Biochimica et Biophysica Acta, 527 (1978) 277—281

BBA 68598

AN ENZYME ELECTRODE FOR ACETYLCHOLINE

PATRICK DURAND, ALAIN DAVID and DANIEL THOMAS

Laboratoire de Technologie Enzymatique, E.R.A. No. 338 du C.N.R.S., B.P. 233-U.T.C.-60206 Compiegne (France)

(Received March 6th, 1978)

Summary

A new enzyme electrode is described to measure continuously acetylcholine concentration. A coating containing active acetylcholinesterase is produced on a pH-glass electrode. The mean thickness of the coating is 50 μm. Optimal operational conditions with respect to buffer concentration, ionic strength, linearity, stability, sensitivity, pH of the bulk solution, and response time are studied and discussed. The use of acetylcholinesterase-containing membranes as sensors could offer several novel advantages.

Introduction

Perhaps the most interesting application of immobilized enzymes has been their use as the active element of an electrochemical probe or sensor. The enzyme electrodes described in the literature were recently reviewed [1,2].

Classical analytical procedures in most cases, require the precipitation or dialytic separation of proteins and particulate material. In contrast, electrochemical monitoring with enzyme electrodes can be carried out on whole blood or biological media, thus eliminating preparation of the sample. Enzyme electrodes can be used not only for medical and industrial applications, but also in basic biological research. It could be possible to measure continuously metabolite (e.g. transmitter, hormone) concentrations in vivo.

The use of an enzyme as a functional element of an electrochemical device was first reported by Clark and Lyons [3]. The earliest electrode incorporation of an immobilized enzyme, however, was described by Updike and Hicks [4] followed by extensive work done by Guilbault and coworkers [5].

The goal of the present paper is to describe an acetylcholinesterase electrode that allows the continuous recording of acetylcholine concentration in complex media. Goodson and Jacobs [6—8] have described a device based on an electrochemical cell containing an immobilized acetylcholinesterase pad. The device

was useful for the collection and detection of enzyme inhibitors from air and water. Guilbault and Iwase [9] have described an assay of cholinesterase in an electrode system with an immobilized substrate. Baum et al. [10] have described work on the liquid membrane acetylcholine electrode.

Methods

Enzyme electrode production. A coating containing active acetylcholinesterase was produced on a pH-glass electrode, by pouring a solution of gelatin and enzyme on the surface of an electrode bulb (Metrohm EA 125) rotating horizontally around its axis at 100 rev./min [11]. The solution was prepared from 240 bloom gelatin (from Bone-Rousselot) solubilized at 50 mg/ml and 50°C in distilled water. After solubilization, 0.25 mg/ml acetylcholinesterase (E.E.L.-Sigma) was added to the gelatin solution at 25°C. The mean thickness of the coating was 50 μm. The coating was dried at 25°C for 4 h and then immersed in 2.5% glutaraldehyde in 0.02 M phosphate buffer (pH 6.8) for 15 min.

The active electrode bulb was rinsed in the same buffer for 1 h and then introduced into a 10 mg/ml glycine solution for 4 h.

Calibration of the electrode and measurements. The enzyme electrode was connected to a pH-meter (E 300 B Metrohm) and introduced into a 2 ml temperature-controlled bath at a constant pH maintained with a pH state (E 525–535 Metrohm). The active electrode was stabilized at pH 7, the pH of the bulk solution. Buffer and salt concentrations are given for each experiment in the results section. Acetylcholine was then introduced into the solution and the variation of pH registered by the active electrode recorded as a function of time (Recorder Ricken Denshi). During the enzyme reaction the substrate is transformed into choline acetate with production of one H^+ per molecule of acetylcholine hydrolyzed. Due to the interaction of the enzyme reaction with membrane diffusion constraints [12], there exist localized regions of varying pH that are dependent upon reaction velocity and, in turn, substrate concentration in the bulk solution.

