The Total Quasi-Steady State Approximation Readily Explains the Loss of Zero-Order Ultrasensitivity at Intermediate and High Enzyme Concentrations

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Abstract

Covalent modification plays a pivotal role in many signal transduction pathways, for example in the activation of enzymes by kinases and their inactivation by phosphatases. Goldbeter and Koshland (Proc. Natl. Acad. Sci. USA (1981) 78:6840-6844) showed that these systems can possess so-called ultrasensitivity if their catalyzing enzymes operate in a regime where they follow approximate zero-order kinetics. This happens when the enzymes have high affinities for their substrates and low concentrations compared to the substrate concentrations, such that the enzymes are saturated. However, experimental data indicate that enzymes *in vivo* are present in concentrations

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similar to or higher than those of their substrates in many cases. In this case ultrasensitivity is lost even for high affinity systems. This is closely related to the breakdown of the Briggs-Haldane approximation of the kinetics, such that one must describe the system by the full set of reactions or the recent total quasi-steady state approximation. We show that the latter approach not only reproduces the presence or lack of ultrasensitivity, but in addition, and more importantly, allows a treatment in line with the one used at low enzyme concentrations thus providing a simplification compared to the use of the full system of reactions.

Keywords: Covalent modification – Goldbeter-Koshland switch – metabolic control analysis – substrate sequestration

Running head: Ultrasensitivity and high enzyme concentrations

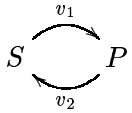
Introduction

Cells respond to external stimuli by changing the activities of enzymes controlling pathways, which transduce the signal to the appropriate targets within the cell. An ubiquitous mechanism for modifying the activity of an enzyme is by covalent modification, such as phosphorylation and dephosphorylation of the enzyme.

Often cells have to respond to a signal in an all-or-none way rather than a graded response, a property that has been termed ultrasensitivity (Goldbeter and Koshland, 1981). This can be obtained by having a steep sigmoidal signal-response curve. Several mechanisms such as cooperativity and feed-forward loops can lead to such a steep response.

In the present work we reinvestigate the covalent modification system, which

possesses ultrasensitivity under the right conditions, as Goldbeter and Koshland (1981) showed more than 25 years ago. The system consists of a substrate S, which can be modified for example by phosphorylation, to the form P. Vice versa, P can be transformed, e.g. by dephosphorylation, back to S. The scenario is summarized as:



(1)

where v_1 and v_2 are the reaction rates. In the following we will use the same symbols for reactants and their concentrations. Moreover, we follow the usual assumption saying that neither synthesis nor degradation of S and P occur. If no significant amounts of intermediate complexes are created in the reactions, we then approximately have $S(t) + P(t) = S_0$ (constant) for every t. However, in general we must include the complexes in the conservation law.

If v_1 and v_2 follow zero order kinetics, i.e., the reactions happen at constant rates independent of the concentrations of S and P, $v_1 = k_1, v_2 = k_2$, then the steady-state of the system will depend on the value of $\alpha = k_1/k_2$, which can be seen as the input signal to the system. For $\alpha < 1$ the steady state is given by all the substrate being unmodified, $S = S_0$, while for $\alpha > 1$ it is given by all the substrate being modified, $P = S_0$. Thus, the system acts like a switch showing a high (infinite) degree of sensitivity to the value of α around $\alpha = 1$, which underlies the term zero-order ultrasensitivity.

If on the other hand v_1 and v_2 follow simple first-order kinetics, i.e., they are proportional to their respective substrates, $v_1 = k_1 S$ and $v_2 = k_2 P$, then the steady state of the system can be found from $v_1 = v_2$, i.e. (if no intermediate complexes are created in the reactions), $k_1(S_0 - P) = k_2 P$, such that the steady

state concentration of modified substrate is given by

$$P = \frac{S_0 \alpha}{1 + \alpha}, \tag{2}$$

which responds weakly to changes of $\alpha = k_1/k_2$.

