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Enzymes as molecular automata: a stochastic model of self-oscillatory glycolytic cycles in cellular metabolism

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Abstract

A stochastic model based on the molecular automata approach was developed to simulate the cyclic dynamics of glycolysis–gluconeogenesis in cell energy metabolism. The stochastic algorithm, based on Gillespie's method, simulates the master equation associated with any network of enzymatically controlled reactions. This model of the glycolytic–gluconeogenetic cycle was developed by assembling the stochastic kinetic reactions controlled by two enzymes: phosphofructokinase (PFKase) and fructose-1,6-biphosphatase (FBPase). In order to obtain the hysteresis behaviour predicted by classical Sel'kov analysis, a minimum complexity is required in the allosteric regulations. This implies that the FBPase cannot have a single binding site for its transition to the inactive state (a minimum of two or three binding sites is necessary). Given the multimeric structure of this enzyme, this kinetic hypothesis is tenable. Other possible applications of the stochastic automata approach for different cases of channels, receptors and enzymatic control are suggested.

Keywords: Molecular automata; Stochastic model; Digital enzyme; Glycolytic cycle; Hysteresis; Stochastic resonance

1. Introduction

Sel'kov and Igamberdiev have cogently emphasized the importance of temporal control in the futile cycles of cell energy metabolism (CEM) (Sel'kov, 1968, 1979; Igamberdiev, 1993; Igamberdiev et al., 1999).

As expressively formulated by Sel'kov: "If I were God designing an optimal scheme for CEM, I should allow for dramatic time-dependent variations of the energy demands of different consumers as well as a very inhomogeneous distribution of the initial substrates in the extracellular medium" (Sel'kov, 1979, p. 166). In this context, the crucial problem involves stabilizing the concentration of ATP and unstable intermediates. One solution is to design a set of energetic units that are each specialized for a given extracellular substrate. Every unit would contain a depot for the intermediate substance in order to buffer

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temporary depletion of the specific substrate. In addition, to prevent persistent shortages of a specific substrate, the various energetic units should be interconnected to allow the ATP-generating steps to reverse. The resulting structure (Fig. 1) interconnects the energetic units through a ring of reactions recalling the Krebs cycle (Sel'kov, 1979).

One problem with this kind of structure is the appearance of *futile cycles*: the uncontrolled and wasteful dissipation of energy that can take place in ATP-dependent cycles like those between I_i and P_i and between I_i and D_i, $i = 1,2,3$ in Fig. 1.

Futile cycles can be temporally controlled if the opposing reactions are reciprocally controlled by some regulator, which may be the reaction product itself, P_i. This occurs in the carbohydrate branch of CEM, where the forward reaction enzyme, phosphofructokinase, is activated by its product fructose-1,6-P_2 (FBP), while the antagonist enzyme, fructose-1,6-biphosphatase, is inhibited by FBP.

In the present work, the molecular automata approach proposed by Sugita (Sugita, 1961, 1963) and Conrad (Conrad, 1972, 1985) and developed by Marijuán and co-workers (Marijuán and Westley, 1992; Alves, 1995; Marijuán and Alves, 1995) was applied to illustrate how the enzymic control of futile cycles could take place. An interesting aspect highlighted by this approach is that the specific kinetic data necessary to produce the hysteresis behavior in the system (more than one binding site at the FBPase) were inferred by the authors from dynamic-systems considerations alone. Actually, the tetrameric structure of FBPase, as reviewed by Hodgson and Plaxton (1995) and Smolen (1995), indicates that this multiplicity of binding sites is a general property of the enzyme, facultatively realized in the metabolic networks of specific tissues by differential iso-splicing.

