REAGENTS FOR THIOL DETECTION

Key Inventor

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State of the Art

Thiols and disulfides are of particular interest in the field of biochemistry, playing important roles in determining protein structure (disulfide linkages) and enzymatic mechanisms (as covalent catalysts), and also determining the redox state of the cell as regulated by the thiol/disulfide statues of intracellular glutathione and protein thiols. Current thiol detection molecules include using symmetrically substituted fluorescent molecules, such as Bodipy-linker-S-S-linker-Bodipy, and also the colorimetric Ellman's reagent (a pair of dithionitrobenzoic acid molecules, linked by a dithiol). Fluorescence detection has widely been used to study cellular proteins because they have high sensitivity and a good dynamic range of detection. However, some drawbacks include photobleaching, stability, and purity of the fluorophore, which can yield unreliable results. Furthermore, there are no reagents to detect sulfides.

Invention

The present invention are fluorescent dithiol probes that could potentially be used to detect thiols inside of living cells, which could be useful since thiol levels are an indicator of disease state, and that there are currently no reagents available for intracellular detection of biologically relevant changes in thiol levels. These probes are useful for detecting any thiol, as demonstrated by the detection of glutathione and ADPβS as well as protein thiols. These probes use an asymmetric pair of molecules attached by a dithiol linker, where one molecule is a fluorescent molecule (donor [D]) and the other is an acceptor [A] or a quencher [Q]: D-S-S-A/Q. Fluorescent detection occurs when the probes react with a present thiol, in which the probes' disulfide bond which joins the donor and acceptor or quencher is broken and results in fluorescence emission changes. This invention has an advantage over the current state of the art in that the probes' acceptor or quencher half can be used to either to (1) quench fluorescence from the donor half for use in a fluorescence assay, or (2) be used as an acceptor in a FRET-based assay (Fluorescence Resonance Energy Transfer), in which the probes have two fluorescence emission bands, coming from both the donor and acceptor molecules and which overlap when the donor and acceptor molecules are tethered together in the probes.

These probes are photostable and a single isomer, which is desirable for fluorescence detection reliability and reproducibility. In addition, these probes have an unexpected and unusually low reduction potential, meaning that they are only reduced by relatively high concentrations of glutathione, and thus can be used to detect biologically relevant changes of glutathione levels within cells. While other probes exist that are more sensitive (i.e., detecting <10μM thiol), none of these probes can detect changes in the 1-20mM range. Furthermore, these probes may be useful for studying disulfides and disulfide exchange reactions as a variation of this reagent has been developed that can detect disulfides and protein thiols. Moreover, the introduction of specific acceptor molecules can also serve to transport or target the probe to specific locations within cells.

Patent Protection

A U.S. utility patent application and a subsequent U.S. provisional patent application have been filed for this invention.