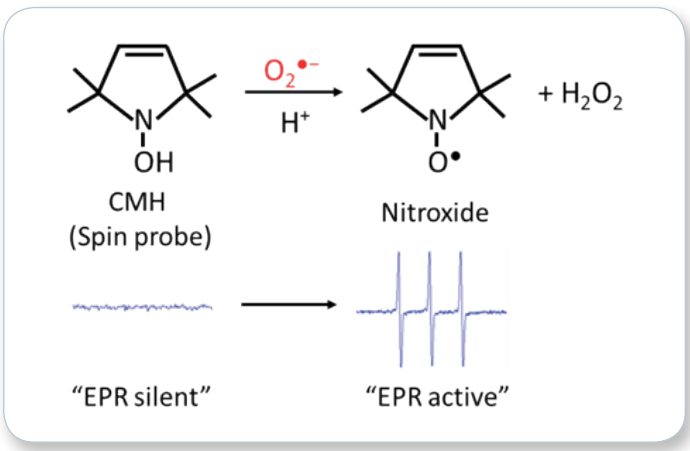
Biological ROS and RNS
Part II. EPR Spin Probes

In vascular cells, increased generation of superoxide ($O_2^{\cdot-}$) has been suggested to occur in hypertension, diabetes, and heart failure. Thus the accurate detection and ability to quantify $O_2^{\cdot-}$ are critically important in understanding the pathogenesis of these various cardiovascular disorders and other non-cardiovascular diseases. As shown here the generation of superoxide over time can be easily monitored with the EMXnano.

Introduction

The major reason to measure Reactive Oxygen Species (ROS) in biological systems is to determine whether they play a role in physiological or pathophysiological processes. Most of the biologically relevant radicals are very short lived and, therefore, impossible to detect in biological samples. For this reason, compounds (spin traps and spin probes) have been used that form stable adducts with radicals. Unfortunately, quite often the spin traps have low reactivity with ROS and the formed radical adducts are very susceptible to bio-reduction when exposed to cells or tissues that converts them to EPR silent species.



Challenge

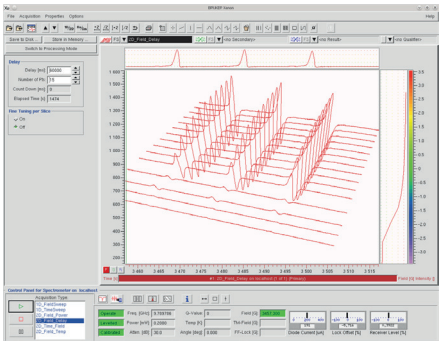
Direct detection of ROS and RNS is very difficult or impossible in solution at room temperature due to their very short half-life.

Solution

Spin probes are not spin traps, in that they do not "trap" radicals, but they are oxidized to form nitroxides (free radicals) with a half-life of several hours, which can readily be detected by EPR. For example, the cyclic hydroxylamine 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine (CMH) can provide quantitative measurements of superoxide ($O_2^{\bullet-}$) with high sensitivity and it has been used for detection of intracellular $O_2^{\bullet-}$ in cultured cells and tissue samples:

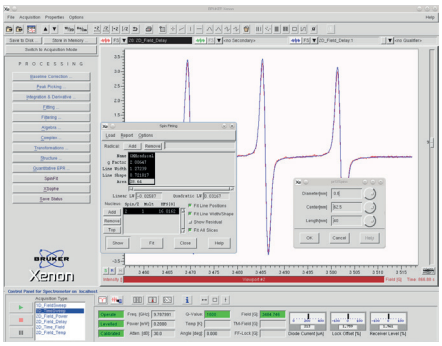
Equipment

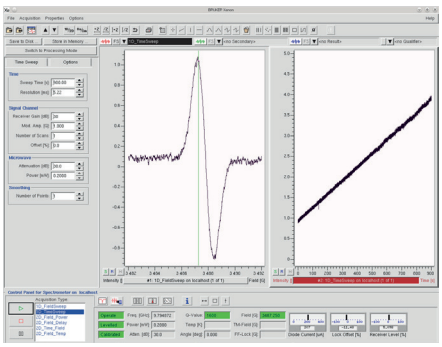
EMXnano can be used effectively for mechanistic studies and kinetic analysis of multiple radicals (ROS and RNS) generated in enzyme reactions. Properly controlled spin probe experiments can verify that the formation of radical adducts is due to free radical production in the reaction system being studied.



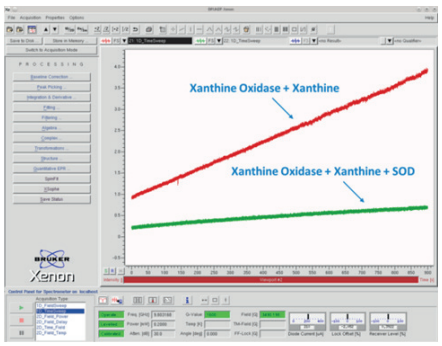
- Formation of CM● nitroxide as a function of time in the 2D Field versus Time experiment due to the reaction:
  CMH + O_2●– ● CM + H_2O_2

- SpinFit and SpinCount modules provide ROS concentration for quantitative EPR analysis at any given time point during the reaction.





- The 1D time sweep experiment is useful for monitoring the kinetics of CM● formation via changes in intensity with time at one particular field value (the top of the line).



- Addition of xanthine and xanthine oxidase to CMH leads to a steady rise in EPR peak-to-peak amplitude of the nitroxide radical CM●.
- Detection of superoxide radical (O_2•⁻) is confirmed by suppression of the EPR signal by superoxide dismutase (SOD).

- The rate of superoxide formation by xanthine oxidase can be obtained from a linear fit of the kinetic trace and presented as nM O_2•⁻ /min.

Key Features include:

- Easy-to-use software (Xenon)
- 2D Field vs Time experiments showing the oxidation of the spin probe due to its reaction with free radicals
- SpinFit module to simulate the nitroxide formation
- SpinCount module to quantify the total number of spins and to determine the nitroxide concentration
- 1D Time sweep experiment to collect kinetics traces which can be fitted.

References for further reading

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2. Mrakic-Sposta S., Gussoni M., Montorsi M., Porcelli S., Vezzoli A. Assessment of a standardized ROS production profile in humans by Electron Paramagnetic Resonance, Oxid Med Cell Longev. (2012) Article ID 973927
3. Xiao D., Powolny A.A., Moura M.B., Kelley E.E., Bommareddy A., Kim S., Hahm E., Normolle D., Van Houten B., and Singh S.V. Phenethyl isothiocyanate inhibits oxidative phosphorylation to trigger Reactive Oxygen Species-mediated death of human prostate cancer cells, J. Biol. Chem. (2010) 285(30) 26558

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