In between these two cases is the more realistic scenario investigated in the ground-breaking work by Goldbeter and Koshland (1981), where the enzymes follow Michaelis-Menten kinetics, given by

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} E + P, \tag{3}$$

$$F + P \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2 \xrightarrow{k_4} F + S, \tag{4}$$

where the first reaction is catalyzed by the enzyme (kinase) E, which binds the substrate S forming the complex C_1 followed by the release of the product P. Similarly, the reverse reaction is driven by the enzyme (phosphatase) F forming the complex C_2 by binding to P followed by the release of S. Several authors have reinvestigated ultrasensitivity in the Goldbeter-Koshland switch (see for example Berg et al. (2000); Blüthgen et al. (2006); Ciliberto et al. (2007)).

In this paper we have assumed for simplicity that the two reactions have the same binding and dissociation constants : $k_1 = k_3, k_{-1} = k_{-3}$ and $k_2 = k_4$. In particular, they have the same Michaelis constant $K_M = \frac{k_{-1}+k_2}{k_1}$. However, their maximal reaction rates, $V_{\max,1} = k_2 E_0$ and $V_{\max,2} = k_2 F_0$, are not necessarily equal because the total enzyme concentrations, E_0 and F_0, are allowed to differ.

When the enzyme concentrations are low, $F_0, E_0 \ll S_0 + K_M$, the complex concentrations are negligible, and thus we have approximately $S_0 = S + P$. In this case, we can suppose that the complexes are in dynamical equilibrium $\left(\frac{dC_1}{dt} \approx\right.$

$0,\ \frac{dC_2}{dt} \approx 0\big)$ and consequently that the reaction rates can be approximated by the Briggs-Haldane expressions (Segel and Slemrod, 1989)

$$v_1 = \frac{k_2 E_0 (S_0 - P)}{K_M + (S_0 - P)}, \quad v_2 = \frac{k_2 F_0 P}{K_M + P}. \tag{5}$$

The steady-state is again found by setting $v_1 = v_2$, i.e.,

$$\frac{\alpha(S_0 - P)}{K_M + (S_0 - P)} = \frac{P}{K_M + P}, \tag{6}$$

where the signal $\alpha = E_0/F_0$ is the ratio of the total enzyme concentrations. For $K_M \ll \min\{S, P\}$ we have approximately zero-order kinetics, because in this case $v_1 \approx k_2 E_0$, $v_2 \approx k_2 F_0$. This condition is satisfied for $K_M \ll S_0$ in our region of interest $\alpha \approx 1$. The steady-state changes swiftly from $P \approx 0$ to $P \approx S_0$ as α passes from below to above 1 (Fig. 1A). On the other hand, when $K_M \gg S_0 \geq \max\{S, P\}$, the reactions are approximately first-order, because $v_1 \approx \frac{k_2 E_0}{K_M} S$ and $v_2 \approx \frac{k_2 F_0}{K_M} P$, and the system no longer possesses ultrasensitivity (Goldbeter and Koshland, 1981). In fact, ultrasensitivity is lost already at $K_M \approx S_0$ (Fig. 1B).

[Figure 1 about here.]

However, even when $K_M \ll S_0$ but at non-negligible complex concentrations, the system is less sensitive to changes in the signal (Goldbeter and Koshland, 1981; Blüthgen et al., 2006; Ciliberto et al., 2007) (Fig. 2).

This happens at enzyme concentrations comparable to or higher than the substrate concentration, due to a large amount of substrate being bound in the complexes, so-called substrate sequestration (Blüthgen et al., 2006). Let us remark that this situation can not be analyzed with the Briggs-Haldane expressions (5), which are invalid at intermediate and high enzyme concentrations (Segel and Slemrod,

1989). Recently, the total quasi-steady state approximation (tQSSA) has been developed in order to treat such scenarios similarly to the Briggs-Haldane approach (Borghans et al., 1996; Tzafriri, 2003). Tzafriri (2003) showed that the tQSSA is always roughly valid, and if $k_{-1} \gg k_2$ the approximation is excellent for any combination of enzyme and substrate concentrations. The tQSSA has been extended to reversible reactions (Tzafriri and Edelman, 2004), fully competitive reactions (Pedersen et al., 2007b) as well as other more complex scenarios (Ciliberto et al., 2007; Pedersen et al., 2007a,c).

