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EQUIVALENCE OF ENZYME REACTIONS AND SIMPLE AND CASCADED FEEDBACK LOOPS

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Abstract: This paper demonstrates the equivalence between simple feedback loops and the biochemical enzyme reactions in confined media that obey Michaelis-Menten kinetics. Single and cascaded reactions are modeled, and general principles to achieve enzyme transfer functions are set out. Reaction rate and concentration difference are identified as the through and across variables of biochemical networks, and it is shown that Kirchhoff's laws can be applied to these systems. Allosteric inhibition is examined as a way of injecting external signals into the biochemical network. *Copyright © 2002 IFAC*

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1. INTRODUCTION

Even a cursory analysis of living systems reveals that they rely heavily on feedback control to maintain function. It has been stated that biology is more akin to the synthetic sciences like engineering and computer science than to the analytical sciences like mathematics and physics (Leland *et al*, 1999). Because biological systems have been optimized through billions of years of evolution, it can be said that they have been "designed". Thus biology should be subject to the conservation laws (conservation of mass, momentum and energy) and the constraints of dynamics (the behavior of potential differences and fluxes). In other words, biology should obey systems theory. The challenge is to identify the elements of biology that correspond to the variables and parameters of system analysis. Once that is done it should be possible to interpret, manipulate and create the structures of biology in much the same way as is done for other engineering systems.

This paper interprets biology as a chemical engineering system, subject to the law of conservation of mass and the laws of chemical dynamics (kinetics). Concentration levels are used to transmit information in the same way as other analog systems, including the values that have to be sensed for automatic feedback.

2. WILHELMY'S EQUATION AND GENERAL MASS CONSERVATION

In 1850 Leonard Wilhelmy proposed a rate law to describe the behavior of a single, first order chemical reaction:

$$r(t) = kX(t) \tag{1}$$

This law is similar to Ohm's Law, which describes the relation between current (flux) and voltage (potential difference) in a single resistor. Wilhelmy's Law describes the relation between reaction rate (mass flux) and concentration difference in a 1st order reacting system with a single substrate leading to a single product.

The general law of mass conservation in a 3 dimensional medium can be stated as follows:

$$\frac{\partial X(x,y,z,t)}{\partial t} = D\nabla^2 X(x,y,z,t) + \mathbf{V}.\nabla X(x,y,z,t) + r(t) \tag{2}$$

This equation states that the change in concentration of a chemical species equals the sum of the contributions from diffusion, convection and chemical reaction.

Without diffusion and convection this equation reduces to:

$$\frac{\partial X(x,y,z,t)}{\partial t} = r(t) \tag{3}$$

For a continually stirred medium x, y and z can be neglected so that we are left with:

$$\frac{\partial X(t)}{\partial t} = r(t) \tag{4}$$

We may be tempted to substitute Wilhelmy's equation and solve for X(t), but here we need to be cautious and examine what actually happens inside living cells.

The application of control theory to biochemical systems started with the work of Jacob and Monod (1961) on the lactose operon. Even then it was clear that the operon was a control element, and that it could only be understood from the perspective of negative feedback.

System analysis and control engineering are general theories that are applicable to any system where the conservation of some property is respected and that conserved quantity (be it mass, energy or momentum) is distributed over lumped parameters. Systems where these theories are used for analysis and design include thermal systems (conservation of energy), electrical systems (conservation of charge and energy), hydraulic, pneumatic and mechanical systems (conservation of mass and momentum) and chemical systems (conservation of mass).

Biochemical systems are clearly control systems (albeit quite complex) because of the presence of feedback. Even more fundamental is the presence of concentration gradients and fluxes caused by forcing functions. These forcing functions differentiate biochemical systems from ordinary chemical reactions that can proceed to chemical equilibrium in any direction depending on initial conditions. Biochemical reactions are driven in a particular direction to an artificial equilibrium through the consumption of energy (using ATP or free energy). In addition to the energy, the maintenance of this dynamic equilibrium requires the presence of concentration gradients and fluxes, similar to electrical voltages and currents. Instead of a single reaction that one tries to stabilize in chemical process control, biochemical systems have many reactions in series and parallel and there are clear attempts to control the fluxes and the concentrations across these reactions as a whole. Biochemical reaction networks therefore exhibit a similarity to electronic circuits that single chemical reactions do not. The added presence of feedback through inhibitor proteins (like calmodulin) only reinforces the impression that a network/feedback model is appropriate.

Parameter sensitivity analysis (Metabolic Control Analysis) is being used by biochemists (Fell, 1996; Hofmeyr *et al*, 1991) but for a better understanding the methods of control engineering will have to be used, as has been the case in other fields.

