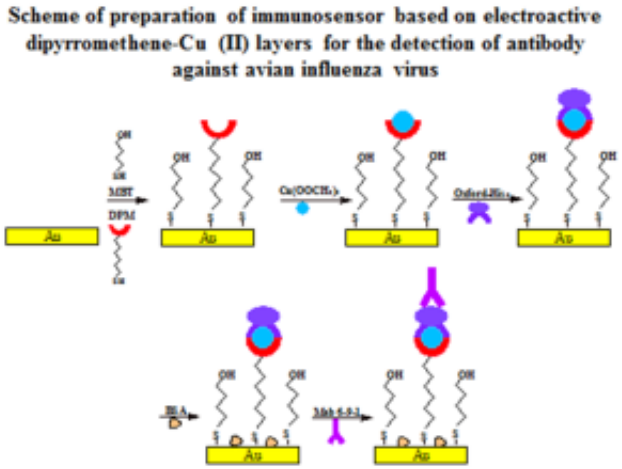
A electrochemical biosensor for the detection of antibodies against avian influenza virus type H5N1 in hen sera

Avian in?uenza (AI) is a highly contagious disease caused by Orthomyxoviridae family viruses. Highly pathogenic avian in?uenza (HPAI) virus can be easily transmitted between poultry, leading to severe disease outbreaks or even pandemics. It is difficult to control the spread, so all chickens in facilities are usually slaughtered. All these causes enormous economic losses in the poultry industry and seriously threatens human health. To prevent and control AI in poultry, vaccination has been employed as a key strategy in many countries since the 1990s. Therefore, methods suitable for sensitive and fast detection of antibodies against AI virus are still desired.



Among a variety of analytical techniques, which are presently applied to detection of antibodies, biosensors are promising tools. They are analytical devices consisting of biological components (e.g., tissues, microorganisms, proteins, enzymes, antibodies, nucleic acids) coupled to transducers and generate analytical responses related to analyte concentrations in samples. Biochemical sensors have attracted considerable attention due to selectivity, sensitivity and possibility of miniaturization. They have found numerous applications for control of food quality, environmental pollution and medical diagnostics.

Here, we present the development of a biosensor for the detection of antibodies against influenza virus hemagglutinin. The steps of biosensor fabrications are as follows: (i) creation of a mixed layer containing the thiol derivative of dipyrromethene and 4-mercapto-1-butanol, (ii) complexation of Cu(II) ions, (iii) oriented immobilization of recombinant hemagglutinin from the HPAI virus type H5N1 (His_6-H5HA) and (iv) filling free spaces with bovine serum albumin (see Scheme).

