

Detection and Imaging of Nitric Oxide with Novel Fluorescent Indicators: Diaminofluoresceins

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Abstract

Nitric oxide is a gaseous, free radical which plays a role as an intracellular second messenger and a diffusable intercellular messenger. To obtain direct evidence for NO functions in vivo, we have designed and synthesized diaminofluoresceins (DAFs) as novel fluorescent indicators for NO. The fluorescent chemical transformation of DAFs is based on the reactivity of the aromatic vicinal diamines with NO in the presence of dioxygen. The N-nitrosation of DAFs, yielding the highly green-fluorescent triazole form, offers the advantages of specificity, sensitivity, and a simple protocol for the direct detection of NO (detection limit 5 nM). The fluorescence quantum efficiencies are increased more than 100 times after the transformation of DAFs by NO. Fluorescence detection with visible light excitation and high sensitivity enabled the practical assay of NO production in living cells. Membrane-permeable DAF-2 diacetate (DAF-2 DA) can be used for real-time bioimaging of NO with fine temporal and spatial resolution. The dye was loaded into activated rat aortic smooth muscle cells, where the ester bonds are hydrolyzed by intracellular esterase, generating DAF-2. The fluorescence in the cells increased in a NO concentration-dependent manner.