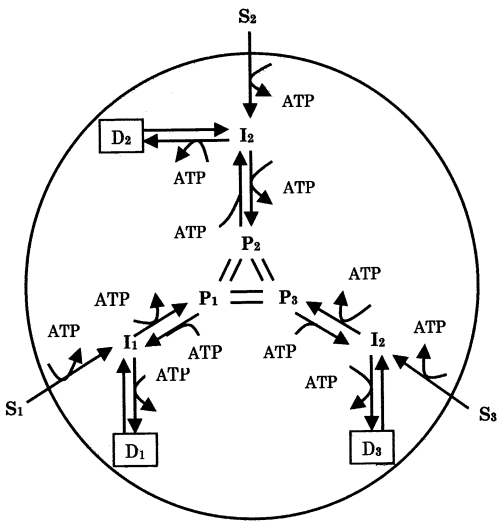

Fig. 1. Multisubstrate structure of the cell energy metabolism according to Sel'kov (1979). In this scheme, three energetic units dependent on their respective substrate S_i are coupled to each other through a central reaction cycle recalling the Krebs cycle.

2. The stochastic molecular automata approach

An automaton is a computing entity which can adopt a variety of internal states, shifting from state to state depending on the values of the state variables and developing a particular function or computation in each state. It can be mathematically expressed using state transition functions and logical tables or by formal languages and generational grammars (Sampson, 1976).

To apply the automaton concept to enzymatic reactions we have to transform the classical system of differential equations into an equivalent automaton whose state changes and command variables must reproduce a dynamics similar to the equations of the system. Fig. 2 represents a generic automata-based model for enzyme activation and function according to Marijuán (1994). Two cases of allosteric regulation are shown: activation (upper) and inhibition (lower).

Enzyme–substrate collisions and eventual reactions are considered stochastic Markov processes generating mean reaction rates in agreement with the observed kinetic constants. Operationally, we define an *occupation number* for any enzyme and chemical substance in the system of reactions and we translate *reaction rates* to *reaction probability per unit time*. This relation is given by the following well known (Gillespie, 1976, 1977) expression:

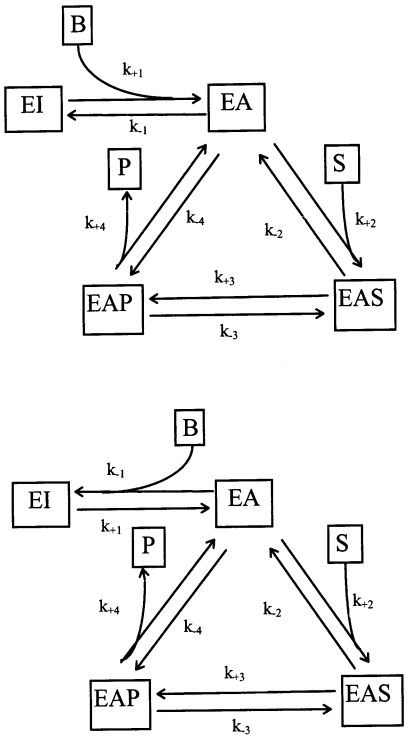

Fig. 2. (a) Formal mechanism of an isomerase regulated by an activator, with a single substrate and a single product. (b) Formal mechanism of an isomerase regulated by an inhibitor, with a single substrate and a single product.

Table 1
Number of molecular reactant combinations for a reaction R_v

Kind of reaction	h_v
External source → reaction products	1
S_i → reaction products	X_i
$S_i + S_j$ → reaction products $i \neq j$	$X_i X_j$
$2S_i$ → reaction products	$X_i(X_i-1)/2$
$S_i + S_j + S_k$ → reaction products $i \neq j \neq k$	$X_i X_j X_k$
$S_j + 2S_k$ → reaction products $j \neq k$	$X_j X_k (X_k-1)/2$
$3S_i$ → reaction products	$X_i(X_i-1)(X_i-2)/6$

$$P(\tau,\mu) = P(\tau)P(\mu)$$

$$= \begin{cases} a_\mu \exp(-a_0\tau) & \text{if } 0 \le \tau < \infty \text{ and } \mu = 1,\ldots,M \\ 0 & \text{otherwise} \end{cases}$$

where $a_\mu \equiv h_\mu c_\mu$ $(\mu = 1,\ldots,M)$ and

$$a_0 \equiv \sum_{v=1}^{M} a_v \equiv \sum_{v=1}^{M} h_v c_v$$

Here $P(\tau,\mu)$ is the probability at time t that the next reaction in the volume V will occur in the differential time interval $(t+\tau,\ t+\tau+\mathrm{d}\tau)$, and that it will be an R_μ reaction, where μ is the number of the reaction. If some statistical *local equilibrium* can be assumed, then

$$c_v = \frac{k_v}{V}$$

where k_v is the deterministic reaction-rate constant for reaction number v.

