological and pathological consequences. H$_2$O$_2$ is a source of oxidative stress, and oxidative damage resulting from cellular imbalance of H$_2$O$_2$ and other ROS oxidants is connected to aging and severe human diseases such as cancer, cardiovascular disorders, and Alzheimer’s and related neurodegenerative diseases. On the other hand, emerging evidence supports a physiological role for H$_2$O$_2$ as a second messenger in cellular signal transduction. For example, peroxide bursts trigger mitogen-activated protein (MAP) kinase pathways that affect cell proliferation and cell death.

Despite the importance of H$_2$O$_2$ to human health and disease, the molecular mechanisms of its production, accumulation, trafficking, and function are insufficiently understood even in the simplest eukaryotic organisms. We are interested in developing new chemical tools to study the physiological and pathological roles of H$_2$O$_2$ and related ROS in living systems. In this regard, fluorescent probes are well suited to meet the need for reagents to interrogate the cellular chemistry of H$_2$O$_2$ at the molecular level. One major challenge to achieving this goal is creating water-soluble systems that report H$_2$O$_2$ selectively over competing cellular ROS like superoxide (O$_2^-$), nitric oxide (NO), and lipid alkylperoxides. Synthetic small molecules offer one approach to such probes, and several types of reagents have been examined for H$_2$O$_2$ detection. Included are dihydro analogues of fluorescent dyes (e.g., 2',7'-dichlorodihydrofluorescein (DCFH), Amplex Red, dihydrorhodamine 123), phosphine-based fluorophores, lanthanide coordination complexes, and chromophores with ROS-cleavable protecting groups. However, limitations of currently available H$_2$O$_2$-responsive probes include interfering background fluorescence from other ROS, the need for an external activating enzyme, lack of water solubility or compatibility, and/or excitation profiles in the ultraviolet region, which can damage living samples and cause interfering autofluorescence from native cellular species. The most commonly used fluorophore for cellular ROS detection, DCFH, is also easily autoxidized and exhibits background fluorescence from native ROS upon continued exposure to light. In this report, we present the synthesis and properties of Peroxyfluor-1 (PF1), a new water-soluble, turn-on optical probe for H$_2$O$_2$ that exhibits high selectivity and dynamic range for this small molecule over other ROS. In addition, we establish its utility for imaging changes in [H$_2$O$_2$] within living mammalian cells with visible wavelength excitation and emission energies.

Our strategy for the optical detection of H$_2$O$_2$ relies on the selective H$_2$O$_2$-mediated transformation of arylboronates to phenols. In particular, we reasoned that installation of boronic ester groups at the 3' and 6' positions of a xanthenone scaffold would force this platform to adopt a closed, colorless, and non-fluorescent lactone form. Upon treatment with H$_2$O$_2$, hydrolytic depredation of the boronates would subsequently generate the open, colored, and fluorescent fluorescein product. Scheme 1 outlines the preparation of PF1 based on this design. Acid-catalyzed condensation of 3-iodophenol and phthalic anhydride affords 3',6'-diiodofluorancarboxylate, which represents a 10- to 100-fold increase in H$_2$O$_2$ selectivity compared to previously reported probes. The xanthenone probe is also more responsive to H$_2$O$_2$ compared to highly reactive oxygen radicals such as O$_2$(O)Bu (>15-fold higher for H$_2$O$_2$) and OH (>3-fold higher for H$_2$O$_2$). We suggest that the observed selectivity of PF1 for H$_2$O$_2$ over more oxidizing ROS is based on its detection mechanism, which relies on depredation rather than oxidation to provide an optical response. We next assessed the ability of PF1 to operate within living cells. HEK cells were incubated with 5 μM PF1 for 5 min at 25 °C and show negligible intracellular background fluorescence (Figure 2A). Prompt increases in cytosolic fluorescence are observed upon addition of physiologically relevant concentrations of exogenous H$_2$O$_2$ (10−100 μM, Figure 2B), as determined from scanning confocal microscopy on live samples. Control experiments performed without dye or H$_2$O$_2$ give negligible fluorescence responses.
and brightfield transmission measurements after PF1 incubation and H$_2$O$_2$ addition (Figure 2C) confirm that the cells are viable and that the probe is membrane-permeable and can respond to micromolar changes throughout the imaging experiments. These data establish that PF1 is a highly sensitive indicator of ROS at 25 °C. The correspondence of the fluorescence and brightfield images (Figure 2C) further confirm that the probe is membrane-permeable and can respond to micromolar changes in H$_2$O$_2$ concentrations within living cells. In addition, subsequent experiments show that fluorescence responses to 100 nM H$_2$O$_2$ are readily detectable in vitro.

To close, we have presented the synthesis, properties, and biological applications of PF1, a new type of probe for optical imaging of intracellular H$_2$O$_2$. This fluorescein-based reagent features excellent selectivity for H$_2$O$_2$ over competing cellular ROS, a large dynamic response range owing to its dual colorimetric/fluorometric detection mechanism, and long-wavelength visible excitation and emission profiles to minimize cell and tissue damage while avoiding interfering autofluorescence from native cellular species. Furthermore, we have demonstrated the value of this probe by measuring changes in intracellular [H$_2$O$_2$] within living mammalian cells. Current efforts are directed toward applying PF1 and related tools for studying the oxidation biology of living systems.

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Supporting Information Available: Synthetic and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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