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ELECTRICAL EXCITABILITY OF ARTIFICIAL ENZYME MEMBRANES

III. HYSTERESIS AND OSCILLATIONS OBSERVED WITH IMMOBILIZED ACETYLCHOLINESTERASE MEMBRANES

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Experimental evidence for memory and oscillations in artificial acetylcholinesterase membranes is presented. When acetylcholine is injected on one side of an artificial proteinic membrane bearing acetylcholinesterase, a potential difference is recorded as a function of time. The steady-state potential due to the enzyme activity for increasing and decreasing substrate concentrations exhibits a hysteresis loop. The non-linearity of the enzyme reaction coupled with the diffusion constraints cause also some instabilities, such as oscillations of the membrane potential.

1. Introduction

Organization in time and space is a fundamental property of living systems. The experimental evidence for instabilities in biochemical systems is mainly concerned with oscillations in homogeneous solutions involving principally the glycolytic system [1–3] and the peroxidase system [4,5].

Such phenomena were described as 'dissipative structures' [6] arising far from the equilibrium.

The problem of information storage in the phase of 'short term memory' has attracted considerable attention. A plausible mechanism for this phenomenon can be based on an all-or-nothing transition, involving hysteresis and metastable states [7]. Such phase transitions are thought to require structural changes in a macromolecular storage unit [8].

Synthetic enzyme membranes have been constructed, in which the internal microenvironment, as determined both by the membrane structure and by the local concentrations of reactants and products, exerts a profound effect on the mode of action of the enzymes [9,10].

By using an artificial urease membrane, Thomas et al. [11] demonstrated the existence of hysteresis phenomena through a simple mechanism moderated by a simple physicochemical process: diffusion. The system exhibited an auto-catalytic effect with reaction product.

An artificial protein membrane bearing papain activity was used by Naparstek et al. [12] to produce experimentally oscillation phenomena. The system also exhibited an auto-catalytic effect with H^+, a reaction product.

The present article is devoted to artificial acetylcholinesterase membranes. The similarity between both papain and acetylcholinesterase kinetic behaviour suggested that hysteresis and oscillations might be achieved with artificial acetylcholinesterase membranes.

2. Materials and methods

2.1. Enzyme membrane production

The artificial acetylcholinesterase membranes were prepared with the co-cross-linking method

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described in the preceding paper [15]. Acetylcholinesterase used was from electric eel (fraction V-S, Sigma; 1040 I.U./mg).

2.2. Potential difference measurement

Experiments were performed in a diffusion cell where the membrane separated two 25-ml compartments. The diffusion surface area was 0.5 cm^2. Identical 10^{-3} M phosphate buffer solutions at pH 7.5 were stirred continuously in both compartments. Acetylcholine chloride was injected into one compartment and the potential difference between the two compartments was then measured using a vibrating-reed electrometer with calomel reference electrodes.

The pH was regulated on each side of the cell with pH stats. The determination of the effect of acetylcholinesterase activity was obtained by comparing the resulting potential differences with membranes, with and without acetylcholinesterase.

2.3. Hysteresis of the membrane potential

The inactive protein used for enzyme membrane production in hysteresis experiments was ossein gelatin. Acetylcholine chloride (1 M) in phosphate buffer (10^{-3} M, pH 7.5) was injected into one compartment. Decreasing concentrations of substrate were obtained by successive dilutions in the same buffer.

2.4. Oscillations of the membrane potential

In these experiments, the inactive protein was bovine serum albumin (Sigma, fraction V). To abolish oscillations, eserine (physostigmine, from Sigma) was added at a final concentration of 5×10^{-5} M.

3. Experimental results

3.1. Hysteresis of the membrane potential

The artificial membrane used was produced with ossein gelatin containing about 50 I.U./cm^2.

Increasing acetylcholine chloride concentrations were injected into only one compartment; decreasing concentrations were obtained by successive dilution.

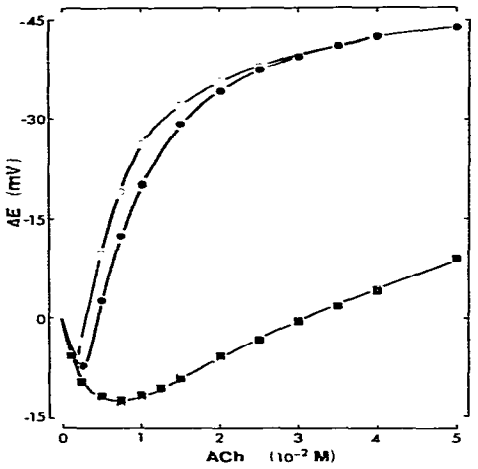

Fig. 1. Ossein gelatin membranes. Membrane potentials under steady-state conditions as a function of increasing acetylcholine concentrations for active (●) and inactive (■) gelatin membranes and of decreasing substrate concentrations for active (○) and inactive (□) membranes. Measurements were performed in phosphate buffer (1 mM, pH 7.5) at 25°C.