The capacity of the electrode for continuous measurements was checked by generating a function of acetylcholine concentration versus time using a gradient pump (Ultrograd, L.K.B.) to control the solution flowing past the enzyme electrode.

Results

The calibration of the electrode was done at pH 8 and 25°C with different phosphate buffer concentrations. In the presence of 0.1 M buffer, a linear response was observed in the range between 0.01 and 0.02 M acetylcholine. With a 0.01 M buffer, there was a linear response to substrate concentration between 0.1 and 2 mM. Without buffer the sensitivity was higher (Fig. 1) and a response was obtained with a concentration lower than 10^{-5} M. Fig 1 present results obtained under steady-state conditions. In sampling experiments, where fast response is desirable, it was better to use transient, rather than steady-state, conditions with enzyme electrodes (Romette, J.L. and Thomas, D., unpublished

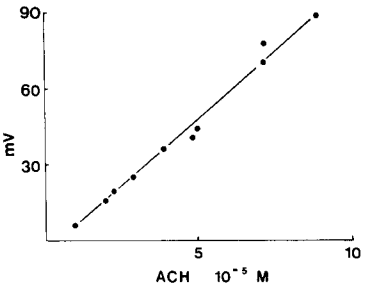


Fig. 1. Calibration curve of an acetylcholinesterase (ACH) electrode. Potential given by the enzyme electrode as a function of the acetylcholine (ACH) concentration under stationary state conditions. Measurements were done at pH 8 in 0.01 M NaCl solution without buffer (25°C).

data). The initial slope of the potential variation is plotted against acetylcholine concentration and a linear relation is observed between 0.01 and 0.1 mM (Fig. 2).

The stability over time of the enzyme electrode response under steady-state conditions was tested (Fig. 3). With a concentration of 1 mM acetylcholine, quantitative stability was observed for 40 days.

The influence of the pH of the bulk solution was studied and calibration was done under steady-state conditions for different pH values in presence of 1 mM phosphate buffer (Fig. 4). Between pH 6 and pH 8, the higher the pH the better the enzyme electrode response. This observation is in good agreement with the pH activity profile of acetylcholinesterase.

The electrode was tested during a continuous process. A function of acetylcholine concentration versus time was generated with a gradient pump (L.K.B., Ultrograd) in the solution flowing past the enzyme electrode. In Fig. 5, both the generated function and the observed response of the enzyme electrode are given. Clearly, the enzyme electrode is useful for studies on the continuous evolution of the process over time.

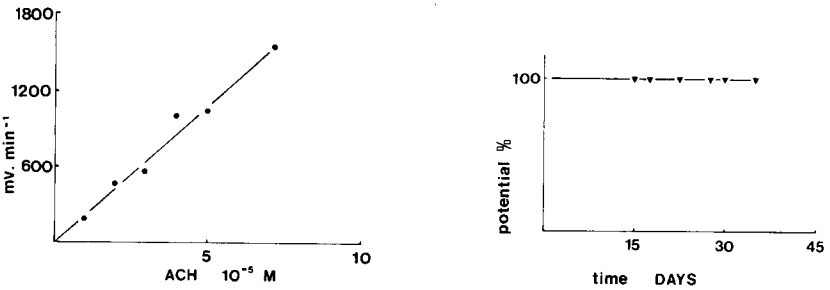
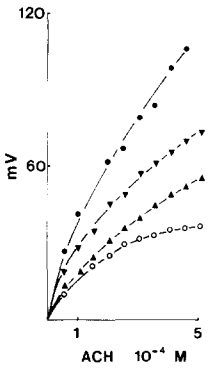


Fig. 2. Initial slope of the variation in time of the enzyme electrode potential as a function of acetylcholine (ACH) concentration during the transient state. Measurements were done at pH 8 in 0.01 M NaCl solution without buffer (25°C).

Fig. 3. Stability in time of the enzyme electrode response under stationary state conditions. Potential given by the acetycholinesterase electrode in presence of 0.05 mM acetylcholine as a function of the time of storage. Measurements were done at pH 8.5 in 0.01 M NaCl without buffer (25°C).



