A rapid, convenient, and highly sensitive electrochemical detection of human hemoglobin in serum using a high-affinity bivalent antibody-enzyme complex

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Antibody-enzyme complexes (AECs) are ideal sensing elements for immunosensors due to their bifunctionality. We have recently established a convenient, universal, and homogeneous preparation method of a bivalent AEC using Catcher/Tag system[1]. EGFR, one of cancer marker, was successfully detected using the AEC as a sensing element, but its sensitivity was not meet clinically required range[2].The bivalent AEC could not exert avidity effects for EGFR with monomeric structure at lower target concentration. Here, we hypothesized that higher sensitivity can be achieved for multimeric target. Human hemoglobin (Hb) has tetrameric structure and is a biomarker for hematologic diseases. An extremely low level of Hb in blood immediately leads to hypoxia. Thus, rapid, convenient, and sensitive Hb detection is highly required.

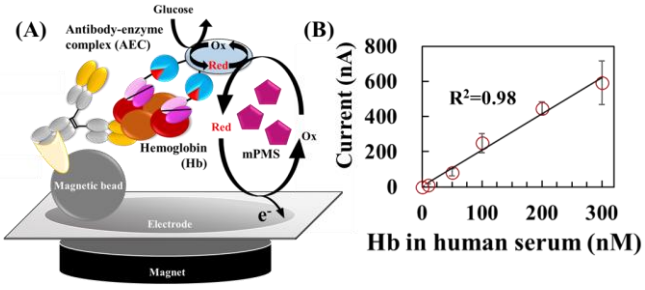

Fig. (A) A schematic illustration of wash-free Hb detection system. (B) Hb detection in human serum.

In this research, a bivalent AEC composed of two anti-Hb single-chain antibodies (scFvs) and a glucose dehydrogenase was prepared using Catcher/Tag system, and applied to wash-free and electrochemical immunosensor. The bivalent AEC retained high enzymatic activity even after AEC formation. Whereas, an affinity to Hb of the bivalent AEC (K_D = 4.3 nM) was remarkably improved compared to scFv itself (13 nM) as we expected. This is due to synergic effect of the bivalency of AEC and the multimeric structure of Hb. Using the bivalent AEC combined with a magnet and magnetic beads, we tried to detect Hb in diluted human serum (Fig.). As a result, Hb-dependent current increase was observed within 25 min of manipulation time. The linear range was 0.97-28 g/dL, which completely covered clinically required range. Hemolysis reagents are often used for pre-treatment of Hb detection and can be considered as contaminants on electrochemical detection, however, in our system there were no influences of the hemolysis reagents. These results strongly suggested that we successfully developed a novel Hb detection system and it can be applied to detection of other multimeric biomarkers.

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