In order to apply control theory biochemical elements will have to be identified as control elements. Once that has been done the many techniques of control engineering can be used for analysis.

3. GENERAL DESCRIPTION OF A METABOLIC SYSTEM

Since feedback is present in metabolic systems it seems reasonable to interpret a cell as an automatic control system rather than just a metabolic systems. That is, it is reasonable to expect the cell to have all the elements of a control system – system transfer functions, outputs, comparators, set points, error functions and controllers. The challenge is to identify these elements inside the living cell. Once identification is complete we could proceed to analysis and perhaps design.

A metabolic system consists of reactions mediated by enzymes that process molecules and pass them to each other. It therefore appears similar to an electrical system, which consists of electric elements that processes charge and energy, or a mechanical system that processes mass and momentum. The links in the network is the liquid in which the reactions take place, interpreted such as that each link is the medium for the transfer of a particular metabolite to the next enzyme. So the reactions and their associated enzymes working together are the system, identifiable with the transfer functions in a classical control system or the "A" matrix of a state space representation.

Individual species are used to represent intermediate variables. At steady state all these intermediate variables are equal. During dynamics they vary and differ from each other.

ATP and the free energy of the reactants drive the enzymes and the transport processes – ATP (and the co-factors) is the power source for biochemical systems. This means that ATP can be ignored in the modeling in the same way that power sources are ignored in other engineering systems. That is, unless we are modeling the power system itself.

A metabolic system is a dynamic system an all variables must therefore be with respect to time. The variables are the concentration difference X(t) between the substrate and the product, and the molar flux r(t) of the products. The concentration difference is similar to the voltage v(t) in electrical systems, the velocity difference v(t) in mechanical systems, the pressure p(t) in fluid systems and the temperature difference T(t) in thermal systems. The concentration difference is the across variable – it differs across the reaction. The flux is the through variable – it retains the same value as it goes through the reaction. Flux is similar to current I(t), force f(t), rate of fluid flow Q(t) and rate of heat flow q(t).

Because pressure and volume do not change very much in biochemical systems and temperature is usually regulated, we do not have to worry too much about changing parameters. This is in stark contrast to chemical engineering where engineers are happy just to control the parameters – no attempts are made to manipulate the variables. (Reactions are left to proceed to maximum conversion or equilibrium because that is all that is required. No further engineering is done).

The concentration of the enzymes is usually kept constant through regulation (feedback) applied to the protein synthesis system of the ribosomes and the genes (Jacob and Monod, 1961). This is a separate, higher order system, which I will ignore to get focus in the discussion. The activation of enzymes through phosphorylation or other means would also add new loops to the network and therefore add extra elements to the transfer function or "A" matrix. This would result in new eigenvalues, new steady states, new outputs etc. So signals that activate enzymes would have an enormous effect, since they are changing the network itself, rather than just changing an input. To keep the representation linear, I will consider only the allosteric effects of the metabolites (outputs) and inhibitor proteins like calmodulin.

To summarize – 3 systems are identifiable – the genetic system, the metabolic system and the power/cofactor system. For reasons given, I will ignore the genetic system and the power/cofactor system.

4. ENZYME REACTIONS AS FEEDBACK LOOPS

When a metabolite is left alone it will eventually transform into another substance. This usually occurs very slowly. The metabolite and the daughter substance will settle into a fixed relationship at equilibrium (the one will be some multiple of the other). Clearly the reaction somehow senses the concentration of the daughter substance and slows down the reaction as the daughter concentration increases. There is thus some feedback relation (reverse reaction) between the metabolite concentration and the daughter concentration. If the metabolite concentration is driven or maintained by some forcing function the following control diagram could represent the process (Jacobs, 2001):

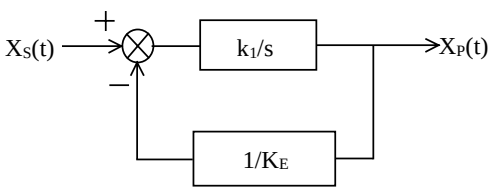

Fig. 1 Michaelis-Menten Reaction as Feedback Loop.

Here K_E is the equilibrium constant, equal to k_1/k_2 (Fogler, 1999). The 1/s term in the system block represents the integration effect of the medium in which the enzyme operates.

