Differential regulation of proteins by bursting calcium oscillations—a theoretical study

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Abstract

Calcium in ionic form is a second messenger connecting several input signals to several target processes in the cell. The question arises how one second messenger can transmit more than one signal simultaneously (bow-tie structure of signalling). Experimental data on calcium dynamics often show patterns of successive low-peak and high-peak oscillatory phases, known as bursting. Here, we propose that bursting calcium oscillations can perform the function of simultaneous transmission of two signals at physiological calcium concentrations, for example, by selective activation of two calcium-binding proteins. This differential regulation by periodic bursting is investigated in a theoretical model. The two proteins are assumed to be activated by calcium, and one of them is assumed to be subject to biphasic regulation due to additional inhibitory binding sites. To explore which characteristics of the complex signal could be responsible for independent regulation of low-peak activated and spike activated targets, different bursting patterns of simplified square pulses are applied. Depending on the change in the bursting pattern, one protein can be gradually activated at a constant level of the other protein’s activity, or the two proteins can be activated simultaneously, or one protein can be activated while the other one is deactivated simultaneously. Thus, the two proteins can be regulated virtually independently.

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1. Introduction

Calcium oscillations play an important role in intra- and intercellular signal transduction (Berridge et al., 1998; Dupont et al., 2000). This phenomenon has been the subject of intense modelling studies (for a monograph, see Goldbeter, 1996; for reviews, see Dupont et al., 2000; Schuster et al., 2002; Falcke, 2004). A widely accepted hypothesis is that in calcium oscillations,
information is encoded mainly by frequency (Goldbeter, 1996; Kummer et al., 2000; Marhl et al., 1998a; Grubelnik et al., 2001; Dupont et al., 2003). However, a possible role of amplitudes and temporal pattern is also considered (Dolmetsch et al., 1997; Prank et al., 2000; Tompa et al., 2001; Rozi and Jia, 2003).

Interestingly, the effect caused by the oscillatory calcium signal is usually a stationary output, for example, upon fertilizing oocytes, generating an endocrine signal or enhancing the transcription of a gene. In many cells, a central role in the process of decoding is played by calmodulin (cf. Van Eldik and Watterson, 1998). This protein can bind both calcium and other proteins, such as calcium/calmodulin-dependent protein kinase type II (CaM kinase II, EC 2.7.1.123, cf. Colbran, 2004), myosin light-chain kinase (EC 2.7.1.117, cf. Stull et al., 1993), phosphorylase b kinase (EC 2.7.1.38, cf. Brushia and Walsh, 1999) and caldesmon kinase (EC 2.7.1.120, cf. Gorene et al., 2004). De Koninck and Schulman (1998) presented experimental results showing that CaM kinase II is well-suited for decoding frequency-modulated calcium signals. The protease calpain involves calmodulin-like domains and is also activated by calcium (cf. Tompa et al., 2001). Moreover, there are proteins that are activated by calcium without involvement of calmodulin. A prominent example is protein kinase C (EC 2.7.1.37, cf. Webb et al., 2000).

Several modelling studies on the decoding of calcium oscillations by calcium-binding proteins have been presented, mainly based on an enzyme cycle made up of a fast kinase activated by cytosolic calcium and a slow phosphatase (Dupont and Goldbeter, 1992; Gall et al., 2000; Salazar et al., 2004) or on the autophosphorylation of CaM kinase II, which enables a “molecular memory” (Prank et al., 1998; Dupont et al., 2003).

All the above-mentioned studies on the decoding of calcium signals concern simple calcium oscillations, i.e., sinusoidal, rectangular, or spike-like regular oscillations. However, experimental data on calcium dynamics often show patterns more complex than simple, regular oscillations (Dixon et al., 1990, 1993; Dixon, 2000; Kummer et al., 2000). A common pattern is a periodic or more or less chaotic succession of low-peak and high-peak oscillatory phases, known as bursting. Such a dynamics has been investigated in modelling studies of transmembrane potential oscillations in nerve cells (Goldbeter, 1996; Chay, 1997; Izhikevich, 2000; Doss-Bachelet et al., 2003) and of calcium oscillations (Borghans et al., 1997; Houart et al., 1999; Marhl et al., 2000, 2004; Kummer et al., 2001, 2003). It is worth noting that often in electric bursting, both the active phase and quiescent phase involve several spikes. In calcium bursting, by contrast, the active phase comprises only one large spike. It may be assumed that experimentalists often do not report complex oscillations because they consider them to be due to experimental “noise” and are in search for regular patterns.

