ELSEVIER

# A cellular automata model of enzyme kinetics

**Lemont B. Kier,* C.-K. Cheng,* Bernard Testa,† and Pierre-Alain Carrupt†**

**Departments of Medicinal Chemistry and Mathematical Sciences, Virginia Commonwealth University, Richmond, Virginia*
*†Institute of Medicinal Chemistry, University of Lausanne, Lausanne, Switzerland*

*We have developed a cellular automata model of an enzyme reaction with a substrate in water. The model produces Michaelis–Menten kinetics with good Lineweaver–Burk plots. The variation in affinity parameters predicts that, in general, hydrophobic substrates are more reactive with enzymes, this attribute being more important than the relationship between enzyme and substrate. The ease of generation and the illustrative value of the model lead us to believe that cellular automata models have a useful role in the study of dynamic phenomena such as enzyme kinetics.* © *1996 by Elsevier Science Inc.*

*Keywords: cellular automata, dynamic models, enzyme kinetics*

## INTRODUCTION

The functioning of enzymes produces phenomena driving the processes that impart life to an organic system. The principal source of information about an enzyme-catalyzed reaction has been from analyses of the changes produced in concentrations of substrates and products. These observations have led to the construction of models invoking intermediate complexes of ingredients with the enzyme. One example is the Michaelis–Menten model, postulating an intermediate enzyme–substrate complex, followed by changes in the substrate leading to a product.

E + S <----> ES ----> EP <----> E + P

Enzyme reactions, like all chemical events, are dynamic. Information coming to us from experiments is not dynamic even though the intervals of time separating observations may be quite small. In addition, much information is denied us because of technological limitations in the detection of chemical changes. Our models would be improved if we could observe and record all concentrations at very small intervals of time. One approach to this information lies in the creation of a model in which we know all of the concentrations at any time and know something of the structural attributes of each ingredient. A class of models based on computer simulations such as molecular dynamics, Monte Carlo simulations, and cellular automata offers such a possibility.

Our goal in this study is to explore the possibility of using cellular automata to model some of the phenomena of enzyme reactions and to determine if there is a potential here for acquiring new information about these processes. We conduct these studies by varying the rules relating the enzyme, substrate, product, and water to themselves and to the other ingredients. We allow the dynamics to proceed and observe the initial velocities and progress of the product concentration. This information is compared to a Michaelis–Menten model. Some general inferences about the influence of substrate–water and substrate–enzyme relationships on rates of reaction are made, illustrating the potential value of cellular automata in this area.

## CELLULAR AUTOMATA

Cellular automata are dynamic systems that are discrete in space, time, and state and whose behavior is specified completely by rules governing local relationships. It is an attempt to simplify the often numerically intractable dynamic simulations into a set of rules that mirror intuition and are easy to compute. As an approach to the modeling of emergent properties of complex systems, it has a great benefit in being visually informative of the progress of dynamic events. From the development by von Neumann[1] a variety of applications ranging from gas phenomena to biological applications have been reviewed by Ermentrout and Edelstein-Keshet.[2] We have begun to use cellular automata to advance our understanding of water and solution phenomena and have embarked on a series of studies with this goal in mind.

The Color Plate for this article is on page 226.

Address reprint requests to: Dr. Kier, Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, Virginia 23298.

Received 29 July 1996; revised 10 September 1996; accepted 17 September 1996.



655 Avenue of the Americas, New York, NY 10010

0263-7855/96/$15.00
PII S0263-7855(96)00073-2

Our model is composed of a grid of square spaces called *cells*. Each cell, *i*, has four adjoining neighbors, *j*, and four extended neighbors, *k*, beyond *j*. This is called an *extended von Neumann neighborhood* (Figure 1). Each cell is assigned a state governing whether it is empty or is occupied by a water molecule or other ingredients in our simulation. The contents of a cell may break away from an occupied neighboring cell or move to join a neighboring cell that is occupied. These trajectories result from probabilistic rules assigned at the beginning of the dynamics to reflect an anticipated relationship among the ingredients in the system. The rules are then applied to each cell at random until all cells have computed their state and trajectory. This is one iteration of time. The rules are applied uniformly to each cell of the same state. The initial state of the system is random, hence it does not determine subsequent configurations at any iteration. The same set of rules does not yield the same configurations except in some average sense. The configurations after many iterations reach a collective organization that possesses a relative constancy in appearance and in reportable counts of cells, called *attributes*. These are the emergent characteristics of a complex system.