The h_v are functions that depend on the occupation numbers of the substances in the reaction number v. The form of h_v depends on the kind of reaction taking place and is defined by the corresponding expression in Table 1 (Gillespie, 1976).

To simulate the stochastic time evolution of the system of reactants, we sequentially construct random pairs (τ,μ) with the pair probability density function 2. This is easily done by taking pairs (r_1, r_2) of random numbers out of the unit-interval uniform distribution and transforming them appropriately. We define the successive values of τ by the following expression (Gillespie, 1976):

$$\tau = \frac{1}{a_0} \ln \frac{1}{r_1}$$

and μ is that integer for which

$$\sum_{v=1}^{\mu-1} a_v < r_2 a_0 \le \sum_{v=1}^{\mu} a_v$$

The stochastic evolution of the system will be obtained by applying this method starting from a given initial state. Moreover, successive repetition of the method with different seeds in the random number generator will simulate the solution of the *master equation* associated with the given model.

We have thereby obtained a compact stochastic solver of metabolic reactions controlled by a single enzyme at a moderate programming cost. This stochastic automaton simulates the time behavior of the (probabilistic) local concentrations of the species in the reactions catalyzed by a single enzyme. Obviously, different stochastic automata of this kind can be assembled to model more complex metabolic reactions and pathways.

3. Modelling the allosteric controlling mechanisms of the fructose futile cycles

Futile cycle control in the reversible conversion of fructose-6-P (F6P) into fructose-1,6-P_2 (FBP) was used to illustrate the application of the above automata.

We used an assemblage of two automata, representing the set of reactions catalyzed by fructose-1,6-biphosphatase (FBPase) and phosphofructokinase (PFKase). The model structure is shown in Fig. 3.

This model is the probabilistic automata-counterpart of the kinetic model discussed by Sel'kov (1979). Here, PFKase is activated by its product FBP, while the antagonist enzyme FBPase is inhibited by FBP.

The enzyme reaction has three successive steps (Fig. 2): (1) the active enzyme reacts with its specific substrates, (2) the bonded substrates are converted into bonded products, and (3) the products are released and the enzyme returns to active state. This succession would generate a delay between substrate reception and liberation. However, we grouped the three corresponding reaction rates into one to avoid the possible delay. The main effect of this approximation would be a trivial delay in the response time of the system.

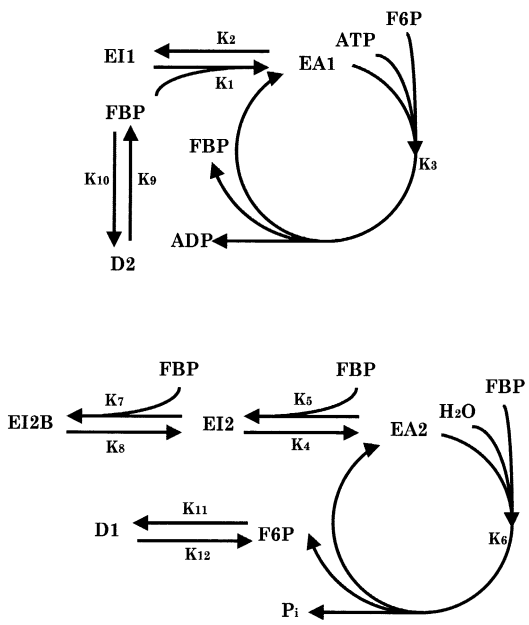

Fig. 3. Formal mechanism of the model used to simulate the futile cycle control between FBP and F6P.

