ABSTRACT

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TITLE: Smart Molecular Probes for Selective Sensing of Nitric Oxide and Nitroxyl with Cell Imaging Applications

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Nitric oxide (NO) is a free radical and also an important biomolecule among various oxides of nitrogen. It plays a keystone role in (a) the central nervous system (b) the inflammatory response, (c) modulations of ion channels, and also in (d) the cardiovascular homeostasis. It also plays an integral role in the immune system. As a result, monitoring of the concentration of NO in a biological system is crucial. Since NO can diffuse rapidly through the cells and tissues, monitoring nitric oxide induced reactions in biological systems is a challenging task. Several analytical methods like chemiluminescence, electro-chemical, fluorescence and electron spin resonance have been proposed for the *in vitro* and *in vivo* analysis of NO. Among all these approaches fluorescence technique is considered as the most favourable one because of its high sensitivity, selectivity and spatiotemporal resolution. Therefore, the design of a suitable fluorescent probe to monitor the formation and migration of NO in living cells is crucial compared to other methods. Similarly, Nitroxyl (HNO) is the protonated and one electron reduced analogue of NO which is a cousin species. HNO and NO are very closely related showing similarities in numerous chemical and biochemical properties. The most effective strategy for detecting HNO is to take advantage of its redox activity. In this research endeavor we have synthesized some organic probes for the selective and sensitive monitoring of NO/HNO without any interference from species like AA, DHA and RNOs, endogenously present in the biological system. All the probes were thoroughly characterized by using standard analytical tools and spectroscopic techniques. A simple N-nitrosation based fluorescent probe (**NDAQ**) displayed high selectivity and sensitivity towards NO in pure aqueous medium with green fluorescence emission (λ_{em}~542 nm) due to ICT effect. The low cytotoxicity, high selectivity, water solubility and excellent detection limit (7 nM) of NDAQ towards NO enhances its potentiality to acts as a good NO sensor for cellular studies. Moreover, the kinetic assay illustrates the second–order dependency w.r.to [NO] and first order with [NDAQ]. The probe NDAQ is capable of detecting exogenous and endogenous NO in A549 cells and RAW 264.7 cells, respectively. The probe **$HqEN_{480}$** recognizes NO selectively over other biological interfering species through the N-nitrosation sensing mechanism in pure aqueous medium. The least cytotoxic effect, high selectivity, good water solubility and excellent detection limit (53 nM) of **$HqEN_{480}$** towards NO enhances its potentiality to acts as a good NO sensor for cellular studies. DFT calculations were also carried out to further strengthen the N-nitrosation sensing mechanism of **$HqEN_{480}$**. Probe **CQME**, containing a dihydropyridine unit as a NO recognition site exhibits highly selective turn-on fluorescence response through the unprecedented C-C bond cleavage between the benzochromene fluorophoric unit and the 1,4 dihydropyridine unit leading to the formation of C-nitrosated product (**$PYNO_2$**) and dimethyl 2,6-dimethylpyridine-3,5-dicarboxylate (**PYMAA**) as major products, thereby eliminating any interference from various endogenous biomolecules including DHA, AA etc. The pH independency, cell permeability, large turn-on fluorescence response (30 fold) along with very low LOD (42 nM) makes it a a premier candidate for *in vivo* monitoring of NO. A **Cu(II) based sensor (1)** was developed by complexation between (quinolin-8-ylamino)-acetic acid hydrazide (L^2) and Cu^{2+} ions for highly sensitive and selective recognition of HNO and S^{2-} over other biologically abundant anions with prominent enhancement in absorption and emission intensities. DFT calculations were also employed to gain insight into the coordination mode of L^2 towards Cu^{2+} ion and to know how the sensing property of L^2 changes on binding with metal.

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