Larsen and Kummer (2003) and Rozi and Jia (2003) were the first to study the decoding of complex calcium oscillations. Rozi and Jia (2003) did so for the model developed by Borghans et al. (1997) and Houart et al. (1999) and considered only one target protein (glycogen phosphorylase kinase, EC 2.7.1.38). Kummer and coworkers analysed in more detail whether decoding of bursting calcium oscillations differs from that of regular calcium oscillations (Larsen and Kummer, 2003; Larsen et al., 2004). They showed by computer simulations that information could be encoded in the shape and complexity of calcium oscillations, on the basis of the model proposed by Kummer et al. (2000). They considered cooperative, activatory binding of calcium to two different effector enzymes and showed that cooperativity offers a simple way to decode different calcium dynamics into different enzyme activity.

A striking feature of many signal transduction systems (such as those based on calcium oscillations) is that several inputs can influence several targets via only one or a few intermediary components. This architecture can be termed bow-tie (or hour-glass) structure (Csete and Doyle, 2004). The question arises how such an architecture can operate (Sommer et al., 2002; Larsen et al., 2004), and whether the multiple signals can be transmitted and decoded not only successively, but also simultaneously. An obvious idea is that the signal pattern contains at least two “structural” elements, such as in bursting oscillations, which are made up of spikes and low peaks.

Here, we tackle the question in which way periodic bursting may transmit two independent signals, like the selective activation of two calcium-binding proteins. We consider two different proteins, both of which are activated by calcium and one of which is characterized...
by a bell-shaped curve of activation due to additional inhibitory binding sites. Particularly, the separate activation by spikes and secondary peaks is investigated. We analyze frequency decoding by taking into account that regular bursting oscillations are characterized by two different frequencies. Moreover, it is analysed how the parameters of the two proteins should differ for this selectivity.

To separate the questions of the generation and decoding of bursting calcium oscillations (and for achieving a controlled change of frequencies), we simulate such oscillations by artificially generated square-shaped patterns. Such square-shaped pulses have also been used in experiments (De Koninck and Schulman, 1998) and in simulations (Li and Goldbeter, 1992; Salazar et al., 2004). Other authors (Gall et al., 2000) used artificially generated sinusoidal patterns.

The paper is structured as follows. In Section 2, the activation and inhibition kinetics of the calcium-dependent proteins are derived. Moreover, it is explained how the bursting signal is simulated, how the levels of active proteins are calculated and which parameters are varied. The results of our simulations are presented in Section 3 and discussed in Section 4.

2. Model description

We consider two different proteins, the first of which is assumed to bind four calcium ions in a cooperative way. By this binding, the proteins switch from an inactive to a (for example, catalytically) active state. An example is provided by calmodulin (cf. Van Eldik and Watterson, 1998). The second protein is assumed to be able to bind, in addition, four further calcium ions fast and cooperatively. These ions, in turn, inhibit the protein. Results by Eshete et al. (1998) indicate that protein kinase C-α (PKC-α) involves both activatory (C2 domain) and inhibitory (modulatory) binding sites for calcium. They found in C2 deletion mutants that PKC activation by other substrates (e.g., fatty acids) is strongly inhibited by calcium at micromolar concentrations. They concluded that the modulatory Ca²⁺-binding site is normally suppressed by the intact activatory C2 domain and regains its Ca²⁺ responsiveness in case of malfunction.

Dependence of protein activation and inhibition on calcium has been reported for protein C, a precursor to a natural plasma anticoagulant (Rezai and Esmen, 1994a,b). Calcium affects protein C activation by thrombin. Whether this effect is activatory or inhibitory depends on the presence of a protein cofactor, thrombomodulin.

Throughout the paper, we assume that the sequesteration of calcium by the calcium-binding proteins is so small that it can be neglected in the calcium balance. This is justified because we keep the total protein concentration so small that at most 1% of calcium can be bound to proteins. Thus, no conservation relation for the amount of calcium was taken into account. In the following, two different cases are distinguished depending on whether the binding of activatory calcium ions is at rapid equilibrium. The binding of calcium at the inhibitory site is assumed to be fast in both cases.

