Nitric Oxide spin traps for Dynamic Nuclear Polarization.

T. Guiberteau, L. Marx*, A. Rassat* and D. Grucker.
Institut de Physique Biologique, URA du C.N.R.S. n° 1173, 4, rue Kirschléger 67085 Strasbourg Cedex.
* Ecole Normale Supérieure de Chimie, URA du C.N.R.S. n° 1110, 24, rue L'homond, 75231 Paris Cedex 05.

Introduction

Nitric oxide (NO) is a hydrophobic, paramagnetic gas that has been shown to be synthesized in biological systems. NO can act as a biological mediator in the regulation of blood pressure and as a neurotransmitter. Despite its paramagnetism, NO is not directly detectable by Electron Paramagnetic Resonance (EPR), the most accurate technique to study free radicals. However the combination of spin trapping techniques and EPR allows to detect indirectly the production of nitric oxide. Dynamic Nuclear Polarization (DNP) is a method that can detect free radicals indirectly by measuring the intensity of the nuclear magnetic resonance signal of protons when an EPR transition of a free radical is saturated. The NMR signal intensity can be enhanced, depending on the coupling between protons and the free radicals. The aim of this work is to present nitric oxide spin traps suitable for DNP experiments. Two kinds of spin traps were studied: the first is a water-soluble iron complex $(MGD)_2Fe^{2+}$, a conventional spin trap used in nitric oxide EPR experiments, and the second is a new biradical (TMP) with two nitroxide functions (Fig. 1).

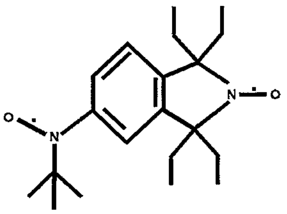

Fig.1: structure of the biradical TMP

Material and Methods

The apparatus used for the DNP experiments were described elsewhere (1). Field cycled dynamic nuclear polarization (2) (FCDNP) experiments were performed in a magnetic field B_0 which was kept constant at 68 Gauss during the detection of the NMR signal (289 kHz), but switched to a different value , B_0', for the duration of the EPR irradiation at 198 MHz. The enhancement of the NMR signal is characterized by the DNP factor calculated from (I-Io)/Io where I and Io are the intensities of the proton NMR signal with and without EPR saturation respectively.

N-methyl-D-glucamine dithiocarbamate (MGD) was synthesized by following the method of Shinobu et al (3). The spin trap was then obtained by mixing MGD (75 mM) and $FeSO_4$ (15 mM) in 20 ml of water. The synthesis of the biradical TMP (Fig. 1) will be published elsewhere. The nitric oxide donor S-nitroso-N-acetylpenicillamine (SNAP) was synthesized from N-acetyl-d,l-penicillamine according to the procedure described by Field et al (4). SNAP was then introduced in excess (30 mg) into the spin trap solutions.

Results and discussion

Fig 2 presents the DNP spectrum of the $(MGD)_2Fe^{2+}$-NO complex. The spectrum exhibits 3 lines with a hyperfine coupling of about 13 Gauss. the X band EPR spectrum of this complex shows a 3.5 Gauss linewidth. In DNP experiments, the signal depends the linewidth of the free radical. In fact, the larger the linewidth (of the free radical), the more difficult it is to saturate the EPR transition, and therefore to detect a DNP signal. This explains the weak DNP factor, about -0.7, observed in experiments involving the $(MGD)_2Fe^{2+}$-NO complex.

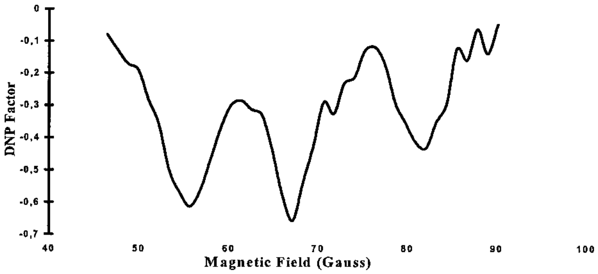

Fig.2: DNP spectrum of the $(MGD)_2Fe^{2+}$-NO complex. DNP parameters are : 1 sec EPR irradiation at 198 MHz (30W), 20 accumulations, 40 points.

Fig 3 presents the DNP spectrum of the free radical resulting from the reaction between TMP and the NO donor. TMP (1 mM) was first dissolved in 20 ml ethanol/water solution (20/80 in vol.). The X band EPR spectrum of the biradical presents a broad line (about 25 Gauss) with unresolved hyperfine splitting. No DNP signal was obtained with the biradical in solution because of the difficulty to achieve the saturation of the EPR transitions. When SNAP is added to the solution, the aromatic nitroxide function of TMP reacts with NO. Then the biradical becomes a single free radical of the nitroxide type, displaying a 3-line EPR spectrum with a hyperfine coupling of about 15 Gauss due to the coupling between the unpaired electron and the nitrogen nucleus of spin 1. The DNP factor is about -2.5. The linewidth of the single radical in solution is about 1.5 Gauss.

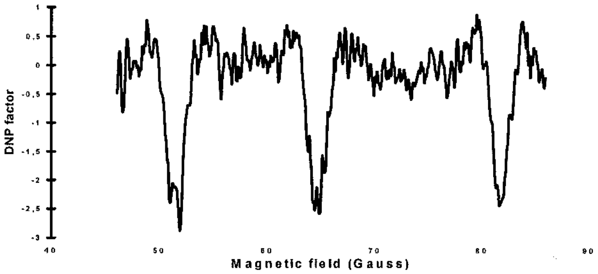

Fig. 3: DNP Spectrum of the nitroxide resulting from the reaction between TMP and NO. DNP parameters are: 1 sec EPR irradiation at 198 MHz (30W), 20 accumulations, 400 points.

Conclusion

This work shows nitric oxide can be detected by combining the spin trapping technique and Dynamic Nuclear Polarization spectroscopy. The spin trap of the iron-complex type is not suitable for DNP experiments because of the broad EPR lines of the free radical. The biradical TMP seems to be the most powerful spin trap to study NO by dynamic nuclear polarization, firstly because it yields no DNP signal when there is no nitric oxide in the solution but yields a nitroxide signal when it reacts with nitric oxide and secondly because it yields a larger DNP factor than $(MGD)_2Fe^{2+}$.

Acknowledgments

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References

(1) T. Guiberteau et al., *J. Magn. Reson.* Ser. B **110**, 47-54 (1996).
(2) D.J. Lurie et al. *J. Magn. Reson.*, **76**, 366 (1988).
(3) L.A. Shinobu et al., *Acta pharmacol. et toxicol.* **54**, 189 (1984)
(4) L. Field, *J. Chem. Soc. Chem. Commun.*, 249 (1978)