

Recombinant fructosyl peptide oxidase expression and purification for HbA1c determination

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Abstract

HbA1c (Hemoglobin A1c) is the major glycosylated hemoglobin in human blood, the HbA1c concentration also reflects the averaged glucose concentration in blood of past few months. Therefore, it is recognized as an important diagnostic indicator of diabetes mellitus. HbA1c is a hemoglobin molecule in which the N-terminal valine residue of its β -subunit is glycosylated with blood glucose. The enzymatic method developed to detect HbA1c concentration in blood consists of three steps: i) proteolysis of the HbA1c β -subunits to release fructosyl valine (FV); ii) oxidation of the released FV by FPO (fructosyl peptide oxidase) to produce H_2O_2 ; and iii) detection of the produced H_2O_2 . FPO is the key enzyme involved in the enzymatic detection of HbA1c concentration in the blood. To obtain enough amount of FPO with high purity, FPO gene was synthesized and inserted into T7 expression plasmid for the production of genetically modified FPO in a recombinant *Escherichia coli*. The recombinant *E. coli* strain cultured at 26°C could produce more active FPO than that incubated at 37°C. The active FPO was effectively purified by immobilized metal affinity chromatography (IMAC). In combination with highly sensitive H_2O_2 detecting dye (DA-64), the purified recombinant FPO can easily measure FV concentration as low as 0.02 mM. In addition to the enzymatic colorimetric method, the recombinant FPO was also employed in amperometric detection of FV by using a Pt-CNT catalyzed single-use H_2O_2 electrochemical sensor strip. FV can be detected with sensitivity of $51.4 \mu A mM^{-1} cm^{-2}$ with linear range of 0.01 to 0.5 mM and detection limit of 0.01 mM. The single-use FV sensor was also employed to determine the concentration of HbA_{1c} in blood. However, the catalase released from the blood cell interfered the detection significantly. After cells lysis and proteolysis pretreatment, the blood sample needs to be filtered by ultrafiltration membrane to remove the interfering compounds. The HbA_{1c} concentration in the blood can be accurately measured by this single-use FV sensor after proper pretreatment.

Keywords: diabetes, fructosyl peptide oxidase, hemoglobin, recombinant protein