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Reaction Kinetics in Intracellular Environments: The Two Proposed Models Yield Qualitatively Different Predictions

Željko Bajzer,[a,*] Miljenko Huzak,[b] Kevin Neff,[a] and Franklyn G. Prendergast[c]

[a]*Department of Biochemistry and Molecular Biology, and Biomathematics Resource, Mayo Clinic College of Medicine, 200 First St. SW, Rochester MN, 55905, USA*

[b]*Department of Mathematics, University of Zagreb, Bijenička 30, HR-10000 Zagreb, Croatia*

[c]*Department of Molecular Pharmacology and Experimental Therapeutics and Department of Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, 200 First St. SW, Rochester MN, 55905, USA*

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A recently proposed model by Schnell and Turner for reaction kinetics in environments crowded by macromolecules is applied to elementary bimolecular binding. It is found that it leads to an unusual equilibrium constant equal to zero. The progress curves are qualitatively different from the prediction of a model based on a non-integer (fractal) power law proposed earlier by Savageau. In the case of the Michaelis-Menten reaction, the two models predict qualitatively similar progress curves and identical equilibrium concentrations. The two models are investigated analytically and numerically, and their differences are discussed in regard to possible validation of the models by use of experimental data.

Key words
macromolecular crowding
law of mass action
fractal kinetics
enzymatic reactions

INTRODUCTION

Biochemical reactions in intracellular environments are characterized by macromolecular crowding. In such circumstances the classical law of mass action is not adequate but should be modified to include excluded volume effects as proposed by Minton.[1–4] This is reflected in the need for correction factors for rate constants, which can depend on the concentrations of all molecular species present in the system.[1–5] The subject was recently thoroughly reviewed by Schnell and Turner.[5] They proposed a modification of the law of mass action in which the rate constant is, in fact, a function of time. This idea is based on the extensive work of Kopelman and coworkers on reaction dynamics in spatially constricted media; they proposed so-called fractal kinetics as a phenomenological description of reactions in media that can be considered fractal.[6–9] In this approach the »rate constant« at long times decreases as a power function of time, *i.e.* $k(t) = k_1 t^{f-1}$, where $0 < f \leq 1$ is the fracton dimension of the corresponding fractal.[9,10] The problem remains, however, how to describe consistently the reaction kinetics for all times and to avoid the singularity at $t = 0$. To solve this problem Schnell and Turner[5] proposed: $k(t) = k_1(t + \tau)^{f-1}$, which is the Zipf-Mandelbrot temporal distribution[11,12] with a characteristic constant τ. They assumed that this function can also describe the reaction kinetics at the beginning of a reaction. In order to justify this as-

* Author to whom correspondence should be addressed. (E-mail: bajzer@mayo.edu)

sumption they have simulated enzyme kinetics obeying Michaelis-Menten mechanism by using the lattice gas automaton, an approach that was previously proposed by Berry.[13] The resulting time course for the concentration of the intermediate complex was found to be in agreement[5] with the proposed $k(t)$.

Schnell and Turner also considered another approach to kinetics for reactions *in vivo*, one first proposed by Savageau[14–16] and further developed and applied by Voit and Savageau.[17–21] In this approach the »rate constant« effectively depends on the concentrations of reactants, rather than explicitly on time. Thus the association rate for the elementary bimolecular reaction $A + B \rightarrow C$ is given by $k_s[A]^\alpha [B]^\beta$, where α and β are parameters which can be larger than 1 and can have non-integer values. Comparing the prediction of Savageau's approach for progress curves of simulated Michaelis-Menten reaction, Schnell and Turner found considerable quantitative disagreement.[5] On the other hand, their model fitted well the progress curves for simulated Michaelis-Menten reaction. These results suggest that their kinetic equations should be preferred over the equations proposed by Savageau.[20]

