3rd International Conference on Scientific and Academic Research
December 25-26, 2023 : Konya, Turkey
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The N-nitrosation approach from fluorescence nitric oxide recognition mechanisms

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Abstract – ROS (Reactive oxygen species) represent free molecules and radicals (the species containing a single unpaired electron) obtained from molecular oxygen. Broadly speaking, ROS include hydrogen peroxide and lipid peroxide without unpaired electrons, and on the other hand, peroxyl ($ROO^{\bullet-}$), superoxide ($^{\bullet}O_2^-$), hydroxyl ($^{\bullet}OH^-$), nitric oxide ($^{\bullet}NO$) radicals, nitrogen dioxide ($^{\bullet}NO_2$), peroxynitrite ($^{\bullet}ONOO^-$), alkoxy ($RO^{\bullet}$) radicals. The ROS are the outcomes of aerobic life. Living things constantly produce, transform, and consume ROS as long as they survive. The general opinion about ROS is that aging occurs with the weakening of organs with the oxidative stress they cause and the damage it causes. The $NO^{\bullet}$, a short-lived and a liberate radical molecule, manages important mechanisms in biological processes. This radical can be carcinogenic with free amines, creating acid rain and potential NO species (NO_2, N_2O_3, N_2O_4). Additionally, NO can cause hypertension and genetic disorders due to DNA mutation. Furthermore, it causes basic biological system diseases. Briefly, it is important to investigate NO in biological processes and to find simple and effective solutions for NO determination. In recent years, fluorescence spectroscopy has attracted attention due to its features such as being suitable for the determination of many species, having high selectivity and sensitivity, and cheap and easy to use. Many different mechanisms are proposed in fluorescence studies for NO. These mechanisms range from the benzotriazoles from the o-phenyldiamine group to N-nitrosation and deamination mechanisms. Within the scope of this study, the N-nitrosation mechanism was evaluated and discussed.

Keywords – Reactive Oxygen Species, Fluorescence Method, Nitric Oxide (NO), Detection Mechanisms, N-Nitrosation

I. INTRODUCTION

Free radicals derived from oxygen (hydroxyl radical, superoxide, nitric oxide) and non-radical oxygen species (hydrogen peroxide, singlet oxygen, hypochlorite, peroxynitrite) are famous as Reactive Oxygen Species (ROS) under the common name. ROS could be both unhealthy and helpful to the body. An imbalance in the formation of liberate radicals could cause oxidative stress, which in turn brings about aging and age-linked sicknesses such as neurodegenerative disorders, cardiovascular diseases, cancer, and other chronic conditions [1].

The production area of ROS and Reactive nitrogen species (RNS) is mitochondria. The reduced formation of these radicals provides benefits by playing an active role in messaging between tissues and homeostasis [2], [3].

Nitric oxide, one of these reactive species, reacts rapidly with other radicals or metalloproteins in organisms. As a result, NO plays critical roles in various processes. In addition, this substance is a colorless, heteronuclear diatomic, paramagnetic and highly reactive gas molecule. In mammals, nitric oxide is involved as a signaling molecule in many physiological and pathological pathways. Production of NO at levels above normal causes cancer and neurodegenerative-related diseases.

Therefore, attempts to develop sensitive and selective methods to analyze NO production and distribution in living cells are very valuable [4].

Among several determination techniques for NO, the fluorescence strategy, which combines the use of a fluorescence microscopy, is a potent device to detect and image intracellular NO production due to its high sensitivity, selectivity and experimental feasibility [5]. Generally, when studies on the determination of Nitric Oxide (NO) are examined, it is shown that fluorimetric determinations of NO proceed via dissimilar mechanisms. In order to better understand the origins, activities, and physiological and pathological functions of the NO agent, it is very significant to improve methods and tools that can be used to detect NO in real time. These mechanisms are basically specific reactions of the NO communication tool with the o-phenylenediamine (OPD) moiety (Nagano research group), metal-ligand complexes (Lippard research group) and other substances. Specific reactions with other substances include nitrosation reaction, formation of diazocyclic compounds from o-amino-3'-dimethylaminophenyl (ADF) aromatics, Se-NO bond formation, N-nitrosation, deamination reaction and aromatization of Hantzsch dihydropyridines and others [6], [7]. Fluorescent probes for NO commonly consist of a trapping (binding) site and an organic fluorophore [8]. So in general, fluorescent probes for NO are improved by coupling an electron riched *o*-diamino-phenyl group with various fluorophores. Because it can effectively quench the fluorescence of fluorophores both by a photo-induced electron transfer (PET) mechanism and react with NO (NO/O_2) under aerobic conditions to turn on the fluorescence of fluorophores and form the corresponding benzotriazole derivatives [5]. Among these, fluorescent probes based on OPD are by far the most versatile for detecting NO [6], [9]. In this study, the N-nitrosation mechanism was examined.

II. Materials and Method

The N-nitrosation mechanism was examined and discussed through publications in the literature.

III. Results and Discussion

In general, compounds containing secondary amine units undergo N-nitrosation reactions under oxygenic conditions and in the presence of NO. Therefore, in this mechanism, the NO sensitive part is the secondary amine group. The fluorescence of the probe related to the binding of NO to nitrogen is stimulated, resulting in a turn-on response. [10].

