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Spontaneous electrical potential oscillation on a filter impregnated with soybean lecithin placed between identical solutions of alanine

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Abstract

Filters impregnated with soybean lecithin, placed between two identical aqueous alanine solutions, display spontaneous electric potential oscillations. Alanine solutions used in a large concentration range from 1 mM to 1 M produce damped oscillatory behaviour with an exponential decay in time. The dependence of decay time on concentration shows saturation behaviour which is well fitted with a sigmoidal curve. Power spectra obtained by Fourier transform show peaks specific for each concentration. When fitted with a Lorentzian curve in the peak domain, the centre of the peak height and width at half height could be extracted. All these parameters depend on alanine concentration in a saturating pattern.

Keywords: Spontaneous potential oscillation; Soybean lecithin; Aqueous alanine solutions

1. Introduction

Fluctuations of physical chemical parameters are phenomena frequently observed in living systems from unicellular to complex organisms. Some of them display an oscillatory behaviour with periods ranging from seconds to years. Several of these fluctuations have been described as self-sustained phenomena unrestricted by external factors. They have been observed in neocortex and thalamus, characterised as a 'unified oscillatory machine' [1] or at the cellular level as pulsatory release of calcium ions from intracellular stores [2]. Other oscillations have been described in response to stimulation by extracellular signals. In this case, oscillations of membrane potential occur in cells as first steps towards more complex phenomena [3,4].

In order to characterise these biological

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rhythms, artificial systems were designed; attempts have been made to create experimental models with biological significance. These models, diverse in construction, helped also in understanding the physical and chemical aspects of biological oscillations [5–11]. Two principal kinds of experimental model have been used: (i) based on liquid interfaces; two or three immiscible liquids, forming one or two interfaces; and (ii) lipid or lipid analogues deposited on filters or polymeric substrates. Both types of experimental model allow either studies of self-sustained electrical potential oscillations, or those induced by application of external factors (electric potential or current).

At liquid–liquid interfaces, fluctuations of electric potential or current have been reported. The most common immiscible solutions used were water/nitrobenzene, water/dodecanol or water/octanol [5,6]. The electric potentials observed in these systems were very sensitive to physical and chemical aspects of the substances added in one of the liquids, usually in the aqueous phase. More 'subtle' characteristics of the added substance, like the degree to which it induces taste or smell sensations in humans, have an influence on the electrical potential. This encouraged the use of liquid–liquid systems as biosensors [7].

Oscillations of electric potential were observed also in polymeric membranes such as tri-block copolypeptide: $(\text{L-Glu})_{0.18}$–$(\text{L-Leu})_{0.64}$–$(\text{L-Glu})_{0.18}$ [8]. The polymeric membrane was placed between two salt solutions. These oscillations, in the frequency range 0.1–5 Hz, were dependent on the anion and cation compositions of the solutions.

Other study used a poly(methyl-L-glutamate) membrane with several lengths of diamine segments [9]. The interest was focused on membrane polymeric composition; the lengths of the diamine segments were modified. The oscillations were better expressed when longer diamine segments were used.

Filters impregnated with lipid analogues (triolein, dioleyl phosphate) produce oscillations in the presence of aqueous salt solutions. The properties of these membranes were improved, in the sense of stability and control of oscillatory behaviour, by application of an electric current [10,11]. Studies have been carried out with two types of methods: in symmetrical systems with identical concentrations of aqueous solution on both sides of the membrane, and in asymmetrical systems with a concentration gradient. The latter experimental method showed a better reproducibility.

A model closer to a biological membrane was obtained with the experimental set-up of Ikematsu et al. [12]. This study used a filter impregnated with soybean lecithin and incorporated with a channel-forming peptide (alamethicin), a light-activated proton pump (bacteriorhodopsin) and a basic protein (protamine) [12]. Oscillations occurred when the stimulating current was varied and the membrane was illuminated; they were observed only when the membrane potential reached approximately 60 mV.

The authors presumed that these oscillations were caused by the alternating change in ion selectivity between anion and cation. The complexity of this system with three integrated proteins makes the interpretation and understating of the phenomenon rather difficult.

We propose in this paper a simple symmetrical model: a filter impregnated with soybean lecithin, placed between two identical aqueous solutions. Spontaneous oscillatory behaviour, without current application, on this model membrane has not been described so far. We used this type of membrane in order to approximate to natural systems: the lipid with which the filter is impregnated (lecithin) is found in the membrane of all living systems and it is very stable in response to external noise or physical stress.