The present paper will show that the tQSSA allows a simple and elegant explanation of the effect of significant enzyme concentrations on ultrasensitivity in system (3), and that approaches such as metabolic control analysis (MCA) are immediately applicable to scenarios with intermediate or high enzyme concentrations described by the tQSSA. A simple criterion which excludes the possibility of ultrasensitivity is presented.

[Figure 2 about here.]

Results

The total quasi-steady state approximation of the Goldbeter-Koshland switch

Following Borghans et al. (1996) we introduce the total substrates $\bar{S} = S + C_1$ and $\bar{P} = P + C_2$, and assume that the complex concentrations are quasi-stationary, $\frac{dC_i}{dt} \approx 0,\ i = 1, 2$, which yields (Tzafriri, 2003; Pedersen et al., 2007c)

$$\frac{d\,\bar{P}}{dt} \approx k_2(C_1^- - C_2^-), \tag{7}$$

where

$$C_1^- = \frac{((S_0 - \bar{P}) + E_0 + K_M) - \sqrt{((S_0 - \bar{P}) + E_0 + K_M)^2 - 4(S_0 - \bar{P})E_0}}{2}, \tag{8}$$

$$C_2^- = \frac{(\bar{P} + F_0 + K_M) - \sqrt{(\bar{P} + F_0 + K_M)^2 - 4\bar{P}F_0}}{2}. \tag{9}$$

At this point it is important to note that we do not risk predicting ultrasensitivity with respect to $\bar{S}$ and $\bar{P}$ when there is no ultrasensitivity with respect to S and P and vice versa: At steady-state $C_1 = C_2$, so if for example $\bar{P} \approx S_0$ then $\bar{S} \approx 0$ implying $C_1 \approx 0$ and therefore also $C_2 \approx 0$ and $P \approx S_0$. On the other hand $P \approx S_0$ implies always $\bar{P} \approx S_0$.

When $\max\{F_0, E_0\} \ll S_0 + K_M$ (low enzyme concentrations) or $S_0 \ll \min\{E_0, F_0\} + K_M$ (high enzyme concentrations) the above expressions can be approximated by (Tzafriri, 2003)

$$C_1^- \approx \frac{E_0(S_0 - \bar{P})}{E_0 + K_M + (S_0 - \bar{P})}, \quad C_2^- \approx \frac{F_0\bar{P}}{F_0 + K_M + \bar{P}}, \tag{10}$$

such that at steady-state $\left(\frac{d\bar{P}}{dt} = 0\right)$ we have

$$\frac{\alpha(S_0 - \bar{P})}{E_0 + K_M + (S_0 - \bar{P})} = \frac{\bar{P}}{F_0 + K_M + \bar{P}}, \tag{11}$$

where $\alpha = E_0/F_0$ as above. Note that we have $\bar{P} \approx P$ for $\max\{E_0, F_0\} \ll S_0 + K_M$, and (11) reduces to (6) in this case. More interestingly, we see that it is not sufficient to have $K_M \ll S_0$ for ultrasensitivity to appear: The constraint $\max\{E_0, F_0\} \ll S_0$ is necessary (see also Ciliberto et al. (2007)). The reason is that the kinetics is no longer approximately zero-order when $E_0, F_0 \gtrapprox S_0 \gg K_M$. Indeed, it is seen from (10) that the reactions are roughly first order for large enzyme concentrations and low K_M value, see also Tzafriri (2003); Tzafriri and Edelman (2007).

