Layer-by-layer films containing peptides of the Cry1Ab16 toxin from Bacillus thuringiensis for future applications in electrochemical sensors of genetically modified organisms

Alexandra Placido, Emanuel Airton de O. Farias, Mariela Marani, Andraeanne G. Vasconcelos, Ana C. Mafud, Yvonne P. Mascarenhas, Carla Eiras, José Roberto S. A. Leite, Cristina Delerue-Matos

Introdução: Cry1Ab16 is a toxin of crystalline insecticidal proteins that has been widely used in genetically modified organisms (GMOs) to gain resistance to pests. Recently, many studies have focused on evaluating the potential environmental impact of this toxin, including the impact on aquatic environments. For the first time, in this study, peptides derived from the immunogenic Cry1Ab16 toxin (from Bacillus thuringiensis) were immobilized as layer-by-layer (LbL) films for future applications in electrochemical sensors for GMOs detection. Materiais e Métodos: Peptides were manually synthesized using a solid phase approach with Fmoc/t-butyl chemistry. Electrochemical measurements were performed using an Autolab PGSTAT204 potentiostat/galvanostat and a three-electrode electrochemical cell with a capacity of 10 mL. A platinum plate (area 1.0 cm2) and silver/silver chloride (Ag/AgCl) were used as the auxiliary and reference electrodes, respectively. LbL films deposited onto indium tin oxide (ITO) (area 1.04 cm2) were used as the working electrode. In an attempt to improve the electrochemical signal of the peptide, it was combined with other polymeric materials in the form of a bilayer film. Chitosan and polyethylenimine (PEI) were tested as positive polyelectrolytes (polycation), whereas agar, alginate, and poly(sodium 4-styrenesulfonate (PSS) were tested as negative polyelectrolytes (polyanion). All the films were characterized by Cyclic Voltammetry, AFM, UV-Vis spectroscopy, XRD and FTIR. Resultados e Discussão: In electrochemical characterization was observed that the PcL342-354C exhibited an irreversible oxidation process at +1.1 V vs. Ag/AgCl in the H2SO4 electrolyte, similar at related studies of literature. The process observed at +1.1 V vs. Ag/AgCl in the acidic medium shifted to lower potential values when employing PBS as the supporting electrolyte. In PBS (pH 7.2), the oxidation process was at +0.87 V, whereas in PBS (pH 8.0) it was at +0.83 V. This decrease of potential is interesting because this peptide is intended for future construction of electrochemical (bio)sensors for detection of GMOs. Besides, PBS is a biologically compatible buffer. Among the polymers used, the PEI and PSS (synthetic polymers) showed a marked improvement in the current obtained for the peptide-based film (100 times higher than that recorded for the peptide adsorbed directly on ITO). The ITO/PEI/PSS/PcL342-354C film is very
promising for future applications in GMO sensors; therefore, we undertook a more thorough characterization of this film (data not showed). Conclusão: In general, the thorough characterization of the ITO/PEI/PSS/PcL342-354C film showed that this system is not only homogeneous and low cost, but is also reproducible, which opens prospects for future application of this film in electrochemical sensors for GMOs and environmental biosensors, among other biotechnological applications in the food safety area.