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A mathematical model of endothelial nitric oxide synthase activation with time delay exhibiting Hopf bifurcation and oscillations

Q1 L.R. Ritter[a,*], C.A. Chrestensen[b], J.C. Salerno[c]

Q2 Q3 [a] Department of Mathematics, Kennesaw State University, 1100 S. Marietta Pkwy, MD #9085, Marietta, GA 30060, USA
[b] Department of Chemistry and Biochemistry, Kennesaw State University, Kennesaw, GA 30144, USA
[c] Department of Molecular and Cellular Biology, Kennesaw State University, Kennesaw, GA 30144, USA

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This paper is dedicated to the memory of John Salerno (May 1949–December 2015), researcher and teacher, mentor and friend.

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ABSTRACT

Nitric oxide (NO) is a gaseous compound that serves as a signaling molecule in cellular interactions. In the vasculature, NO is synthesized from endogenous agents by endothelial nitric oxide synthase (eNOS) where it plays key roles in several functions related to homeostasis, adaptation, and development. Recent experimental studies have revealed cycles of increasing and decreasing NO production when eNOS is stimulated by factors such as glucose or insulin. We offer a mathematical model of a generic amino acid receptor site on eNOS wherein this species is subject to activation/deactivation by a pair of interactive kinase and phosphatase species. The enzyme kinetic model is presented as a system of ordinary differential equations including time delay to allow for various intermediate, unspecified complexes. We show that under conditions on the model parameters, varying the delay time may give rise to a Hopf bifurcation. Properties of the bifurcating solutions are explored via a center manifold reduction, and a numerical illustration is provided.

1. Introduction

Nitric oxide (NO) is a significant biological signaling molecule in intracellular and cell–cell interactions. In the vasculature, for example, NO is used by endothelial cells for regulatory and developmental processes including maintenance of vascular tone, vasodilation, insulin secretion, and angiogenesis [1,2]. Within a variety of organisms, NO is synthesized from endogenous L-arginine, oxygen and NADPH by a class of enzymes called nitric oxide synthases. Among these, endothelial nitric oxide synthase (eNOS) is the primary integrator for the signaling network in the human vasculature. The eNOS isoform is known as a key element in vaso-protective NO production for smooth muscle cell relaxation, inhibition of platelet aggregation, and suppression of immune cell adhesion at the arterial wall. Understanding the regulatory network in NO production and endothelial function and dysfunction has direct application to the study of diabetes and cardiovascular health and disease [3–5].

Endothelial NOS is an important enzyme in a complex regulatory network involving kinases and phosphatases, signal inducing hormones and growth factors (e.g. insulin, estrogen, VEGF), as well as mechanical stress. The discovery of eNOS activation via phosphorylation by various kinases opened a new window on the understanding of NO production [2,6]. And several studies have focused on identification of reaction sites to specific agonists and elucidation of signaling pathways (see for example the review papers [6–8] and the references therein). More recently, experimental and computational approaches have been implemented in an effort to study the time course of related biological signaling. Complex dynamics, in particular bistability and oscillatory behavior, have been observed in some kinase cascade models characterized by phosphorylation mediated activation [9–11]. In [10] for example, it was observed in vitro that competition between substrates and phosphatase gives rise to oscillation in activation of extracellular signal-regulated kinase (ERK). The mathematical analysis carried out by Liu et al. [9] is based on substrate dependent control of phosphorylation observed in both in vitro and

Q4 * Corresponding author. Fax: +1 470 578 9812.
E-mail addresses: lritter@kennesaw.edu, lritter67@gmail.com (L.R. Ritter), cchreste@kennesaw.edu (C.A. Chrestensen).

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in vivo studies. Experimental results involving single dose insulin stimulation following serum starvation of bovine aortic endothelial cells by Wang et al. [11] showed a time course characterized by cycles of rapid increase and decline in NO production. The current authors have observed oscillations of phosphorylation at the amino acid site serine 1177 (S1177) in response to insulin and fetal bovine serum treatment (unpublished). These dynamic processes lend themselves to mathematical models of both the discrete and continuous variety. Most notably, enzyme kinetic models formulated as systems of differential equations provide insight into feedback mechanisms and the dependence of observed behavior on key parameters [9,10,12–14].

In this paper, we propose a model of a generic amino acid residue in eNOS (representing for example any of several serine amino acids) wherein this species is subject to activation or deactivation by a pair (in general a pair of families) of interactive kinase and phosphatase species. This is a dramatic simplification of eNOS regulation, since it has multiple regulatory phosphorylation sites both activating and inhibitory [8,15]. We note that this simplification is further enhanced because of the long list of candidate kinases to phosphorylate those targets. For phosphorylated S1177 (the most studied site), AKT, PKA, AMPK, PKG and CaMKII are all candidates[1]. Less is known about phosphatase action on eNOS, but PP2A, PP2B and PTEN[2] have all been shown to dephosphorylate eNOS [8,16]. The genesis of this mathematical analysis came from work initiated with the neuronal isoform of nitric oxide synthase (nNOS) [17] that looked specifically at pulse generation of NO caused by feedback inhibition of nNOS by NO. Direct feedback inhibition of eNOS by NO is less potent but eNOS has been shown to produce NO in a transient fashion peaking at 1 and 5 min post stimulation with insulin [11]. In our simplification we attempt to understand a mechanism by which this could be plausible for a single phosphorylation modification as well as create a general model for eNOS and other signaling pathways.

