

Development of a peptide-based electrochemical biosensor for juvenile idiopathic arthritis diagnosis

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Juvenile idiopathic arthritis (JIA) is a wide group of autoimmune and inflammatory diseases that affect children and adolescents under the age of sixteen. In the absence of proper diagnosis and treatment, irreversible damage may occur in the joint tissues. Biosensors emerge in the clinical scenario as promising analytical alternatives to molecular diagnosis. In previous work, PRF+1 peptide (ACSSWLPRGCGGGS) has been selected from random phage-display libraries and shown to react against antibodies from patients with JIA. This work presents the development of an electrochemical biosensor for the diagnosis of JIA in human serum using screen-printed carbon electrodes (SPCE) functionalized with PRF+1 peptide. Electrochemical methods and/or structural analysis were employed to investigate PRF+1 immobilization and its interaction with the antibody target through the monitoring of an oxidation peak. The SPCE surface was cleaned by cyclic voltammetry in perchloric acid (0.5 mol.L⁻¹) prior to immobilization of the peptide. Electrochemical detections were conducted by differential pulse voltammetry in phosphate buffer (0.1 mol.L⁻¹, pH 7.4). Moreover, PRF+1 three-dimensional structure was predicted with I-Tasser suite. Cyclic voltammograms in phosphate buffer showed a well-defined oxidation peak in $E_p = +0.49$ V for the electrode functionalized with the peptide (SPCE/PRF+1) and no peaks in this potential range for bare SPCE. Among the common 20 amino acids that compose peptides and proteins, methionine, tyrosine, histidine, tryptophan and cysteine are oxidized on carbon electrodes. However, only cysteine and tryptophan residues are present in PRF+1 sequence. Structural analysis was employed to investigate which of them was related with the detected oxidation peak. As the two cysteine residues form a disulfide bond, which is the oxidized form of these amino acids, the oxidation peak is probably due to tryptophan residue oxidation. This peak was also monitored for this system after blocking with bovine serum albumin (BSA) and incubation with positive or negative serum for JIA. The biosensor was able to discriminate samples from these different groups of patients. Therefore, a simple, miniaturized and functional platform was developed as a promising strategy for JIA molecular diagnosis.

Funding support: FAPEMIG, CAPES, CNPq.