MAGNETIC ENZYME MEMBRANES AS ACTIVE ELEMENTS OF ELECTROCHEMICAL SENSORS. LACTOSE, SACCHAROSE, MALTOSE BIENZYME ELECTRODES*

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1. Introduction

In the field of immobilized sequential enzymes Mosback and co-workers [1–3] have immobilized several multienzyme systems on to particules and studied their kinetic behavior. Hervagault et al. [4] and Lecoq et al. [5] have published theoretical and experimental studies on protein membranes bearing bienzyme systems. Enzyme electrodes were introduced in the preceeding paper [6] but the present one is the first dealing with bienzyme membranes as active elements of an electrochemical sensor. The results presented were obtained with a sensor based on a pO_2 electrode. Lactose, maltose and saccharose are measured with respectively β-galactosidase, maltase and invertase coupled to glucose-oxidase. In 1967 an electrode incorporating an immobilized glucose-oxidase membrane was described by Updike and Hicks [7]. The method depending upon the measurement of a differential pO_2 was used by Haynes and Siegelman [8] on whole blood and by Notin et al. [9] with a Clark oxygen electrode. Clark has described an oxidase electrode based on the polarographic oxidation of H_2O_2 and the same method has been used by Guilbaut and Lubrano [11] for producing a glucose electrode. Wingard et al. [12] described glucose electrochemical measurements with immobilized glucose-oxidase by constant current voltammetry.

In the device described here a magnetic film bearing the bienzyme system is fixed on a Clark pO_2 electrode bearing a cylinder magnet. The active bienzyme membrane is situated on the external surface of the gas permeable membrane. When the electrode is in contact with a solution containing the substrate to be measured an intramembrane production of glucose occurs, the glucose is then transformed into gluconic acid with a local oxygen consumption. A well defined relationship exists between local pO_2 level and substrate concentration in the bulk solution. It is important to note that with a polarographic electrode the current exhibits a linear variation as a function of the concentration.

2. Materials and methods

2.1. *Membrane production*

The procedure for the manufacture of magnetic enzyme membranes was described in the preceeding paper [6]. 10 mg of β-galactosidase (Kyowa Hakko) or 16 mg of invertase (Rapidase, from *Saccharomyces cerevisiae*) or 10 mg of maltase (Sigma, from *Aspergillus niger*) with 40 I.U. of glucose oxidase (Sigma from *Aspergillus niger*) were added to 2.5 ml of a solution containing 6% plasma albumin and 0.7% glutaraldehyde in a 0.02 M P_i buffer pH 6.8, for producing a 20 cm^2 membrane, thickness 40 μm.

2.2. *Enzyme activity measurements*

Lactase, invertase and maltase kinetics were measured coupled with glucose oxidase. Maltase activity measurement is given as an example. The

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activity was studied at 25°C with a maltose solution in a 0.1 M phosphate buffer at pH 6. The activity was measured by observing the formation of glucose by enzyme titration [13].

The glucose oxidase activity was measured using a 'pH-stat' in presence of 0.55 M glucose solution in 0.1 M phosphate buffer at pH 7.5. The pH value was maintained constant with 1N sodium hydroxide.

2.3. *Fixation of the magnetic enzyme membrane on a pO_2 electrode*

The magnetic enzyme membrane is maintained by the magnetic field on a pO_2 electrode (E 5040 Radiometer) modified according to the design described in the preceeding paper [6]. Magnetic materials were purchased from La Radiotechnique.

2.3. *Calibration of the electrode and measurements*

The coated electrode was calibrated by oxygen or air bubbling for the 100% point and by sodium sulfate and copper sulfate method for zero. During enzyme electrode measurements the bulk solution pO_2 is maintained by oxygen or air bubbling. The local pO_2 value along the electrode gas permeable membrane was recorded as a function of time for miscellaneous values of substrate concentrations in the bulk solution.

2.5. *Continuous testing of the bienzyme electrode*

Functions of substrate concentration in time are generated in a solution flowing along the active electrode. These functions are produced by an ultrograd pump (L.K.B. 11 300)

3. Theoretical section

Let us consider a coating (thickness e) between a bulk solution and the electrode surface. The reference axis is perpendicular to the membrane surface and the continuity equations for the lactase–glucose oxidase system choosen as an example, can be written as

$$\frac{\partial A}{\partial t} = D_A \frac{\partial^2 A}{\partial x^2} - V_{M1}\, f(A) \qquad (1)$$

$$\frac{\partial B}{\partial t} = D_B \frac{\partial^2 B}{\partial x^2} + V_{M1}\, f(A) - V_{M2}\, f(B, C) \qquad (2)$$

$$\frac{\partial C}{\partial t} = D_C \frac{\partial^2 C}{\partial x^2} - V_{M2}\, f(B, C) \qquad (3)$$

A, B, and C are saccharose, glucose and oxygen concentrations respectively. D_A, D_B and D_C are diffusion coefficients of the substances.

