Bulletin of Electrochemistry 8 (4) April 1992, pp 186–188

0256-1654/92/$ 3-50

A SIMPLE APPROACH FOR THE MODIFICATION OF GLUCOSE OXIDASE WITH A REDOX MEDIATOR

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[Received: 1992 APril]

The modification of glucose oxidase with ferrocene by a simple route has been carried out. The modified enzyme was studied using cyclic voltammetry.

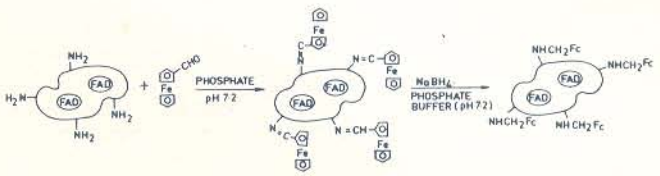
Key words: Glucose oxidase, modified glucose oxidase, ferrocene

INTRODUCTION

Oxidoreductases are enzymes, which catalyze redox reactions in living systems and having molecular weight in the range 40 KDa to 800 KDa [1]. Glucose oxidase (GOx) falls in this category with flavine adenosine dinucleotide (FAD) as the prosthetic group. The molecular weight of GOx is 50 KDa with a hydrodynamic radius of 21Å [2,3]. It is known that the electron transfer rate decreases with increase in the distance between the sites of interest, in this case viz. the FAD of the enzyme with the electrode surface. The prosthetic group is located deep inside the glycoprotein of the GOx and hence making it electrically inaccessible [3]. Electron transfer at such an enzyme is the subject of a number of recent investigations [3–6]. Different approaches have been proposed to effect a facile electron transfer in the above. According to one strategy, modification of electrode surface is done which facilitates the protein to approach the electrode surface at the right orientation conducive for the electron transfer [7]. This electron transfer on modified electrode is effective in the case of low molecular weight enzymes such as cytochromes, wherein the prosthetic group is along the edges of the protein matrix. In another approach, the enzyme is modified with a redox mediator, having the required redox characteristics by linking it covalently. The redox mediator with appropriate redox characteristics linked to the enzyme offers a convenient and favourable path for electron transfer between the enzyme and the electrode. In the case of GOx, modification with ferrocenyl derivatives [3–5] and tetrathiofulvalene [6] has been reported recently. The reported procedure, however, involves the opening of the ternary structure of the enzyme as the first step, followed by its modification [3–5]. The ternary structure of the enzyme was finally reconstituted after the modification. However, modification of GOx using this procedure was found to result not only in the decreased activity of the modified enzyme but also in its life time considerably. This prompted us in examining an alternate approach of modification of GOx without perturbing the original globular structure, thus attempting to arrest the loss in activity and lifetime of the enzyme noted above. The details of such a study are reported in this note. The free amino groups of lysine residues of the GOx can be utilised for bond formation with the redox mediator by Schiff base formation or esterification. We have opted for the reaction of these free amino groups available, with an aldehydic functional group attached to the ferrocene molecule to form the Schiff's base which can be reduced to make the covalent linkage stable and investigated the properties of this modified enzyme for its stability, activity and electro-catalytic properties for glucose oxidation.

EXPERIMENTAL

GOx (2 mgs.ml^{-1} obtained from Sigma Chemicals, type VII) was treated with ferrocene carboxaldehyde (procured from Aldrich Chemicals) in phosphate buffer (pH 7.2) and the mixture was allowed to react for a period of two hours at 277K to form the Schiff base with the lysine/arginine residue of the enzyme. The Schiff base was reduced with sodium borohydride to the aminomethyl ferrocene derivative of the enzyme. During the course of the above reduction, the unreacted ferrocene aldehyde gives hydroxymethylferrocene as byproduct [Scheme 1].



SCHEME .1

Scheme 1: The various chemical transformations associated with the modification of GOx

The resultant reaction mixture, which is golden yellow in colour, was separated by dialysis against phosphate buffer for a period of 4 h to remove the hydroxymethylferrocene. The dialysed solution containing the modified enzyme with ferrocenyl derivative was studied using cyclic voltammetry for its electrochemical properties. All the electrochemical experiments were carried out in a three electrode assembly using a glassy carbon working electrode (BAS, 0.3 cm dia), a Pt wire counter electrode and a normal calomel reference electrode (NCE).

RESULTS AND DISCUSSION

The cyclic voltammetric response of unmodified GOx on GC electrode did not show the redox characteristics corresponding to that of FAD. In contrast to this, two redox processes are observed in the cyclic voltammogram, one at −0.3 V and the other around 0.18 V in the case of modified enzyme prepared as described under experimental section (Fig. 1). The redox wave at 0.18V may be attributed to that of ferrocenyl group appended to the enzyme based on the known electrochemical response of this system. Here, we may quote for example, that methylferrocene derivatives give a reversible redox process around 0.18V vs SCE [8]. The broad

redox wave around −0.3 V (indicated by arrows in Fig. 1) may be attributed to that of FAD in the modified enzyme from its known similar behaviour, such as when entrapped in a conducting polymer matrix [9] or molecular wired enzyme on a Pt electrode with a conducting polymer. [10]. Further, the peak currents for the process at 0.18V and −0.3V show square root dependency on potential sweep rate, indicating that the process is diffusion controlled [11].