2.1. Rapid equilibrium of calcium binding

The model system and the rapid equilibrium equations are exemplified with that protein that is subject to inhibition (in the following denoted as “Prot₂”). The four calcium ions bound to the inhibitory sites are denoted as Ca₄I (Fig. 1). For the solely calcium-activated protein (“Prot₁”), binding to inhibitory sites does not occur. We consider noncompetitive inhibition, that is, the binding affinity of calcium to the inhibitory site is independent of whether or not the activatory site is occupied. The mass action law implies:

\[ \frac{\text{Prot}_2 \times \text{Ca}^4}{\text{Prot}_2 \text{Ca}_4} = K_1 = \frac{\text{Prot}_2 \text{Ca}_4 \times \text{Ca}^4}{\text{Prot}_2 \text{Ca}_4 \text{Ca}_4} \]  
\[ K_2 = \frac{\text{Prot}_2 \times \text{Ca}^4}{\text{Prot}_2 \text{Ca}_4} \]  

Fig. 1. Reaction scheme of calcium binding to a protein with activatory and inhibitory Ca²⁺ binding sites (Prot₂). For the other protein (Prot₁) the two inhibitory binding reactions do not occur.
$K_2$ denotes the dissociation constant. From Eq. (1a), we obtain:

$$\mbox{Prot}_2 \text{Ca}_4 \text{I} = \frac{\text{Ca}_4 \times \mbox{Prot}_2}{K_1}$$

(2)

$$\mbox{Prot}_2 \text{Ca}_4 \text{Ca}_4 \text{I} = \frac{\text{Ca}_4 \times \mbox{Prot}_2 \text{Ca}_4}{K_1}$$

(3)

For each protein, a conservation relation holds. For protein 2, it reads

$$\mbox{Prot}_2 \text{I} = \mbox{Prot}_2 + \mbox{Prot}_2 \text{Ca}_4 \text{I} + \mbox{Prot}_2 \text{Ca}_4 + \mbox{Prot}_2 \text{Ca}_4 \text{Ca}_4 \text{I}$$

(4)

with $\mbox{Prot}_2 \text{I}$ standing for the total concentration of protein 2. So the amount of active protein is given by the following relation:

$$\mbox{Prot}_2 \text{Ca}_4 = \frac{\mbox{Prot}_2 \text{Ca}_4 \times \text{Ca}_4}{(K_2 + \text{Ca}_4)\left(1 + \frac{\text{Ca}_4}{K_1}\right)}$$

(5)

This is the well-known Hill equation for cooperative binding (cf. Segel, 1993). Fig. 2 shows the binding curves of the two proteins according to Eqs. (5) and (6).

2.2. Differential equations

For a slower protein activation process, which does not fulfill rapid equilibrium conditions, the protein response can be calculated by differential equations. The reaction scheme is as depicted in Fig. 1, where the dissociation constants $K_1$ and $K_2$ are the ratios of the rate constants of dissociation ($k_{\text{off},i}$) and binding ($k_{\text{on},i}$), respectively ($i = 1, 2$).

Because active and inactive proteins are in rapid equilibrium with their inhibited complexes (for inhibition still a rapid equilibrium is assumed), the differential equations are written for their sums:

$$\frac{d(\mbox{Prot}_2 + \mbox{Prot}_2 \text{Ca}_4 \text{I})}{dr} = k_{\text{off},2} \times \mbox{Prot}_2 \text{Ca}_4 - k_{\text{on},2} \times \mbox{Prot}_2 \times \text{Ca}_4$$

(7)

$$\frac{d(\mbox{Prot}_2 \text{Ca}_4 + \mbox{Prot}_2 \text{Ca}_4 \text{Ca}_4 \text{I})}{dr} = k_{\text{off},2} \times \mbox{Prot}_2 \times \text{Ca}_4 - k_{\text{on},2} \times \mbox{Prot}_2 \text{Ca}_4$$

(8)

Fig. 2. Binding curves for both classes of proteins ($\mbox{Prot}_1$, solid line; $\mbox{Prot}_2$, dashed line). The protein activation is calculated by rapid-equilibrium approximation at constant calcium.
In the latter equation, the inhibited protein–ligand complex is substituted using Eq. (3):

$$
\frac{d}{dt} \left[ \text{Prot}_2 \text{Ca}_4 \times \left( 1 + \frac{\text{Ca}_4}{K_I} \right) \right] = k_{\text{on}} \times \text{Prot}_2 \times \text{Ca}_4 - k_{\text{off}} \times \text{Prot}_2 \text{Ca}_4
$$