In view of these findings, we wished to determine, how and to what extent the predictions of the Schnell-Turner (ST) and Savageau-Voit (SV) kinetic models differ in the case of elementary bimolecular binding. It turned out that the difference is not only quantitative but also qualitative. First, ST model with $f < 1$ predicts a zero equilibrium concentration for the complex C in the reaction $A + B \rightleftharpoons C$, while the SV model predicts a non-zero equilibrium concentration. Second, the concentration $[C]_t$ in the Schnell-Turner approach (for $f < 1$) shows a distinct maximum at a finite time, while in the Savageau-Voit approach the maximum (equilibrium) is achieved at infinity. We believe that these findings will facilitate the design of experiments that could reveal which of the two kinetic models represents better description of reaction kinetics in heterogeneous media, crowded by macromolecules.

In the present paper we first consider the two models for bimolecular association and the complete conditions for their equivalency.[10,20,5] Then, we discuss in detail equilibrium concentrations in bimolecular binding (when both association and dissociation ocur), and progress curves as predicted by the two models for various initial conditions. We also consider to what extent, and within what time range, the two models predict similar progress curves when the parameters are adjusted for maximal possible agreement. Finally, we consider the Michaelis-Menten reaction and confirm some of the results obtained by Schnell and Turner.[5] We remark that the ST and SV models predict the same equilibrium concentrations. However, the Michaelis-Menten type equations based on the pseudo steady-state conditions turn out to be different for the two models.

In the following text we will refer to reactions in well-stirred, homogeneous media as »reactions in ideal conditions«. The reactions in heterogeneous intracellular media, crowded by macromolecules, which results in effects of excluded volume, will be referred to as »reactions in crowded media«.

BIMOLECULAR ASSOCIATION

In the model proposed by Schnell and Turner the rate of change of concentration of the bound complex in an elementary association reaction

$$A + B \rightarrow C \tag{1}$$

is given by[5]

$$\frac{d[C]}{dt} = k(t)[A][B], \quad k(t) = \frac{k_1}{(t+\tau)^h}, \quad 0 \le h < 1, \tag{2}$$

where $k_1 > 0$ is the rate coefficient for ideal conditions (*i.e.* when $h = 0$), $h = 1 - f$ is a fractal parameter, related to the fracton dimension,[9,10] f, and τ is a time constant which determines when the rate coefficient $k(t)$ becomes driven by the effects of macro-molecular crowding. At the beginning of the reaction ($t << \tau$) the molecules which happen to be the most accessible to each other will interact in a manner similar to the binding in ideal conditions, *i.e.*, with essentially constant rate coefficient. Later ($t > \tau$) less accessible molecules will bind with ever decreasing rate, which is determined both by a decreasing function $k(t)$ and by the decreasing concentrations $[A]_t$ and $[B]_t$.

In the approach of Savageau and Voit[14–21,5] the rate of change of concentration of the bound complex for the elementary reaction (1) does not depend explicitly on time, but on the concentrations of reactants:

$$\frac{d[C]}{dt} = \kappa([A],[B])[A][B],$$
$$\kappa([A],[B]) = \kappa_1[A]^\eta [B]^\zeta . \tag{3}$$

The form of function κ is a consequence of the assumption that $\log[C]$ considered as function of $\log[A]$ and $\log[B]$ can be approximated by the linear terms in the Taylor expansion.[21] κ_1 is the rate coefficient for an ideal solution (*i.e.* when $\eta = \zeta = 0$), while the exponents $\eta \ge 0$ and $\zeta \ge 0$ characterize the effects of macromolecular crowding. The rate coefficient $\kappa([A],[B])$ also decreases with time because concentrations $[A]$ and $[B]$ decrease with time. This begs the question whether the ST and SV models can yield the same function for concentration $[C]_t$? It has been suggested,[20,5] that this is indeed true when $[A]_{t=0} = [B]_{t=0}$ and the respective parameters obey the following relations:[20,5]

