

## Characterization of electrochemically modified proteins by use of NMR: nitration of myoglobin

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Electrosynthesis as a means for the selective modification of residues in proteins and other bioactive molecules is a relatively unexplored methodology, but is one that offers the production of novel proteins <sup>(1, 2)</sup>, and which could lead to a better understanding of protein behaviour *per se* and particularly the processes involved in oxidative stress.

Myoglobin, used as model protein, has been smoothly electro-oxidatively nitrated at both platinum and BDD electrodes in the presence of sodium nitrite as source of the nitro-species. Nitration occurs site-specifically at tyrosine residues and has been probed by mass spectrometry. A benefit of electrosynthetic methodology is that control of electrolysis parameters offers advantages not easily attained by use of, for example, chemical reagents in protein modification. Here we report a study on the effects of reaction conditions, in particular pH, applied potential, nitrite concentration and electrode pre-treatment. Distinct nitrated products obtained at different electrode surfaces have been compared in respect of their protein activity.

Additionally, we investigate more generally the production of electrosynthetically nitrated tyrosine residues in myoglobin at BDD electrodes, correlate enzymatic function and structure by enzymatic assay and nuclear magnetic resonance NMR studies respectively. Comparison of 1D and 2D dimensional NMR, TOCSY, NOESY, and HSQC experiments together with the effects of temperature for samples of the native and the differently-nitrated myoglobin will be discussed. Finally, molecular dynamics calculations will be used to determine the 3D-structural changes between the native and the nitrated protein.

<sup>1</sup> D. Matters, H.J. Cooper, L. McDonnell, J. Iniesta, J. Heptinstall, P. Derrick, D. Walton, I. Peterson, *Analytical Biochemistry* (356) **2006**, 171-181

<sup>2</sup> J. Iniesta, H.J. Cooper, A.G. Marshall, J. Heptinstall, J. Walton, and I.R. Peterson, *Archives Biophysics and Biochemistry*, *impressed*.

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