

Determination of viral nucleic acids by electrochemical detection array using paramagnetic nanoparticles

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Abstract

Simple and express determination of dangerous viruses is one of the most important precautions of their pandemic spreading. Paramagnetic nanoparticles in combination with electrochemical detection methods belong to the group of techniques fulfilling these requirements. We were able to isolate and subsequently detect 100 pg/ μ l of viral nucleic acid by using 1 μ l of paramagnetic nanoparticles.

Keywords: Viral DNA, Electrochemical, Nanoparticles, Nanobiosensor,

Introduction

Development of the simple and fast detection devices like nanobiosensors is one of the main goals in the area of nanotechnology. Application of this technology can be beneficial for

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effective identification of dangerous viruses such as HIV, influenza and hepatitis. This work is focused on the detection of the hepatitis B virus, which attacks liver and may lead to the cancer. Epidemics all around the world caused that up to one third of the world's population has been exposed to this disease and about 3-6% is currently infected. Magnetic particles responding to an external magnet field are providing an elegant method of separation of targeted molecule from the solution (Huska et al. 2009a, 2009b, 2008).

Taking advantage of their magnetic characteristic and low cost of synthesis, magnetic particles (MPs) have been widely used as a universal separation tool for biologically active compounds such as nucleic acids (i.e., DNA and RNA), proteins and peptides, as well as intact cells. Streptavidin modified paramagnetic particles in conjunction with electrochemical detection have the potential to create highly sensitive and selective detection device

Materials and Methods

Electrochemical DNA analysis was carried out by AUTOLAB analyzer (EcoChemie, Netherlands) in connection with VA-Stand 663 (Metrohm, Switzerland). Viral nucleotides were purchased from Sigma – Aldrich (U.S.A.). Square wave voltammetric (SWV) measurements were carried out in the presence of acetate buffer pH 5.0. SWV parameters: potential step 5 mV, frequency 260 Hz. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999%), saturated with water for 120 s.

Isolation of nucleic acids proceeded on paramagnetic nanoparticles (MNPs) from Department of Microelectronics, Faculty of Electrical Engineering and Communication, Brno University of Technology. MNPs were biotinylated by viral oligonucleotides (ODNs) and subsequently hybridization with complementary sequences took place. This step was followed by the separation using external magnetic field and releasing of the complementary sequences from the MNPs by thermal denaturation. Isolated oligonucleotides were analyzed by square-wave voltammetry using hanging mercury drop electrode and by chip based capillary gel electrophoresis (Biorad Experion, USA).

Results and discussion

Suggested method is based on the biotin – streptavidin interaction between biotinylated ODNs used as probes for viral DNA and MNPs modified by streptavidin. Conditions of the isolation were optimized. The optimal hybridization time of the viral nucleic acid to MNPs - 5 minutes - was determined. Eight different sequences of the hepatitis B (responsible for various molecular-biological processes) were analyzed. The dependence of the amount of isolated nucleic acid on the amount of MNPs was studied. For isolation of 100 pg/ μ l of viral DNA 1 μ l MNPs was used. Maximal amount of MNPs was 20 μ l, which was able to isolate 2 μ g/ml of viral nucleic acid. Both capacities were enough for subsequent electrochemical detection. Isolated viral nucleic acids were detected by electrochemical detection. Linear calibration curves were constructed ($R^2 = 0.99$).

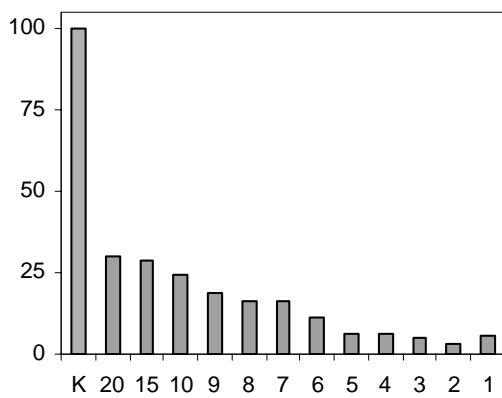


Figure 1: Effect of the amount of MNPs to the total extraction of the viral nucleic acid – electrochemical analysis. (K) bar represents the signal of the control viral DNA sample (5 μ g/ml).

Chip-based capillary gel electrophoresis was used for qualitative verification. Finally, experimental electrochemical array was constructed and electrochemical detection as well as electrophoretic analysis of the isolated viral nucleic acids mixture was performed.

Conclusion

An early identification of the rapidly spreading viruses is one of the preventive actions to protect the world population. Nanobiosensors employing paramagnetic nanoparticles in combination with electrochemical detection methods may be potentially effective way to prevent pandemic spreading of these diseases

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