$$\eta + \zeta = h/(1-h), \quad k_1 = \kappa_1^{1-h}(1-h)^{-h}. \tag{4}$$

However, one can show[22] that the additional condition

$$[A]_{t=0} = (1-h)k_1^{-1}\tau^{h-1} \tag{5}$$

should also be satisfied when $0 < h \leq 1$. Thus, only for a particular initial concentration these two models lead to the same progress curves. Equations (4) and (5) are obtained by solving differential equations derived from (2) and using the conservation equations:

$$[A]_t + [C]_t = [A]_{t=0} + [C]_{t=0}\,,$$

$$[B]_t + [C]_t = [B]_{t=0} + [C]_{t=0}\,. \tag{6}$$

The same conditions (4) and (5) for equivalence of the considered models apply to homodimeric reaction[5] $A + A \rightarrow A_2$ or exciton-fusion reaction $T + T \rightarrow S$, because those reactions are described by differential equation of the same form.[5–7,9]

BIMOLECULAR BINDING AND EQUILIBRIUM CONSTANTS

Now we consider more realistic binding in crowded media when both association and dissociation occur:

$$A + B \underset{k_1}{\overset{k_{-1}}{\rightleftharpoons}} C\,. \tag{7}$$

The Schnell-Turner model[5] in this case yields the following equation:

$$\frac{d[C]}{dt} = k(t)(a-[C])(b-[C]) - k_{-1}[C]\,, \tag{8}$$

where $k(t)$ is given in (2) and we used the conservation equations (6) to express [A] and [B]. The following notation $a = [A]_{t=0} + [C]_{t=0}$, $b = [B]_{t=0} + [C]_{t=0}$ is employed. Schnell and Turner assumed that dissociation of complex C is not influenced by macromolecular crowding and heterogeneity of the medium and is therefore described just by the rate constant k_{-1}. This assumption could be considered reasonable as long as one can assume that C molecules are not trapped in such a way that they cannot dissociate.

The peculiar characteristic of this model is that at equilibrium the C complex is present at zero concentration. To see this, one can rewrite equation (8) as an autonomous system of two differential equations for $[C]_{t=0}$ and $k(t)$*:*

$$\frac{d[C]}{dt} = k(a-[C])\,(b-[C]) - k_{-1}[C],$$

$$\frac{dk}{dt} = -k_1^{-1/h}hk^{1+1/h}. \tag{9}$$

It is easy to verify that the stationary point of this system (*i.e.* when $d[C]/dt = dk/dt = 0$) is given by $[C] = k = 0$. Thus, the equilibrium concentration is $[C]_e = 0$. Consequently, the association equilibrium constant is zero and the dissociation equilibrium constant is not defined. On the other hand equilibrium concentrations of A and B are the initial concentrations $[A]_{t=0}$ and $[B]_{t=0}$, respectively (*cf.* equation (6)).

The Savageau-Voit approach[14–21] for the above reaction, however, yields well defined equilibrium constants. The rate equation is given by

$$\frac{d[C]}{dt} = \kappa([A],[B])\,[A]\,[B] - k_{-1}[C] =$$

$$\kappa_1[A]^{\alpha}[B]^{\beta} - k_{-1}[C]\,, \tag{10}$$

where κ is given in (3) and $\alpha = 1 + \eta$, $\beta = 1 + \zeta$. The apparent association equilibrium constant is

$$K_c = \frac{[C]_e}{[A]_e^{\alpha}[B]_e^{\beta}} = \frac{k_{-1}}{\kappa_1} = \Gamma K,$$

$$K = \frac{[C]_e\,\gamma_C}{(\gamma_A[A]_e\,\gamma_B[B]_e)}\,. \tag{11}$$

We denote equilibrium concentrations with the subscript e, and K represents the equilibrium constant for the same reaction in ideal conditions; γ_C, γ_B and γ_A are the respective activity coefficients. The factor

