

Electrochemical Study of B11 Glucose Oxidase from Direct Protein Evolution

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In this study, electrochemistry study of glucose oxidation has been carried out using modified glucose oxidase obtained by direct protein evolution.

Progress in medical science raises demands for implantable electronic devices. Enzymatic biofuel cells and biosensors could offer specific advantages for these purposes. However, current developments are limited by enzyme stabilities and activities in complicated physiological environment and reactions. Direct protein evolution has become a powerful method for biocatalyst engineering to tailor enzyme properties to application demands, which could provide a promising solution for the current problems for enzymatic bioelectronics.

Wild type glucose oxidase (WtGOx) and mutant B11 glucose oxidase (B11GOx) obtained from direct evolution expressed in *Pichia pastoris* were purified by chromatography. Biological activities of the enzymes and kinetic parameters were determined by ABTS assay. Mutant B11GOx showed improved K_m and V_{max} values.

Enzyme electrodes were prepared by immobilizing WtGOx and B11GOx on the polymeric mediator, poly(vinylferrocene-co-2-hydroxyethylmethacrylate) (poly(VFc-co-HEMA)), on glassy carbon substrates. Figure 1 shows the cyclic voltammograms of the enzyme electrodes in pH 7 phosphate buffer solution. With similar amount of polymer and enzyme loading, the electrode immobilized with B11GOx exhibited higher current response indicating better electron transfer than the WtGOx. It proves that the electron transfer property with ferrocene mediator for B11GOx was improved by directed evolution. Glucose oxidation was also conducted on the enzyme electrodes. Linear sweep voltammograms in Figure 2 demonstrate a higher glucose oxidation activity obtained on B11GOx, which was also in consistent with the ABTS activity assay. Further characterisation on electrochemical properties of glucose oxidation with B11GOx will be studied with electrochemical methods of CV, LSV and electrochemical impedance spectroscopy.

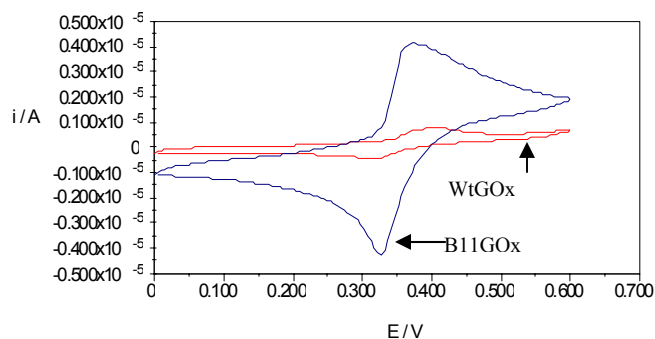


Figure 1 Cyclic voltammograms for enzyme electrodes immobilised with WtGOx and B11GOx on poly(VFc-co-HEMA), scan rate 25 mV/s, surface area 0.07 cm², in 0.1 M phosphate buffer, pH 7.0

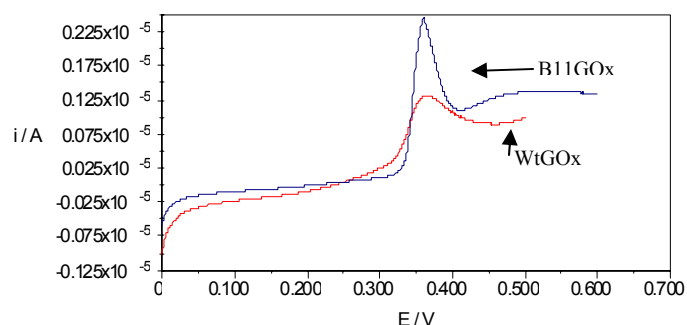


Figure 2 Linear sweep voltammograms for enzyme electrodes immobilised with WtGOx and B11GOx on poly(VFc-co-HEMA), scan rate 1 mV/s, surface area 0.07 cm², in 0.1 M phosphate buffer, pH 7.0 with 1mM glucose