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Electrochemical deposition of artificial recognition unit for gluten epitope selective determination

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Gluten, which chemically corresponds to storage proteins deposited in the starchy endosperm cells of the developing grain, is the allergen that triggers autoimmune reactions in people suffering from celiac sprue (CS). It is estimated that CS affects 1-2% of the European population with direct healthcare costs estimated at about 3 bn EUR/year. The only effective therapy is strict dietary abstinence from gluten. In fact, even a small contamination of food with gluten can cause serious adverse reactions from digestive system. Food considered as gluten-free, thus safe for CS suffering people, should contain less than 20 mg/kg of gluten. Molecularly imprinted polymers (MIPs) are artificially made receptors with the ability to bind reversibly and therefore, to recognize the target analytes. The fabrication of MIPs against small molecules or peptides is now straightforward whereas imprinting of large molecular structures, such as proteins, is still challenging. A possible solution is to imprint just defined epitopes instead of the whole protein. In the present study, a toxic gluten epitope, PQQPFPQQ, was chosen as a template for imprinting. The MIP film was prepared by electrochemical polymerization of bis(bithiophene) derivatives, bearing either cytosine or carboxylic acid substituent, in the presence of the template and a cross-linker. After deposition, the template was extracted from the polymer film. Subsequently, the film composition was characterized by x-ray photoelectron spectroscopy (XPS) as well as its morphology and thickness was studied by atomic force microscopy (AFM). Performance of this chemical sensor was tested under laboratory conditions. Extended-gate field-effect transistor (EG-FET) sensor signals were measured for an aqueous solution of the PQQPFPQQ analyte, as well as its interferences with 1 or 2 mismatched amino acids. Moreover, the sensor responses were measured toward the PQQPFPQQ analyte in gluten samples digested with pepsin at pH ≈ 2. Finally, analytical parameters of the devised chemosensor were evaluated.

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