Biological ROS and RNS Detection
Part III. Nitric Oxide

Human skin contains photolabile nitric oxide (NO) derivatives which upon UVA radiation decompose under high-output NO formation and exert NO-specific biological responses such as increased local blood flow or reduced blood pressure. The intradermal increase of free NO due to blue light irradiation of human skin can be monitored and quantified using the EMXnano.

Introduction

Nitric oxide (NO) is a highly reactive regulatory molecule which has many important physiological roles, such as a neurotransmitter in the central nervous system, a regulator of vasomotor tone in the cardiovascular system, and a cytotoxic mediator of the immune system.

Challenge

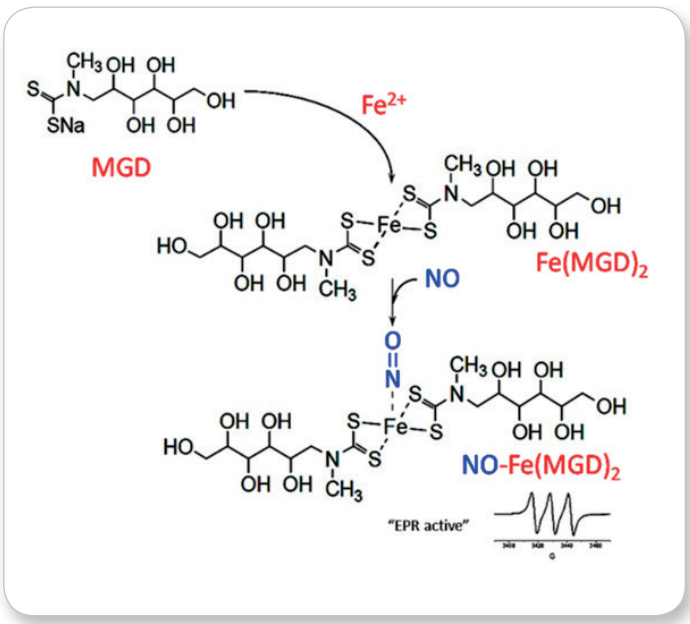
NO is a free radical with a very short half-life (< 30 sec), and this makes direct measurement difficult in most cases.

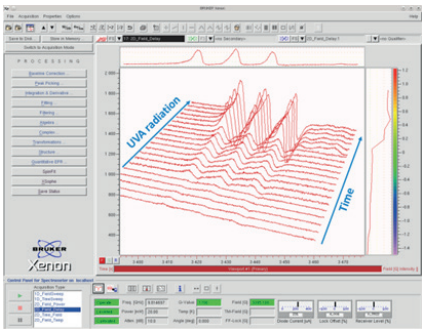
Solution

The instability of NO can be overcome by using a NO-trapping technique, in which a more stable complex is formed and subsequently detected by EPR.

Equipment

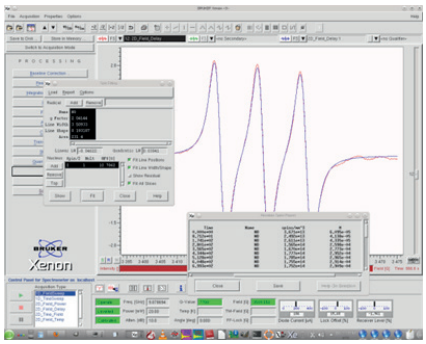
The EMXnano provides the means to detect NO by both room temperature and low temperature techniques with high sensitivity. With accessories for room temperature and low temperature measurements, detection of NO with MGD or hemoglobin as a spin trap is easy to achieve.

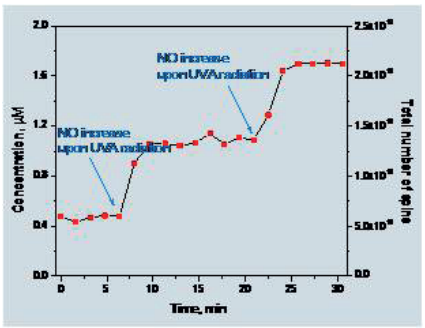




- Experimental data (in red) of the $(MGD)_2$-Fe(II)-NO complex as a function of time in the 2D Field versus Time experiment.

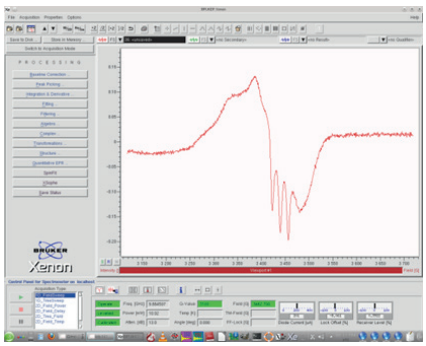
- For spin adducts not in the spin-trap library, you can manually input the simulation parameters to create your own library.
- Simulating the spectrum of the NO-$(MGD)_2$-Fe(II) spin adduct using SpinFit by entry of the parameters in the dialog box.





- SpinCount provides a report showing the time evolution of the concentration of the radical adducts.
- The report can be saved as an ASCII file for further evaluation.

- Nitric oxide (NO) plays a central role in a multitude of diseases such as intravascular hemolysis and hemolytic anemias. The reaction of NO scavenging by Hb is recognized to play a major role in several of these diseases.
- Trapping of the HbNO complex with the finger dewar or VT accessories permit the direct detection of NO-hemoglobin complex.



Key Features include:

- Easy-to-use software
- 2D EPR spin trapping experiments showing the trapped nitric oxide
- Spin trap library – a wide database collection of previously simulated spectra for many trapped radicals
- SpinFit module to simulate the spectra of $(MGD)_2$-Fe(II)-NO complex
- SpinCount module to quantify the total number of spins and to determine the radical concentration

References for further reading

1. Opländer C., Deck A., Volkmar C.M., Kirsch M., Liebmann J., Born M., van Abeelen F., van Faassen E.E., Kröncke K., Windolf J., Suschek C.V. Mechanism and biological relevance of blue-light (420-453 nm)-induced nonenzymatic nitric oxide generation from photolabile nitric oxide derivatives in human skin in vitro and in vivo, Free Rad. Biol. Med. (2013) 65 1363
2. Hawkins C.L., Davies M.J. Detection and characterisation of radicals in biological materials using EPR methodology, Biochim. Biophys. Acta (2014) 1840(2) 708
3. Li H., Hemann C., Abdelghany T.M., El-Mahdy M.A., and Zweier J.L. Characterization of the mechanism and magnitude of cytoglobin-mediated nitrite reduction and nitric oxide generation under anaerobic conditions, J. Biol. Chem. (2012) 287(43) 36623

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