The above expressions (10) can be invalid at intermediate or moderately high enzyme concentrations, $E_0, F_0 \gtrapprox S_0$, for example if K_M is not too high. We investigate the case of $K_M \ll S_0 \lessapprox \min\{F_0, E_0\}$ rewriting (calculations for C_1^- are similar)

$$\begin{aligned} C_2^- &= \bar{P} + \frac{(F_0 - \bar{P} + K_M) - \sqrt{(F_0 - \bar{P} + K_M)^2 + 4K_M\bar{P}}}{2} \\ &= \bar{P} + \frac{1}{2}(F_0 - \bar{P} + K_M)\left(1 - \sqrt{1 + \frac{4K_M\bar{P}}{(F_0 - \bar{P} + K_M)^2}}\right) \\ &\approx \bar{P} + \frac{1}{2}(F_0 - \bar{P} + K_M)\left[1 - \left(1 + \frac{1}{2}\frac{4K_M\bar{P}}{(F_0 - \bar{P} + K_M)^2}\right)\right] \\ &= \bar{P} - \frac{K_M\bar{P}}{F_0 - \bar{P} + K_M} \\ &= \bar{P}\frac{F_0 - \bar{P}}{F_0 - \bar{P} + K_M} \end{aligned} \tag{12}$$

so that

$$C_1^- = \bar{S}\frac{E_0 - \bar{S}}{E_0 - \bar{S} + K_M}, \quad C_2^- = \bar{P}\frac{F_0 - \bar{P}}{F_0 - \bar{P} + K_M}, \tag{13}$$

where we have assumed $\frac{4K_M\bar{P}}{(F_0 - \bar{P} + K_M)^2} \ll 1$ in order to substitute the square root with its linear approximation.

This is satisfied if for example $F_0 \geq 2\bar{P}$, or less restrictive, $F_0 \geq 2S_0$. Note that the kinetics is almost first-order in this case, since from our assumptions $K_M \ll S_0 = 2S_0 - S_0 \leq F_0 - S_0 \leq F_0 - \bar{P}$, such that the fraction is close to 1. This merely says that almost all substrate will be bound to a high affinity enzyme present at sufficiently high concentrations. Recently, Tzafriri and Edelman (2007) found a similar result in the limit $K_M \to 0$.

Metabolic Control Analysis

Metabolic control analysis (Fell, 1992) links global properties of a system to local ones. Global properties are described by response coefficients, which describe

the response of the entire system to changes in parameters. For example, the relative change in the concentration A in correspondence to a small change in the parameter p is described by the response coefficient $R_p^A = \frac{p}{A}\frac{\partial A}{\partial p}$. Local properties are described by the so-called elasticities, which describe the relative change of reaction rate v in response to a small change in the concentration of one of the biochemical species or parameters (B) involved in the reaction, and are defined as $\epsilon_B^v = \frac{B}{v}\frac{\partial v}{\partial B}$.

Small and Fell (1990) developed metabolic control analysis for covalent modification systems with general rate expressions. Their approach is immediately applicable to our scenario with $\bar{S}$ and $\bar{P}$ being the modified quantities. The response coefficient of the modified substrate concentration ($\bar{P}$ for tQSSA) to a change in the total enzyme concentration E_0 is given by (Small and Fell, 1990)

$$R_{E_0}^{\bar{P}} = \frac{\bar{S}}{\epsilon_{\bar{P}}^{v_2}\bar{S} + \epsilon_{\bar{S}}^{v_1}\bar{P}}. \tag{14}$$

$R_{E_0}^{\bar{P}} = 1$ corresponds to a linear response, while $R_{E_0}^{\bar{P}} > 1$ is needed for an ultrasensitive response. Thus, the elasticities must be close to zero, which happens when the rates v_1 and v_2 are nearly constant with respect to the substrates, i.e., in the zero-order regime.

