Biophysical Chemistry 15 (1982) 9–13
Elsevier Biomedical Press

ON EXPLORING THE BASIS FOR SLOW AND FAST OSCILLATIONS IN CELLULAR SYSTEMS

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Received 23rd July 1981

Key words: Immobilized enzyme; Acetylcholinesterase; Substrate inhibition; Cellular excitation; Membrane potential; (Oscillation)

We show that interesting oscillatory patterns may arise from an immobilized enzyme system, when the enzyme has the properties that it is inhibited by a substrate, produces H^- and has a pH-activity curve of bell-shaped form. In agreement with experimental observations in cellular excitations, the system generates fast oscillations that are superimposed on a slow cycle, at certain salt and enzyme concentrations.

1. Introduction

During stimulation of the electric organ of *Torpedo*, oscillations of acetylcholine were observed, with a slow wave and a rapid wave superposed on a slow wave [1]. Two oscillatory modes (spikes and bursts) are common in other cellular excitations [2,3]. Thus far, all the models proposed assume more than one self-oscillator to generate two different modes of oscillations [3–5]. On this note, we show that only one self-oscillator is sufficient to simulate an oscillation that has many different modes, if an oscillator has a property resembling that of acetylcholinesterase.

2. Model

We use a modified multi-barrier kinetic model [6] to write a mathematical expression for the time-dependent change in concentration involved in an oscillatory phenomenon:

$$\frac{dC_i^{(n)}}{dt} = k_n C_{i-1}^{(n)} F_{i-1}^{(n)}(\Lambda_i) - k_n C_i^{(n)} \times \left[F_i^{(n)}(\Lambda_i^{-1}) + F_i^{(n)}(\Lambda_{i-1}) \right] + k_n C_{i+1}^{(n)} F_{i-1}^{(n)}(\Lambda_{i-1}^{-1}) - R_i^{(n)}, \quad (1)$$

where $C_i^{(n)}$ is the concentration of the species (i.e., K^+, Cl^-, buffer, or substrate) at the ith site, and $R_i^{(n)}$ the consumption of the nth species at the ith site. In our case, $R_i^{(n)}$ is the enzyme activity R_i when n represents a substrate and is equal to zero when n represents a species other than the substrate. In the above equation, k_n is the rate of jumping for the nth species and is related to the diffusion constant D_n by:

$$k_n = D_n N_M^2 / L_M^2,$$

where L_M and N_M are, respectively, the length and number of multi-barriers that the molecule has to jump to reach the last site. In the same equation, $F_i^{(n)}(\Lambda)$ contains the sum of all the dissociated ions of the nth buffer and takes the following form:

$$F_i^{(n)}(\Lambda) = \frac{\Lambda^{Z_n} + \frac{K_{a1}}{[H^+]_i}\Lambda^{Z_n-1} + \frac{K_{a1}K_{a2}}{[H^+]_i^2}\Lambda^{Z_n-2} + \dots}{1 + \frac{K_{a1}}{[H^+]_i} + \frac{K_{a1}K_{a2}}{[H^+]_i^2} + \dots},$$

where K_{a1}, K_{a2},... are the first, second,... H^+ dissociation constants, and Z_n the charge of the nth buffer at the lowest pH. For $F_i^{(n)}(\Lambda)$ of pro-

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0301-4622/82/0000–0000/$02.75

tein, we write [7]:

$$F_i^{(\mathrm{Pr})}(\Lambda)=\sum_j \frac{N_j K_j^{(\mathrm{Pr})}}{[\mathrm{H}^+]_i}\Lambda^{Z_{\mathrm{Pr}}-j}\Big/\left(1+\frac{K_j^{(\mathrm{Pr})}}{[\mathrm{H}^+]_i}\right),$$

where Z_{Pr} is the protein charge at the lowest pH, N_j the number of the jth titratable group, and $K_j^{(\mathrm{Pr})}$ the intrinsic H^+ dissociation constant. The Λ appearing in the above equation is the partition coefficient defined as:

$$\Lambda_i = e^{\frac{F(\phi_i-\phi_{i-1})}{2RT}},$$

where F is the Faraday constant, ϕ_i the electrostatic potential at the ith site, and RT has its usual meaning.

