A New Amperometric Sucrose Biosensor Based on Fructose Dehydrogenase

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Abstract

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A new, ferricyanide mediated amperometric biosensor for estimation of sucrose in biological fluids is proposed based on fructose dehydrogenase coimmobilized with baker's yeast as a source of invertase on the surface of glassy carbon electrode. Potential +420 mV was applied to the electrode against saturated calomel electrode. Maximum current was observed at pH 6, linearity of response ranged from 0.01mM to 0.29mM. Concentration of potassium ferricyanide 2mM was found as the best value.

Key words: biosensor; sucrose; fructose dehydrogenase; baker's yeast

Disaccharides are important constituents of foods and are often determined in routine analysis. However, measurement of sugars in foodstuffs presents some particular problems to analysts. Conventional methods are time- and laborconsuming and suffer from non-selectivity. Use of enzymatic methods is a possible way to overcome these problems. Among various enzymatic methods, there is a growing interest to use biosensors as a tool for analysis of sugars. Estimation of sucrose by biosensors is usually based on its hydrolysis by invertase, followed by oxidation of glucose by glucose oxidase, or another oxidase immobilized on the surface of Clark type (FILIPIAK *et al.* 1996) or H_2O_2 (MATSUMOTO *et al.* 1995) measuring amperometric sensors. These biosensors often posses various disadvantages coming from problems typical of Clark or hydrogen peroxide estimating electrodes, i.e., dependence on barometric pressure, high interferences due to high electrochemical potentials, as well as high cross interference of glucose occurring in tested materials. Moreover, the use of mutarotase as the third enzyme is obvious to accelerate the biosensor response via mutarotation of liberated glucose.

Fructose dehydrogenase (D-fructose:[acceptor] 5-oxidoreductase, EC 1.1.99.11, FDH), a paraquinoline quinone (PQQ) dependent oxidoreductase catalyzes oxidation of fructose to 5-oxofructose. Potassium ferricyanide or other substances like *p*-benzoquinone could serve as final electron acceptors in this process (MATSUMOTO *et al.* 1986; IKEDA *et al.*1990). High substrate specificity of this enzyme makes it an ideal tool for determination of fructose in various materials containing also other saccharides. Similarly, it is possible to use this enzyme for sucrose estimation, when connected with invertase in one sensor. Up to now, only a spectrophotometric assay (HOLMES 1997) and a chromatography column – enzyme reactor system with coulometric detection (KIBA *et al.* 1991) have been developed based on this principle.

The aim of this paper is to combine fructose dehydrogenase with sucrose hydrolyzing biocatalyst and to demonstrate our approach to the construction of sucrose biosensor based on the consequent reaction. To our knowledge, this is the first time when fructose dehydrogenase was used for sucrose estimation in an amperometric biosensor.

MATERIAL AND METHODS

Chemicals and Reagents. Fructose dehydrogenase 19 IU per mg solids from Sigma Chemical Company, St. Louis (USA), commercial dry baker's yeast from S. I. Lesaffre (France), potassium ferricyanide, sucrose and salts for buffer solutions were p.a. grade and purchased from domestic suppliers.

Preparation of Electrode: A rod of glassy carbon (diameter 7 mm) was cut off to obtain a disk which after polishing was connected to a copper wire by conductive epoxy

resin containing carbon powder. Connected disc electrode was attached into the teflon tubing by low molecular epoxy resin.

Preparation of Biocatalytic Layer: 5 µl of fructose dehydrogenase (190 IU/ml) and 5 µl of baker's yeast suspension (300 mg dry weight suspended to final volume of 5 ml of 0.1M Mc Ilvain buffer) were applied to the surface of the working electrode and such a biocatalytic layer was allowed to dry on air at ambient temperature. When dry, the layer was covered with wet dialysis membrane (MWCO 12,000) and fixed with teflon ring.

Experimental Set-up: A calibrated oxymeter from laboratory fermenter LF-2 (Vývojové dílny ČSAV, Czech Republic) was used in the electrochemical experiments together with two-electrode cell configuration consisting of glassy carbon working electrode and saturated calomel electrode. All measurements were performed at +420 mV in a thermostated cell with a magnetic stirrer. The cell was filled with 20 ml of appropriate buffer solution (0.1M Mc Ilvain pH 4.5–6 or 0.2M phosphate pH 6–7) doped with potassium ferricyanide as the mediator. Measurements were started by addition of 20 µl of sucrose solution, 20 g/l.

