Sensing nitric oxide with a fluorescent protein

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Abstract

Real-time quantitative visualization of nitric oxide (NO) concentration at the single cell level is fundamental to achieve a direct and accurate determination of NO dynamics. To this aim, development of genetically encoded fluorescent sensors (GES) is of fundamental relevance. The field is still poorly explored and only a few GES have been so far developed and proposed for monitoring intracellular NO levels (1).

The recent finding that the blue-emitting fluorescent protein mTagBFP2 is sensitive to NO in the micromolar range suggested new development strategies (2). Protein mutants and mass spectrometry demonstrated that S-nitrosylation of Cys residues is at the basis of the observed reduction in emission intensity and lifetime in response to NO exposure. The potential of this GES for monitoring intracellular NO was shown on HeLa cells transiently expressing mTagBFP2.

In this work, we present a more accurate study on the dynamics of S-nitrosylation of Cys residues in mTagBFP2. S-nitrosylation of mTagBFP2 was induced by the NO donor MAHMA nonoate and the changes in fluorescence intensity and lifetime were monitored as a function of time. By following the process on long time scales (ca. 1h), it was possible to show that when the reaction reaches equilibrium, changes in fluorescence intensity and lifetime are larger than previously thought. From the overall change, it was possible to obtain a better estimate of the Kd for the reaction. Similar results were obtained when experiments were conducted under nitrogen atmosphere or in air□equilibrated samples.

We have also explored the response of mTagBFP2 to the NO donors S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP). Interestingly, while results with GSNO were quite similar, except for a slower kinetics, to those obtained with MAHMA nonoate, no S-nitrosylation was observed in the presence of SNAP. Experiments on mTagBFP2 mutants containing a single Cys residue show that two of the three Cys residues in mTagBFP2 are responsible for the changes in fluorescence emission. Further experiments on mammalian cells, transiently expressing mTagBFP2 and its mutants, are underway.

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