In addition to eq. 1, the pH change with time can be obtained from the following expression by using a chain rule:

$$\frac{dq_i}{dt}=\Delta_{i-1}(\Lambda_i)-\Delta_i(\Lambda_i^{-1})-\Delta_i(\Lambda_{i+1})+\Delta_i(\Lambda_{i+1}^{-1})+R_i. \quad (2)$$

where q_i and Δ_i contain all the ions involved in the protonation process:

$$q_i=[\mathrm{H}^+]_i-[\mathrm{OH}^-]_i+[\mathrm{B}]_i\left(\partial F_i^{(\mathrm{B})}/\partial \ln\Lambda\right)_{\Lambda=1}+[\mathrm{Pr}]\left(\partial \ln F_i^{(\mathrm{Pr})}/\partial \ln\Lambda\right)_{\Lambda=1},$$

and

$$\Delta_i(\Lambda)=k_{\mathrm{H}}[\mathrm{H}^+]_i\Lambda-k_{\mathrm{OH}}[\mathrm{OH}^-]_i\Lambda^{-1}+k_{\mathrm{B}}[\mathrm{B}]_i\left(\partial F^{(\mathrm{B})}/\partial \ln\Lambda\right)_{\Lambda=\Lambda_i},$$

where [Pr] and [B] are, respectively, the immobilized buffer (i.e., proteins) and mobile buffer concentration.

We are particularly interested in an immobilized enzyme system, where the enzyme has the properties that it is inhibited by a substrate, produces H^+ and has a pH-activity curve of bell-shaped form. Acetylcholinesterase which is a key enzyme in cellular excitations has the above properties. An expression for the enzyme activity of acetylcholinesterase is known in the literature and is given by [8,9]:

$$R=\frac{V_{\max}[\mathrm{S}]}{[\mathrm{S}]+K_{\mathrm{sa}}+[\mathrm{S}]^2/K_{\mathrm{ssa}}}. \quad (3)$$

where

$$V_{\mathrm{m}}=k_2k_3[\mathrm{E}]/(k_2+k_3),$$

$$K_{\mathrm{ssa}}=(k_2+k_3)K_{\mathrm{ss}}/k_2,$$

$$K_{\mathrm{sa}}=k_3K_{\mathrm{s}}/(k_2+k_3).$$

In the above equations, k_2, k_3, K_{s} and K_{ss} take the following values [8,9]:

$$k_2=6\times10^4\Big/\left(1+\frac{[\mathrm{H}^+]}{10^{-7.2}}+\frac{10^{-10}}{[\mathrm{H}^+]}\right)\ \mathrm{s}^{-1},$$

$$k_3=10^4\Big/\left(1+\frac{[\mathrm{H}^+]}{10^{-6.5}}+\frac{10^{-10}}{[\mathrm{H}^+]}\right)\ \mathrm{s}^{-1},$$

$$K_{\mathrm{s}}=1.25\times10^{-4}\ \mathrm{M},$$

$$K_{\mathrm{ss}}=3\times10^{-3}\Big/\left(1+\frac{[\mathrm{H}^+]}{10^{-7.35}}+\frac{10^{-9.2}}{[\mathrm{H}^+]}\right)\ \mathrm{M}.$$

Here, [S] and [E] in eq. 3 are the substrate and enzyme concentrations, respectively.

