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Short communication

Stabilization of ferrocene leakage by physical retention in a cellulose acetate membrane. The fructose biosensor

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Abstract

The prevention of ferrocene leakage from an electrode by physical retention of mediator in a matrix of cellulose acetate membrane is reported. Five types of the cellulose acetate membranes were prepared, containing 1.8%, 5.3%, 8.5%, 20.0% of ferrocene and a membrane containing 1.8% of ferrocene and 0.05 % of Nafion® in the matrix. Ferrocene embedded membranes were successfully applied in the construction of a fructose biosensor by immobilization of PQQ-dependent fructose dehydrogenase (FDH). The biosensor comprising a cellulose acetate membrane with 1.8% of ferrocene and 0.05% of Nafion® had good stability characteristics, retained almost 40% of the initial response after 8 h of continuous use with an initial sensitivity of 226 nA mM^{-1} and response time of 75 s.

Keywords: Biosensor; Cellulose acetate membrane; Ferrocene; Fructose dehydrogenase

1. Introduction

The use of PQQ-dependent dehydrogenases represents a very interesting way to overcome problems associated with the use of oxidases and NAD-dependent dehydrogenases. Such enzymes are insensitive to oxygen fluctuations and do not need cofactor addition to the reaction mixture [1].

Fructose dehydrogenase is a complex of three different subunits with a molecular weight of 140 kDa and is able to oxidize fructose exclusively. This enzyme was isolated and partially characterized for the first time by Yamada et al. [2] in 1966. The enzyme permits determination of fructose in an easy and simple manner without using cofactors or other enzymes, comparing to other procedures which involve three coupled reactions requiring ATP and $NADP^+$. The oxidation of D-fructose to 5-keto-D-fructose results in the reduction of tightly bound PQQ to $PQQH_2$ [2]. External mediator is necessary for regeneration of the reduced cofactor ($PQQH_2$). Since the first use of fructose dehydrogenase for fructose determination in 1986 [3], amperometric detection based on either direct bioelectrocatalysis or indirect (mediated) bioelectrocatalysis is so far the most used.

Several mediators were employed for efficient fructose dehydrogenase regeneration, but ferricyanide is the most frequently used one. Ferrocene is also a mediator often used for biosensor construction [4], but the ferricinium as the water soluble oxidized form of ferrocene is easily leached out from the electrode surface.

Recently, very interesting approach for dichlorphenol–indophenol retention in a cellulose acetate membrane was invented and successfully applied in ascorbic acid detection [5]. This way of mediator incorporation leads us into the stabilization of ferrocene leaching by its incorporation into the cellulose acetate membrane. Ferrocene-embedded cellulose acetate membrane was further successfully applied in a fructose biosensor construction, when fructose dehydrogenase was spread onto this membrane.

2. Experimental

2.1. Reagents

Fructose dehydrogenase (FDH), cellulose acetate (CA), Triton X-100, dialysis membrane and fructose were supplied

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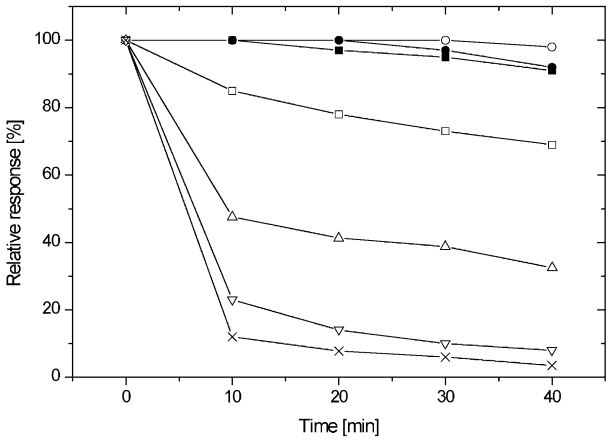

Fig. 1. Leakage of ferrocene from Fc-embedded CA membrane in time measured by cyclic voltammetry ($0 \rightarrow +500$ mV, scan rate 50 mV s^{-1}) in stirred McIlvaine buffer pH 5.5 at 25 °C. The sum of cathodic and anodic peak in 0 min was expressed as 100%. Legend: ○, CA membrane containing 1.8% of ferrocene (11 nmol cm^{-2}); ●, CA membrane containing 5.3% of ferrocene (33 nmol cm^{-2}); ■, CA membrane containing 1.8% of ferrocene (11 nmol cm^{-2}) and 0.05% Nafion®; □, CA membrane containing 8.5% of ferrocene (56 nmol cm^{-2}); △, CA membrane containing 20.0% of ferrocene (149 nmol cm^{-2} of ferrocene); ▽, glassy carbon electrode containing 190 nmol cm^{-2} of ferrocene without CA; ×, glassy carbon electrode containing 11 nmol cm^{-2} of ferrocene without CA.

by Sigma. Ferrocene was purchased from Merck. Nafion® was obtained from Aldrich. All other reagents were supplied by Lachema (Brno, Czech Republic).

2.2. Apparatus

Biosensor measurements were carried out on Amperometric Detector ADLC2 (Laboratorní přístroje, Prague, Czech Republic) using a glassy carbon electrode (GCE; Hochtemperatur-Werkstoffe, Therhaupten, Germany) as a working electrode (diameter 6 mm) and saturated calomel electrode (SCE) as a reference electrode.