By using dimensionless parameters with e and K_M (Michaelis constant of the first enzyme) as space and concentration units, under stationary state conditions (1), (2), (3) can be written as:

$$\frac{\partial^2 a}{\partial x^2} = \sigma\, f(A) \qquad (4)$$

$$\frac{D_b}{D_a}\frac{\partial b}{\partial x^2} = -\sigma f(A) - \frac{V_{M^2}}{V_{M^1}}\,\sigma\, f(B, C) \qquad (5)$$

$$\frac{D_c}{D_a}\frac{\partial c}{\partial t} = -\frac{V_{M^2}}{V_{M^1}}\,\sigma\, f(B, C) \qquad (6)$$

The definition of the system is completed by the boundary conditions. The concentrations of saccharose, glucose, O_2 are constant (A_0, B_0 C_0) in the bulk solution and along the electrode surface the fluxes are

$$D_i \frac{dI}{dx\,1} = O$$

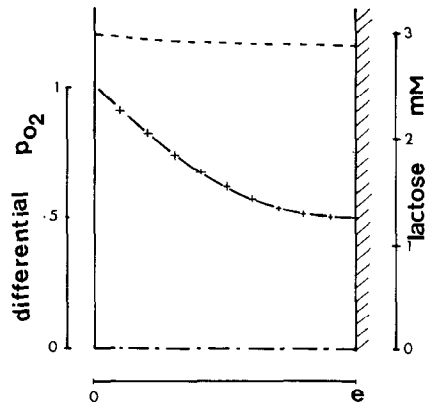

Fig.1. Calculated concentration profiles for lactose (----), glucose (—·—) and oxygen (—) within an active coating of a bienzyme electrode (σ = 0.1).

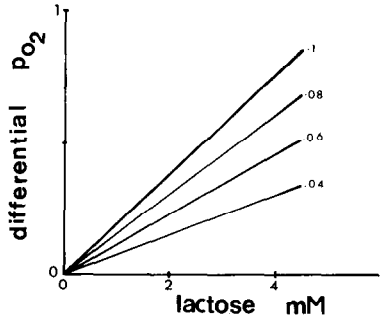

Fig.2. Calculated concentration along the electrode as a function of lactose concentration in the bulk solution for different values of the parameter σ.

These equations were solved numerically by computer according to the previously described methods [14]. Examples of calculated concentration profiles of substances inside the active coating are given in fig.1. The important point for applications is the relationship between the local pO_2 measured by the sensor and the saccharose concentration in the bulk solution. Calculated pO_2 values along the electrode surface as a function of the lactose concentration in the bulk solution are given fig.2 for several σ values.

4. Experimental results

The glucose oxidase was tested alone first. The output of that glucose oxidase electrode was studied as a function of glucose concentration in the bulk solution with different enzyme densities in the active layer (fig.3). A minimum level of activity is needed for getting the linear response of the sensor. Above this value, the glucose electrode can be used easily for a large range of glucose concentration (0 to 3 mM).

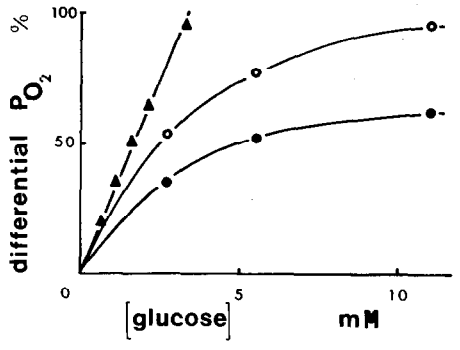

Fig.3. Calibration curves of a glucose oxidase electrode. Differential pO_2 as a function of glucose concentration in the bulk solution. Several calibration curves are given for increasing values of the enzyme activity within the active coating.

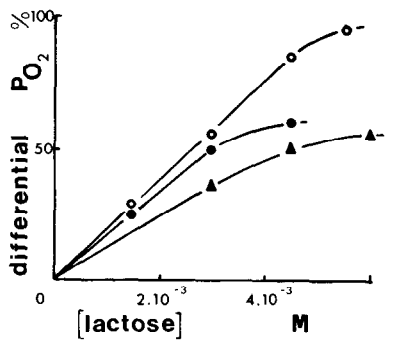

Fig.4. Calibration curves of a lactose bienzyme electrode. Differential pO_2 measured as a function of lactose concentration in the bulk solution. Calibration curves are given for different glucose oxidase activities within the bienzyme membrane:
$V_{M2} = 5.10^{-8}$ mol cm^{-3} min^{-1} (-▲-);
$V_{M2} = 2.10^{-7}$ mol cm^{-3} min^{-1} (-●-);
$V_{M2} = 2.10^{-6}$ mol cm^{-3} min^{-1} (-○-).