This corresponds to the reaction:

$$X_S(t) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X_P(t) \tag{5}$$

The overall transfer function will be:

$$\frac{X_P(s)}{X_S(s)} = \frac{\frac{k_1}{s}}{1+\frac{k_1}{sK_E}} \tag{6}$$

Notice that if the input equals one (so that $X_S(s)=1/s$ after application of the Laplace Transform):

$$X_P(s) = \frac{\frac{k_1}{s^2}}{1+\frac{k_1}{sK_E}} \tag{7}$$

The final value theorem (Raven, 1995) states:

$$\lim_{t\to\infty} X_P(t) = \lim_{s\to 0} sX_P(s) \tag{8}$$

Therefore:

$$\lim_{t\to\infty} X_P(t) = \lim_{s\to 0} \frac{\frac{k_1}{s}}{1+\frac{k_1}{sK_E}} = K_E \tag{9}$$

So:

$$\lim_{t\to\infty} X_P(t) = K_E \tag{10}$$

So if $X_S(t)=1$, $X_P(t)$ will be K_E, as expected from Michaelis-Menten.

Changing the input concentration will change the output concentration (once equilibrium has been reached). The substrate concentration is the set point and the product concentration is the output. So the reaction with the enzyme, substrate and product is a feedback system where the input concentration controls the output concentration, mediated by the enzyme, which settles the system to a new, forced equilibrium. It doesn't matter if the enzyme is non-linear – the feedback linearises the reaction.

5. CASCADES

If two reactions are cascaded we would get the following situation:

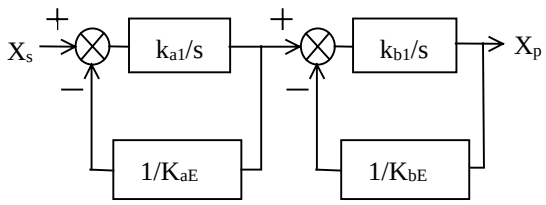

Fig. 2. Two Cascaded Enzyme Reactions

In this case we would get the following transfer function:

$$\frac{X_p}{X_s} = \frac{k_{a1}k_{b1}}{s^2(1+\frac{k_{a1}}{sK_{aE}})(1+\frac{k_{b1}}{sK_{bE}})} \tag{11}$$

Using the final value theorem on this equation, with a step input function for $X_s(t)$, we get:

$$X_{pfinal} = \lim_{s\to 0} \frac{k_{a1}k_{b1}}{s^2(1+\frac{k_{a1}}{sK_{aE}})(1+\frac{k_{b1}}{sK_{bE}})} = K_{aE}K_{bE} \tag{12}$$

So the final value of for $X_p(t)$ is $K_{aE}K_{bE}$, as expected.

The general transfer function for enzyme cascade reactions would then be:

$$\frac{X_p}{X_s} = \frac{\prod_1^N k_{n1}}{s^N \prod_1^N (1+\frac{k_{n1}}{sK_{nE}})} \tag{13}$$

where N is the number of enzymes in the cascade reaction. The time response could then be worked out from Laplace transform inversion. It can be seen that the final value of the cascade product (for a step input) would be the product of all the individual equilibrium constants.

6. KIRCHHOFF'S LAWS

It should be noted that in general chemical mass action is left alone to operate without being driven into any particular direction. The only cause of transient dynamic behavior is the initial substrate, interpreted as a constant (the total mass in the system). What we have done here is insert an external forcing function corresponding to processes that consume energy and maintain the substrate concentration. As a result certain metabolites have concentrations and fluxes that are independent of the results of mass action. Concentration gradients are set up and steady state fluxes are possible. The chemical system becomes an engineering network, obeying Kirchhoff's laws. Instead of a series of mass action differential equations, the equations are now in the form of engineering network equations with forcing functions. The concentrations and fluxes caused by the forcing functions turn the cell into a reaction network.

To illustrate (for a single enzyme):

$$\frac{\partial X(t)}{\partial t} = r(t)$$

from (4)

So:

$$X(t) = \int r(t)dt \tag{14}$$

But:

$$X(t) = X_S(t) - X_P(t) \tag{15}$$

So:

$$X_S(t) = \int r(t)dt + X_P(t) \tag{16}$$

This states that the total concentration is equal to the concentration drop across an individual component plus the concentration drop following – i.e. the total concentration is equal to the sum of the concentrations in the loop. These are normal engineering network equations, (like Kirchhoffs Voltage Law). Also, it can be seen that the concentration drop across a reaction is equal to the integral of the reaction rate. This is similar to the situation for an electrical capacitor, where the voltage drop across the capacitor equals the integral of the current through it, multiplied by the inverse of the capacitance. Because an enzyme drastically reduces the time needed to attain equilibrium, a dynamic (time varying) substrate concentration can now be used to drive a dynamic product concentration. Without the enzyme there would be no control i.e. because of the long time lapse there would be no effect of the substrate variable on the product variable.

Such a system could be described as a (biochemical) feedback amplifier. It is similar to the feedback amplifier described by Black in 1927, using vacuum tubes. The modern common emitter amplifier is the electrical equivalent.