The potential difference measured under steady-state conditions with membranes, with and without acetylcholinesterase, is shown in fig. 1. Steady potential difference as a function of substrate concentration exhibits hysteresis loop when

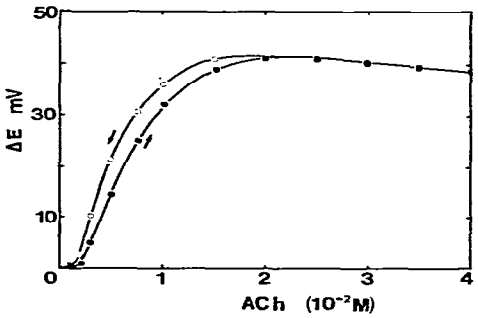

Fig. 2. Membrane potential due to acetylcholinesterase activity in ossein gelatin membranes as a function of increasing (●) and decreasing (○) acetylcholine concentration.

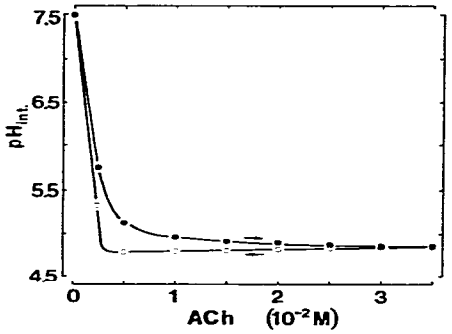

Fig. 3. Calculated intramembranal pH of acetylcholinesterase/gelatin membranes as a function of increasing (●) and decreasing (○) acetylcholine (ACh) concentration.

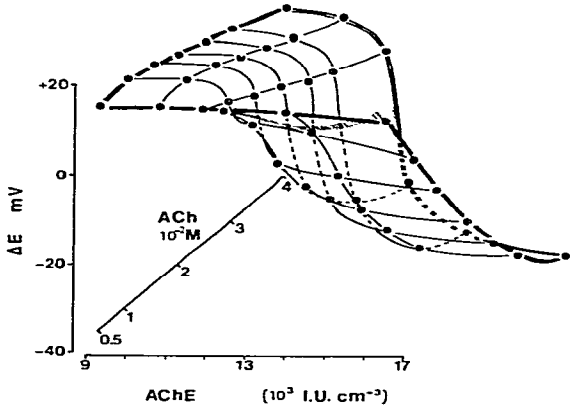

Fig. 4. Bovine serum albumin membranes. Membrane potentials of acetylcholinesterase/albumin membranes as a function of acetylcholine concentration and enzyme concentration immobilized in the membrane. Enzyme concentration is expressed as I.U. per membrane volume unit. Membrane thickness was 50 μm. When the system crossed the area depicted by hatching, oscillations occurred.

the membrane bears acetylcholinesterase. The contribution of the enzyme activity to the potential difference (fig. 2) is sigmoid shaped for increasing and decreasing substrate concentrations.

By comparing the resulting potential differences for membranes with acetylcholinesterase at pH 7.5 and for membranes without acetylcholinesterase at different external pH values, it is possible to calculate approximately the corresponding internal membrane pH. Results are given in fig. 3.

3.2. *Oscillations of the membrane potential*

The artificial membranes used were produced with bovine serum albumin containing varying enzyme concentrations. The potential difference

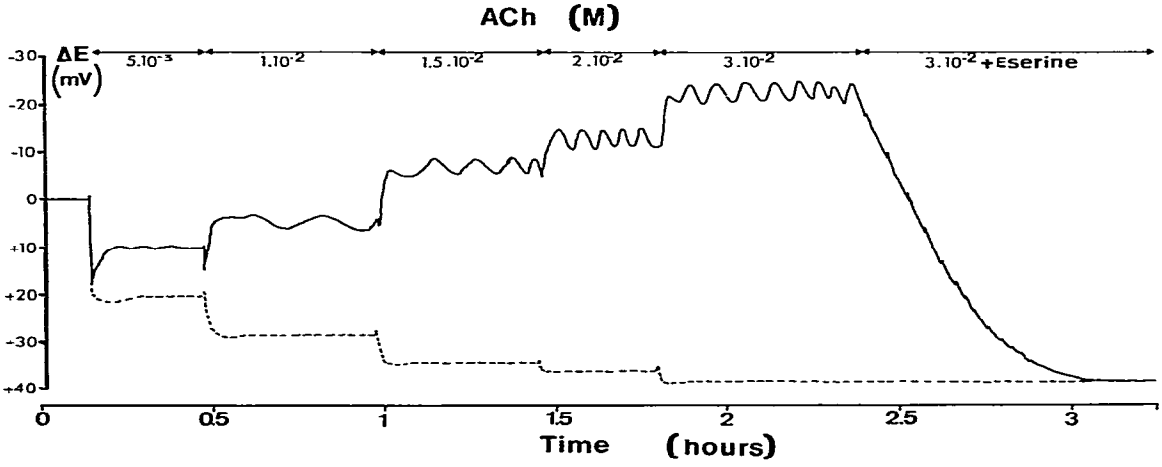

Fig. 5. Membrane potential recorded as a function of time when oscillations occurred with an acetylcholinesterase/albumin membrane. Dashed lines indicate the potential recorded with inactive membranes. Upon addition of eserine (5×10^{-5} M) oscillations vanish.

was studied as a function of both substrate and membrane enzyme concentrations.