2.3. Procedures

An unmodified cellulose acetate membrane was prepared according to the procedure published in our previous paper [6]. Ferrocene embedded cellulose acetate membrane (Fc-CA) and ferrocene/Nafion® modified cellulose acetate membrane (Fc-CA/Nafion®) were prepared by dissolving ferrocene or both ferrocene and Nafion® (5% alcoholic solution) in a cellulose acetate solution. Membranes composition was referred to dry weight.

For cyclic voltammetry study of mediator modified CA, the surface of GCE was covered either with Fc-CA or Fc-CA/Nafion® membrane and subsequently with a dialysis membrane rubbed with an O-ring. For biosensor construction, the stock solution of FDH in McIlvaine buffer of pH 4.5 containing 0.1% Triton X-100 (40 U ml^{-1}) was pipetted on GCE covered with Fc-CA membrane. Water was allowed to evaporate and a dialysis membrane was applied and rubbed with an O-ring.

3. Results and discussion

3.1. Basic characteristics of ferrocene embedded cellulose acetate membranes

Firstly, we tried to find out, whether ferrocene-doped CA membrane exhibits similar performances as the unmodified one. Our observations confirmed that besides the expected behaviour, the mediator-modified cellulose acetate membrane also efficiently prevents ascorbate access to the electrode.

Leaching of ferrocene from the CA membrane was monitored every 10 min by cyclic voltammetry from 500 to 0 mV with a scan rate of 50 mV s^{-1} for a period of 40 min under stirred conditions (Fig. 1). Increasing amount of ferrocene embedded in the membrane resulted in increased leakage of ferrocene. Leaching of the mediator from the modified CA membrane is probably caused by the limited ability of the membrane to physically retain ferrocene. Incorporation of Nafion® into the cellulose acetate membrane decreased the stability of the Fc-CA membranes. This fact can be ascribed to the presence of larger pores in the matrix of CA.

In the case of adsorbed ferrocene (11 nmol cm^{-2}), only 3.5% of initial response was observed after 40 min, compared to 98% for ferrocene fixed in the CA membrane (11 nmol cm^{-2}). A detailed work concerning the loading efficiency of the CA membrane and the behaviour of different compounds with different molecular weight on the sensor response was recently published [7].

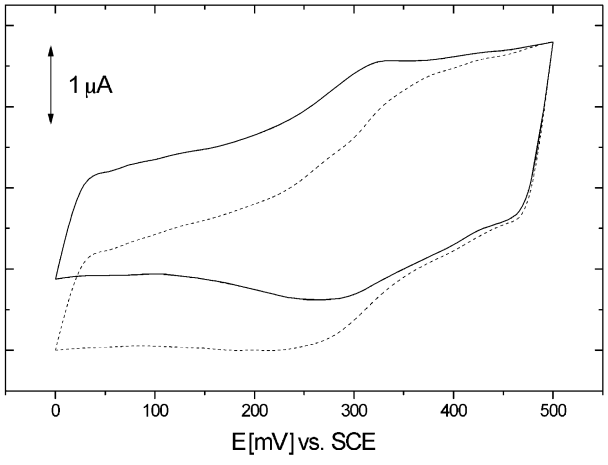

Fig. 2. Cyclic voltammetry of glassy carbon electrode covered with 20.0% Fc-embedded CA membrane on which 1.6 U of FDH was spread and covered with a dialysis membrane. Scan rate 50 mV s^{-1}, McIlvaine buffer pH 4.5. Legend: —— without fructose, ---- with 50 mM fructose. Measured at temperature 25±0.2 °C.

3.2. The effect of working potential and pH on the biosensor performance

Fig. 2 revealed a very efficient communication of ferrocene, retained in the CA membrane, between the electrode and the active site of the FDH. The sensitivity of the biosensor to the fructose addition was negligible up to 0 mV, then the sensitivity rose very rapidly and at +250 mV it levelled off. The working potential of +300 mV was used in further experiments.

With increasing negative charge in the CA membrane, optimal pH was shifted from 5.5 (1.8% Fc-CA) to 6.5 (1.8% Fc-CA/Nafion®). FDH is stable in the rage of pH from 4.5 to 6.0, so pH 6.0 was used also for Fc-CA/Nafion® membrane in further experiments.

3.3. Basic fructose biosensor characteristics

Our fructose biosensor based on 20.0% Fc-CA was very sensitive to fructose, with the detection limit of 3 μM. The biosensor stability decreased in the order: 1.8% Fc-CA > 1.8% Fc-CA/Nafion®>20.0% Fc-CA. This decrease of the biosensor activity is caused by enzyme inactivation and by ferrocene leakage. Then the enzyme inactivation seems to be the more important factor for the decrease of the sensor stability than the ferrocene leaching. We have observed that the most excessive mediator leakage occurred in the first 30 min of sensor operation and then the signal remained almost stable.

4. Conclusion

According to our results, the 1.8% Fc-CA/Nafion® membrane is the most useful one for the fructose biosensor construction, because of acceptable analytical characteristics and operational stability. The fructose biosensor could be very useful in the determination of fructose in fruits or juices or for the detection of lactulose after its enzymatic hydrolysis in milk samples.

Acknowledgements

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