Synopsis of Original Research Paper

Detection and Functional Analysis of Nitric Oxide on Living Cells Using Novel Fluorescence Probe

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Nitric Oxide (NO) is produced by a variety of cells and is involved in a broad array of physiological and pathophysiological processes. It is reported that NO perform in vasodilation, immunoregulation and neurotransmission. However, many proposed physiological roles of NO were not proved directly by measuring NO. One of the reasons for this is the difficulty of direct, real-time detection of this gaseous, free radical species. Although several methods of detecting NO, which is unstable and produced at low concentration, have been developed, a new method is required which is satisfactory for studies in living cells in terms of selectivity, sensitivity, and experimental feasibility. In order to obtain direct evidence for NO functions in cultured dermatological cells, I designed and synthesized fluorescent NO indicators to detect NO in living cells as a means to examine the physiological functions of NO. First, the reactivity of NO was examined in order to find a suitable reaction for selective NO trapping. I found that aromatic amines react with NO in the presence of dioxygen to produce the corresponding triazenes.

We started to investigate the design and synthesis of fluorescent compounds based originally on 2,3-diaminonaphthalene as a vicinal diamine. Based on these findings, we designed diaminofluoresceins (DAFs, Figure 1) as indicators for NO. 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). Fluorescence detection with visible light excitation and high sensitivity enabled the practical assay of NO production in living cells. These dyes were applied to NO detection from cultured macrophages, which have inducible NO synthase. The macrophages were cultured in a plate reader wells and the extracellular NO production was measured by their fluorescence intensity. These results were successfully shown to establish the basic fundamental of detectiong NO from cultured dermatological cells.