According to Sel'kov, two alternative quasi-steady states of the production reaction should be obtained: the glycolytic state in which the net flux is directed to the right and the gluconeogenetic state in which the flux is reversed. A state with 100% recycling of intermediates is thus highly improbable.

To obtain this kind of behaviour, the FBP production rate could be a rising function of the FBP concentration, but the F6P production rate has to reach a maximum for some intermediate value of the FBP concentration and then tend to zero when FBP tends to infinity.

One interesting feature is that this second condition was not satisfied when FBPase was modelled by the simple automata shown in the lower part of Fig. 2.

In fact, when k_{+2}, k_{-2}, k_{+3}, k_{-3}, k_{+4} and k_{-4} were grouped into a single constant k_c, we obtained a simplified deterministic system associated with the automaton. Using this system, the F6P production rate is:

$$\frac{\mathrm{d[F6P]}}{\mathrm{d}t} = k_c[\mathrm{EA}][\mathrm{FBP}][\mathrm{S_4}]$$

$$\frac{\mathrm{d[EA]}}{\mathrm{d}t} = k_{+1}[\mathrm{EI}] - k_{-1}[\mathrm{EA}][\mathrm{FBP}] \quad (1)$$

The EA steady state is therefore:

$$[\mathrm{EA}] = \frac{k_1[\mathrm{EI}]}{k_2[\mathrm{FBP}]}$$

and the steady production of F6P is FBP-independent:

$$\frac{\mathrm{d[F6P]}}{\mathrm{d}t} = \frac{k_c k_{+1}}{k_{-1}}[\mathrm{S_4}][\mathrm{EI}]$$

In fact, FBPase is too complex an enzyme to be adequately represented by this model. A simple way to increase the complexity is by introducing one more inhibitory receptor in the automata. With two inhibitory receptors the model behaves adequately. If there is initially a number EI(0) of inactive enzymes, the associated deterministic model would be:

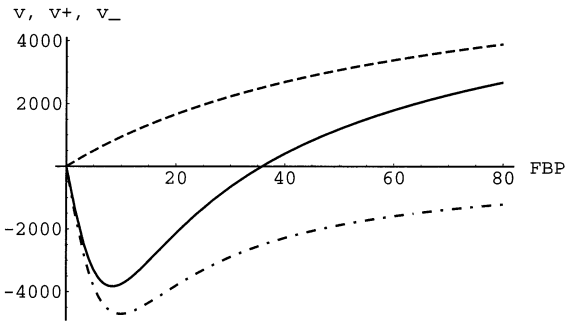

Fig. 4. Velocities v_+ and v_- of the opposing reactions in the futile cycle (2), (3) as a function of FBP at a certain non-zero F6P. Glycolytic state is represented with a dashed line, gluconeogenetic state with a dot–dashed line and the net velocity $v_+ - v_-$ is represented with a continuous line. Point 2 is unstable as discussed in Sel'kov (1979).

$$\frac{\mathrm{d}[\mathrm{EI1}]}{\mathrm{d}t} = k_5[\mathrm{EA}][\mathrm{FBP}] - k_4[\mathrm{EI1}] + k_8[\mathrm{EI2}] - k_7[\mathrm{FBP}][\mathrm{EI1}]$$

$$[\mathrm{EI(0)}] = [\mathrm{EI1}] + [\mathrm{EI2}] + [\mathrm{EA}]$$

$$[\mathrm{EI2}] = \frac{k_7}{k_8}[\mathrm{FBP}][\mathrm{EI1}] \qquad (2)$$

This makes it possible to obtain the steady state production as:

$$\frac{\mathrm{d}[\mathrm{F6P}]}{\mathrm{d}t} = k_6[\mathrm{S4}][\mathrm{EA}][\mathrm{FBP}] = k_6[\mathrm{S4}]\frac{k_4[\mathrm{EI(0)}][\mathrm{FBP}]}{k_4k_5[\mathrm{FBP}] + \frac{k_5k_7}{k_8}[\mathrm{FBP}]^2}$$

where the k-subscripts are those used in Fig. 3b.