(9)

For calcium square waves, calcium can be considered to be constant during each plateau. Therefore, the active protein obeys the equation:

$$
\frac{d}{dt} \text{Prot}_2 \text{Ca}_4 = \frac{1}{1 + \left( \frac{\text{Ca}_4}{K_I} \right)} \times (k_{\text{on}} \times \text{Prot}_2 \times \text{Ca}_4 - k_{\text{off}} \times \text{Prot}_2 \text{Ca}_4)
$$

(10)

Analogously, the inactive protein obeys the same equation with a negative sign.

For protein 1, the derivation is simpler because inhibition need not be considered. We obtain:

$$
\frac{d}{dt} \text{Prot}_1 \text{Ca}_4 = k_{\text{on},1} \times \text{Prot}_1 \times \text{Ca}_4 - k_{\text{off},1} \times \text{Prot}_1 \text{Ca}_4
$$

(11)

If calcium obeys a given temporal regime, one can transform the differential Eqs. (10) and (11) into non-autonomous differential equations, from which the concentration of active protein can be calculated.

### 2.3. The bursting signal

The bursting signal was simulated by a square pulse oscillation with spikes of two different heights (see Fig. 3).

This oscillation is characterized by the following parameters: the baseline, $h_0$, height of the high and low spikes, $h_1$ and $h_2$, respectively, durations of high and low peaks, $t_1$ and $t_2$, respectively, interpeak interval, $t_0$, and the number of low peaks occurring between two high peaks, $n$. In accordance with experimental results (Dixon et al., 1990; Dixon, 2000; Kummer et al., 2000), the number of high peaks per burst is set equal to one. Thus, $n$ can also be defined as the frequency ratio of high- and low-peak oscillations. The experimental results also show that usually, there is some refractory period between the last low peak and the next high peak. In some of our simulations, we include this period (denoted by $t_r$). The period, $T_1$, of high-peak oscillations and the period of low-peak ($T_2$) oscillations are given by:

$$
T_1 = t_0 + t_1 + n(t_0 + t_2) + t_r
$$

(12)

![Fig. 3. Bursting Ca$^{2+}$ signal used in all calculations. $t_0 = t_1 = t_2 = 2\,s, h_0 = 0.05\,\mu M, h_1 = 1.0\,\mu M, h_2 = 0.2\,\mu M, T_1, t_r$, and $n$ are varied.](image-url)
\[ T_2 = t_0 + t_2. \]  

(13)

The corresponding frequencies are:

\[ f_1 = \frac{1}{T_1} \]  

(14a)

\[ f_2 = \frac{1}{T_2} \]  

(14b)

Moreover, an effective (averaged) frequency, \( f^*_2 \), for the low-peak oscillations can be defined, which takes into account the refractory time:

\[ f^*_2 = \frac{n}{T_1} \]  

(15)

In this definition, the number of low peaks occurring in one period \( T_1 \) is counted. For the high-peak oscillations, an analogous definition is not necessary because there is only one spike per period.

2.4. Calculation of the level of active proteins

Bursting signals with varying parameter values are applied to the two model proteins described above. In the first series of simulations, the rapid-equilibrium approximation is used. This implies that the time course of protein activity is a square pulse oscillation with spikes of two different heights as well. The time approximation is used. This implies that the time course of protein activity is a square pulse oscillation. The average levels of protein activity are calculated in the same way as in the case of rapid-equilibrium approximation, but using numeric computation of integrals.

The calculations for the rapid-equilibrium case were performed with the program MS EXCEL. The numerical solving of differential equations was carried out by using the software MADONNA (University of Berkeley, CA) with the Rosenbrock (STIFF) integration method.

2.5. Parameter values

The parameter values concerning the bursting Ca\( ^{2+} \) signal that are kept constant (except in Fig. 6) are: \( t_0 = t_1 = t_2 = 2 \) s; \( h_0 = 0.05 \) \( \mu \)M, \( h_2 = 1.0 \) \( \mu \)M, \( h_3 = 0.2 \) \( \mu \)M.