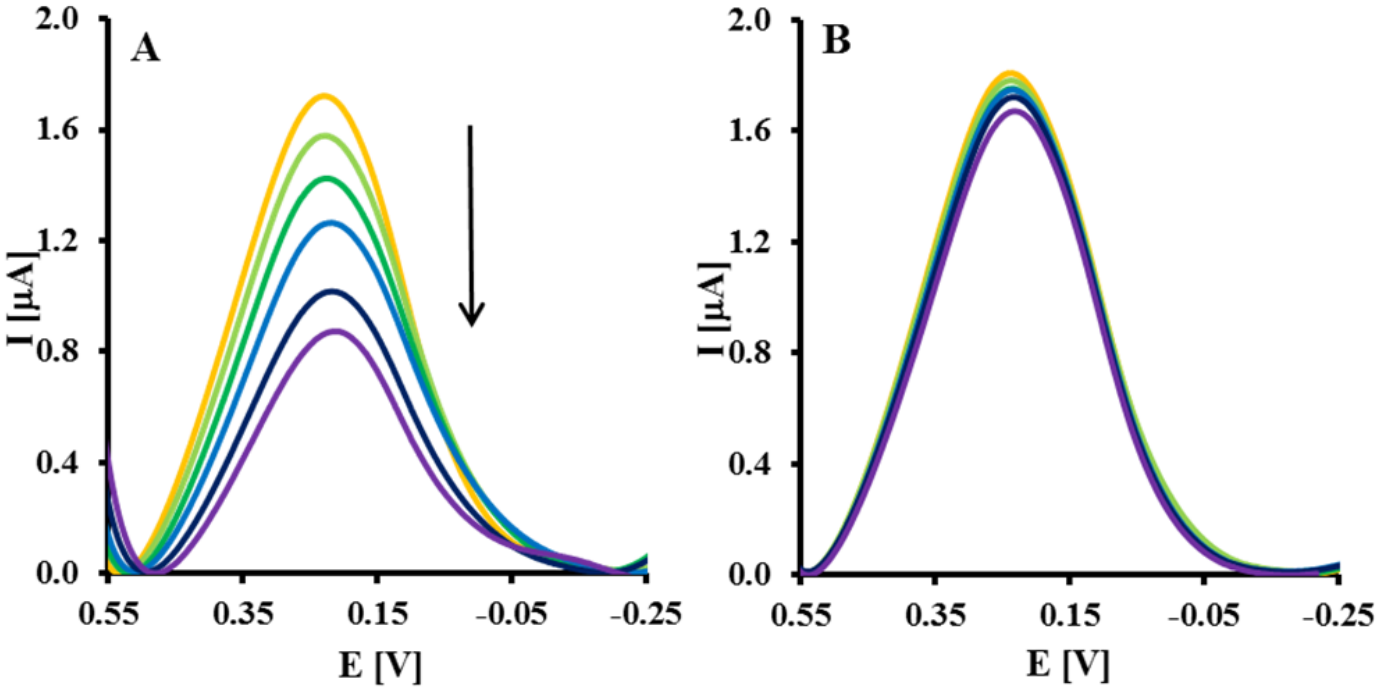


Fig. 1. OSWV recorded for electrode Au–MBT/DPM–Cu (II)–His6-H5 HA–BSA in the presence : (A) serum 1 (from high responder), (B) serum 4 (pathogen free)

The interactions between His_6-H5HA and specific anti-H5HA antibodies was observed using Osteryoung square-wave voltammetry (OSWV). Quantitative assessment of the sensitivity of the biosensor based on the redox active DPM–Cu(II) layer was done with serial dilutions of antibodies in PBS buffer. Typical responses of the biosensor registered using OSWV are shown in Fig. 1. The Cu(II) ions redox current decreasing is observed with an increasing of the antibodies concentration in buffer (Fig. 1A). A linear range of analytical response was observed between 4.0 and 100.0 pg/mL. The limit of detection was 2.4 pg/mL. The negative control (anti-IL-2 antibodies), with no affinity to the His_6-H5HA, generated a weak response (Fig. 1B). This confirmed the selectivity of the biosensor.

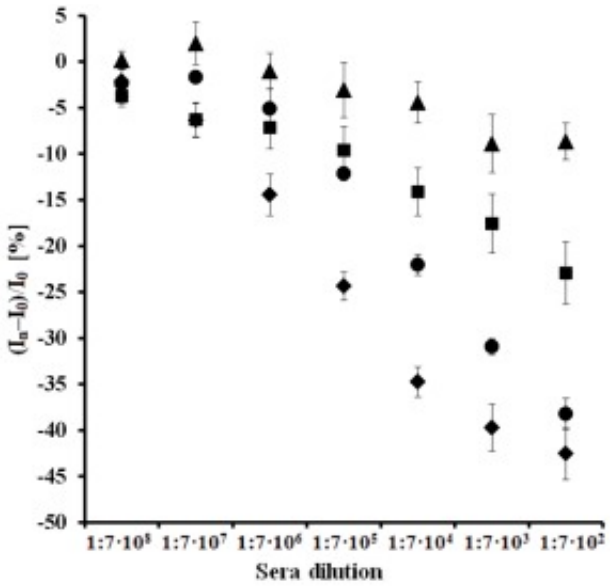


Fig. 2. The relationship of (In–I0)/I0 vs. dilutions of: (?) serum 1 from a high responder; (?) serum 2 from a low responder; (?) serum 3 from non-vaccinated; (?) serum 4 (pathogen free) (n = 5 ÷ 8).

In addition, this device was applied for the detection of specific antibodies in biological samples. A series of dilutions of the hen sera from: vaccinated (serum 1 from a high responder and serum 2 from a low responder), non-vaccinated (serum 3), and chickens serum hatched from the specific pathogen free (serum 4) were prepared in PBS buffer. Figure 2 shows the relationships of $(I_n–I_0)/I_0$ [%] vs. dilutions of different sera. The biosensor was able to detect anti-H5HA antibodies in serum 1 diluted $1:7\times10^6$ and serum 2 diluted $1:7\times10^4$. These results indicate that the sensitivity of our biosensor is nearly 200 and 20 times better than the enzyme-linked immunosorbent assay (ELISA). The slight decrease of peak current in the case of serum 3 could be caused by the matrix (different antibodies, which could be generated by hens contact with an environment). On the other hands, in the presence of serum 4 (totally free from any antibodies), weak responses were observed.

The system presented detected specific antibodies in a selective way. This biosensor was able to detect a humoral response in sera of hens immunized with DNA vaccine based on the sequence of HA from the H5N1. Therefore, the high sensitivity and selectivity of the system presented allowed distinguishing hens vaccinated from non-vaccinated ones against AI virus. Consequently, it could be very effective in detection of antibodies for immune surveillance and monitoring of the efficiency of poultry vaccination programs. The detection limit of proposed system is 200 better then ELISA.

Publication

A biosensor based on electroactive dipyrromethene-Cu(II) layer deposited onto gold electrodes for the detection of antibodies against avian influenza virus type H5N1 in hen sera.
Jarocka U, Sawicka R, Stachyra A, Góra-Sochacka A, Sirko A, Zagórski-Ostoja W, S?czy?ska V, Por?bska A, Dehaen W, Radecki J, Radecka H
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