In our previous work on a triphasic liquid membrane (water/nitrobenzene/water), alanine produced relatively high amplitude electric potential oscillations. These electrical oscillations were presumed to be due to the equilibrium distribution of the alanine between water and an organic solvent [13]. This type of behaviour was considered as a combination of various weak damped harmonic oscillations, which in the Fourier spectrum was characterised by a set of peaks at frequencies specific for the substance at a given concentration.

In the present study, we describe the spontaneous behaviour of alanine on impregnated filters and we characterise it with respect to the decay of amplitude in time, as well as the dominant peak in power spectra obtained by fast Fourier transform. This report could be the basis for a systematic study of oscillations induced by amino acids in contact with lecithin.

2. Experimental

Cellulose ester filters with 0.2 μm nominal pore size (type GS, Millipore) were immersed in soybean lecithin (200 mg/1 ml hexane). Immersion time is arbitrary, until lecithin is absorbed on the filter surface. Zones which were completely and homogenously impregnated were chosen for experiments by visual inspection. After drying, the impregnated filter was placed between the two halves of a modified Ussing chamber (WPI) with a diameter of 4 mm and a volume of 3 ml. The Ussing chamber allows a stable position of the filter and the electrodes, and it is easy to avoid air bubbles, which is an advantage in our type of experiments. The halves were filled with alanine solutions (Sigma) of different concentrations. After each alanine experiment a control with pure water was done, which determined the baseline potential. Measurements were made with alanine at six different concentrations: 1 M, 0.1 M, 0.5 M, 50 mM, 5 mM and 1 mM.

Ag/AgCl electrodes were connected by 1 M KCl-agar bridges to a high-input impedance (10^{10} Ω) voltage amplifier (WPI) equipped with filters to reduce noise. The entire set-up was placed in a grounded Faraday cage to minimise electrical interference.

A PC analogue/digital acquisition card (Plc 812) with 16 bits precision was used to record the electric potentials. The acquisition rate was 10 samples/s and analysis was done with software written by ourselves. Fig. 1 shows the experimental set-up used in this study.

Approximately 20 experiments were performed at each concentration, and values are expressed as averages for each concentration. Oscillatory behaviour was followed in the concentration range

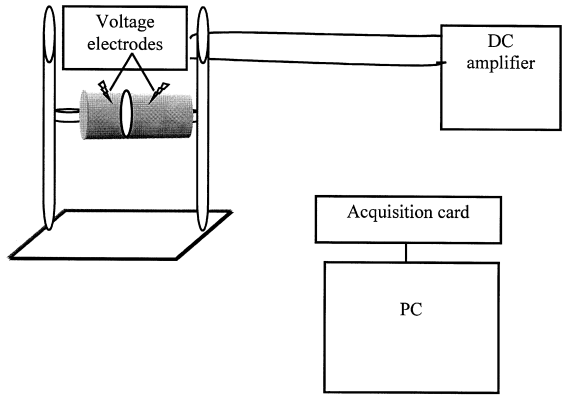

Fig. 1. Experimental equipment.

from the highest concentration that alanine is soluble (1 M) to the last alanine dilution we still observed them (1 mM). All experiments were performed at room temperature (20 ±2°C).

3. Results and discussions

3.1. Electric potential oscillations: amplitude and shape

When the impregnated filter was placed between the two halves of the Ussing chamber filled with distilled water, the potential was between 2 and 3 mV. For future analyses, we considered this the base line. When water was

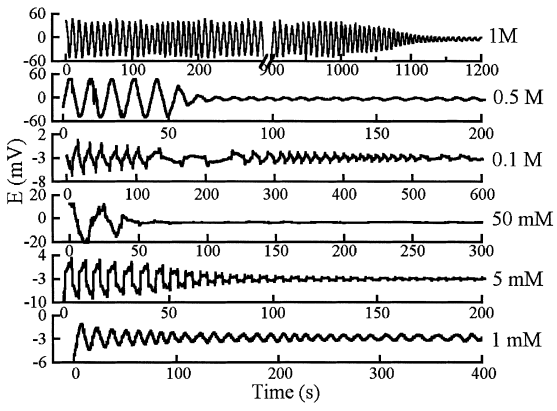

Fig. 2. Typical recordings of electrical potential oscillations for six different concentrations of aqueous alanine solutions.

replaced by aqueous alanine solutions, time-dependent electric potentials appeared. The amplitude of the potential was between 6 and 50 mV and decreased in time to the base line. As a control, all filters were tested with water after each alanine experiment in order to check for baseline drift.

