A yeast biosensor for glucose determination

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Received 18 December 1989/Accepted 23 July 1990

Summary. A yeast potentiometric biosensor for glucose determination is described. After induction of glycolytic enzyme synthesis a cell suspension of the yeast Hansenula anomala is retained in calcium alginate gel on the surface of a glass electrode. This biosensor gives a Nernstian response in glucose concentration of 5·10^-4-5·10^-3 mol/l with a response time of 5 min and a life-time of at least 2 months. Mannose and fructose are the only significantly interfering substances. The biosensor was used for measurement of glucose concentration in urine with results comparable to those obtained by a photometric enzymatic method.

Introduction

Determination of glucose in biological material is of a great importance both in medicine and the food industry. Electrochemical methods, based on glucose oxidase immobilization on surfaces of special electrodes, are common in many laboratories (Vadgama 1981). The possibility of measuring glucose concentration in turbid samples such as blood is their greatest advantage. Recently several glucose biosensors with immobilized whole cells have been described. In comparison with enzyme biosensors they do not need enzyme isolation, purification and stabilization on the electrode surface and their preparation is very simple and cheap. Glucose-metabolizing bacteria (Grobler and Rechnitz 1980; Grobler and van Wyk 1980; Karube et al. 1979; Vais et al. 1985), yeasts (Mascini and Memoli 1986) or a mould mycelium (Vincké et al. 1984) with various kinds of detection have been used for preparation of these biosensors. In our study we describe a yeast biosensor based on the aerobic yeast Hansenula anomala and a glass electrode as the most common type of electrode in clinical laboratories.

Materials and methods

Chemicals. Standard glucose solutions were prepared by dissolving D-glucose in working buffers: imidazole and sodium phosphate buffers (0.5 mmol/l), pH 7.4 and 8.0 were tested. All chemicals were purchased from Lachema (Brno, ČSFR) except for imidazole (Serva Feinbiochemica, Heidelberg, FRG) and sodium alginate (Belfco Biotechnology, Vineland, N.J., USA). All chemicals used were of analytical grade.

The glucose concentration in urine was measured photometrically after dilution with distilled water (1:100); using Oxochrom Glucose (Lachema) as a reagent kit.

Yeast culture. The aerobic yeast Hansenula anomala, purchased from the Institute of Chemical Technology in Prague, was cultured on Sabouraud's agar and then inoculated into liquid medium. The culture medium used was that of Baudras and Spyridakis (1971) except that L-lactate was replaced by D-glucose (0.3 mol/l) and trace elements were added (Racek and Musil 1987). After incubation at 25°C for 48 h, glucose (0.3 mol/l in 0.1 mol/l sodium phosphate buffer, pH 6.0) was added (1:4) to the culture and incubation continued for 12 h. During growth the culture was shaken on a linear shaker (50 beats/min). The cells were recultured three times to get the highest induction of glucose-convert- ing enzymes. The yeast cells were harvested by centrifugation (1000 g for 15 min), washed three times with physiological saline and then resuspended in the same volume of sodium alginate solution (3.2% in physiological saline). This viscous suspension was stored at +4°C.

Biosensor preparation and procedures. The cell suspension in sodium alginate solution (ca. 50 μl) was applied by brush on the surface of the bulb of a glass electrode GA 50N (Research Institute, Meinsberg, GDR) to give a thin compact layer. Then the bulb was immersed into a CaCl₂ solution (50 mmol/l in physiological saline) for 2 h at room temperature and at +5°C for a further 22 h. During this time a solid calcium alginate gel developed (Ogbonna et al. 1989).

Measurements were carried out in a thermostatted vessel with 20 ml imidazole buffer (0.5 mmol/l), pH 7.4, with CaCl₂ (50 mmol/l) under constant stirring. The temperature was kept at 25°C. The decrease in pH after addition of 0.2 ml glucose solution was observed using a digital pH meter, MV 870 (Präctronic, GDR), and registered by a linear recorder, TZ 4200 (Laboratorní přístroje, Prague, ČSFR). When biological material was used for glucose determination, the pH change of a second auxiliary electrode with heat-inactivated cells (60°C for 15 min) was subtracted from the result obtained by the working electrode. After the response had reached steady state, the biosensor was immersed into a new portion of working buffer, which was used for the next measurement as soon as the pH value returned to its original level.
After measurements the biosensor was stored either in working buffer at +4°C or in the same solution containing glucose (10 mmol/l) at room temperature; this solution was renewed daily.

Results

Analytical performance

Figure 1 shows a calibration graph obtained with the yeast biosensor under the finally recommended conditions. The lower limit of glucose detection is $5 \times 10^{-5}$ mol/l. The biosensor gives a Nernstian type of response in the concentration range $5 \times 10^{-4}$ to $5 \times 10^{-3}$ mol/l. Glucose concentrations higher than $2 \times 10^{-2}$ mol/l do not lead to a further increase in the biosensor response. Reproducibility defined as a coefficient of variation was 5.7% when a sample with a glucose concentration of 5 mmol/l in the measuring vessel was measured 25 times in a series.

The time course of the biosensor response is presented in Fig. 2. The steady state was reached in approximately 5 min; after rinsing the measuring vessel with a new volume of working buffer, 3–5 min were necessary for the pH to return to its original value.

The glucose concentration in 35 samples of urine of diabetic patients was estimated with both the yeast biosensor and a routine photometric method (with glucose oxidase, reagent kit Oxochrom Glukosa). The results were compared by linear regression (Fig. 3) and a t test for pair values. No significant differences between the results were observed.

Influence of buffer concentration and initial pH

Two different buffers were tested: sodium phosphate and imidazole buffer, both at pH 7.4 and 8.0. Their concentration increased from $5 \times 10^{-4}$ to $10^{-2}$ mol/l. In