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FLUORESCENCE-BASED SENSORS FOR THE DETECTION OF BIOLOGICALLY AND ENVIRONMENTALLY RELEVANT MOLECULES

Silvia Mirra

TUTOR
Prof. Claudio Pellecchia

CO-TUTORS
Dott.ssa Mina Mazzeo
Prof. Gerard Canters

COORDINATORE
Prof. Gaetano Guerra

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Abstract

Hydrogen sulfide (H_2S) has been known for long time as a toxic molecule in biological systems. More recent studies have shown that mammals can produce H_2S in a controlled fashion, suggesting that it's important in maintaining normal physiology. In particular, recent efforts are mostly devoted to implement sensitive and selective detection techniques to monitor the distribution and function of this molecule in complicated biological systems. Depending on the mechanism by which the recognition event occurs there are three main approaches in the literature. 1) Azide-to-amine reduction, 2) Nucleophilic addition and 3) Copper displacement. The idea behind this doctoral thesis project is based on a different approach: a coordinative mechanism. In this way one may, in principle, be able to remove H_2S from the metal center of the sensor and ensure a reversible H_2S binding process. This would be advantageous for practical sensing applications allowing reusability of the sensing device.

Knowing that vitamin B12 is involved in the H_2S transport, was decided to use vitamin's models: cobaloximes. These complexes are water soluble and easily synthesized. Both these conditions are highly desirable when implementing a sensing device. ^{1}H-NMR and UV-Vis studies suggested that the H_2S coordination to the cobalt center occurs through the displacement of the pyridine fragment. The complexes have also proven to be selective towards H_2S. No UV-Vis response was observed in the presence of physiological thiols, such as cysteine and glutathione, and of a series of inorganic anions.

It is well known that H_2S can bind to heme proteins, inducing different responses that in turn modulate its cytotoxic and cytoprotective activities. To improve an H_2S sensors I focused on designing a simple copper porphyrin complex (Copper(II) Protoporphyrin IX), which I reasoned could work by a coordinative-based approach. The probe can selectively and sensitively detect HS^- anions in water over other anions, biothiols, and common oxidants such as H_2O_2. ^{1}H NMR and ESI-MS experiments provide clear evidence that the turn-on response in the presence of H_2S is ascribed to binding of the target analyte to the copper center.

Exploiting the previous experience on the copper complexes as H_2S sensors, I decided to focus my attention on the Azurin, a copper protein expressed in a very easy way and very stable in nature. Atto620 labelled azurin wild type showed a good fluorescence response but has not shown selectivity and the system was not reversible. The best results has been obtained with Cobalt-azurin-Alexa 350. This system, nevertheless working by turn-off mechanism, showed a good fluorescence response when adding KSH, a dependence of the fluorescence intensity on the KSH concentration, a detection limit in micromolar range and a good selectivity.

In the last years have been reported few H_2S fluorescent sensor developed on the method of copper sulfide precipitation utilizing dyes-functionalized cyclam-copper(II) complexes. Despite showing good sensibility and excellent selectivity, this sensors work as simple chemodosimeter and are not reusable, since the recognition process is not reversible. To achieve a coordinative approach, I designed a cyclam-based fluorescent sensor in which a dimethylpyridine moiety could allow a further coordination and a greater steric hindrance to the copper ion. Moreover, a pendant amide-pyrene moiety which acts as a fluorophore, was chosen to avoid the coordination of bigger analytes than H_2S. This sensor showed a 4.5-fold fluorescence enhancement with 10 equivalents of KSH in methanol. The probe can highly selectively detect H_2S even in the presence of millimolar concentrations of glutathione. To gain insights in the mechanism of recognition of H_2S ESI-MS and mono-dimensional NMR experiments were performed.