The parameters concerning the Ca\( ^{2+} \) association, dissociation, and inhibition of Ca\( ^{2+} \) binding to proteins have been chosen in such a way that the maxima of the binding curves (Fig. 2) are sufficiently separated: \( K_{1} = 0.67 \mu \)M\(^{-1}\), \( K_{2} = 3.33 \times 10^{-4} \mu \)M\(^{-1}\), \( K_{3} = 0.005 \mu \)M\(^{-1}\), \( \text{Prot}_{1} = 10^{-4} \mu \)M, \( \text{Prot}_{2} = 10^{-4} \mu \)M. In the dynamic simulations, the following kinetic constants are used: \( k_{1,\text{on}} = 0.007 s^{-1} \mu \)M\(^{-1}\), \( k_{1,\text{off}} = 0.0047 s^{-1}\), \( k_{2,\text{on}} = 60 s^{-1} \mu \)M\(^{-1}\), \( k_{2,\text{off}} = 0.02 s^{-1}\).

To analyse the differential regulation of two proteins, we vary parameters \( T_1, t_r, \) and \( n \), in separate simulations:

- \( T_1 \) (at \( n = 0 \)) and herewith \( f_1 \) and \( t_r \);
- \( n \) at \( T_1 = \) constant, which changes \( f_2 \) and \( t_r \);
- \( t_r \) at \( n = \) constant, which changes \( f_1 \) and \( f^*_2 \) in the same ratio;
- \( n \) and \( T_2 \) at \( t_r = 0 \), which changes \( f_1 \) and \( f^*_2 \) in the opposite way.

3. Results

The binding curves for the two classes of proteins (Fig. 2) indicate that a selective regulation of proteins 1 and 2 is possible. First, we show that one protein can be gradually activated whereas the other protein remains inactive. The independent regulation of protein 1 can be achieved by a signal with an amplitude corresponding to the maximum of the binding curve of protein 1 (see Fig. 2). As this binding curve tends to its maximum
value in an asymptotic way and the calcium level in the cytosol is limited for various reasons by about 1 μM (cf. Goldbeter, 1996), we have chosen this value for the amplitude of the high peaks, $h_1$. This enables a selective regulation of protein 1 because protein 2 is inhibited in this concentration range (see Fig. 2). Taking $n=0$, we have a regular oscillation involving high peaks only and, thus, only protein 1 is activated. The level of this activation can be regulated by changing the frequency of such an oscillation (Fig. 4). The calculations were performed using the rapid-equilibrium approximation both for the activatory and inhibitory binding sites, cf. Eqs. (5) and (6). It can be seen that protein 1 can be regulated keeping protein 2 in a virtually inactive state.

The independent regulation of protein 2 is depicted in Fig. 5. A gradual activation of protein 2 whereas protein 1 remains inactive can be achieved by a bursting oscillation upon increasing the number of low peaks, $n$, at constant period $T_1$, thus more and more reducing the refractory period $t_r$ (see Eq. (12)). Such a variation, which was observed experimentally (Fig. 4 in Dixon, 2000), implies a variation of the average frequency of low peaks, $f_2^*$, whereas keeping frequency $f_1$ constant. This selective regulation of protein 2 is optimised if the amplitude of the low peaks corresponds to the maximum of the corresponding binding curve (see Fig. 2).

To investigate whether the two proteins can both be gradually activated by one signal, we have analysed a bursting pattern with $n=1$ (i.e., a 1:1 ratio of high and low peaks) and varied the refractory period $t_r$ and, hence, the frequencies $f_1$ and $f_2^*$ (Fig. 6a, thick lines). It can be seen that a simultaneous activation of both proteins can indeed be achieved by simultaneous increasing of both frequencies $f_1$ and $f_2^*$ (which are equal in this case). To compare the efficiency of the regulation by bursting oscillations and a regulation by simple spiking oscillations, in Fig. 6a, also the dependence of protein activity on the frequency of simple oscillations is plotted (thin lines). For the amplitude of simple spiking oscillations we take the value of 0.5 μM, because this corresponds to the intersection point of the two binding curves in Fig. 2. Note that the average calcium level is then nearly equal to that in the bursting signal. In Fig. 6b, protein activation is plotted versus the average calcium level. It can be seen that a simultaneous activation of both proteins is achieved more efficiently by bursting than by simple oscillations. This is understandable because the high and low peaks in a bursting pattern correspond to the activation maxima of proteins 1 and 2, respectively. By contrast, the peaks in simple spiking oscillations cannot correspond to both maxima simultaneously.
A selective up- and down-regulation of the two proteins can be achieved by changing the frequency ratio, $n$, of the low and high peaks ($n = f^*_2/f_1$; see Eqs. (14a and b) and (15)) in the signal with $t_2 = 0$ and variable $T_1$. Thus, as $n$ increases, the frequency, $f_1$, of high spikes decreases, whereas the effective frequency, $f^*_2$, of low peaks (averaged over the period $T_1$) increases. Therefore, the contribution of the low peaks, which activate protein 2 while not activating protein 1, increases, while the contribution of the spikes, for which the opposite holds true, decreases. Therefore, with increasing $n$, the activity of protein 1 monotonically decreases while the activity of protein 2 monotonically increases (see Fig. 7). Moreover, as can be guessed from Fig. 7, the relationships between the average active protein concentrations and $f_1$ and between $f_1$ and $f^*_2$ are linear. This can be proved as follows.