## THE MODELING RELATIONSHIP

Using cellular automata dynamics we have studied models of water,[3] solution phenomena,[4] the hydrophobic effect,[5] solute dissolution,[6] immiscibility and solute partitioning,[7] and micelle formation.[8] As a continuation of these studies we model the dynamics of enzyme reactions. It should be made clear what the cells, the configurations generated, and the cellular automata model represent. This is important in order to derive any understanding of the results of a simulation and to dispel misunderstanding based on direct comparisons with molecular models. A cell with a state value encrypting occupation by a particular ingredient is not a model of a molecule with specific electronic and steric features. It is a statement of the existence in space–time of an object that has certain rules governing its trajectory. These rules govern the transactions of the ingredient in a cell with all other ingredients in neighboring cells, the von Neumann neighborhood. The ingredient is interpreted to be a molecule, specified or not, that is defined only by its state and transition functions. The rules are considered sufficient to allow the dynamics to proceed. Electronic and topological characterizations are considered to be subsumed into these rules.

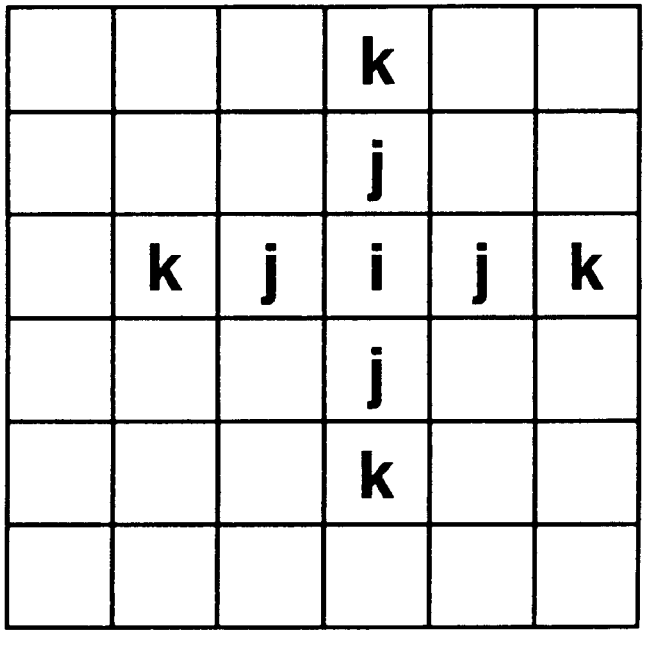


*Figure 1. von Neumann neighborhood of cell* i.

The transactions in the dynamics result in configurations after several iterations that involve more than a single molecule. There is simulated the emergent behavior of a *molecular system*. We define a molecular system as the minimum number of molecules (or their modeled surrogates) necessary to model a phenomenon that is recognizable as emergent behavior. This emergent behavior is epitomized by visual patterns or calculable attributes such as the average counts or size of configuration features. The molecular system is intermediate between the molecular level and the bulk-phase models of systems. Molecular systems may be modeled with molecular dynamics, Monte Carlo, or cellular automata dynamics. Molecular-level phenomena are modeled by quantum mechanical-based methods while bulk-phase phenomena are modeled by statistical and thermodynamic methods.

## THE RULES

The cellular automata dynamics are run on the surface of a torus to eliminate a boundary condition. The grid of cells is $110 \times 110 = 12{,}100$ cells. The water-designated cells are 69% of the total, in agreement with the fraction of space occupied by water, used and verified in our earlier studies. The cavity space represented by empty cells is thus 31% of the total cell complement. When additional ingredients are introduced they are assumed to displace the water cell count on a one-for-one basis, thus the 31% cavity complement is always maintained. These ingredients and their designations are enzyme, E, substrate, S, and product, P. The rules govern the movement, joining, and breaking of cells ingredients with each other and with neighboring cell ingredients. The rules take the form of probabilities. Each cell type, *X*, has a set of parameters governing its relationship to itself and to all other cell ingredients, *Y*. The joining parameters, *J*(*XX*) and *J*(*XY*), determine the extent of cell *X* moving toward another cell *X* or *Y* in a von Neumann neighborhood. The breaking parameters $P_B(XX)$ and $P_B(XY)$ determine the extent of disruption of adjacent cells occupied with like or unlike ingredients. The movement probability, $P_m$, determines the extent of any movement, thus for an enzyme cell, $P_m = 0$ would designate a stationary enzyme.

The enzyme, E, is allowed to join with only one molecule of either S, P, or *W*, but not another E. Thereafter further joining with any other ingredient is not possible. The E cells are constrained to remain some designated minimum distance from any other E cell. A parameter, $P_c$, is employed that describes the probability of an ES pair of cells changing to an EP pair of cells. This is chosen to be an irreversible event in these studies.