In the following section, we present our simple model in the form of a system of differential equations with time delays. The time delays in particular allow for complex behavior induced by intermediaries that are allowed to remain unspecified. Taking a further simplification of a single delay parameter, the third section is devoted to the establishment of stability criteria of an equilibrium state. Stability of the steady state is then analyzed further in Sections 3 and 4 where we consider the delay as a bifurcation parameter. The existence and resulting properties of periodic solutions – local oscillations about the constant equilibrium – are provided in Section 4 via a center manifold reduction. A numerical example, using a set of test parameter values, is provided. We close with a brief discussion of our system as a model of the eNOS activation process and potential implications of the mathematical findings.

2. Mathematical model

The activation and deactivation of an eNOS amino acid site occurs downstream of a sequence of interactions between various kinase and phosphatase species typically initiated by some chemical or mechanical stimulus (e.g. insulin, glucose, bradykinin, etc.). Fig. 1 shows a schematic of the eNOS regulatory network depicting the signal cascade resulting in NO production. Kinases and phosphatases may activate, deactivate, and inhibit activation of one another via phosphorylation and dephosphorylation. And at the final step considered herein, an eNOS site is phosphorylated by a kinase (such as AKT) and dephosphorylated by a phosphatase (such as PP2A) to influence NO production.

We introduce a three species model including a single kinase **A**, a single phosphatase **B**, and one eNOS receptor site **C** whose interactions are illustrated in Fig. 2. These species are taken as a simplified model of the final stage of a complex signal cascade (e.g. the PP2A–AKT–eNOS interactions seen in Fig. 1). Consideration of the multi-step interactions could be accounted for by allowing each of **A** and **B** to be matrix valued functions of time whose rows represent individual enzyme types and having two columns to differentiate between inactive and active forms of each specific enzyme. Preliminary numerical investigations (unpublished) show that such a multi-step system involving a large number of ordinary differential equations is capable of producing the oscillatory behavior expected. Here however, we will assume that each species is vector valued of the form $\mathbf{A} = (a_1, a_2)$, $\mathbf{B} = (b_1, b_2)$, and $\mathbf{C} = (c_1, c_2)$ where the subscript 1 denotes the inactive form and 2 the active form of the respective enzyme. To account for the unspecified upstream reactions, the obligatory formation and decay of various protein complexes, diffusion, mobilization to other compartments, and the interactions of intermediaries, we allow for time delays. In addition, these biochemical processes are conservative so that $a_1 + a_2 = const$, $b_1 + b_2 = const$, and $c_1 + c_2 = const$. Hence our model presents as a limited system of delay differential equations.

Each of the interactions is assumed to be of Michaelis–Menton type with cross species interactions appearing as factors in the related reaction rates. The kinase **A** can convert from inactive to active $a_1 \to a_2$ and vice versa $a_2 \to a_1$, and these conversions can be influenced by the active phosphatase b_2 through both direct dephosphorylation as well as through inhibition of phosphorylation of **A** by upstream kinases (Fig. 2, cell (i)). Combining the regulatory and feedback terms, the equations for the kinase are

$$\frac{da_1}{dt} = -K_{a1} f_p(b_2(t-\tau_b)) \frac{a_1}{K_{an} + a_1} - K_{a2} g_p(b_2(t-\tau_b)) \frac{a_1}{K_{am} + a_1} + K_{a3} b_2(t-\tau_{b'}) \frac{a_2}{K_{ao} + a_2} + K_{a4} \frac{a_2}{K_{ap} + a_2}, \quad (1)$$

$$\frac{da_2}{dt} = -\frac{da_1}{dt}. \quad (2)$$

Each parameter K_{ai}, $i = 1, \ldots, 4$ is a positive rate constant. The effective Michaelis constants $K_{an}, \ldots, K_{ap}$ are positive and allow for up to four distinct substrates. The inhibition of activation of kinase by the active phosphatase b_2 appears in the first two terms on the right hand side of the a_1 equation which is further fractionated to account for multiple substrates [18] by the steady state partition functions

$$f_p(x) = \frac{r_1}{x + r_1 + r_2}, \quad \text{and} \quad g_p(x) = \frac{x + r_2}{x + r_1 + r_2} = 1 - f_p(x).$$

Parameters r_1 and r_2 are effective reaction rate ratios for kinase activation and deactivation processes. This inhibition depends on a previous state of the active phosphatase, $b_2(t - \tau_b)$, with fixed delay time τ_b. (Here, and throughout this paper, the time dependence of nondelayed terms is suppressed. So for example, $a_1 = a_1(t)$ and $a_2 = a_2(t)$ in (1) and (2).) Direct dephosphorylation of active kinase ($a_2 \to a_1$) by active phosphatase at its previous state $b_2(t - \tau_{b'})$ appears in the third term of (1). In this general model, we allow for two distinct delay times for the different phosphatase actions. The final term on the right side of Eq. (1) accounts for the intra-species regulation of the kinase.

We note here that there are roughly 500 kinase proteins in the human genome as contrasted with approximately 200 phosphatases accounting for less interactive specificity of any one protein phosphatase [19–22]. Hence we allow for the active phosphatase to both inhibit activation and to directly deactivate the

[1] AKT – protein kinase B, PKA – protein kinase A, AMPK – activated protein kinase, PKG – protein kinase G, CaMKII – calmodulin-dependent protein kinase II.

[2] PP2A – protein phosphatase 2, PP2B – protein phosphatase 2b, PTEN – phosphatase and tensin homolog.