$$\Gamma = \gamma_A\gamma_B[A]_e^{-\eta}\,[B]_e^{-\zeta}/\gamma_C \tag{12}$$

represents a correction factor, so that this expression generally agrees with the ideas proposed by Minton.[1,3,5] This correction factor explicitly depends on concentrations of reacting species while through exponents η and ζ and activity factors, the correction factor could depend on the concentration of other molecules which cause obstacles to binding. Equilibrium concentrations can be obtained by solving the equation

$$(a-[C]_e)^{\alpha}\,(b-[C]_e)^{\beta} - K_c[C]_e = 0\,, \tag{13}$$

while

$$[A]_e = a-[C]_e, \quad [B]_e = b-[C]_e\,. \tag{14}$$

In general Eq (13) can only be solved numerically. Analytical solutions exist when α and β are such rational numbers that finding solution of (13) can be reduced to finding the zeros of polynomials with degree ≤ 4. Obviously this includes the case of reaction in ideal conditions ($\alpha = \beta = 1$). It can be shown that the unique positive solution exists.[22]

The two considered models exhibit very different behavior, not only at equilibrium, *i.e.* at infinite time, but also for finite times. There are essentially two cases depending on initial concentrations and model parameters.

Case 1. $[C]_{t=0}$, the initial concentration of C is zero.

The ST model for the considered reaction exhibits (for $h > 0$) an initial increase of concentration $[C]_t$ which terminates in the maximum, and then it monotonously decreases

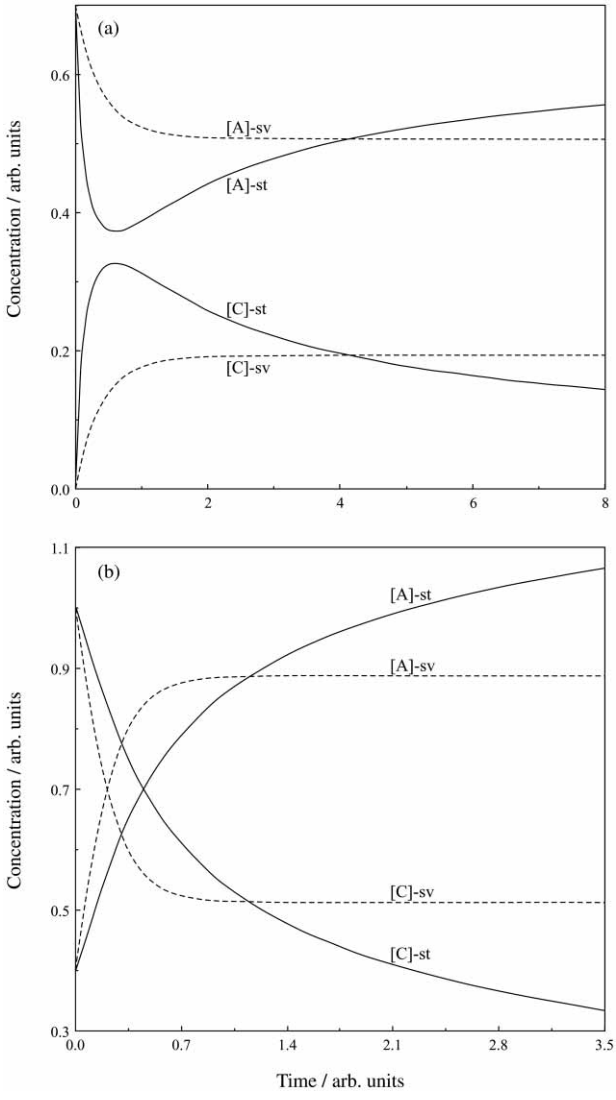

Figure 1. Progress curves for concentrations [A] and [C] for bimolecular reaction (7). a) Case $[C]_{t=0} = 0$, $a = [A]_{t=0} = 0.7$, $b = [B]_{t=0} = 1$. ST model (8): curves [A]-st and [C]-st; parameters are: $k_1 = 1$, $k_{-1} = 1$, $\tau = 0.05$, $h = 0.4$. SV model (10): curves [A]-sv and [C]-sv; parameters are $k_1 = 1$, $k_{-1} = 1$, $\alpha = 1.6$, $\beta = 2.6$. b) Case $[C]_{t=0} > 0$, $a = [A]_{t=0} + [C]_{t=0} = 1.4$, $b = [B]_{t=0} + [C]_{t=0} = 1.6$. ST model (8): curves [A]-st and [C]-st; parameters are: $k_1 = 1$, $k_{-1} = 2$, $\tau = 0.05$, $h = 0.4$. SV model (10): curves [A]-sv and [C]-sv; parameters as in a).