An expression for the partition coefficient Λ which enters in eqs. 1 and 2 may be obtained by using the charge-neutralization condition and a boundary condition. In the absence of an applied voltage, the charge neutralization condition leads to

$$\sum_I k_I[\mathrm{I}^+]_{i-1}\Lambda_i-\sum k_I[\mathrm{I}^-]_{i-1}\Lambda_i^{-1}+k_{\mathrm{B}}[\mathrm{B}]_{i-1}\left(\partial \ln F_{i-1}^{(\mathrm{B})}/\partial \ln\Lambda\right)_{\Lambda=\Lambda_i}-\sum k_I[\mathrm{I}^+]_i\Lambda_i^{-1}+\sum k_I[\mathrm{I}^-]_i\Lambda_i-k_{\mathrm{B}}[\mathrm{B}]_i\left(\partial \ln F_i^{(\mathrm{B})}/\partial \ln\Lambda\right)_{\Lambda=\Lambda_i^{-1}}=0. \quad (4)$$

where $[\mathrm{I}^+]$ and $[\mathrm{I}^-]$ are the concentrations of singly charged positive and negative ions, respectively.

To simulate closely enzymes in vivo, we assume that the enzymes are surrounded by other proteins (in the present work, we used bovine serum albumin) and are immobilized uniformly over a matrix. The number and value of the pK_a of titratable groups in bovine serum albumin are known in the literature [10] and are: 1 α-carboxyl with $pK_a=3.75$, 99 β,γ-carboxyl with $pK_a=4.02$, 16 imidazole with $pK_a=6.9$, 1 α-amino with $pK_a=7.75$, 57 ϵ-amino with $pK_a=9.8$, 19 phenolic with $pK_a=10.35$, 22 guanidine with $pK_a=12.5$.

3. Computation and results

A two-time-step procedure was used to solve eqs. 1 and 2. A predictor method which is more

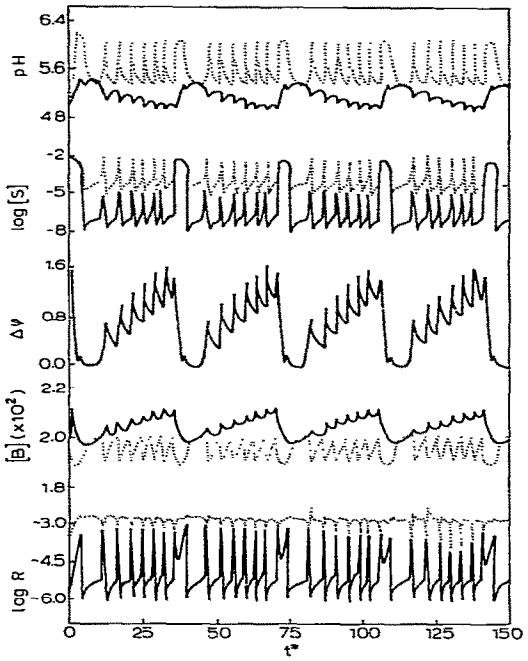

Fig. 1. The pH of the matrix, substrate concentration, membrane potential, phosphate buffer concentration, and enzyme activity versus t^*. Dotted and solid lines are, respectively, those at the site neighboring the bath and reflecting barrier. The parameters used are $N_M=2$, $V_{max}=32D_B/L_M^2$, $D_B=D_S=D_H=D_{OH}=D_K=D_{Cl}$, and $\Delta t=5\times10^{-4}$. The pK_a values of phosphate buffer were taken to be 2 and 7. The initial conditions and bath concentrations were as follows: $pH_o=7.5$, $pH_M=5.0$, $[KCl]_o=0.1$ M, $[KCl]_M=0.1$ M, $[B]_o=[B]_M=0.02$ M, $[S]_o=0.015$ M, $[S]_M=0$, and $[Pr]=2.5$ mM, where the subscripts o and M refer to the bath and matrix, respectively.

