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Oxidação electroquímica directa de proteínas e interações proteína-DNA

Protein direct electrochemical oxidation and protein-DNA interactions

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Abstract: Electrochemical studies in proteomics of individual protein domains and mutants of proteins, which do not contain a centre with fast-reversible redox reactions, are still very few. The surface of a protein is often complex in nature, and a major factor influencing protein adsorption is the surface properties, the adsorption process being dynamic. The adsorption of native proteins without prosthetic groups on an electrode solid surface is weaker due to the rigidity of the 3D-structure. The unfavourable orientation of protein molecules on electrode surfaces may hinder electron exchange between the electrode and the electroactive amino acids in the interior of the proteins, which are tyrosine, tryptophan, histidine, cysteine and methionine residues, and that all present a pH-dependent electron transfer mechanism. The direct electrochemical oxidation behaviour of the enzyme peptide methionine sulfoxide reductase A (MsrA), native and denatured protein monoclonal antibodies rituximab (RTX) and bevacizumab (BEVA), and human cytochrome P450 (CYP1A2) adsorbed on glassy carbon electrode (GCE) or on boron doped diamond electrode (BDDE) surfaces, and their interactions with DNA have been investigated. MsrA is a ubiquitous protein, highly conserved, one of the major targets of reactive oxygen species, an antioxidant repair enzyme which reduces the oxidized form of methionine to methionine, preventing irreversible protein damage and, as a consequence, extending the organism's life span. The oxidation of native RTX and BEVA was studied using a protein antibody monolayer film deposited on the GCE surface. At physiological pH, native RTX and BEVA presented only one oxidation peak corresponding to tyrosine and tryptophan residues. Significant differences were observed between the oxidation of native and denatured forms of RTX and BEVA due to morphological changes and unfolding of the protein antibody native structures. RTX or BEVA-electrochemical biosensors, prepared by depositing a RTX or BEVA thick multilayer onto a GCE, were incubated for different time periods with the denaturing agent sodium dodecyl sulfate (SDS), or with the reductancts tris(2-carboxyethyl)phosphine (TCEP) or dithiothreitol (DTT), in order to unfold the RTX or BEVA tertiary and quaternary structures. Three oxidation peaks, corresponding to the oxidation of tyrosine, tryptophan and histidine residues, were detected. A multilayer dsDNA-electrochemical biosensor was used for the *in situ* electrochemical evaluation of RTX and BEVA interaction with double-stranded DNA.
The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism as well as the synthesis of cholesterol, steroids and other lipids. The interaction of cytochrome P450 with DNA showed a decrease of the DNA oxidation peak currents with time, and the appearance of free guanine and 8-oxoGua and/or 2,8-oxoAde oxidation peaks, demonstrating that the P450-DNA interaction caused DNA condensation and DNA oxidative damage. *In situ* DNA oxidative damage by electrochemically generated hydroxyl free radicals has been directly demonstrated at a BDDE. The dsDNA-electrochemical biosensor was used to study the interaction between dsDNA immobilized on a BDDE surface and *in situ* electrochemically generated $OH^{\bullet}$. The relevance of the dsDNA-electrochemical biosensor in the evaluation of the dsDNA-hydroxyl radical interactions was clearly demonstrated. Proteins are essential components of organisms and are involved in a wide range of biological functions. Oxidative damage to proteins is considered to be one of the major causes of aging and age-related diseases. The interaction of proteins with solid electrode surfaces is not only a fundamental phenomenon but also a key to important and novel applications in biosensors, biotechnology, medical devices and drug-delivery schemes.

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