Fig. 2 shows electrical potential oscillations recorded at different alanine concentrations. The oscillations had a lifetime of 2–15 min and most of them had a regular form.

The duration of the self-sustained oscillations is comparable with that obtained in the liquid system [13]. Oscillations with very long periods from several hours to days have been reported on impregnated filters, but only when a continuous current was applied [10,11].

The general behaviour of the time dependency of the electric potentials is that of a damped harmonic oscillation. We checked this by fitting the amplitudes of the oscillations in the decay region with an exponential function:

$$y = y_0 + P\mathrm{e}^{-(t-t_0)/\tau}$$

where t_0 is the time offset, y_0 is the electric potential offset, P the initial amplitude of the electrical potential oscillations and τ the time decay.

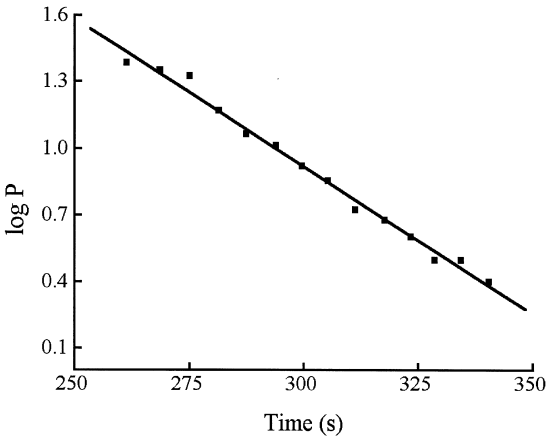

Fig. 3. Typical curve of logarithmic amplitude (expressed in mV) in time in the decay region. The points are fitted with a straight line by linear regression.

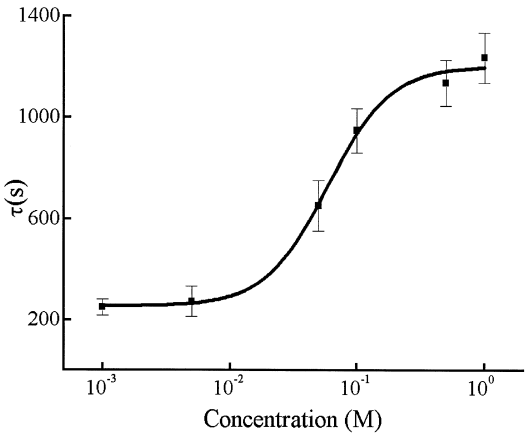

Fig. 4. Dependence of decay time on alanine concentration. Data are fitted with a sigmoidal curve: initial value of τ, Init (A1) = 254 ± 34 s; final value of τ, Final (A2) = 204 ± 39 s, half maximal concentration (C_m) is 50 mM.

Fig. 3 shows a typical curve for logarithmic values of amplitudes in the decay region in time. The points are fitted with a straight line, with a regression coefficient better than 0.96. We can therefore conclude that the type of behaviour is one of damped oscillation.

In Fig. 4 we show the dependence of decay time on alanine concentrations; decay time increases with increasing concentration. The concentration dependence shows saturation behaviour, and fitting the points with a sigmoid curve we can extract the concentration at half of the maximal decay time, which is 50 mM.

3.2. Frequency of oscillations

For a complementary discrimination between alanine concentrations, fast Fourier transform of each sample at every concentration was carried out. Although not often used in interpretation of fluctuations in artificial system, fast Fourier transform is still a convenient tool for the investigation of regular oscillations [8,13]. The power spectra obtained by fast Fourier transform showed maximal values in the low-frequency domain of 0.03–0.19 Hz. Although noisy, a distinct peak could be observed as showed in Fig. 5.