This solution has a relative maximum in some critical value $[\mathrm{FBP}]_c$ as required.

The corresponding deterministic system for the F6P steady production is the following:

$$\frac{\mathrm{d}[\mathrm{FBP}]}{\mathrm{d}t} = k_3[\mathrm{F6P}][\mathrm{ATP}][\mathrm{EA1}]$$

$$k_1[\mathrm{EI1}][\mathrm{FBP}] = k_2[\mathrm{EA1}]$$

$$[\mathrm{EI(0)}] = [\mathrm{EI1}] + [\mathrm{EA1}] \qquad (3)$$

where [EI(0)] now represents the initial inactive PFKase concentration.

The solution of the system is the FBP production in the steady state:

$$\frac{\mathrm{d}[\mathrm{FBP}]}{\mathrm{d}t} = k_3[\mathrm{F6P}][\mathrm{ATP}]\frac{k_1}{k_2}[\mathrm{EI(0)}]\frac{[\mathrm{FBP}]}{1 + k_1/k_2[\mathrm{FBP}]}$$

The FBP production rate is therefore a logistic function of the FBP concentration and the difference between the FBP and F6P production rates is the net velocity of the futile cycle.

Fig. 4 shows the theoretical FBP and F6P mean production rates as well as the net velocity of the futile cycle as a function of FBP. As discussed by Sel'kov (1979), point 2 is unstable since a spontaneous increase (decrease) in FBP results in further accumulation (depletion) of FBP, thereby minimizing the periods in which the system remains in that state of 100% recycling. Consequently, a minimal model that can break futile cycles would look like Fig. 3.

An important property of this kind of regulation is that it shows a hysteretic dependence of the quasi-stationary net velocity v on F6P concentration, which results in autonomous oscillations around an unstable stationary state between the two extrema of the characteristic v. To illustrate this property, depots of FBP and F6P were added to the model ('D_1' and 'D_2' in Fig. 3).

4. Numerical results

Some remarks are necessary from a system dynamics point of view regarding the kinetic constants and initial concentrations of the model:

1. The FBP depots must have kinetic constants (k_9 and k_{10} in Fig. 3) such that the FBP concentration outside the depot can be lower than the inside concentration. In this way, the FBP only efficiently excites and inhibits the corresponding enzyme when its flow is coming from the enzymatic activity (and not from the depot).
2. k_{11} and k_{12} must be such that the F6P concentration outside the depot can be moderate, in order to allow some few reactions in the first automaton when the F6P depot is full but the reaction is not yet favored. Constants k_3 and k_6 must be large enough to allow the 'tun-

nelling' of F6P when its concentration is high but its elimination is not yet favored. This tunnelling acts as a *trigger* of the change in reaction direction.

3. When FBP is steadily exciting and inhibiting the two enzymes, the depot of FBP (D_2) fills up and the F6P depot empties. This occurs until the concentration of F6P in the environment becomes scarce. Then the FBP production flow decreases as well as its environmental concentration. FBP is no longer able to continue acting as an inhibitor and starts to (slowly) empty the FBP depot, which produces F6P that fills up the depot D_1.
4. This production process continues until the F6P concentration in the environment returns to high values, which only occurs when its depot reaches large enough concentrations. Then the 'tunneling' of FBP production takes place, even though the direct process is not yet excited, when the large population of F6P takes advantage of the stochastic appearance of a single F6Pase enzyme from time to time. This increase of the tunneling is sensitively detected by the system in the form of an instability that reverts the reaction direction.
5. In order to make this 'tunneling effect' possible, the capacity, that is, the initial concentration in the F6P depot ('D_1' in Fig. 3), must be large enough.