In a study with a BODIPY-based probe, the probe responded to turn-on via N-nitrosation of the secondary amine under NO/O_2 conditions. [10]. (Fig.1).

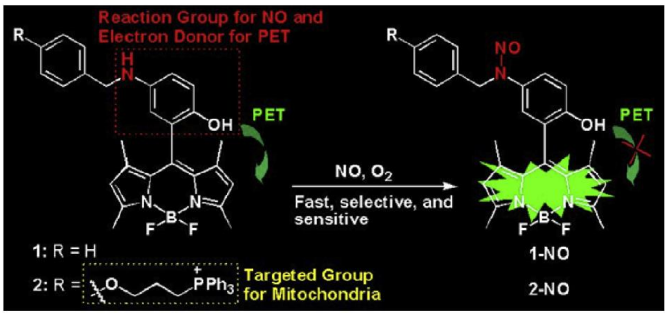


Fig. 1

In a study conducted with methyl-amino substituted Silicon-Rhodamine (SiR) derivative, NO was identified in cells by near infrared (NIR) fluorescence as a result of N-nitrosation of methylamino in the presence of NO/O2.individual interpretations in isolation are particularly discouraged. [7]. (Fig. 2).

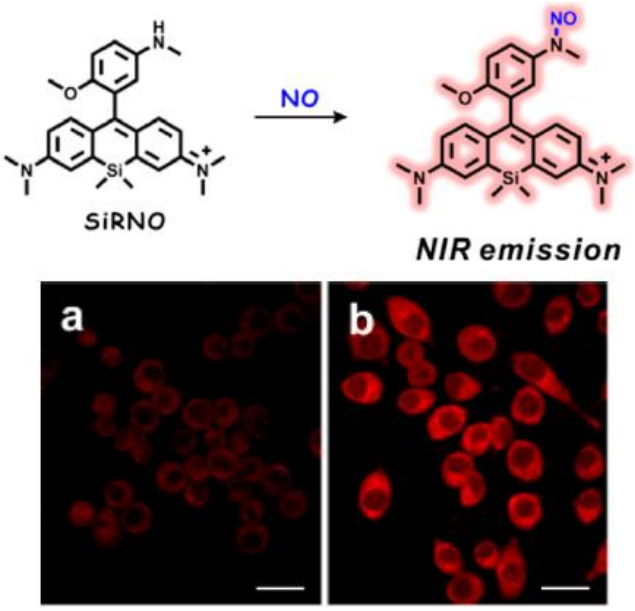


Fig. 2

In the study using the cyanine dye-based probe, NIR fluorescence response was reported with the formation of N-nitrosa by N-nitrosation of the methyl amino group in the meso position of the relevant dye structure with NO [11]. (Fig. 3).

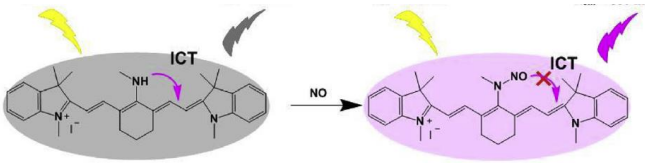
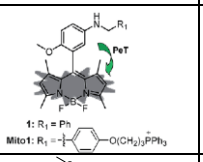
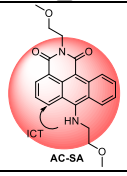
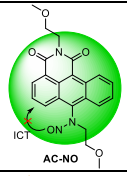
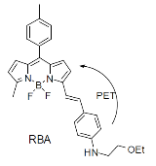
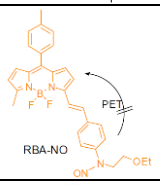
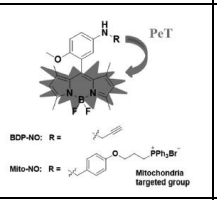
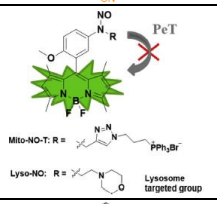
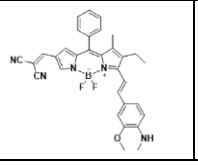
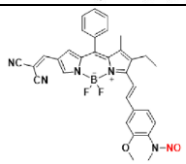


Fig. 3

Different studies on the N-nitrosation mechanism for the detection of NO are summarized below. (Table 1).

Table 1. Examples of Probes with N-nitrosation mechanism

Probe	Probe-NO	Reference
		[12]
		[13]
		[14]
		[15]
		[16]

IV. CONCLUSION

To sum up, since developing nitric oxide sensors is very important for biological processes, finding the most appropriate and accurate method for nitric oxide determination has become very critical. The most potential method for nitric oxide determination is the use of fluorescent probes. The basic mechanism in nitric oxide recognition is that recognition groups attached to fluorophores identify NO. Of the many mechanisms reported for the recognition of nitric oxide, N-nitrosation has been known as one of the most suitable methods. Therefore, the N-nitrosation mechanism was discussed and examined in this study. The study presented here could contribute insight into the design and advancement of many fluorescent probes for the monitoring of nitric oxide with this mechanism.

ACKNOWLEDGMENT

The examination work detailed here was supplemented by TÜBİTAK (Project numbered 120Z914).

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