We now use the fact that at steady state $v_1 = v_2$ such that

$$\begin{aligned} R_{E_0}^{\bar{P}} &= \frac{\bar{S}}{\frac{\bar{P}}{v_2}\frac{\partial v_2}{\partial \bar{P}}\bar{S} + \frac{\bar{S}}{v_1}\frac{\partial v_1}{\partial \bar{S}}\bar{P}} \\ &= \frac{v_2}{\bar{P}}\left(\frac{\partial v_2}{\partial \bar{P}} + \frac{\partial v_1}{\partial \bar{S}}\right)^{-1} \\ &= \frac{C_2}{\bar{P}}\left(\frac{\partial C_2}{\partial \bar{P}} + \frac{\partial C_1}{\partial \bar{S}}\right)^{-1} \\ &\leq \left(\frac{\partial C_2}{\partial \bar{P}} + \frac{\partial C_1}{\partial \bar{S}}\right)^{-1}. \end{aligned} \tag{15}$$

Note that $R_{E_0}^{\bar{P}} \leq 1$ if

$$\min\left\{\frac{\partial C_1^-}{\partial \bar{S}}, \frac{\partial C_2^-}{\partial \bar{P}}\right\} \geq \frac{1}{2}, \tag{16}$$

and ultrasensitivity is then excluded.

Calculating

$$\frac{\partial C_2^-}{\partial \bar{P}} = \frac{1}{2}\left(1 + \frac{F_0 - (\bar{P} + K_M)}{\sqrt{(\bar{P} + F_0 + K_M)^2 - 4\bar{P}F_0}}\right), \tag{17}$$

it is readily shown that $\frac{\partial C_2^-}{\partial \bar{P}}$ is a decreasing function of $\bar{P}$ hence taking its minimum at the maximum allowable value of $\bar{P}$. Thus, to estimate an upper value of $R_{E_0}^{\bar{P}}$, we must find an upper bound for $\bar{P}$ (and $\bar{S}$, which similarly minimizes $\frac{\partial C_1^-}{\partial \bar{P}}$).

In the limit $E_0 = F_0$, such that $\bar{S} = \bar{P} = \frac{S_0}{2}$, we therefore find the following criterion, which excludes ultrasensitivity:

$$E_0 = F_0 \geq K_M + \frac{S_0}{2}. \tag{18}$$

For the general case we need some observations. At steady state $C_1 = C_2$, and hence we have the conservation law

$$S_0 = \bar{P} + \bar{S} = 2C_2 + P + S, \tag{19}$$

which implies $C_2 \leq \frac{S_0}{2}$. Moreover either $\bar{P} \geq \frac{S_0}{2} \geq \bar{S}$ or $\bar{S} \geq \frac{S_0}{2} \geq \bar{P}$. In the following we assume the former condition, calculations for the second case being identical. Motivated by the above considerations and in particular by condition (16), we make the assumption

$$\min\{E_0, F_0\} \geq K_M + \frac{S_0}{2}. \tag{20}$$

which is the natural generalization of criterion (18), from which we immediately see

$$\frac{\partial C_1^-}{\partial \bar{S}} \geq \frac{1}{2}, \tag{21}$$

since $\bar{S} \leq \frac{S_0}{2}$. In addition, using the fact that C_2^- is an increasing function of both F_0 and $\bar{P}$ and recalling that $F_0 \geq K_M + \frac{S_0}{2}$ and $\bar{P} \geq \frac{S_0}{2}$, we obtain

$$\begin{aligned} \frac{S_0}{2} \geq C_2 &\geq \frac{1}{2}\left[2\left(K_M + \frac{S_0}{2}\right) - \sqrt{4\left(K_M + \frac{S_0}{2}\right)^2 - 4\left(K_M + \frac{S_0}{2}\right)\frac{S_0}{2}}\right] \\ &= \frac{S_0}{2} + K_M - \sqrt{K_M}\sqrt{K_M + \frac{S_0}{2}}. \end{aligned} \tag{22}$$