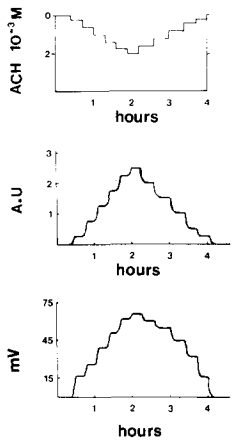


Fig. 4. Enzyme electrode response as a function of acetylcholine (ACH) concentration in phosphate buffer (1 mM) at pH 8 (○), 7.5 (▽), 7 (△), 6 (○), at 20° C.

Fig. 5. From the top to the bottom: Acetylcholine (ACH) concentration vs. time programmed in the 'Ultrograd' pump; Actual concentration (A.V.) generated by the pump as a function of time. The difference is due to the time-lag of the mass transfer; Electrode response as a function of time. The curves were generated within 4 h. The full scale deals with a concentration of acetylcholine from 0 to 2 mM. During the experiment the solution was continuously flowing along the electrode.

Conclusion

The product and the study of an acetylcholinesterase electrode is presented. The enzyme electrode was sensitive to concentrations lower than 0.01 mM and was stable for several weeks.

Enzyme electrodes which possess the important enzyme characteristics of specificity and high sensitivity constitute new tools for the analytical biochemist. In the analysis of blood components, for example, these methods promise to be much more convenient than the classical methods. Although many spectrophotometric methods make use of enzyme reactions (in free solution) because of their specificity, these procedures, in most cases, require the elimination of particulate material, a tedious procedure which can be bypassed using enzyme electrodes.

The use of acetylcholinesterase-containing membranes as sensors could offer several novel advantages: (a) it is a simple device made from a currently manufactured electrode, slightly modified by the use of an enzyme coating, (b) there is no consumption of enzyme thanks to immobilization, (c) consumption of substrate during measurements is negligible, (d) the sensitivity of the electrode can be sharpened by a systematic study of the coating parameters, (e) there is a continuous response by the electrode as long as it is in contact with the substrate solution, (f) the electrode has the potential to be refined for use as a miniature sensor.

The two last points are important for biological applications and in our group we are working toward manufacturing such an acetylcholine microelectrode (less than 1.5 μm) to be used continuously, in vivo, at a cellular level. One limitation of the method is the effect of buffer capacities and pH values on

the response of the electrode. It will be important to calibrate the electrode in the biological media in order to reduce the effect of the interference. These problems are the most important in the use of miniature sensors in vivo.

References

1 Gough, D.A. and Andrade, J.D. (1973) Science 180, 380
2 Thomas, D. and Caplan, S.R. (1976) in Membrane Separation Processes (Meares, P., ed.), 351, Elsevier, Amsterdam
3 Clark, L.C. and Lyons, C. (1962) Ann. N.Y. Acad. Sci. 102, 29
4 Updike, S.J. and Hicks, J.P. (1967) Nature 214, 986
5 Guilbault, G.G. (1976) Methods Enzymol. 44, 579
6 Goodson, L.H. and Jacobs, W.B. (1974) in Enzyme Engineering II (Pye E.K. and Wingard, Jr., L.B., eds.), p. 393, Plenum Press, New York
7 Goodson, L.H., Jacobs, W.B. and Davies, A.W. (1973) Anal. Biochem. 51, 362
8 Goodson, L.H. and Jacobs, W.B. (1976) Methods Enzymol. 44, 647
9 Guilbault, G.G. and Iwase, A. (1976) Anal. Chim. Acta 85, 295
10 Baum, G., Lynn, M. and Ward, F.B. Anal. Chim. Acta 65, 385
11 Quennesson, J.C. and Thomas, D. (1977) French Patent No. 77 15, 616
12 Thomas, D. and Kernevez, J.P. (1976) in Analysis and Control of Immobilized Enzyme (Thomas, D. and Kernevez, J.P., eds.), North-Holland Publishing Co., Amsterdam