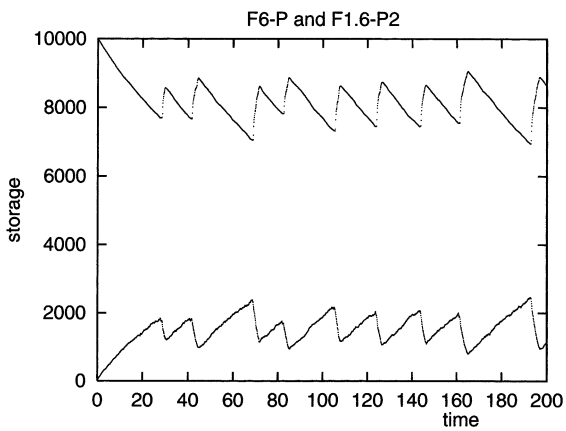

Fig. 5. FBP and F6P concentrations in the depots as a function of time obtained by the model when the data shown in Table 2 are used to simulate the futile cycle control. Sustained oscillations are observed due to a hysteresis process around one unstable node.

Table 2
Relative values of the parameters used in the model[a]

Parameter	Meaning	Value
k_1	Rate of reaction 1	0.1564
k_2	Rate of reaction 2	10
k_3	Rate of reaction 3	0.01
k_4	Rate of reaction 4	80
k_5	Rate of reaction 5	1
k_6	Rate of reaction 6	0.1
k_7	Rate of reaction 7	8
k_8	Rate of reaction 8	10
k_9	Rate of reaction 9	10
k_{10}	Rate of reaction 10	0.01
k_{11}	Rate of reaction 11	1
k_{12}	Rate of reaction 12	0.3
EI1	Initial inactive F6Pases number	100
EA1	Initial active F6Pases number	0
B1	Initial FBP's number	100
S1	Initial F6P's number	70
S2	ATP's number	100
P2	Initial ADP's number	0
EI2	Initial FBPases with one occupied receptor	0
EA2	Initial number of active FBPases	100
EI2B	Initial FBPases with two occupied receptors	0
S4	Number of H_2O molecules	100
P4	Initial number of P_i molecules	0
D1	Initial F6P's number in the depot	0
D2	Initial FBP's number in the depot	10 000
t_0	Initial time	0
t_f	Final time	100
dt	Time step	0.1

[a] Units of reaction rates are the inverse of a time.

The results obtained by the model for the FBP and F6P concentrations in the two respective depots are shown in Fig. 5, when the initial values given in Table 2 are used.

In the simulation, the ATP number is maintained constant as well as the number of H_2O molecules. The P_i and ADP molecules are reaction products and thus do not have a direct influence on the reactions.

There appears a sustained oscillation of F6P and FBP concentrations in the two respective depots, as a consequence of the hysteresis between the two quasi-steady states around the unstable

point. As expected, when one depot is filled, the other is emptied.

The stochastic nature of the simulation is evident in two main features: (1) the discrete nature of the dependent variables, which fluctuate around a mean cyclic behaviour, and (2) the precise time of system destabilization (from its current quasi-steady state) that becomes unpredictable. As mentioned above, a stochastic burst of several tunnelling reactions will cause this phenomenon. Since the appearance of this triggering effect is stochastic in nature, the period of the sustained oscillation is unpredictable.

In dynamic systems theory, this kind of phenomena is known as *stochastic resonance* (Benzi et al., 1982; Nicolis, 1982). It has been used to explain such things as the unpredictability of glaciations and the clear statistical periodicity of glaciations in the Quaternary scale, as shown by the Fourier transform of many climatic series.

According to Sel'kov, the observed glycolytic oscillation has three main functions: (1) it represents a solution for the temporal control of futile cycles and the corresponding waste of ATP, (2) it maintains a finite storage of several substrates or intermediate substances for cell metabolism to avoid any momentary shortages, and (3) it can be used as a time-keeping mechanism. Even though the period obtained is not stable, it can be stabilized when a multiplicity of chemostatic negative feedback mechanisms are introduced into the energetic structure (Sel'kov, 1979).