Eqs. (16) and (17) are of the form:

$$\langle \text{Prot}_{i \text{Ca}_4} \rangle = a_i n + b_i \frac{T_1}{T_1},$$

where $i = 1, 2$ and $a_i, b_i = \text{constant}$. According to Eq. (12), $T_1$ is a linear function of $n$:

$$T_1 = c n + d,$$

where $c, d = \text{constant}. \quad (19)$$

Inserting Eq. (19) into Eq. (18) gives:

$$\langle \text{Prot}_{i \text{Ca}_4} \rangle = \left( b_i - \frac{a_i d}{c} \right) f_1 + \frac{a_i}{c}. \quad (20)$$

which shows that the relationships between the average active protein concentrations and $f_1$ is indeed linear.

To find the relation between $f_1$ and $f^*_2$, we start with the ansatz:

$$f^*_2 + A f_1 = B,$$

where $A, B = \text{constant}. \quad (21)$$

Taking into account the definitions of $f_1$ and $f^*_2$ (Eqs. (14a) and (15)), Eq. (21) can be written as:

$$n + A = T_1 B \quad (22)$$

According to the definition of $T_1$ (Eq. (12)), we obtain:

$$n + A = n B(t_2 + t_0) + B(t_1 + t_0 + t_r) \quad (23)$$

From a comparison of the terms in Eq. (23), coefficients $A$ and $B$ can be determined:

$$A = \frac{t_1 + t_0 + t_r}{t_2 + t_0} \quad \text{and} \quad B = \frac{1}{t_2 + t_0}. \quad (24a,b)$$
Fig. 6. Activation of proteins Prot 1 (thick solid line) and Prot 2 (thick dashed line) vs. (a) frequency $f_1 = f^*_2$, (b) average calcium concentration of the Ca$^{2+}$ signal. Both in (a) and (b) a bursting Ca$^{2+}$ signal with $n = 1$ (i.e., a 1:1 ratio of high and low peaks; see inset) is used. The frequencies $f_1$ and $f^*_2$, and subsequently the average Ca$^{2+}$ concentration of the signal, are enlarged by shortening the refractory period $t_r$. Thin solid and thin dashed lines represent the activation of Prot 1 and Prot 2, respectively, vs. the frequency of simple spiking oscillations with the amplitude 0.5 μM (see inset). For all calculations the rapid-equilibrium approximation is used.
Fig. 7. Opposite regulation of Prot$_1$ (solid line) and Prot$_2$ (dashed line) by varying the frequency ratio, $n = f_2/f_1$, of the low and high peaks in the signal with $t_r = 0$ and variable $T_1$. Each vertical line, which serves for 3D visualisation, corresponds to one value of $n$. For the calculations the rapid-equilibrium approximation is used.

Fig. 8. Time course of the activation of Prot$_1$ (dashed line) and Prot$_2$ (solid line) obtained by numerically integrating the differential Eqs. (10) and (11). Parameters: $n = 10, t_r = 0$. 
Fig. 9. Opposite regulation of Prot 1 (thick solid line) and Prot 2 (thick dashed line) by varying the frequency ratio, $n = f_2^*/f_1$, of the low and high peaks in the signal with $t_r = 0$ and variable $T_1$. The results were obtained by numerically integrating the differential Eqs. (10) and (11). For comparison the calculations using rapid-equilibrium approximation (results presented in Fig. 7 are added to the figure (thin lines)).