RESULTS AND DISCUSSION

The sensor was proposed for estimation of sucrose by oxidation of fructose liberated from disaccharide. Since simple application of commercial invertases to the electrode surface led to sensors with very short time of storage stability (few hours), commercial dry baker's yeast was chosen as the source of invertase. In this case, the enzyme is naturally stabilized by its immobilization on cell wall surface. Eventual consumption of glucose by the yeast does not cause any problem, since liberated fructose is the detected compound. This is also the reason why the sensor does not need mutarotase for transformation of α-D-glucopyranose to its β-anomer acceptable by glucose oxidase which is used in other types of sucrose biosensors. Potassium ferricyanide was chosen as a cheap and widespread material suitable for electrochemical mediation.

The maximum current response 390.5 nA/mM was found at pH 6.0 and concentration of ferricyanide 2mM (Figs 1 and 2). No current increase was observed above this concentration of mediator. Response of the sensor was relatively fast, 4–5 min. Linearity of the sensor ranged from 0.01mM up to 0.29mM of sucrose in the buffer solution (Fig. 3). This linearity is sufficient for at least 8 measurement equivalents corresponding to addition of 20 µl of sucrose solution, 20 g/l to the analytical system. The sensor was highly selective for sucrose, in a way that lactose, lactulose and glucose were not oxidized. One must, however, take into account that this sensor will oxidize also fructose, so parallel connection with fructose sensor containing only fructose dehydrogenase is proposed for samples containing honey, fruits or glucose-fructose syrups. Our simple arrangement without parallel connection was however suitable for sucrose estimation in evaporated sweetened milk (the results not shown).

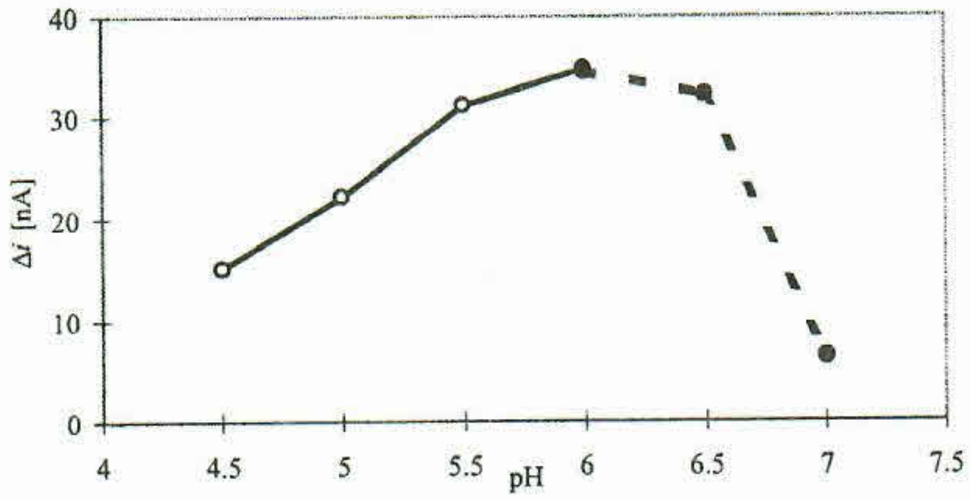

Fig. 1. pH profile of sucrose biosensor. Values Δi correspond to addition of 20 µl of sucrose solution (20 g/l) to 20 ml of 0.1M Mc Ilvain (o) or 0.2M phosphate (•) buffer solution containing 2mM potassium ferricyanide at 25°C