The results obtained with a magnetic protein membrane bearing both glucose oxidase and β-galactosidase are given on fig.4. The local pO_2 is measured as a function of the lactose concentration in the bulk solution with different glucose oxidase–

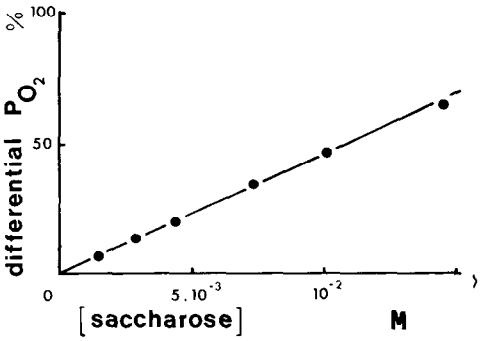

Fig.5. Calibration curve of saccharose bienzyme electrode. Differential pO_2 measured as a function of saccharose concentration in the bulk solution.

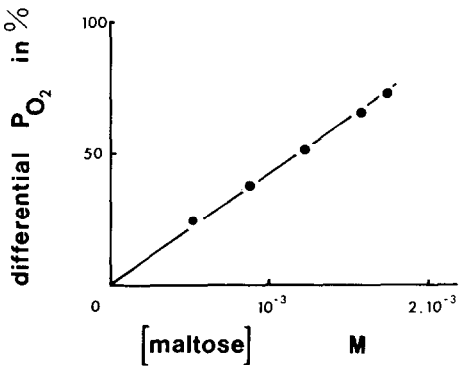

Fig.6. Calibration curve of a maltose bienzyme electrode. Differential pO_2 as measured as a function of maltase concentration in the bulk solution.

β-galactosidase activity ratios. A minimum ratio is needed in order to get a linear relationship between the sensor output and the lactose concentration. The behavior of maltose and saccharose electrodes is similar. The calibration curves dealing with maltase–glucose oxidase and invertase–glucose oxidase systems are given on figs.5 and 6 respectively. The linearity is obtained in both cases. The feasibility of using continuously the above described bienzyme electrodes was checked. A function in time of lactose concentration was generated with an 'Ultrograd pump' (LKB) in a solution flowing along a lactase-glucose oxidase electrode. The lactose concentrations generated and measured with the bienzyme electrode are given fig.7. The stability of the analytical system was checked.

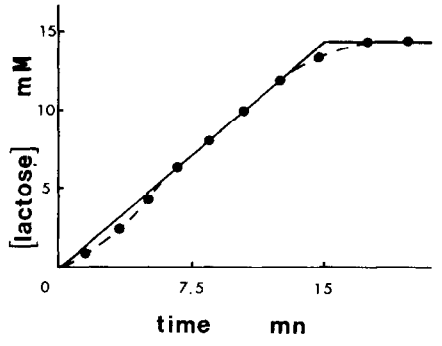

Fig.7. Lactose concentration actually generated (–) by the 'Ultrograd' pump and measured (-•-) with the enzyme electrode as a function of time. The solution was continuously along the active electrode.

5. Conclusion

The direct monitoring of sugars such as lactose, maltose, saccharose is not only useful at the applied point of view but also at the fundamental point of view for studying enzymology, especially in microbiology and fermentation. Bienzyme systems were extensively used in solution for analytical applications in industry and medicine. The progress in the field of immobilization of bienzyme systems [1–3], especially within membranes [4–5], makes possible the production of new analytical devices. From the studies dealing with concentration profiles in artifical enzyme membranes [14], evidence was obtained for a well defined relationship between the local concentration of a metabolite and concentration of the first substrate in the bulk solution. In the described systems a substrate is transformed into glucose within a membrane, the glucose is then transformed in gluconic acid with a local oxygen consumption. The local pO_2 level is linked to the glucose oxidase velocity, which is only linked to the glucose production, that is to say to the concentration of the first substrate. The enzyme electrode is based on the transformation of kinetic phenomena (reaction rates) into absolute values (local concentrations) through the diffusion–reaction coupling process.

The manufacture of magnetic enzyme electrodes [6] allows convenient use of the active sensors. The pO_2 electrode has some advantages, namely the specificity based on the selectivity of the gas permeable membrane and the linear relationship between the oxygen and the output of the electrode. pCO_2, pH, ion electrodes give a logarithmic response as a function of the concentration. The grafting of a multienzyme system on a sensor allows a study of sequential systems in a defined context with a measurement of the local concentration of the metabolites. The tool is useful for both kinetics [4] and regulation studies [5].

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