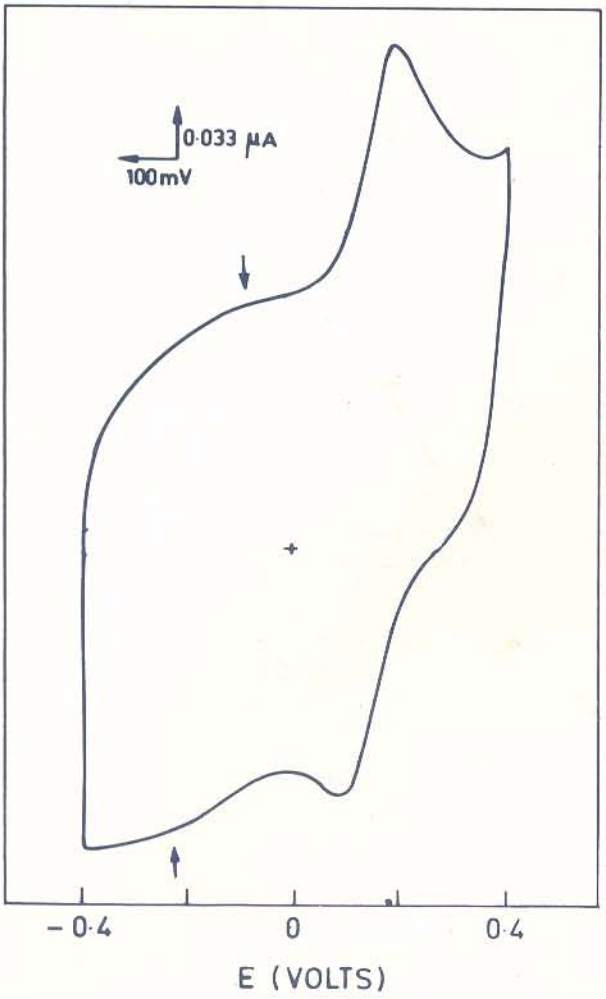


Fig 1: Cyclic voltammogram of modified GOx enzyme. The scan rate is 1000 mV.s^{-1} and 1 mg of enzyme.ml^{-1} in phosphate buffer (pH 7.0)

The cyclic voltammogram of the modified GOx in presence of glucose (20 mM) is recorded and given in Fig. 2 (curve A). For comparison, the cyclic voltammogram of modified enzyme alone and unmodified GOx with glucose are also given (Fig. 2, curves B & C. It can be seen from the figure that the redox peak at 0.18V changes into a pleateau with electrocatalytic behaviour and the kinetic behaviour of the response. This behaviour arises due to the ferroceneium ion, formed by the electrochemical oxidation, accepting an electron from the reduced enzyme and regenerating GOx(oxidised) which further oxidises glucose in the solution in a catalytic mode. The plausible mechanism is depicted in Scheme 2.

To substantiate further the involvement of a modified enzyme and not the unmodified ferrocene present in solution, a control experiment as described below has been carried out. A mixture of GOx and ferrocene alcohol (2 mg.ml^{-1} each) in phosphate buffer was dialysed for a period of 4 h. The cyclic voltammmetric curve of

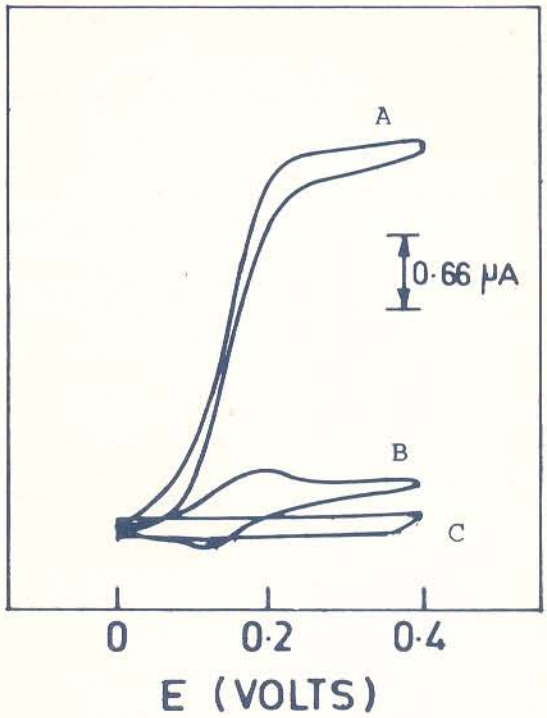
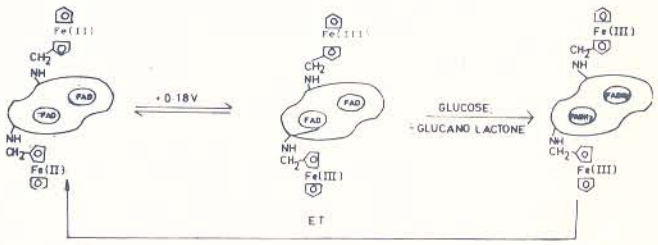


Fig 2: Cyclic voltammogram of modified GOx (A) with 20 mM glucose (B) without glucose and (C) GOx with 20 mM of glucose. 1.0 mg of enzyme.ml^{-1} of phosphate buffer (pH 7.0). The scan rate is 5 mV.s^{-1}

the dialysed solution showed the presence of only trace quantities of the ferrocene alcohol, which in the presence of glucose gave a current of 0.04 μA as compared to 3.2 μA obtained with the modified enzyme described above.



Scheme 2: The possible mechanism of enzymatic oxidation of glucose by the modified enzyme.

From the foregoing results, it can be concluded that facile electrocatalytic oxidation of glucose takes place in the case of ferrocenyl derivative linked to enzyme molecule covalently and such oxidation is insignificant in the case of unmodified enzyme.

The lifetime of the modified enzyme is about 15 days without loss in the activity of the enzyme as observed by cyclic voltammetry. Preliminary experiments are being carried out to fabricate enzyme sensor for glucose using the modified enzyme by electrochemical adsorption onto an electrode surface and wiring the modified enzyme by polypyrrole film.

Aknowledgement: *One of the authors S.V. thanks CSIR for the fellowship awarded.*

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