An interesting side remark is that this hysteretic effect would not be produced if the enzyme involved in the gluconeogenetic state (FBPase) had a single binding site for inhibitory control, as is the case in Fig. 2b. In fact, the complex nature of FBPase was inferred by the authors exclusively from the dynamic-system considerations shown in section 3. Later on, looking at the literature (Hodgson and Plaxton, 1995; Smolen, 1995) and at the WWW libraries (PDB Protein Databank) for the sequence and molecular structure of this enzyme, we found that it constitutes a multidomain aggregate composed of four identical protein chains. Therefore, different iso-enzyme variants could be obtained by differential splicing in specific tissues, containing the kinetically required number of binding sites in accordance with the oscillatory needs of the system.

The execution time of the overall model mainly depends on the magnitude of the reaction rate constants. In the present case, it was on the order of a few seconds (on an IBM-6000 system) which allows the interactive utilization of the model. This execution time will, of course, be proportional to the number of automata assembled in the study.

5. Final comments

Allosterism is the key enzymatic device to create networking controls. As Sols (1981) comments: "Allosteric regulation of enzyme activity is the central and more basic mechanism of physiological modulation of enzyme activity. Its discovery and conceptualization opened a third dimension in physiological enzymology, dramatically recognized by Monod with the confession: 'j'ai decouvert le deuxieme secret de la vie'".

Very possibly, the theoretical elaboration of these findings has not yet reached a complete maturity. In our opinion, the molecular automata approach developed by Marijuán and Westley (1992) may be a promising general language to describe the organization and collective emergence of allosteric networks of enzymes.

Heuristically, automata formulations of activation–inhibition patterns seem more general than rate equations: very often in biological systems we are in a position to infer patterns of activation and inhibition between observable quantities, but not directly to write down a system of rate equations. In addition, an automaton takes into account the probabilistic nature of the transitions between states in one enzyme and the biochemical reactions in a much more straightforward way. In fact, the discrete-stochastic nature of cellular metabolism processes is a striking feature that has not gone unnoticed by classical enzymologists (Sols and Marco, 1970).

We have applied the automata approach to demonstrate the emergence of self-oscillatory glycolytic cycles and how the cell uses them as a basic metabolic control. Carefully controlling the

metabolic network is crucial as it represents the productive infrastructure of the cellular system. Through its reaction pathways and control feedbacks, the system achieves a permanent capacity of (self) construction and primary problem-solving. That is to say, the self-organization implicit in the overall interconnection of metabolic paths allows the system to steadily accumulate nutrients, components, etc., and provides the raw material needed to solve the foreign problems encountered in the environment. Every cellular solution has to be finally carried away by enzyme 'work horses' running in metabolic pathways and blindly following the control signals mandated by the allosteric-automata changes of state.

Actually, most of the control mechanisms that appear in the reviews of metabolic enzyme mechanisms seem consistent with the automata approach: competitive inhibition, substrate inhibition, allosterism in general, many cases of homotropic and heterotropic kinetics, molecular cofactors, and even covalent modification could be modelled. This approach can also be applied to operon and gene network regulation, which has been done classically by Thomas (1991). Capstick et al. (1992) and Marijuán (1994) have also developed the digital representation of small enzyme pathways using automata theory and equivalent digital circuits, with flip-flops and logic gates.

We are currently simulating membrane receptors, channels, and other components of the cellular signalling system following this approach based on stochastic assembled automata, and this will be the object of a future report. To the extent that kinetic 'anomalies' of these cellular signaling components are amenable to more formal treatment, more ambitious simulation goals could be envisioned —in particular, the molecular adaptation of receptors by methyl or phosphate groups, the amplifying chain of protein-kinases and phosphatases, the vertical organization of signaling pathway components in 'transducisomes', the generalized cross talking among such signaling pathways, etc.

Due to its great modularity and efficiency, we think that this approach can potentially model many self-organization and information processes taking place in the intracellular medium.

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