We have learned from our previous studies that the values

**Table 1. Initial concentrations of substrate and initial reaction velocities**

| $[S]_0$[a] | $v_0$[b] |
|---|---|
| 0.0415 | 0.76 |
| 0.0830 | 1.41 |
| 0.1245 | 2.02 |
| 0.1660 | 2.42 |
| 0.2075 | 2.76 |

[a]Concentration expressed as S cells/12,100.
[b]Velocity expressed as P cells/time, where time is expressed as iterations.

of the parameters selected for the dynamic simulations impart attributes to ingredients or pairings of ingredients that are familiar in physical terms. For example, we have demonstrated that the choice of the water-breaking parameter, $P_B(WW)$, influences the extent of association of water cells. This attribute can be likened to the influence of temperature on the water structure. The general influence of $P_B(XY)$ is to create a degree of similarity between $X$ and $Y$. Low values of this parameter encode high similarity while high values relate to dissimilar properties. This relationship was important in our simulation of relative lipophilicity and the modeling of the hydrophobic effect. The collection of rules associated with an ingredient is thus a profile of the structure of that ingredient and its relationship with other ingredients, within our definition of a molecular system. By systematically varying the rules we can develop a profile of configurations reflecting the influences of different structures.

## RESULTS

### Michaelis–Menten kinetics

Our objective in this initial study was to determine if Michaelis–Menten kinetics are observed from our cellular automata model of an enzyme reaction. In this study we use a grid of 12,100 cells. Fifty of these cells are designated as enzymes and a variable number of cells are designated as substrates (Table 1). The remaining cells of the grid are designated as water or cavities, the latter comprising 31% of the grid space. The $P_c$ value was arbitrarily chosen to be 0.1 and the $P_B(WW) = 0.375$ was chosen to simulate water at body temperature. The five concentrations in Table 1 were run for 100 iterations, 100 times to determine an average initial velocity, $v_0$, shown in Table 1. From the Michaelis–Menten equation there is a high degree of linearity between the reciprocals of the concentration and the initial velocity:

$$1/v_0 = 0.050(/[S_0]) + 0.110$$
$$r^2 = 0.98 \qquad n = 5$$

The plot in Figure 2 shows the Lineweaver–Burk representation of the data. From this we calculate the $v_{max}$ to be 9.09, the $K_m$ to be 0.455, and the $k_{cat}$ for the process $ES$ ----> $EP$ to be 0.182. The influence of the $P_c$ probability on this process was evaluated using five different values of this parameter (Table 2). The Lineweaver–Burk plots of these data are shown in Figure 3. The relationship of $k_{cat}$ to $P_c$ is

$$k_{cat} = 629.5\ P_c + 168$$
$$r^2 = 0.98 \qquad n = 5$$

**Table 2. Values of kinetic properties due to $P_c$ parameter variation**

$1/v_0 = a/[S]_0 + b$

| $P_c$ | $a$ | $b$ | $v_{max}$ | $K_m$ | $k_{cat}$ |
|---|---|---|---|---|---|
| 0.9 | 61 | 0.034 | 29 | 0.147 | 725 |
| 0.7 | 84 | 0.040 | 25 | 0.175 | 625 |
| 0.5 | 118 | 0.052 | 19 | 0.192 | 475 |
| 0.2 | 306 | 0.085 | 12 | 0.294 | 300 |
| 0.1 | 601 | 0.110 | 9 | 0.455 | 225 |

### Parameter influence

A systematic variation of the rules governing the interactions of ingredients is presented in Table 3. The + entry in Table 3 reflects a high degree of affinity or similarity between any two ingredients and is encoded into a low value of a $P_B(XY)$ rule. Thus a water–substrate ($WS$) rule with a + sign in Table 3 reflects a rule that describes a substrate with