In our previous study on a triphasic system

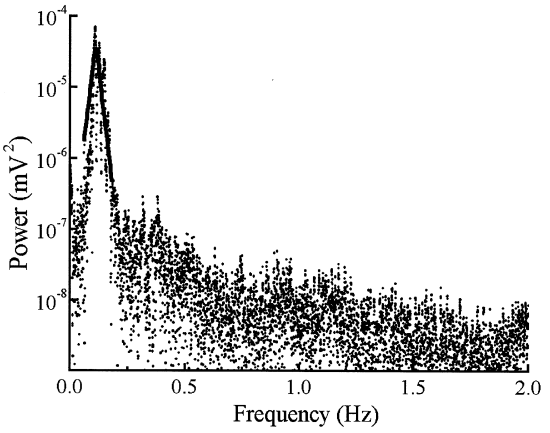

Fig. 5. Power spectrum obtained by fast Fourier transform at 0.5 M alanine. The domain of power spectra is fit with a Lorentzian curve.

(water/nitrobenzene/water) specific peaks were observed in the power spectra at each concentration used [13]. Substances studied from the point of view of taste were: sweet (alanine, glucose, sucrose) salt (NaCl, NaI, KCl) and bitter (atropine, caffeine, strychnine). From all of them alanine produced the most reproducible oscillatory behaviour. The frequency of the first maximum in the power spectra was found to be characteristic for each substance and concentration added in the passive phase [13]. We named the power spectra obtained by Fourier transform the 'fingerprint' of the substances used at a given concentration. In the concentration domain from 1 M to 1 mM characteristic frequencies were from 0.1 to 0.19 Hz, decreasing with rising concentration.

Peaks with lower amplitudes in the high-frequency domain are probably due to electrical noise, so the electrical oscillation with frequencies in the low domain is appropriate to characterise this system. We can extract important properties of the peak (centre, width at half height and height) by fitting the domain from the power spectra with a Lorentzian curve described by:

$$y = y_0 + \frac{2A}{\pi}\frac{w}{4(x - x_0) + w^2}$$

where y_0 is baseline offset, A is the total area under the curve from the baseline, x_0 the centre of the peak, and w the width of the peak at half height.

In Fig. 6 is shown the dependence of the peak centre frequency on alanine concentration. We show results obtained in the present study and in the previous one [13] on the triphasic system.

Centre values are spread over a narrow range (0.03–0.09 Hz) but still it can be seen that the frequency of the electrical potential oscillations increases with concentration. Fitting with a sigmoid, the half-maximal frequency is at 0.18 M. The frequency values obtained for the two systems (ref. 13 and the present study) are significantly different. Also it can be seen in Fig. 6 that the two systems have opposite behaviour. In the triphasic liquid system, the frequency decreases with alanine concentration; in the system using filters impregnated with lecithin, the frequency increases with concentration. Also, for the second system the behaviour shows saturation, and can be fitted with a sigmoidal curve.

Another parameter extracted from Lorentzian fitting is width at half height. The dependence of

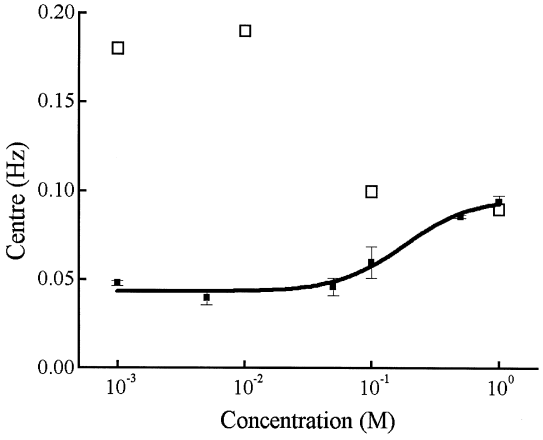

Fig. 6. Dependence of centre frequency on alanine concentration. (a) Black squares represent measurements in filters impregnated with soybean lecithin. The centre frequency of the peak was extracted by fitting a Lorenzian curve to power spectra as shown in Fig. 5. The fitted sigmoid curve (see legend to Fig. 4) shows a half-maximal concentration of 0.18 M: Init (A1) = 0.040 ± 0.003 Hz; Final (A2) = 0.10 ± 0.01 Hz. (b) White squares represent measurements in a water/nitrobenzene/water system (replotted from Cucu et al. [13]). Errors are smaller than symbol size.