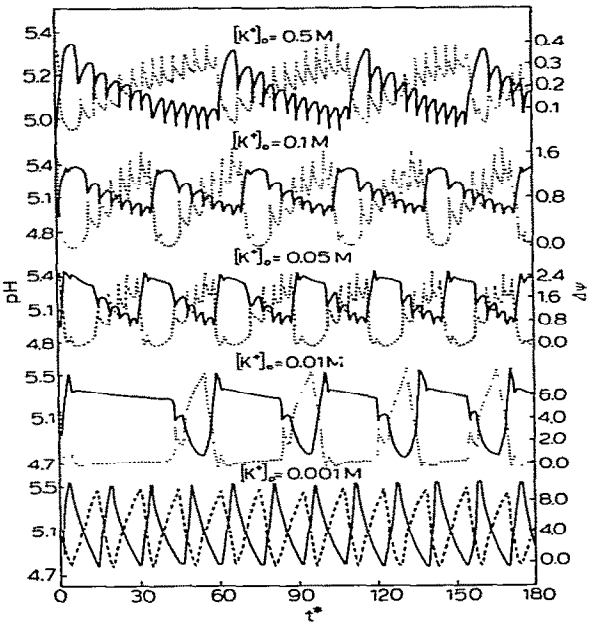

Fig. 2. Salt effects on the pH and membrane potential. Dashed and solid lines, are, respectively, the membrane potential and pH at the site neighboring the reflecting barrier. The parameters used are the same as those in fig. 1 except that $\Delta t=2.5\times10^{-4}$ was used for the computation. The initial conditions and bath concentration were the same as those in fig. 1 with the exception of added salt concentration, as indicated in the figure.

accurate than the two-time-step procedure was also used to check the accuracy of the former. Eq. 4 was solved by using a Newton-Raphson procedure. The bath concentration of all the species involved in the oscillation was kept constant. A reflecting boundary was placed at the last site. In order to facilitate the numerical integration, we assume that the product carries no charge. As in the case of acetylcholine, we assume that the substrate carries a positive charge.

Fig. 1 shows oscillations of the pH, substrate concentration, membrane potential, total concentration of phosphate buffer, and enzyme activity, at 0.1 M KCl added and $N_M=2$. Here, dotted and solid lines are, respectively, those at the site neighboring the bath and reflecting barrier. In this figure and the following three figures, t^* is a dimensionless quantity and is related to the actual time t by:

$$t^*=tD_B/L_M^2,$$

where D_B is the diffusion constant of the phosphate buffer. Here, the membrane potential is defined as the difference in potential between the last site and the bath; i.e.,

$$\Delta\psi=59.2\sum_{i=1}^{N_M}\log\Lambda_i^2$$

Fig. 2 compares effects of added salt on the mode of oscillation. Note that as the added salt

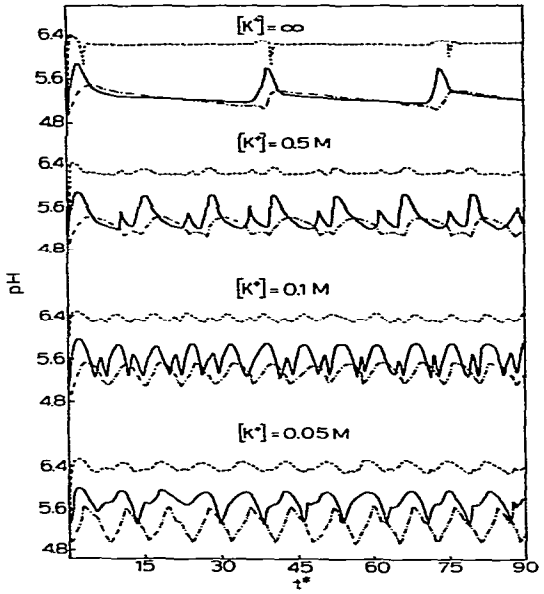

Fig. 3. Oscillation of pH with t^* when $N_M=3$. The initial condition and bath concentration were the same as fig. 1. The parameters used are the same as those in fig. 1 with the exceptions that $V_{max}=20D_B/L_M^2$ and $\Delta t=0.001$ were used in this computation. Dashed, solid and dashed dotted lines are the pH values at $N_M=1$, 2 and 3, respectively.

concentration becomes low, the period of the slow phase becomes shorter, while the number of spikes per burst becomes smaller. We find that at very low salt concentration the oscillations depend on the initial condition used. For example, at an added salt concentration of 1 mM, oscillations do not exist if the pH of the matrix is initially at 5.5, while this is not so at pH 5.0, as shown in this figure. At 5 mM added salt, on the other hand, no oscillation exists when the initial pH of the matrix is at 5.0.

