**Modification of Tyrosine Residues in lysozyme by Electrochemical, Sonochemical and Sonoelectrochemical Nitration**

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INTRODUCTION AND OBJECTIVES

The electrochemical and sonochemical modification, separately or combined in sonoelectrochemistry, of residues in proteins and other bioactive molecules offers the production of novel proteins, enzymes and other bioactive species, in comparison with traditional methodologies such as protein engineering and the use of chemical reagents. The aim of this presentation is to investigate the production of electrochemically nitroylated tyrosine residues in proteins, and to manipulate the selectivity and effectiveness of the modification either by irradiation with ultrasound during the electrolysis or by exposure of the aqueous protein solution to the ultrasonic field without electrolysis. The results have important consequences for the labelling of proteins, specific immobilisation, production of novel modified proteins for pathophysiology in diseases involving oxidative dysfunction, use in biosensors and microcapsulation of proteins in polymeric microspheres.

**ELECTROCHEMICAL MODIFICATION OF LYSOZYME HEWL**

Cyclic voltammetry for the electrochemical behaviour of 50 mM di-sodium tetraborate, pH 9.4 (aq.) in the presence of lysozyme (1 mg mL⁻¹, 0 h) at the presence of 10 mM sodium nitrite. Onset of scan at –0.3 V vs SCE. Potential limits between –1.0 and 0.9 V. Scan rate: 0.050 V.s⁻¹. Limit of detection 10 ppm.

**EXPERIMENTAL SET-UP FOR ELECTROCHEMICAL, SONOELECTROCHEMICAL AND SonoCHEMICAL PREPARATIVE REACTIONS**

Working: Basket Pt (electrode) Reference: SCE electrode. Abs at 420 nm = 0.20 for nitration of lysozyme.

**CONVENTIONAL PROCEDURES FOR PROTEIN MODIFICATION**

**DRAWBACKS**

- Protein Engineering
  - Requires expensive facilities
  - Sophisticated methodology
  - Restricted to naturally occurring amino acids.
- Sonoelectrochemistry
  - Could produce hazardous modified species in the environment.

Sonoelectrochemical modification of lysozyme were performed using an ultrasonic cleaning bath (40 kHz, 180 w power output). Prior to the insonation of lysozyme, the cleaning bath is kept at 5 °C and protein solution equilibrated for 2 min at the same temperature. Ultrasound field is applied through the glass cell for intervals of 5 min each in order to keep the temperature below 12 °C. Tetraborate buffer solutions were de-oxygenated by bubbling argon before starting experiments.

**IDENTIFICATION OF NITRATED LYSOZYME BY ELISA**

(a) An effective procedure has been developed for selective electrochemical nitration of tyrosine in proteins such as lysozyme and myoglobin.

(b) Appropriate electrochemical parameters and selectivity allow retention of activity of the enzyme with consequent labelling, immobilisation and biosensors.

(c) Nitrated at low levels can be achieved sonoechemically in lysozyme but with consequent loss of enzymatic activity reflecting a change in conformation although this does not lead to availability of nitrotyrosine for antibody binding in an ELISA assay.

**CONCLUSIONS**

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