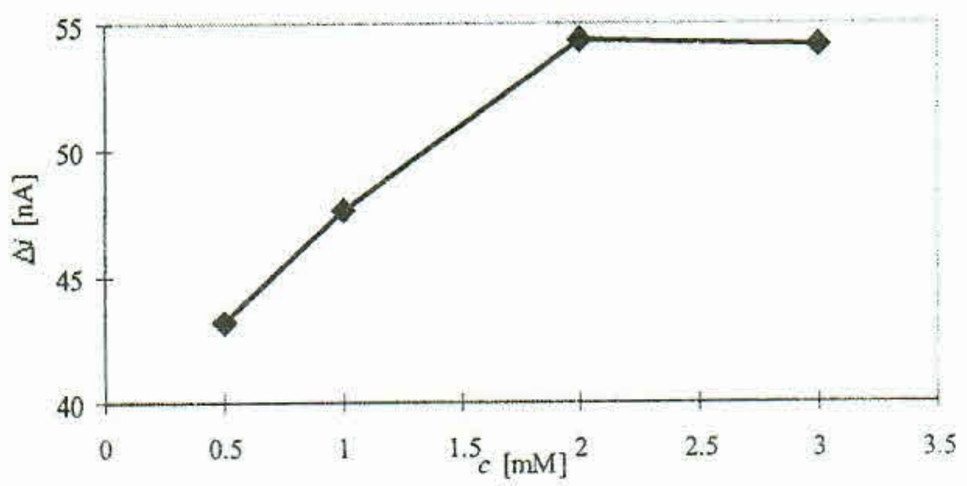

Fig. 2. Influence of potassium ferricyanide concentration on the response of sucrose sensor. Values Δi correspond to addition of 20 µl of sucrose solution (20 g/l) to 20 ml of 0.1M Mc Ilvain buffer solution pH 6 at 25°C

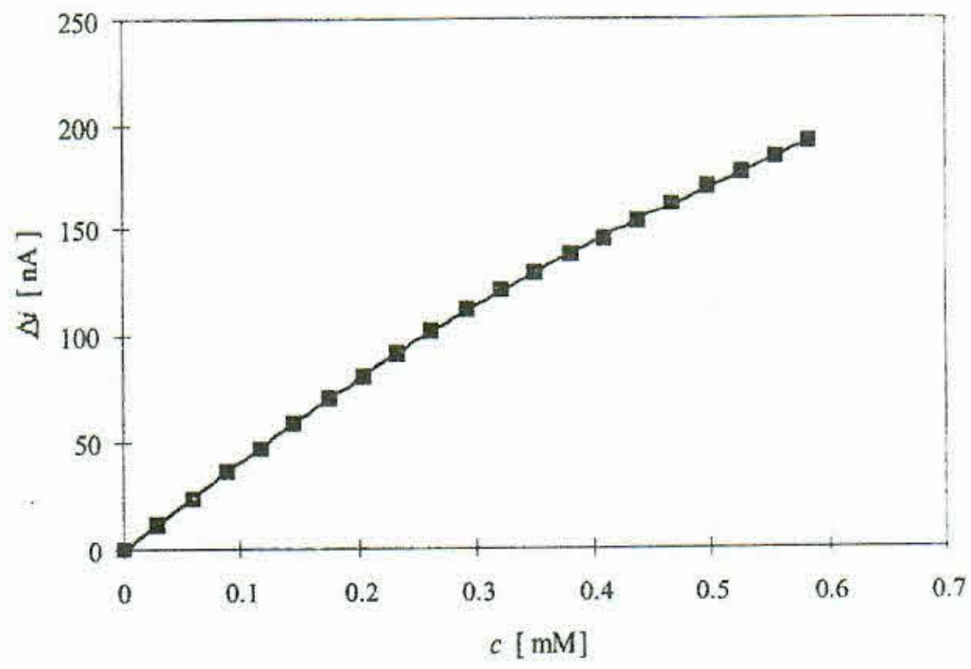

Fig. 3. Dependence of sensor response on sucrose concentration in 0.1M Mc Ilvain buffer solution pH 6 containing 2mM ferricyanide at 25°C

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Súhrn

MASTIHUBA V., LEPORISOVÁ B. (2000): **Nový ampérometrický senzor pre stanovenie sacharózy založený na fruktóza dehydrogenáze.** Czech J. Food Sci., **18**: 121–123.

V práci je opísaný nový amperómetrický biozenzor pre stanovenie sacharózy v biologickom materiáli založený na použití fruktóza dehydrogenázy koimobilizovanej s pekárenskými kvasnicami ako zdrojom invertázy na povrchu elektródy zo sklovitého uhlíka. Pracovný potenciál oproti nasýtenej kalomelovej elektróde bol +420 mV, ako mediátor bol použitý hexakyanoželezitan draselný. Maximálna prúdová odozva bola pozorovaná pri pH 6, linearita signálu bola v rozsahu koncentrácií substrátu 0,01 až 0,29 mM. Ako najvhodnejšia koncentrácia mediátora bola nájdená hodnota 2mM.

Kľúčové slová: biosenzor; sacharóza; fruktóza dehydrogenáza; pekárenské kvasnice

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