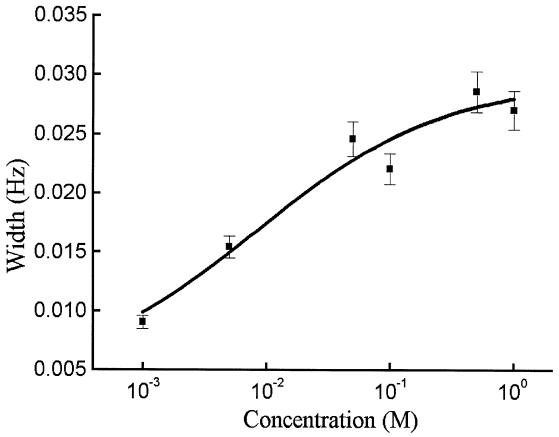

Fig. 7. Dependence of peak width at half-maximal power on alanine concentration. Values for the sigmoid fit are: Init (A1) = 0.003 ± 0.020 Hz; Final (A2) = 0.029 ± 0.006 Hz; C_m = 0.008 ± 0.022 M.

this parameter on alanine concentration was fitted with a sigmoidal in Fig. 7. The increase in width with concentration is not so obvious as for the centre frequency, and the half-maximal concentration is very low, 8 mM.

The height of the peak showed a clear dependence on alanine concentration also fitted with a sigmoidal. Saturation behaviour is also evident and the half-maximal concentration is 0.16 M (Fig. 8).

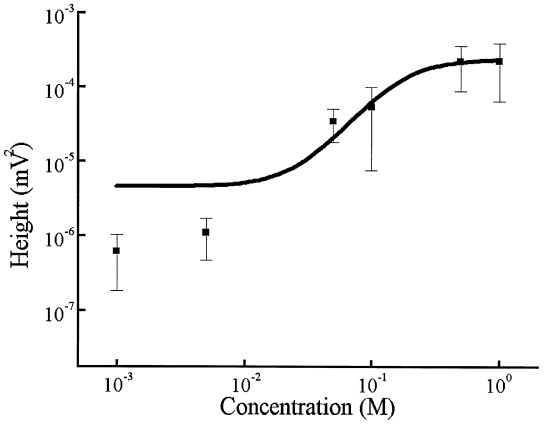

Fig. 8. Dependence of peak power on alanine concentrations. The sigmoid fit (see legend to Fig. 4) has the values: Init (A1) = $4 \pm 9 \times 10^{-6}$ mV^2; Final (A2) = $2.4 \pm 0.2 \times 10^{-4}$ mV^2; $C_m = 0.17 \pm 0.04$ M.

4. Conclusions

Filters impregnated with soybean lecithin placed between two identical aqueous alanine solutions produced spontaneous oscillations in electrical potential. Although simple, this system is a model close to natural membranes. Even though biological membranes have very complex structures, studying the influence on separate components will allow an appropriate interpretation [14].

External physical chemical factors like electrochemical gradients or continuous current were used in other studies in order to stabilise the oscillatory behaviour. In these systems the electric potential varied for at least several hours, in some cases for 10 days [10].

Despite the advantage of longer and more stable oscillation this system was complicated and interpretation became less accurate.

In the present study aqueous alanine solutions used in a large concentration range from 1 mM to 1 M produce a damped oscillatory behaviour with an exponential decay in time. Oscillatory behaviour had a shorter duration (3–20 min) compared to the systems where external stimuli were applied.

The dependency of time decay (τ) on concentration has a saturated pattern well fitted with a sigmoidal curve. From this curve we can observe that for higher concentrations oscillations damped faster than at low concentrations.

The fast Fourier transform method was used to investigate specific frequencies of the electrical potential time series. In a previous study on triphasic system we applied also fast Fourier transform in order to extract the characteristic frequency of each concentration of substances used. In the present study the position of the first peak in the frequency domain is more noisy than in our previous report. Fitting the peak domain with a Lorentzian curve we extracted the centre frequency of the peak, the width at half height and the height. This fact produced uncertainty in estimates of further characteristics of the peak (width at half height, height) — see values reported in the legends to Figs. 4–8. Nevertheless, the centre frequency of the peak in the low-

frequency domain (< 1 Hz) is clearly dependent on the alanine concentration.

Plotting height and width at half height parameters vs. alanine concentrations, values are not significantly different, but they can still be fitted with a sigmoidal curve describing saturation behaviour.

We can conclude that the process of interaction between alanine and lecithin deposed on a filter produces electrical potential oscillations with low frequencies in a specific range of concentrations, which is between 8 mM and 0.18 M for alanine.

Further studies are now in progress to systematically investigate electrical potential oscillations between amino acids and filters impregnated with soybean lecithin.

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