Accordingly, $A$ and $B$ are constants and, therefore, the relation between $f_1$ and $f_2^*$ is linear (see Eq. (21)).

From the linear relation between $f_1$ and $f_2^*$ and Eq. (20), it follows that the relationship between the average active protein concentrations and $f_2^*$ is also linear.

When using the differential Eqs. (10) and (11) rather than the rapid-equilibrium approximation, the time course of protein activity is no longer a pure square-wave pattern. Fig. 8 depicts the time course obtained in this way. It can be seen that after a given time the protein activity is nearly constant. This smoothening effect is a consequence of the dynamics taking into account realistic on and off rate constants. In particular, the off rate constant causes an exponential decay of protein activity in the inter-spike intervals, and hence the smoothening effect. If the on and off rate constants are increased, the binding and dissociation gets faster and faster, so that the time course tends more and more to the square-wave pattern obtained by the rapid-equilibrium approximation. To see the effect of the dynamics, it is interesting to compare the results shown in Fig. 7 obtained on the basis of the rapid-equilibrium approximation with the corresponding calculation obtained on the basis of differential equations. The average concentrations of activated proteins are shown in Fig. 9. Note that the averaging should be done after the initial transient. We see that the proteins are more efficiently (about two-fold) activated by the real, slow (in comparison to the rapid-equilibrium approximation) kinetics. The experimental data show that for many calcium-binding proteins, the average residence time of calcium bound to the protein can take values from several microseconds to some seconds (Falke et al., 1994).

4. Discussion

A theoretical model for the decoding of regular bursting calcium oscillations has been established. The model is based on the binding of cytosolic calcium to two proteins with different characteristics. In particular, both proteins are able to cooperatively bind calcium at activatory sites with different binding constants. Extending the work by Larsen and Kummer (2003) and Larsen et al. (2004), we assume that one of the two proteins can cooperatively bind calcium, in addition, at inhibitory sites. Thus, this protein can be “designed” to respond to the low peaks of the bursting pattern, while
the other protein responds to the high peaks. This inhibition might occur indirectly, by another protein (for example, protein 1 in our analysis) that is activated by calcium. In fact, many calcium-dependent signalling cascades involve numerous proteins (Stull et al., 1993; Webb et al., 2000; Gorenne et al., 2004). Activation of a protein at low calcium levels and inhibition at high levels (biphasic regulation) is well-known for the IP3 receptor channel in the endoplasmic reticulum membrane (Bezprozvanny et al., 1991; De Young and Keizer, 1992). Its activity curve has a bell shape and is, thus, similar to the curve shown in Fig. 2. The IP3 receptor is composed of four subunits, each of which has one activatory and one inhibitory binding site for calcium. This was analysed in a model for bursting calcium oscillations by Borghans et al. (1997). Although the IP3 receptor is not likely to function as a decoder of calcium oscillations, our model is inspired by the activatory and inhibitory properties of calcium with respect to this protein.

Our analysis is not concerned with the transformation of oscillations into a stationary signal, although the simulations with differential equations (Figs. 8 and 9) give some indications for this. We averaged the time-dependent protein activity to compute the output level. Interestingly, the kinetic properties of proteins appear to have evolved towards optimal values. If the on and off rate constants were too large, the activity curves of the proteins would be less smooth; and if they were too small, the transient time to reach the stationary oscillation would be too long.

To investigate which characteristics of the calcium signal could be responsible for a differential regulation of protein activities, simplified square pulses were applied to simulate bursting. In constructing these patterns, we started from knowledge about usual bursting patterns observed experimentally or generated in theoretical models of calcium oscillations. Such models show, for example, that an increase in the number of low peaks, \(n\), occurs at relatively constant interpeak interval, \(t_0\), and peak durations \(t_1\) and \(t_2\) (Habrichter et al., 2001). The effect on the two proteins was calculated by differential and rapid equilibrium equations.

Our results show that a distinction between specifically “high-peak activated” and “low-peak activated” proteins is possible at physiological calcium concentrations. By a bursting pattern, protein activation can be tuned much more effectively than by a non-oscillatory signal or by regular oscillations because the high and low peaks can be adjusted to correspond to the activation maxima of proteins 1 and 2, respectively.

