

A NOVEL SORBITOL BIOSENSOR BASED ON SORBITOL DEHYDROGENASE LINKED TO GOLD BY CYSTEINE

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Abstract

- Elevated sorbitol levels in red blood cells are associated with severe complications of diabetes. A sorbitol biosensor could provide early diagnosis.

- A novel bioelectronic interface was developed that uses cysteine to link sorbitol dehydrogenase (SDH), its required cofactor (NAD⁺), and an electron mediator (toluidine blue O) to a gold electrode in an arrangement that provides efficient electron transfer.

- Cyclic voltammetry indicated that the interface functions as an effective sorbitol biosensor, in which the peak current varies linearly with sorbitol concentration.

Introduction

- Elevated levels of sorbitol in red blood cells can signal diabetic complications before other symptoms appear.¹

- A sensor able to measure sorbitol concentrations could allow early diagnosis, treatment, and prevention of vision loss and nerve damage.²

- Sorbitol dehydrogenase enzymatically oxidizes sorbitol to fructose. Electrons produced in the reaction could be transferred to an electrode, resulting in a electrochemical biosensor for sorbitol.

- Sorbitol dehydrogenase transfers electrons from sorbitol to an electron-carrying cofactor (NAD⁺). Achieving efficient electron transfer from NAD⁺ to the electrode requires an electron mediator.

- Our lab recently developed a novel approach that uses the amino acid cysteine (Fig. 1) to electrically couple NAD⁺ and an electron mediator to a gold electrode.³ The goal of this study is to apply this approach to develop a sorbitol biosensor.

Materials and Methods

- A novel bioelectronic interface was fabricated that includes cysteine, SDH, NAD⁺, toluidine blue O (TBO), and a gold electrode.

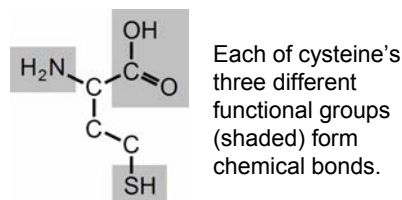


Fig. 1: Molecular Structure of Cysteine

- Cysteine's three functional groups allow the molecular components of the interface to be bound in a unique conformation (Fig. 2).

- Cysteine:** Cysteine's sulfhydryl group was attached to the gold electrode by a thiol bond.
- Toluidine Blue O:** An ester bond was formed between TBO and cysteine's carboxylic acid group.
- 3-Carboxyphenyl Boronic Acid:** An ester bond was formed between CBA and cysteine's amine group.
- NAD⁺:** NAD⁺ was attached to CBA through the cis-diol group on both molecules.
- Sorbitol Dehydrogenase:** SDH was attached to NAD⁺ by an affinity linkage.

Results and Discussion

- The electrochemical activity of the interface was studied using cyclic voltammetry (CV) in aqueous sorbitol solutions. Each curve in Fig. 3 is for a different sorbitol concentration.

- Peak CV current varied linearly with sorbitol concentration, indicating that the interface serves as an effective sorbitol biosensor.

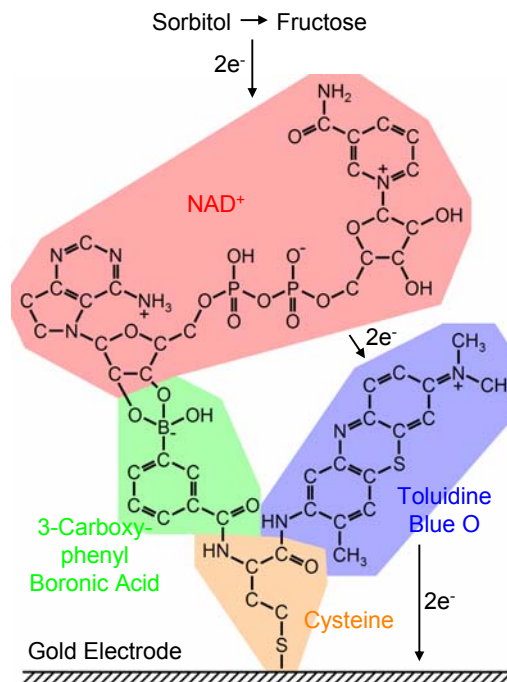


Fig. 2: Schematic Diagram of Biosensor

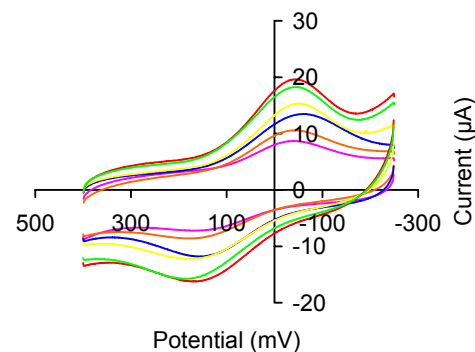


Fig. 3: CV Curves for Sorbitol Biosensor

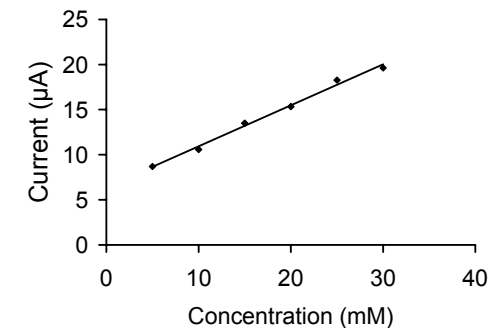


Fig. 4: Calibration Curve for Sorbitol Biosensor

Conclusions

Cysteine was used to attach SDH, NAD⁺, and TBO to a gold electrode in a unique conformation. The resulting bioelectronic interface functions as a sorbitol biosensor.

References

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