The three-dimensional surface obtained by plotting the potential difference vs. substrate and enzyme activity (fig. 4) exhibits a break. In a narrow area (hatched zone) instabilities and oscillations occurred.

The oscillations obtained are given in fig. 5. These oscillations were sustained for at least 3 h. The amplitude remains constant (3–5 mV) and the period depends strongly on substrate concentration: for 10^{-2} and 2×10^{-2} M the period was 13 and 4 min, respectively. Upon addition of eserine, an enzyme inhibitor, oscillations vanish.

4. Discussion

Experimental and theoretical studies reveal that the catalytic properties of bound enzymes can be quite different from those in solution. Memory in enzyme membranes was described by Naparstek et al. [13]. The authors demonstrate the existence of hysteretic phenomena, through simple kinetic mechanisms which are shown by most enzymes. They refer to the two simple cases: auto-catalysis by the product and inhibition by excess substrate.

Acetylcholinesterase artificial membranes exhibit an auto-catalytic effect with the enzymatic production of H^+. When coupled with diffusion of the product and substrate, respectively, there exists, because of the non-linearities in the reaction rate, the possibility of three stationary states, of which two are stable and one instable, over a limited range of parameter values. Due to the amphoteric properties of the membrane, the hysteresis of the internal pH is transformed into a hysteresis of membrane potential.

So, enzyme reaction rate depends not only on the metabolite concentrations but also on the history of the system.

The non-linearity of the enzyme reaction coupled with the diffusion constraints can also cause some instabilities such as oscillations of the membrane potential. The enzyme acetylcholinesterase which has been extensively studied does not oscillate under any conditions of pH and substrate concentration when in solution. The period varies with substrate concentration from 4 to 13 min and the oscillations are abolished by the introduction of an enzyme inhibitor. The phenomenon can be explained by the auto-catalytic effect and by a feed-back action of buffer diffusion in from the outside solution.

5. Conclusion

The aim of the present paper is to show that an enzyme immobilized within a simple and defined membrane can give rise to such sophisticated behaviour as memory and oscillations.

The steady-state potential due to the enzyme activity for increasing and decreasing substrate concentrations exhibits hysteresis loop. Numerical simulations are now in progress to show that the present results can be interpreted in terms of a coupling between the enzyme reaction and diffusion process, without taking into account molecular effects.

The non-linearity of the enzyme reaction coupled with the diffusion constraints can also, over a limited range of parametric values, cause some instabilities, such as oscillations of the membrane potential. No oscillations were shown with the enzyme in solution, but it is interesting to note that oscillations of acetylcholine level during stimulation of the electric organ of the *Torpedo* were observed by Israël et al. [14].

References

1 B. Chance, E.K. Pye, A.K. Ghosh and B. Hess, Biological and biochemical oscillators (Academic Press, New York, 1973).
2 R. Frenkel, Arch. Biochem. Biophys. 125 (1968) 151.
3 B. Hess, A. Boiteux, H.G. Busse and G. Gerisch, Adv. Chem. Phys. 23 (1975) 137.
4 S. Nakamura, K. Yokata and I. Yamazaki, Nature 222 (1969) 794.
5 L.F. Olsen and H. Degn, Biochim. Biophys. Acta 523 (1978) 321.
6 I. Prigogine, Thermodynamics of irreversible processes, 3rd edn. (Wiley Interscience, New York 1967).
7 J.P. Changeux and J. Thiery, Biochim. Biophys. Acta Library 11 (1968) 116.
8 A. Katchalsky and R.A. Spangler, Q. Rev. Biophys. 1 (1968) 127.

9 D. Thomas, G. Broun and E. Selegny, Biochimie 54 (1972) 229.
10 F. Katchalski, I. Silman and R. Goldman, Adv. Enzymol. 34 (1971) 445.
11 D. Thomas, J.N. Barbotin, A. David, J.F. Hervagault and J.L. Romette, Proc. Natl. Acad. Sci. U.S.A. 74 (1977) 5314.
12 A. Naparstek, D. Thomas and S.R. Caplan, Biochim. Biophys. Acta 323 (1973) 643.
13 A. Naparstek, J.L. Romette, J.P. Kernevez and D. Thomas, Nature 249 (1974) 490.
14 M. Israël, B. Lesbats, J. Marsal and F.M. Meunier, C.R. Acad. Sci. Paris 280 (1975) 905.
15 A. Friboulet and D. Thomas, Biophys. Chem. 16 (1982) 145.