It can be seen that the enzyme is the analog of the transconductance device. The enzyme is the biochemical transistor.

7. ALLOSTERIC INHIBITION

With the above in mind I would like to interpret structures that are under external feedback (allosteric enzymes) as follows:

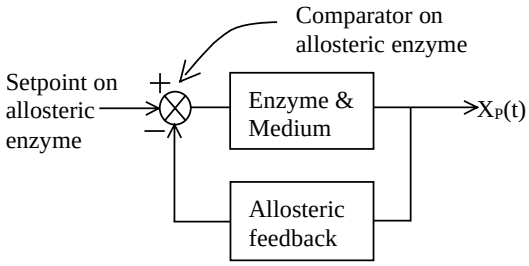

Fig. 4. General Allosteric Feedback

The question now arises – where is the set point and the comparator in this new feedback system? And how could we change the set point?

Negative feedback in, say pyrimidine synthesis achieved when cytidine triphosphate (CTP), the final product in the pathway, inhibits aspartate transcarbamoylase (ATCase), which starts the pathway by forming N-carbamoylaspartate and carbamoyl phosphate (Stryer, 1997). Where is the set point? Clearly the set point is the concentration at which the ATCase accepts CTP, deforms and becomes inactive. Theoretically, by changing the design of ATCase to deform at a different concentration of the end product we could change the set point. In this way we could increase the concentration of CTP – and the feedback system would maintain the increased concentration. The feedback diagram looks as follows:

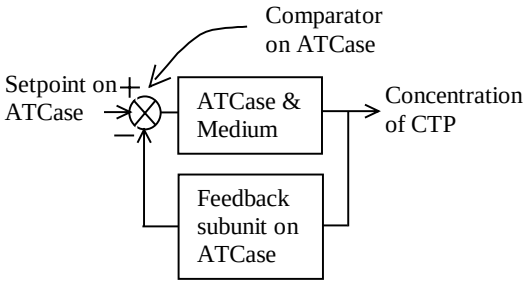

Fig. 5. Allosteric Feedback of ATCase

To change the set point we would have to change the design of the regulatory unit of ATCase or the calmodulin module. Perhaps living organisms already have these subunits, which respond to different concentrations. Notice also that the set points are fixed. It will be difficult to perform step tests or frequency tests on such loops. Perhaps we could use light sensitive subunits.

Where is the comparator? The comparator is also on the allosteric enzyme, which compares the sensed concentration with the concentration at which it must deform and become inactive. When there is no difference (error), the enzyme becomes inactive. The resulting system looks as follows:

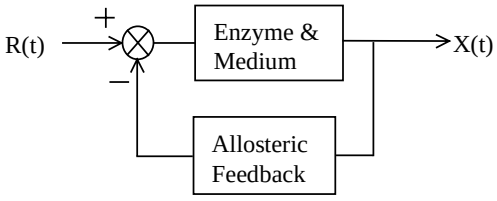

Fig. 6. Allosteric Feedback with External Set Point

R(t) could be any theoretical function. It could be linked to light levels or temperature variations. So concentration variations could be linked to light levels, and the information about that light level would be fed into the biochemical network. Or if the set point were fixed by a genetic operon (Jacob, et al 1961) we would have a steady, robust concentration source, ready to be used if needed. If R(t) is a constant it will change as the species evolves or the organism changes during development. Thus the set points will change, changing the metabolite concentrations inside the organism. Organisms with the right set points will be able to survive in their environment.

If the flux of a metabolite needs to be controlled, forcing a constant concentration through a passive transport system to generate a steady flux will do this. The concentration source is a forcing function, and since it uses energy it is an active element.

If the set point were a constant structure on the regulatory subunit and if the substrate reservoir were very large we would have a constant concentration source (a chemical battery) but if the subunit were e.g. sensitive to light or temperature we would have a variable chemical signal.

These set points would be the real inputs into the system (the independent variables). Material flows would no longer be inputs – that would only apply to systems where regulatory subunits are absent and only dynamic equilibrium is present. Material sources would become reservoirs used to top up the concentration sources when needed – like the unused portions of a battery or buffer.

The concentration of the enzymes themselves would also be maintained in the same way – with the set points and activator/repressor molecules being applied to the genes and the ribosomes. But since feedback isolates subsystems from each other, enzyme concentration regulation can be considered separately.

It can be seen that chemical reactions have been used to process information, and not just to transform chemical species from one identity to another (although this is also done, especially to maintain the reservoirs). This is the fundamental way in which living systems differ from passive chemical reactions.

In addition, the genome is perhaps best described as a genetic program, so that elements like algorithms and state machines become relevant. To summarize: it is good to think of biological organisms as "chemical robots", and to interpret them accordingly.

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