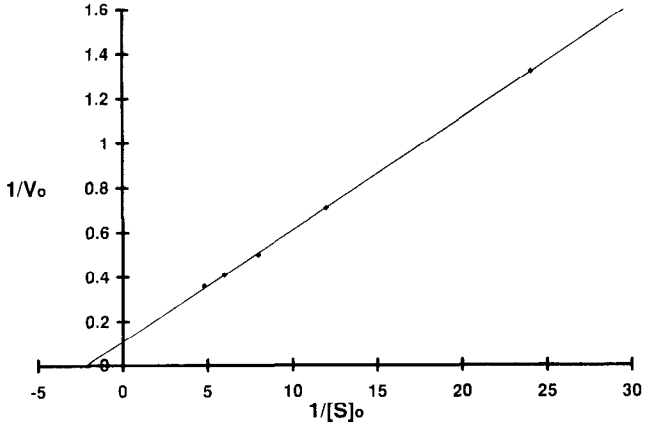


*Figure 2. Lineweaver–Burk representation of data in Table 1.*

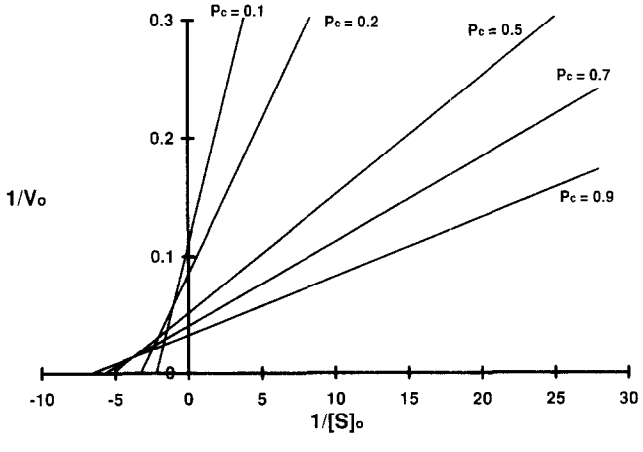


*Figure 3. Lineweaver–Burk representation of data in Table 2.*

**Table 3. Parameter sets and reaction progress**

| | Relative affinities[b] | | | | Concentration[c] | | |
|---|---|---|---|---|---|---|---|
| Set No.[a] | W–S | W–P | E–S | E–P | $[P]_{80}$ | $[ES]_{80}$ | $[EP]_{80}$ |
| 13 | – | – | + | – | 134.8 | 0.82 | 6.6 |
| 11 | – | + | + | – | 121.8 | 0.69 | 6.5 |
| 6 | – | – | + | + | 110.8 | 0.72 | 27.1 |
| 2 | – | + | + | + | 102.8 | 0.48 | 25.6 |
| 14 | – | + | – | – | 87.5 | 0.83 | 4.4 |
| 16 | – | – | – | – | 82.2 | 0.85 | 4.0 |
| 12 | – | – | – | + | 76.6 | 0.82 | 17.7 |
| 10 | + | – | + | – | 75.4 | 0.14 | 2.8 |
| 9 | – | + | – | + | 72.0 | 0.57 | 18.8 |
| 5 | + | + | + | – | 69.1 | 0.07 | 1.7 |
| 3 | + | – | + | + | 62.9 | 0.20 | 13.8 |
| 1 | + | + | + | + | 60.3 | 0.08 | 8.4 |
| 15 | + | – | – | – | 51.0 | 0.14 | 2.9 |
| 8 | + | + | – | – | 50.3 | 0.09 | 1.7 |
| 4 | + | + | – | + | 47.1 | 0.19 | 4.9 |
| 7 | + | – | – | + | 43.8 | 0.08 | 11.0 |

[a]Ordered according to $[P]_{80}$ values, expressed as average counts of cells.
[b]+, High affinity; –, low affinity.
[c]Concentrations at 80 iterations.

water-like or polar characteristics. A – sign for a pair of ingredients in Table 3 is characteristic of a relatively nonpolar or lipophilic characteristic. The model used 50 enzyme cells, all with a moving probability of one, 1,000 substrate molecules and 7,350 water-designed cells, all of these ingredients on a 12,100 grid positioned on the surface of a torus. All recorded values are 10-run averages shown in Table 3. These concentrations are expressed as the count relative to the total number of cells. The data obtained from the runs were from the progress curve at 80 iterations. This was chosen as a point well into the steady state phase of the dynamics but not so far that multiple runs could be obtained in a reasonable length of time. Using the $[P]_{80}$ concentration as a measure of the extent of the reaction at a common time, it is possible to derive some understanding of the influence of interingredient relationships. Inspection of Table 3 reveals that the reaction progresses to a higher $[P]_{80}$ value when the water–substrate encounters are minimal. The dynamics reveal less influence on the time course of the reaction from the enzyme–substrate relationship. Here a high affinity between the two, encoded with a + entry in Table 3, tends to promote the reaction. A modest influence comes from the relationship of the enzyme and the product, where a low affinity tends to promote the reaction.

One consequence of the influence of the water–substrate affinity rules is the effect on the enzyme–substrate concentration, $[ES]_{80}$. Low affinity between water and substrate favors a higher $[ES]_{80}$ value. A close look at the data in Table 3 shows that the influence of the water–substrate affinity on the $[ES]_{80}$ is greater than the influence of the enzyme–substrate affinity. The data also reveal that the enzyme–product affinity directly influences the $[EP]_{80}$ complex concentration, a higher affinity leading to a higher concentration.

