

Supplementary data for article:

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SUPPLEMENTARY DATA

Electrochemical Oxidation of Glucose using Mutant Glucose Oxidase from Directed Protein Evolution for Biosensor and Biofuel Cell Applications

In this study, structure-function relationships of amino acid substitutions are investigated by comparing amperometric glucose sensors based on entrapping redesigned glucose oxidase (B11-GOx) and wild type enzyme (wt-GOx) into conductive polymers, polyethylenedioxythiophene (PEDOT). PEDOT was chosen due to the commercial availability of monomer EDOT, as well as the long-term electrochemical stability of the PEDOT.

Experimental

Preparation of polymer electrode using PEDOT

D(+)-glucose, 3,4-ethylenedioxythiophene (EDOT) and Ferrocenemethanol were purchased from Sigma Aldrich (Steinheim, Germany). α,ω -Bis(2-carboxymethyl)polyethylene glycol (PEG MM 600) was supplied by Fluka (Steinheim, Germany). All reagents were used as supplied without further purification. All solutions were prepared with MilliQ water, ddH₂O (Millipore, UK). Phosphate buffer (5×10^{-2} M K₂HPO₄; 5×10^{-2} M KCl; pH 6.0) were used in all experiments. Glucose stock solution (0.25 M) was allowed to mutarotate for at least 24 h and stored at 4°C prior to use.

Preparation of enzyme electrodes were achieved as it was previously described (1, 2). Enzymes were first covalently bonded to a polyethylene glycol bearing a carboxylic acid group. The modified enzymes (PEG-B11-GOx and PEG-wt-GOx) were then entrapped in the poly(3,4-ethylenedioxythiophene) (PEDOT) matrix during electropolymerization of 3,4-ethylenedioxythiophene (EDOT) in phosphate buffer. PEG plays the role of surfactant for the EDOT monomer (PEG enhances EDOT solubility) (2).

Electrochemical study and glucose calibration curves

The amperometric responses of the enzyme electrodes to glucose injections in a phosphate buffer (5 ml; 5×10^{-2} M K_2PO_4 ; 5×10^{-2} M KCl; pH 6.0) solution containing FeOH (3×10^{-4} M) were examined by measuring the anodic current at a potential of 0.33 V (vs. SEC) at room temperature under anaerobic conditions. Solution used in the amperometric studies were deaerated by bubbling nitrogen for 15 min prior to use and nitrogen blanket was kept over the solution during the experiments. The background current was allowed to settle to a constant baseline, and successive additions of 40 μ L stock glucose solution (0.25 M) were injected, with gentle stirring for 5 s after each addition. The current as a function of time was monitored continuously until a steady state value was reached.

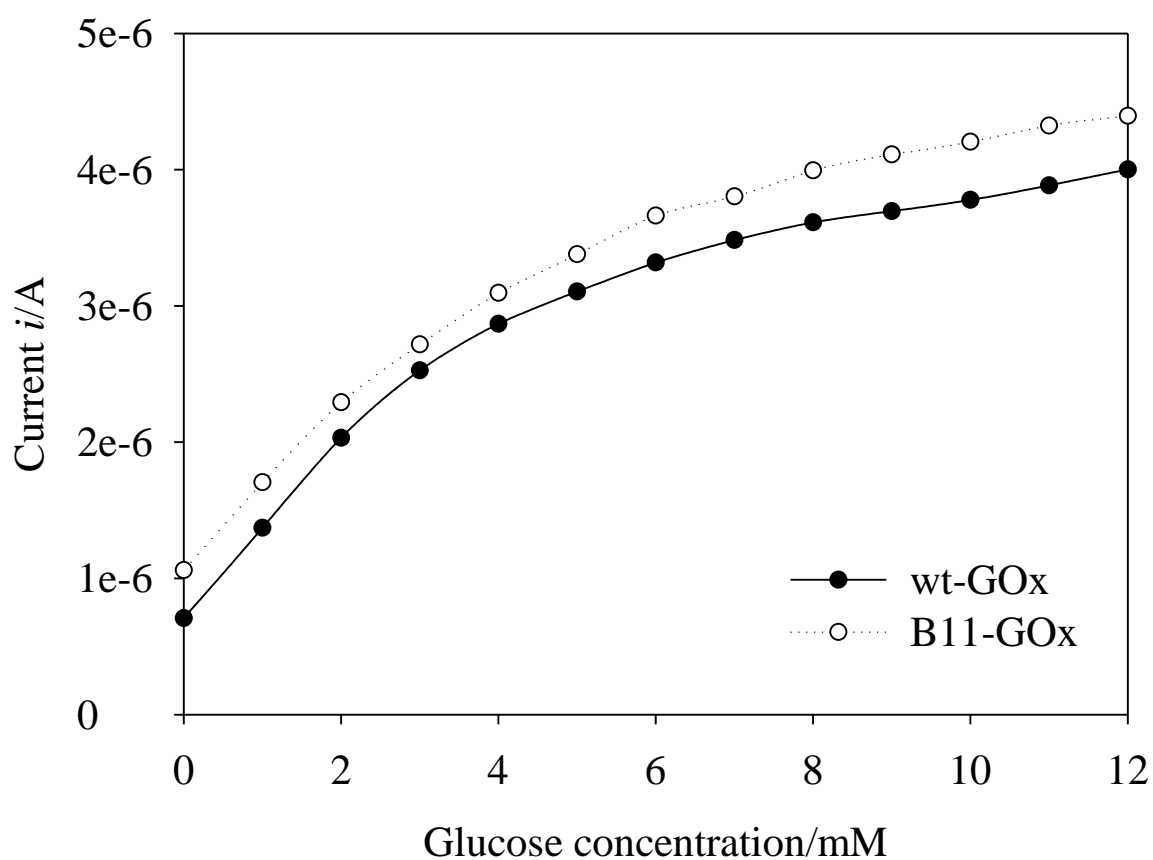
Results and Discussion

Figure 1 shows typical calibration curves for the amperometric response of glucose at enzyme electrodes. The linear relationship between the glucose concentration and the response current was found within the glucose concentration range of 0 to 2 mM for both electrodes. The sensitivities of the electrochemical biosensors that have a 0.07 cm² surface area attain values of 4.3 and 5.0 nA cm⁻² M⁻¹ for PEG-B11-GOx/PEDOT and PEG-wt-GOx/PEDOT electrodes, respectively. This result shows that the sensor with B11-GOx responded more rapidly to the change of glucose concentration than the sensor with wt-GOx. The linear region for the current response for B11-GOx was 6 mM and 4 mM for wt-GOx also showing an improved detection limitation obtained from mutant GOx. These results showed that mutant enzyme has an improved activity towards electrochemical oxidation of glucose, which signifies the feasibility of using engineered glucose oxidase in amperometric glucose sensors.

References

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2. Piro, B., Dang, L. A., Pham, M. C., Fabiano, S. and Tran-Minh, C. (2001) A glucose biosensor based on modified-enzyme incorporated within electropolymerised poly(3,4-ethylenedioxythiophene) (PEDT) films. *Journal of Electroanalytical Chemistry*, **512**, 101-109.

Figure



Caption

Figure 1 Calibration curve of PEG-GOx/PEDOT electrodes: the amperometric response versus glucose concentration