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Thresholds, long delays and stability from generalized allosteric effect in protein networks

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Abstract

Post-transductional modifications tune the functions of proteins and regulate the collective dynamics of biochemical networks that determine how cells respond to environmental signals. For example, protein phosphorylation and nitrosylation are well known to play a pivotal role in the intracellular transduction of activation and death signals. A protein can have multiple sites where chemical groups can reversibly attach in processes such as phosphorylation or nitrosylation. A microscopic description of these processes must take into account the intrinsic probabilistic nature of the underlying reactions. We apply combinatorial considerations to standard enzyme kinetics and in this way we extend to the dynamic regime a simplified version of the traditional models on the allosteric regulation of protein functions. We link a generic modification chain to a downstream Michaelis–Menten enzymatic reaction and we demonstrate numerically that this accounts both for thresholds and long time delays in the conversion of the substrate by the enzyme. The proposed mechanism is stable and robust and the higher the number of modification sites, the greater the stability. We show that a high number of modification sites converts a fast reaction into a slow process, and the slowing down depends on the number of sites and may span many orders of magnitude; in this way multisite modification of proteins stands out as a general mechanism that allows the transfer of information from the very short time scales of enzyme reactions (milliseconds) to the long time scale of cell response (hours).

Keywords: Multisite phosphorylation; Nitrosylation; Threshold effect; Biochemical model; Network dynamics

1. Introduction

With the advancement of biochemical techniques a huge amount of data on the post-transductional modification of proteins and on its role in the regulation of signal propagation through biochemical networks is now available. This knowledge is challenging our understanding of the cells' behavior, and efforts by experts

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from different disciplines are now required to sort the basic dynamic principles out of experimental observations. As it has been recently pointed out [1], this process is not obvious at all because of cultural differences that drive scientists from different disciplines either to prefer the deep molecular details of biochemical networks or to tackle the generic underlying principles. Here we follow this latter perspective, and investigate the general dynamic consequences of multiple chemical modifications of proteins within biochemical networks.

Proteins can be modified by the attachment and detachment of various chemical groups and by means of different mechanisms. Reversible chemical modifications of proteins are now recognized to play a pivotal role in the regulation of the dynamic behavior of biochemical networks, and therefore in the response of cells to environmental signals. The phosphorylation/dephosphorylation of tyrosine residues, for example, allows the propagation of signals from the cell surface to the nucleus thus enabling the transcription of specific genes in response to the presence of environmental activation molecules [2–4]; another example is the nitrosylation/denitrosylation of cysteine residues as a consequence of the redox state of the intracellular environment which can activate enzymes, such as caspase-3, that participate in the activation of the apoptotic program that leads to controlled cell death [5]. Cell activation, proliferation and death, in turn, are at the basis of animal physiology and pathology. For example, the control of the cell cycle through protein phosphorylation is important for the activation of an immune response against foreign antigens [6]; the activation of the apoptotic program regulates tissue homeostasis and prevents the onset of autoimmune diseases and cancer [7].

Central to most biological networks is the existence of biochemical paths that behave dynamically as on–off switches [8,9]. The chemical modification of proteins on multiple aminoacid residues, like e.g., multisite phosphorylation, has been argued to confer a switch-like character to these proteins. One outstanding example is the retinoblastoma protein (Rb) with its 16 putative phosphorylation sites, where at least 10 of them must be phosphorylated by cyclin-dependent kinases to promote the abrupt and irreversible G1-S transition along the cell cycle [10,11]. The switch-like behavior of proteins within biochemical networks has been traditionally modeled using the phenomenological Hill function [12,13]:

$$v = \frac{kX^n}{1 + kX^n}, \tag{1}$$

where v is a production rate (i.e., the time derivative of the concentration of the reaction product), X is the concentration of some biochemical species, k is a positive constant and n is the so-called Hill coefficient. The Hill function provides switch-like sigmoidal behaviors and models phenomenologically cooperative molecular interactions. And yet, while the Hill function is quite good for phenomenological work it is well known to be physically untenable for the following reasons: (1) the Hill equation implies *simultaneous* molecular interactions and this does not reflect a possible reaction scheme [14]; (2) in the real world, the concentration of proteins, enzymes and substrate fluctuates randomly as a consequence of molecular diffusion processes. In addition, molecules are randomly distributed between daughter cells at mitosis and this introduces an additional stochasticity in the molecular concentrations through cellular generations [15]: the Hill function does not incorporate the stochastic nature of the microscopic physical world and cannot be modified in this sense; (3) the sigmoidal Hill function becomes a step function, and thus describes a true threshold, only for $n \to \infty$, but experimentally, the chemical modification of just one key protein residue can turn the function of that protein on or off. For example, each subunit of the methionine adenosyl transferase (MAT)—a member of the caspase family of cysteine proteases—has 10 free cysteines but only cysteine 121 inhibits the activity of the enzyme when targeted by nitrosylation [5]. The Hill function with $n = 1$ reduces to a standard Michaelis–Menten function which is no longer sigmoidal in the biophysically meaningful range ($X \geqslant 0$) [13], and therefore does not model a threshold behavior at all. The conclusion is that the Hill phenomenology must be replaced by deeper microscopic models to obtain a better understanding of the biochemical thresholds.

From a chemical perspective, the processes leading to the chemical modification of proteins are equivalent to the allosteric regulation of enzymes whereby the substrate itself (homotropic interaction) or molecules other than the substrate (heterotropic interactions) can tune the activity of an enzyme. The allosteric theory dates back to the 1960s when detailed models unifying both type of interactions where also developed, such as the