The visualization of the dynamic events described here is of significant value from a didactic point of view. It is possible to see the model evolve over time as a function of concentrations and interingredient relationships. In Color Plate 1 a view of the dynamics is shown. The coloration chosen highlights the relationship among the enzyme, substrate, product, and water cells.

## DISCUSSION

We have shown that a cellular automata model of an enzyme reaction can produce outcomes characteristic of experimentally observed results. This includes models of the relationships between ingredients and the solvent, water. By choosing rules describing cell-joining and -breaking probabilities, we can obtain numerical counts of product and complex concentrations that may be expressed with familiar equations. These are shown to follow the Michaelis–Menten equation and to give good reciprocal plots using the Lineweaver–Burk formalism. A cellular automata model of an enzyme reaction makes it possible to follow concentrations of all ingredients at each time (iteration) interval. Thus some picture of an entire process is available if the model can be trusted to mirror experimental results. These models impart a level of confidence since Michaelis–Menten kinetics are shown.

A value of any model lies in the creation of some understanding of a process beyond just a simple reproduction of observables. In these studies, because we can choose rules depicting different enzyme and substrate characteristics, we can model their influence on the extent of the reactions. A systematic variation in the rules leads us to some understanding of these influences. We see in these models that a lower affinity between a substrate and water leads to a greater extent of the $S$ ----> $P$ reaction at a common time on

the progress curve. This influence is greater than the modeled affinity between the enzyme and the substrate. Putting this prediction in other terms, we would say that the substrates that are more lipophilic may be more reactive in the $S$ ----> $P$ conversion. This agrees with a number of observations of enzyme and metabolic reactions.[9,10] This relationship is more influential than the affinity between the enzyme and the substrate. The consequences of the influence of the water–substrate affinity appear to be directed toward the $[ES]_{80}$ value, which remains constant through much of the steady state phase of the reaction. A low affinity favors a high $[ES]_{80}$ value, thus the availability of substrate to encounter an enzyme is strongly mitigated by the entrapment of the substrate into the water continuum. When the substrate is more lipophilic, it presages a higher probability for more enzyme–substrate complex to form.

## CONCLUSION

In conclusion, we demonstrate here that cellular automata models, generated from suitable rules, have the capacity to reflect some of the useful characteristics of enzyme kinetics. This approach opens up opportunities for the study of a general enzyme model and the effects on its behavior, which includes the interaction of ingredients with the solvent. The influence of substrate concentration has been examined here, along with the effect of changing its relative lipophilicity and enzyme affinity. Many other aspects of this complex system are now open to this approach including effects of temperature, enzyme association, and the presence of inhibitors.

## ACKNOWLEDGMENTS

The authors thank R. Westkaemper for helpful discussions and M. Dowd and F. Billois for technical assistance. The author (L.K.) thanks the A.D. Williams Foundation at VCU for a grant supporting the computer facilities and the Fondation Herbette for sabbatical support at the University of Lausanne. The program DING-HAO was used to compute the cellular automata dynamics.

## REFERENCES

1 von Neumann, J. In *Theory of Self-Reproducing Automata* (Burks, A., ed.). University of Illinois Press, Urbana, Illinois, 1966

2 Ermentrout, G.B. and Edelstein-Keshet, L. Cellular automata approaches to biological modeling. *J. Theor. Biol.* 1993, **160,** 97–113

3 Kier, L.B. and Cheng, C.-K. A cellular automata model of water. *J. Chem. Inf. Comput. Sci.* 1994, **34,** 647–652

4 Kier, L.B. and Cheng, C.-K. A cellular automata model of an aqueous solution. *J. Chem. Inf. Comput. Sci.* 1994, **34,** 1334–1337

5 Kier, L.B., Cheng, C.-K., Testa, B., and Carrupt, P.-A. A cellular automata model of the hydrophobic effect. *Pharm. Res.* 1995, **12,** 615–620

6 Kier, L.B. and Cheng, C.-K. A cellular automata model of solute dissolution. *Pharm. Res.* 1995, **12,** 1521–1525

7 Cheng, C.-K. and Kier, L.B. A cellular automata model of oil–water partitioning. *J. Chem. Inf. Comput. Sci.* 1995, **35,** 1054–1059

8 Kier, L.B., Cheng, C.-K., Testa, B., and Carrupt, P.-A. A cellular automata model of micelle formation. *Pharm. Res.* 1996 13,1419–1422

9 Hansch, C. A quantitative approach to structure–activity relationships. *Acc. Chem. Res.* 1969, **2,** 232–254

10 Hansch, C. and Leo, A. *Exploring QSAR.* American Chemical Society, Washington, D.C., 1995