Thus, using $\bar{S} \geq C_1 = C_2$,

$$\bar{P} = S_0 - \bar{S} \leq S_0 - C_2 \leq \frac{S_0}{2} + \sqrt{K_M}\sqrt{K_M + \frac{S_0}{2}} - K_M. \tag{23}$$

Hence, if we require

$$\min\{E_0, F_0\} \geq \frac{S_0}{2} + \sqrt{K_M}\sqrt{K_M + \frac{S_0}{2}}, \tag{24}$$

we see that not only does (18), and therefore also (21) and (23), hold, but in addition we find from (17), (23) and (24) that

$$\frac{\partial C_2^-}{\partial \bar{P}} \geq \frac{1}{2}, \tag{25}$$

and therefore, from (15), (16) and (21), that $R_{E_0}^{\bar{P}} \leq 1$.

The relation (24) is an important criterion for determining the lack of ultrasensitivity in covalent modification cycles. In the limit $K_M \to 0$, we see that a sufficient condition to exclude ultrasensitivity is $\min\{E_0, F_0\} \geq \frac{S_0}{2}$, which corresponds well to the region ($E_0/F_0 > 0.5$) where the curve is nearly horizontal

in Fig. 2. However, (24) is only a sufficient condition for determining the lack of ultrasensitivity; the violation of (24) does not guarantee that $R_{E_0}^{\bar{P}} > 1$ nor ultrasensitivity, as seen from the example $F_0 \approx E_0 \ll S_0 = K_M$, which clearly violates (24) but does not have ultrasensitive behavior (Goldbeter and Koshland, 1981) (Fig. 1B).

Discussion

A significant part of the success of the Briggs-Haldane approximation lies in the insight and simplification that it provides compared to the full set of equations describing the enzymatic reactions. This reduction was crucial for many decades before the easy access to computer simulations that characterize modern Systems Biology. The entry of computers has in some sense foregone the total quasi-steady state approximation, which can seem complicated to work with.

However, as shown here the total quasi-steady state approximation has the advantage that it directly allows comparison to previous results obtained using the Briggs-Haldane approximation. Thus, decades of obtained knowledge and methods can be extended to intermediate and high enzyme concentrations. We showed how the tQSSA directly yields the fact that, at high enzyme concentrations, the kinetics becomes roughly first order and that ultrasensitivity therefore is lost in the covalent modification system.

The conclusion was further supported by applying previously existing formulas from metabolic control analysis (Small and Fell, 1990) to the tQSSA description. Metabolic control analysis can also be used on the full system of equation (Blüthgen et al., 2006), but a new formula is necessary in this case. Blüthgen

et al. (2006) found such a formula expressed in terms of the elasticities of the Briggs-Haldane reaction term. However, since the Briggs-Haldane approximation is known not to hold at high enzyme concentrations, it can be questioned how to interpret these elasticities, and therefore the formula, under such conditions. The use of the tQSSA sidesteps this issue. In the previous section, we found the simple relation (24), which excludes the possibility of ultrasensitivity.

Goldbeter and Koshland (1981) suggested that ultrasensitivity could be rescued at high enzyme concentrations by assuming that not only P but also the complex C_2 is active. However, the tQSSA works directly with the combined term $\bar{P} = P + C$, and we found that the response coefficient $R^{\bar{P}}_{E_0} < 1$ for sufficiently high enzyme concentrations (24), and that ultrasensitivity therefore is excluded in agreement with Blüthgen et al. (2006).

As pointed out by Blüthgen et al. (2006), the lack of ultrasensitivity at enzyme concentration higher than or similar to the substrate concentrations, a scenario expected to be of importance *in vivo*, means that the cells must possess other means to create a steep signal-response curve, for example signaling cascades. We (Pedersen et al., 2007a) and others (Blüthgen et al., 2006) have shown that high enzyme concentrations can modify the response of the MAPK cascade greatly, and make an otherwise oscillatory response (Kholodenko, 2000) non-oscillatory.

