Electroactive Ferrocenyl Dendrimers as Transducers for Fabrication of Label-Free Electrochemical Immunosensor

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Abstract : Highly branched dendrimers provide structural homogeneity, controlled composition, comparable size to biomolecules, internal porosity and multiple functional groups for conjugating reactions. Electro-active dendrimers containing multiple redox units have generated great interest in their use as electrode modifiers for development of biosensors. The electron transfer between the redox-active dendrimers and the biomolecules play a key role in developing a biosensor. Ferrocenes have multiple and electrochemically equivalent redox units that can act as electron "pool" in a system. The ferrocenyl-terminated polyamidoamine dendrimer is capable of transferring multiple numbers of electrons under the same applied potential. Therefore, they can be used for dual purposes: one in building a film over the electrode for immunosensors and the other for immobilizing biomolecules for sensing. Electrochemical immunosensor, thus developed, exhibit fast and sensitive analysis, inexpensive and involve no prior sample pre-treatment. Electrochemical amperometric immunosensors are even more promising because they can achieve a very low detection limit with high sensitivity. Detection of the cancer biomarkers at an early stage can provide crucial information for foundational research of life science, clinical diagnosis and prevention of disease. Elevated concentration of biomarkers in body fluid is an early indication of some type of cancerous disease and among all the biomarkers, IgG is the most common and extensively used clinical cancer biomarkers. We present an IgG (=immunoglobulin) electrochemical immunosensor using a newly synthesized redox-active ferrocenyl dendrimer of generation 2 (G2Fc) as glassy carbon electrode material for immobilizing the antibody. The electrochemical performance of the modified electrodes was assessed in both aqueous and non-aqueous media using varying scan rates to elucidate the reaction mechanism. The potential shift was found to be higher in an aqueous electrolyte due to presence of more H-bond which reduced the electrostatic attraction within the amido groups of the dendrimers. The cyclic voltammetric studies of the G2Fcmodified GCE in 0.1 M PBS solution of pH 7.2 showed a pair of well-defined redox peaks. The peak current decreased significantly with the immobilization of the anti-goat IgG. After the immunosensor is blocked with BSA, a further decrease in the peak current was observed due to the attachment of the protein BSA to the immunosensor. A significant decrease in the current signal of the BSA/anti-IgG/G2Fc/GCE was observed upon immobilizing IgG which may be due to the formation of immune-conjugates that blocks the tunneling of mass and electron transfer. The current signal was found to be directly related to the amount of IgG captured on the electrode surface. With increase in the concentration of IgG, there is a formation of an increasing amount of immune-conjugates that decreased the peak current. The incubation time and concentration of the antibody was optimized for better analytical performance of the immunosensor. The developed amperometric immunosensor is sensitive to IqG concentration as low as 2 ng/mL. Tailoring of redox-active dendrimers provides enhanced electroactivity to the system and enlarges the sensor surface for binding the antibodies. It may be assumed that both electron transfer and diffusion contribute to the signal transformation between the dendrimers and the antibody.

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Keywords : ferrocenyl dendrimers, electrochemical immunosensors, immunoglobulin, amperometry

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