STRUCTURAL STUDIES BY NMR OF SELECTIVELY ELECTROCHEMICAL MODIFICATION OF PROTEINS: TYROSINE NITRATION OF HEN EGG WHITE LYSOZYME

Mª Deseada Esclapez-Vicente, Jesús Iniesta, Verónica Sáez, Frutos C. Marhuenda, Encarnación Martínez, A. Donaire and Mario Piccioli

The electrochemical modification 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 results have important consequences for the labelling of proteins, specific immobilisation, production of novel modified proteins for pathophysiology in diseases involving oxidative dysfunction, and use in biosensors.

Recently, the relatively robust, stable protein hen egg white lysozyme (HEWL) was exposed in mildly-alkaline aqueous buffer solutions (50 mM disodium tetraborate, pH 9.0) in the presence of sodium nitrite, and electrochemical nitration methods was carried out using a platinum electrode. Much better results in terms of activity retention of HEWL have been achieved when a carbonaceous working electrode like boron doped diamond (BDD) for the different nitrated lysozymes. For both, platinum and BDD electrodes, mass spectrometry showed that electrochemical nitration of HEWL was selective for tyrosine 23 in the initial phase of reaction, followed by bisnitration at tyrosine 20 and 23 at longer times.

The aim of this communication is to investigate the production of electrosynthetically nitrated tyrosine residues in proteins at BDD electrodes, using lysozyme as a model protein, and to correlate enzymatic function and structure by enzymatic assay and NMR studies respectively. Comparison of one-dimensional NMR, TOCSY and NOESY experiments together with DOSY measurements of the native and the different nitrated lysozymes will be shown in this communication. An attempt for determining 3D-structure changes between the native and the nitrated proteins will be presented.


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