The usefulness of the tQSSA for theoretical considerations is starting to appear in literature. Recently, Ciliberto et al. (2007) used the first order tQSSA (10) for a theoretical investigation of covalent modification in line with the treatment presented here. However, (10) does not hold at comparable enzyme and substrate concentrations (Tzafriri, 2003) as noted by Ciliberto et al. (2007). The present paper thus extends those results.

Dynamic computer simulations are easily done for the full set of reactions and equations, but theoretical analysis as presented here is greatly aided by the reduction resulting from the tQSSA, in the same way that the Briggs-Haldane approximation simplifies the analysis at low enzyme concentrations. Such simplifications also help parameter estimation; however the use of an invalid approximation, e.g., the Briggs-Haldane approximation at high enzyme concentrations, can lead to incorrectly estimated parameters (Pedersen et al., 2007c).

Acknowledgments

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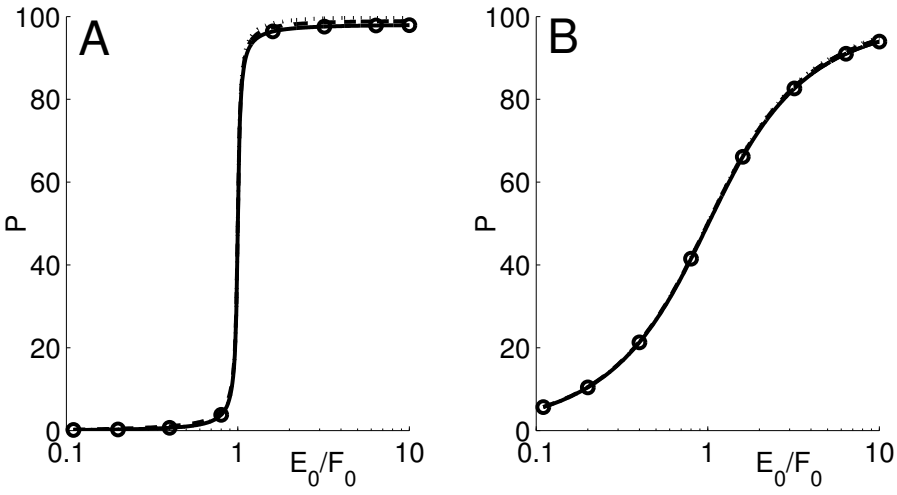

Figure 1.

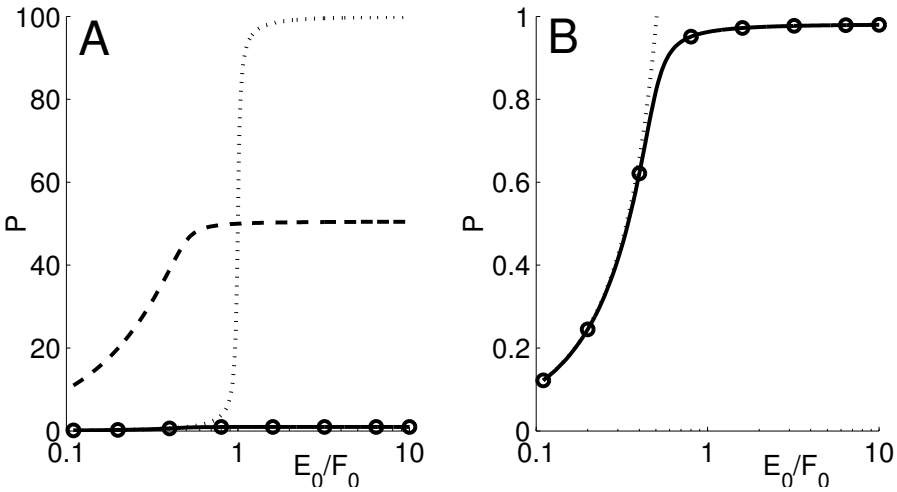

Figure 2.