Many interesting patterns of oscillations may arise as the number of jumping sites increases. We show an $N_M=3$ case in fig. 3 and an $N_M=6$ case in fig. 4.

It is interesting to note that in the β-cell oscillations [3], the burst period (i.e., period of slow cycles) increases as the added salt level increases [3]. This is consistent with our finding in fig. 2. It

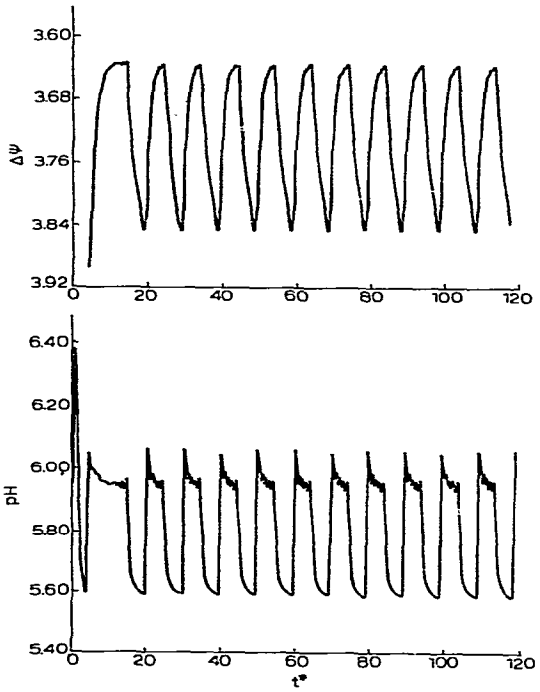

Fig. 4. Oscillations of membrane potential and of pH at the site neighboring the right bath when $N_M=6$. Left and right baths contained 1.85 mM phosphate buffer and 0.02 M KCl at pH 7.5. The left bath, however, contained 0.03 M substrate. Initially, the membrane contained the same amount of buffer and salt with no substrate as the right bath but the pH was at 5.5. The parameters used for this computation are: $V_{max}=10D_B/L_M^2$, $D_B=D_H=1$, $D_S=0.22D_H$, $D_{OH}=D_{Cl}=0.58D_H$, and $\Delta t=0.01$. Note that the reflecting boundary condition (which corresponds to the two baths having the same substrate, salt, buffer and H^+ concentration) is not used for this figure.

is also interesting to note that the membrane potentials shown in fig. 2 resemble the action potential observed in the experiments [2–4]. Thus, we hope our model may lead to an elucidation of mechanisms underlying the oscillatory phenomena observed in β-cells and neurons.

Acknowledgements

This work was supported by NSF Grant PCM 79 22483.

References

1 M. Israël, Y. Dunant, B. Lebats, R. Manaranche, J. Marsal and F. Meunier, J. Exp. Biol. 81 (1979) 63.
2 R.W. Meech, J. Exp. Biol. 81 (1979) 93.
3 I. Atwater, C.M. Dawson, A. Scott, G. Eddlestone and E. Rojas, in: Biochemistry and biophysics of the pancreatic β-cell, ed. W.J. Malaisse (Georg Thieme Verlag, New York, 1980).
4 E.K. Mathews and M.D.L. O'Connor, J. Exp. Biol. 81 (1979) 75.
5 V.V. Dynnik and E.E. Selkov, FEBS Lett. 37 (1973) 342.
6 T. Chay, Biophys. J. 30 (1980) 99.
7 C. Tanford, Physical chemistry of macromolecules (John Wiley and Sons, New York, 1961).
8 T.L. Rosenberry, Adv. Enzymol 43 (1975) 103.
9 J.F. Hervagault, A. Friboulet, J.P. Kernevez and D. Thomas, in: Kinetics of physicochemical oscillations, vol. 2, Sci. Comm. U.F. Franck, B. Hess, F. Schlögl and E. Wicke (1979) p. 379.
10 C. Tanford, S.A. Swanson and W.S. Shore, J. Am. Chem. Soc. 77 (1955) 6414.