Our simulation results show that by bursting calcium oscillations, two proteins can be differentially regulated. All four qualitatively different cases can be achieved (protein 1 and/or 2 being active or inactive). In an earlier modelling study (Larsen and Kummer, 2003; Larsen et al., 2004) only three cases could be realized: both proteins being inactive, one specific protein becoming active while the other being still inactive, and both proteins being active. Moreover, our results show that either protein can be gradually activated while the other one is fixed at a rather constant activity level. This implies that the two proteins can be gradually activated to a different extent or even in the opposite way.

The decoding of bursting oscillations has been studied in much more detail for excitable neurons than for calcium oscillations. It has been postulated that neurons can behave as integrators (cf. Izhikevich, 2001). They perform a time integration of incoming pulses and “prefer” a high frequency of the bursting input: the higher the bursting frequency, the sooner they fire. An alternative view is that neurons act as resonators; they “prefer” inputs having a certain resonant frequency: bursts with specific frequencies are more likely to cause a postsynaptic cell to fire than are bursts with higher or lower frequencies (Izhikevich, 2001). Consequently, a certain burst can resonate only with specific synapses or cells, depending on their inherent resonance frequencies. Hence, bursts, as opposed to a single spike, might provide selective communication via resonance between neurons (Izhikevich, 2002; Izhikevich et al., 2003). This could be considered as a distinct case of frequency encoding. Recently, it has been shown that synapses can be tuned to preferentially respond to specific burst durations, which demonstrates the decodability of a neuronal code based on burst duration (Kepecs and Lisman, 2004).

We analysed frequency encoding rather than amplitude encoding because the former is more robust to noise (Goldbeter, 1996; Berridge, 1997). However, in the case of bursting, no sharp distinction between encoding by frequency, amplitude and time shape can be made. A change in the frequency ratio of high and low peaks might also be considered as a change in the amplitudes.
Marhl et al. (1998b) made a distinction between signalling proteins and buffering proteins. The role of the signalling proteins is just to sense the oscillatory signal without affecting the oscillation while the buffering proteins do affect it by shaping the oscillatory pattern (sometimes to an extent that no oscillation is possible without buffering proteins, see Schuster et al., 2002). The proteins considered here both belong to the class of signalling proteins. In future studies, it will be of interest to consider the effect of buffering proteins on bursting calcium oscillations. Also the analysis of calcium sequestration by mitochondria (Marhl et al., 1998a; Fall and Keizer, 2001) may be further developed by considering bursting oscillations.

For the dependence of the concentration of active protein on cytosolic calcium, we derived two equations. One of these is based on a rapid-equilibrium approximation for the activatory site, while the other equation is based on a kinetic approach for this site. These equations, which are in agreement with the mass action law and the conservation relation for total protein, improve a rate law used by Larsen et al. (2004) for enzyme activation. Moreover, a difference to the study by Larsen et al. (2004) is that we include an inhibitory site in one of the two proteins. Whether such an inhibitory site, for which first indications exist (Eshete et al., 1998; Rezaie and Esmon, 1994a,b), is actually of biological relevance for the decoding of calcium signals needs to be verified in further work.

Our simulations apply to proteins that bind calcium in a reversible way without utilization of energy. In future studies, it will be worth making similar simulations for actively driven kinase/phosphatase cycles such as those studied by Dupont and Goldbeter (1992), Gall et al. (2000) and Salazar et al. (2004).

Another possible mode of differential signal transduction by calcium was studied by Dolmetsch et al. (1997). They showed experimentally that the activation of two transcription factors is controlled by the amplitude of a transient calcium signal and the activation of another transcription factor, by a low stationary concentration of calcium.

In Section 1, we have mentioned the so-called bow-tie structure of signalling. In the present paper, we have analysed one side of the "bow-tie", namely the output side. An interesting point for future studies is to analyse the input part. In particular, the question arises how bursting can be generated by two or more (concomitant or alternative) inputs, in such a way that specific changes in the input selectively affect specific features of the bursting oscillation (e.g., the number, $n$, of low peaks). An interesting experimental observation is that the hormone phenylephrine can cause regular oscillations while, in the same system, ATP can cause chaotic oscillations (Kummer et al., 2000). Moreover, recent experimental methods allow one to vary amplitudes and frequency independently (Tompa et al., 2001).

In the present theoretical study, we have shown that differential regulation by bursting calcium signals is possible. This is in support of the "bow-tie" concept of signalling (Csete and Doyle, 2004). In the future, experimental studies are desirable to check our prediction.

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