ESR SPIN TRAPPING OF THE REACTION PRODUCTS OF URATE AND PEROXYNITRITE

A. Angerhofer (UF, Chemistry), W. Imaram (UF, Chemistry), C. Gersch (UF, Nephrology), R. Johnson (UF, Nephrology)

Introduction

Uric acid is a metabolic product of purines and is present in most mammals in low concentrations (1 mg/dL). Studies have found that an elevated serum uric acid level is an independent risk factor for predicting whether or not a person will develop hypertension, cardiovascular disease and renal disease [1]. This raises the possibility that uric acid may contribute to the progression of these diseases, which are characterized by a loss of endothelial function. Experimental evidence could provide a mechanism by which uric acid, when reacted with peroxynitrite, contributes to the development of these diseases via the generation of a pro-oxidative free radical species capable of inflicting endothelial damage thus causing endothelial dysfunction. In search of a possible mechanism by which elevated serum uric acid levels can trigger the proliferation of vascular smooth muscle cells we have revisited an earlier study on the reaction between peroxynitrite and uric acid *in vitro* using ESR spin trapping methods [2].

Experimental

ESR spectra were recorded at room temperature with a Bruker Elexsys E580 spectrometer using quartz capillaries of 1 mm ID. 20 mW microwave power was used at 9.87 GHz with 1 G modulation amplitude and 100 kHz modulation frequency.

Results and Discussion

The reaction between urate and peroxynitrite in 0.3 M phosphate buffer at pH7.4 causes a trapped radical with the spin traps DMPO, PBN, POBN, and DEPMPO (see fig. 1 for PBN). In all cases a sextet is observed. The hyperfine coupling constants are a(H) = 23.0 G, a(N) = 16.15 G for DMPO, and a(H) = 4.16 G, a(N) = 15.7 G for PBN. The concentration of the trapped radical after completion of the reaction was determined by comparison with a standard TEMPOL solution to be of the order of 1μM (starting conditions: 0.70 mM urate and 1.8 mM peroxynitrite). The observed hyperfine couplings indicate a carbon-centered radical in the reaction mixture. The trapped radical can be extracted using methylenechloride or benzene. According to the NIEHS spin trap database, the radical has an oxygen atom bound in β position (*e.g.*, a $CH_2OH^{\bullet}$ or $CO_2^{-\bullet}$ radical). However, $CO_2^{-\bullet}$ is not consistent with the splitting of 14.9 G of the PBN-adduct in benzene. It is also not consistent with the high a(H) in the DMPO adduct (assuming that PBN and DMPO trap the same radical). Santos *et al.* [2] found an aminocarbonyl radical adduct to DMPO in a similar reaction system which yielded hyperfine coupling constants of a(H) = 19.5 G and a(N) = 15.6 G in aqueous environment. These values are well outside our error margins (approx. ±0.2 G). The aminocarbonyl-PBN adduct is expected to show an a(H) = 3.2 G which is too small for our experimental data. This radical could have implications in the mechanisms of the onset of hypertension and kidney disease.

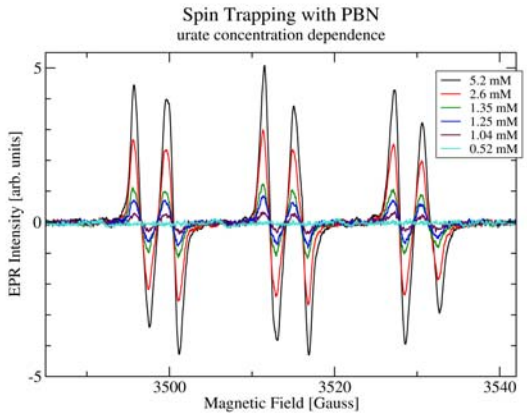

Figure 1: ESR spectrum of the urate-peroxynitrite reaction using PBN as the spin trap.

Acknowledgements

This work was supported by the University of Florida (DSR). WI thanks the Thai government for his stipend.

References

[1] R. J. Johnson, *et al.*, Hypertension **41**, 1183-1190 (2003).
[2] C. X. C. Santos, *et al.*, Archives of Biochemistry and Biophysics **372**, 285-294 (1999).