FLOW-THROUGH IMMUNOSENSOR BASED ON DIRECT ELECTRON TRANSFER DETECTION OF PEROXIDASE LABEL

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The electrocatalytic properties of redox enzymes permit their application as labels for potentiometric immunosensors. Attachment of the electrocatalytic active enzyme to the electrode surface in the presence of substrate initiates an electrocatalytic reaction resulting in a potential shift. Antigen immobilized on the electrode surface interacts with enzyme-labeled antibody, resulting in attachment of the enzyme to the electrode surface. Therefore, the formation of antigen-labeled antibody complex on the electrode surface is accompanied by an electrode potential shift. The presence of free antigen in the solution leads to competition in the binding of labeled antibodies (conjugate) with free and immobilized antigen. The competition results in a decrease of the rate of potential shift. The decrease in rate is proportional to free antigen concentration in the solution.

A potentiometric immunosensor based on the direct electron transfer detection of the conjugate-target analyte complex formation is described. The enzyme peroxidase is used as a label. The ability of peroxidase to catalyze the electrode reaction of hydrogen peroxide electroreduction by a direct (mediatorless) mechanism is utilized. Peroxidase attached to the electrode surface in the presence of hydrogen peroxide results in a significant (hundreds of mV) increase of potential towards the equilibrium H_2O_2/H_2O potential due to catalytic removal of overvoltage. The immunosensor is arranged as a flow through device that is suitable for continuous monitoring of the changes in target analyte concentration. Rabbit IgG has been used as a model analyte. The method allows determination of IgG in the concentration range up to 400 µg/ml. The direct electron transfer detection of immunointeraction suggests a single stage analysis scheme thus providing a basis for a separation-free immunoassay. Analysis time does not exceed 25 min.

The principle of operation of the immunoelectrode is illustrated in Fig. 1. The electrode surface is modified by immobilized antigen (rabbit IgG), and the peroxidase-antibody conjugate associates with the antigen on the electrode surface. Once added to the media, and on reaching the electrode surface, the antibody-conjugated peroxidase starts to catalyze electroreduction of hydrogen peroxide which results in an increase in the electrode potential. When the target analyte is added, displacement occurs resulting in a decrease in electrode potential.

Figure 2 demonstrates the protocol for measurement of potential change (E) due to consecutive additions of conjugate into the cell. In the presence of hydrogen peroxide, a background electrode potential is established. The addition of solution containing anti-rabbit antibodies labeled with peroxidase (conjugate) results in an increase of electrode potential. This indicates that antigen-conjugate complex formation on the surface of electrode leads to peroxidase catalyzed electroreduction of hydrogen peroxide (reaction 1). The increase of conjugate concentration leads to an increase in the rate of electrode potential change. This rate expresses the electrode response, (dE/dt).

The optimal pH for immunointeractions is in the neutral pH range. However, an increase of pH higher then 5.5 leads to a significant increase in the drift of electrode background potential. An increase of the concentration of hydrogen peroxide higher than 0.001 % also results in increase of the background drift of electrode potential. Hence measurements were performed at pH 5.5 and concentration of hydrogen peroxide 0.001%. BSA was added to the reaction media to prevent nonspecific binding.

The immunosensor based on direct electron transfer detection of immunointeraction described above does not need phase separation of immunoagents. The second substrate of the enzyme reaction is an electron. Electrons can be provided by the electrode only after the enzyme label attaches to the electrode surface. The enzyme label does not catalyze any reaction in the absence of a second substrate (electron). Therefore, immunointeraction can be detected directly by recording a potential shift. Most of the immunoanalytical techniques are based on the measurement of antigen-antibody interaction after the interaction is complete (an equilibrium state is established). The immunosensor based on direct electron transfer detection of immunointeraction indicates the rate of immunointeraction. Detection of the immunointeraction is conducted in a kinetic mode resulting in a significant decrease in the time of analysis. The direct electron transfer detection of immunointeraction suggests a single stage analysis scheme and the signal can be detected continuously with increasing and decreasing concentrations of analyte. This allows automation of the measurement procedure and construction of continuously operating immunosensor devices. The basic detection device is a high impedance voltmeter. Potentiometric detection does not depend on electrode surface area, as electrode response is a function of the fraction of electrode surface covered by the electroactive label. This approach allows miniaturization of the immunosensors.

This work demonstrates the potential capabilities of immunosensors based on direct detection of electron transfer catalyzed by peroxidase label. The increase of capacity of immobilized antigen on electrode surfaces and optimization of conditions of assay procedure is the main direction to develop the sensitivity of analysis. The future development of the described immunoelectrodes is focusing on the improvement of the assay sensitivity. The immunoelectrode described above is the subject of a development of immunoassay for applications requiring fast, simple, single-stage analyses.

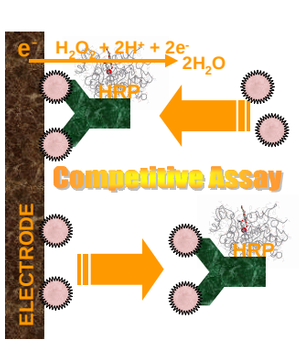

Fig. 1

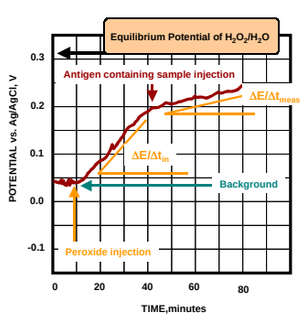

Fig. 2

Schematic of competitive immunoassay for detection of low-molecular weight antigens using antibody labeled by Horseradish Peroxidase (Fig. 1).
Experimental protocol of potentiometric detection of HRP enzyme label based on the elimination of overvoltage reduction (Fig. 2).