Symposium 13: In vivo analysis with biosensors

In vivo-Analytik mit Biosensoren

Development and some applications of amperometric biosensors and related analytical devices

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Entwicklung und Anwendungen von amperometrischen Biosensoren und verwandte analytische Systeme

Manuscript not received
Manuskript nicht eingegangen

Fresenius Z Anal Chem (1988) 330:335
© Springer-Verlag 1988

Potentiometric enzyme sensors

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Potentiometrische Enzymsensoren

An (electrochemical) enzyme sensor consists in general of an electrochemical detector (electrode) of some sort on which a layer of enzyme is immobilized. The enzyme catalyzes the reaction of an organic or inorganic substance (substrate) to produce a species (product) to which the electrode responds.

The detection of the product is possible with a number of techniques such as amperometry (which will be covered in the paper by Higgins at this conference), conductometry [1] or potentiometry. This paper is concerned with the latter category.

Potentiometry based on ion-selective electrodes has become a widely used electroanalytical technique over the past two decades. Next to the well-known pH glass electrodes, electrodes are available for various cations and a limited number of anions, based on either glass, solid state or liquid (plastic) membranes. Also, electrodes for protolytic gases like CO₂ and NH₃ are available, based on an indirect measurement with a pH electrode. The construction of enzyme sensors is not limited to the use of these "classical" ion-selective electrodes. During the past 5 years a considerable number of papers has been published concerning enzyme sensors based on ion-sensitive field effect transistors (ISFETs) [2]. The use of ISFETs may turn out to be advantageous as they can potentially be mass-produced at low cost. This, of course, is a prerequisite for the development of disposable enzyme sensors.

Potentiometric electrodes can in principle be used for the detection of any enzyme reaction in which ions or proteolytic gases are involved. Among others, sensors have been reported for urea, glucose, various amino acids, penicillin etc. [5]. In this paper, special attention will be given to enzyme sensors for urea.

The first potentiometric enzyme sensor was reported in 1969 by Guilbault and Montalvo [4] who immobilized the enzyme urease in a polyacrylamide gel held over the surface of a monovalent cation electrode. Urease catalyzes the following reaction:

The electrode of Guilbault and Montalvo responded to the change in ammonium ions and had an analytically useful range of 5×10^{-5} to 1×10^{-1} mol/l. Because a cation-sensitive electrode was used, interference was observed from sodium and

potassium in the sample solution. This interference can be corrected by the application of a second unmodified cation electrode in a differential measurement.

Ammonium and bicarbonate ions will always be in equilibrium with NH₃ and CO₂ respectively and thus, sensors in which the enzyme is immobilized on the permeable membrane of potentiometric gas-sensitive electrodes were developed [4]. From the reaction as given above it will be obvious that also a pH-sensitive electrode can be used for the detection of enzyme activity [4]. The first urea sensor based on a pH-sensitive ISFET was reported in 1983 [5].

A serious limitation of the sensors mentioned so far is the dependence of the response on the buffer capacity of the sample solution. If the sensor is based on the measurement of pH, this is quite obvious. However, as the equilibria NH₄⁺/NH₃ and CO₂/HCO₃⁻ are pH-dependent, also the sensors based on the detection of these species will heavily depend on the nature of the sample and the response will be non-linear.

As a possible solution to this buffer-dependent response, an ISFET-based urea sensor will be presented in which the pH in the immobilized enzyme layer is controlled via the generation of protons at a noble metal electrode that is integrated with the sensor [2]. In this way, the hydroxyl ions that are produced in the enzymatic reaction are constantly neutralized and the pH in the enzyme layer is kept equal to that in the sample. The magnitude of the proton-generating current that is required to obtain this situation is now a measure of the enzyme activity. The response is linear and independent from the buffer capacity of the sample. An important advantage of this system is that the enzyme is operating at a constant pH level. As a result the dynamic range of the sensor is increased.

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Fresenius Z Anal Chem (1988) 330:335
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