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Electrochemical detection of Avian Influenza Virus genotype using ssDNA probe modified gold electrode

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Avian Influenza H5N1 is a common infectious disease spreading among wild and domestic birds. It is caused by a virus of the family Orthomyxoviridae, belonging to the type A. Highly-Pathogenic Avian Influenza type H5N1 virus (HPAI) is not only extremely lethal to domestic fowl, but also constitutes a threat to humans including mammals and can cause death. Therefore, the methods suitable for early and fast detection of the highly pathogenic forms of virus are much needed. In recent years, intensive research has been seen in the development of biosensors for a wide range application in medical, food, agricultural and environmental areas. The detection and analysis of specific DNA sequences become an important approach in molecular diagnosis. The growing requirements concerning the quality of DNA sensors (called genosensors), in particular sensitivity and selectivity, induce the significant progress in their development.

Here we report two types of electrochemical genosensors devoted for detection of influenza virus H5N1 gene sequence. The first one is a sensor based on ion-channel mechanism. In this case the ss-DNA probe conjugated with amino group was attached on the gold electrode via amide bond derived from thioacid. The signals generated as a result of hybridization were registered in techniques: Osteryoung square wave voltammetry (OSWV) and electrochemical impedance spectroscopy (EIS) in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox marker. The genosensor sensitivity and selectivity were tested with two types of targets, a short (20 bp) single-stranded DNA and long (181 bp) double stranded PCR products. Genosensor is capable to determine 20-mer and 180-mer (PCR products) oligonucleotides complementary sequences with detection limit in the FM range. The genosensor display good selectivity and sensitivity. The 20-mer as well as 180-mer oligonucleotides without a complementary sequence generate very low signal. The second type of sensor is on the base of one electrode system for simultaneous determination of two sequences of DNA of Avian Influenza Virus (AIV) type H5N1. To face of the need of systems for simultaneous determination of few markers of one disease coming from medical diagnosis, we have developed a novel dual DNA electrochemical sensor with "signal-off" and "signal-on" architecture for simultaneous detection of two different sequences of DNA derived from Avian Influenza Virus (AIV) type H5N1 by means of one electrode. Two sequences of ssDNA characteristic for hemagglutinin decorated with ferrocene (ss-DNA-Fc) and characteristic for neuraminidase decorated with methylene blue (ss–DNA–MB) were immobilized covalently together on the surface of one gold electrode. The changes in the faradic current coming from redox reaction of ferrocene and/or metylene blue before and after hybridization processes were used as an analytical signal registered by Osteryoung Square Wave Voltammetry (OSWV) technique.

Biography

Jerzy Radecki, obtained his M.Sc. degree at the Department of Organic Chemistry of Nicholas Copernicus University in Toruń in 1973. In 1980, he received a Ph.D. degree at the same University. Since 1980 till 1998, he was worked at the Olsztyn University of Agriculture and Technology. In 1993, he received D.Sc. degree at this niversity. In 1985 he visited St. John's College of Oxford University. In 1990, he was granted with a fellowship in the National Institute for Environmental Studies in Tsukuba, Japan. Since 1998, he is working as a head of Department of Biosensors in the Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn. Jerzy Radecki is the editor-in-chief of the "Polish Journal of Environmental Studies" from 1992. Currently, he is Polish representative of COST Action CM1005 "Supramolecular Chemistry in Water".

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