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Potentiometric DNA sensor based on electropolymerized phenothiazines for protein detection

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Abstract

Novel method of potentiometric detection of DNA-protein interactions has been proposed. For this purpose, polymeric phenothiazine dyes, methylene blue (MB) and methylene green (MG), were electrochemically deposited onto the glassy carbon electrode and covered with double stranded DNA (dsDNA) as a target for antibodies (DNA-sensor) or DNA aptamer specific to human α-thrombin (aptasensor). The biosensors were consecutively incubated at pH 7.5 and 3.0 and the difference in potentials, ΔE, was used as a measure of protein concentration. The potentiometric DNA-sensors were tested in standard serum of autoimmune disease patients (systemic lupus erythemathosus (SLE) and autoimmune thyroidites). It was shown, that the ΔE value of DNA-sensor depends on the dilution of serum in the range from 1:1 to 1:100. Nonthermostated serum exhibited bell-shape dependence of ΔE on serum dilution due to interfering effect of serine proteins at maximum dilution between 1:20 and 1:50. For SLE serum thermostated at 56°C the ΔE linearly decreased as a function of serum dilution and reached saturation at dilution 1: 20. Similarly the changes in the potential of aptasensor allowed us to determine the α-thrombin in the range from 1 nM to 1 μM. The Faradic impedance spectra measured at presence of redox probe [Fe(CN)6]4-/3- revealed changes in the resistance and capacitance attributed to the shielding effect of anti-DNA antibodies and an increase in the electron transfer. The developed potentiometric biosensors can be used for preliminary diagnostics of autoimmune diseases and thrombin detection with sensitivity comparable to traditional methods. The developed assay is, however simpler and cheaper in comparison with commonly used methods.

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Keywords

Anti-DNA antibody, Aptasensor, DNA sensor, Electropolymerization, Human thrombin, Polyphenothiazine