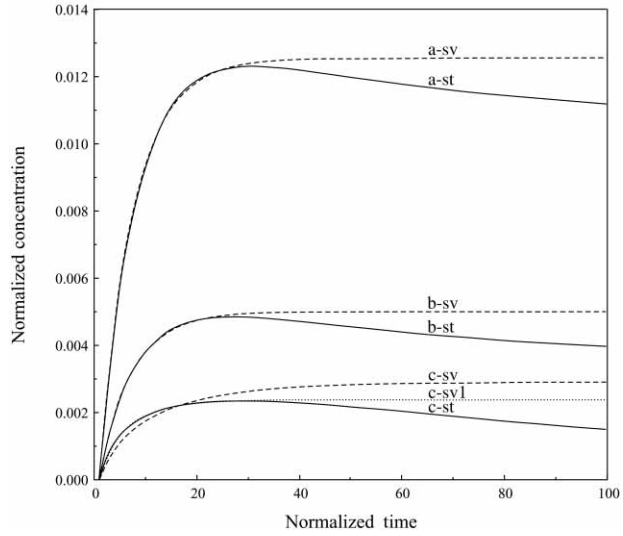

Figure 2. Progress curves for normalized concentration of reaction (7) for ST model and for SV model with parameters adjusted to best coincide (in the least square sense) with the increasing part of the ST progress curves. For the ST model the normalized concentration $Y(x) = [C]_{t=0}/b$ satisfies the non-dimensional equation $Y' = \tilde{k}_1 x^{-h}(R - Y)(1 - Y) - \delta Y$ where $x = (t + \tau)/\tau$ is the normalized time and $k_1 = k_1\tau^{1-h}$, $\delta = k_{-1}\tau$. For the SV model the normalized concentration $Z(x) = [C]_t/b$ satisfies the non-dimensional equation $Z' = \tilde{\kappa}_1(R - Z)^{\alpha}(1 - Z)^{\beta} - \delta Z$ with $\tilde{\kappa}_1 = \kappa_1\tau$. The initial conditions are $Y(1) = Z(1) = [C]_{t=0} = 0$. Curve a-st (ST model) is defined by parameters $\tilde{k}_1 = 0.02$, $R = 0.1$, $\delta = 0.1$, $h = 0.1$. Curve a-sv corresponds to fitted SV model with best fit parameters $\tilde{\kappa}_1 = 3.498$, $\alpha = 3.25$, $\beta = 1$ Curve b-st is defined by parameters $\tilde{k}_1 = 0.03$, $R = 0.03$, $d = 0.08$, $h = 0.2$. Curve b-sv corresponds to SV model with best fit parameters $\tilde{\kappa}_1 = 428.2$, $\alpha = 3.7415$, $\beta = 15.6281$. Curve c-st is defined by parameters $\tilde{k}_1 = 0.025$, $R = 0.03$, $\delta = 0.02$, $h = 0.8$ Curve c-sv corresponds to SV model with best fit parameters $\tilde{\kappa}_1 = 1.0865 \times 10^{20}$, $\alpha = 15.375$, $\beta = 143.58$. Curve c-sv1 corresponds to SV model fitted with respect to four parameters: $\tilde{\kappa}_1 = 1.265$, $\alpha = 2.196$, $\beta = 500$. $\delta = 0.061$.

toward zero (Figure 1a, progress curve [C]-st). One can mathematically analyze equation (2) and generally prove such a behavior.[22] Due to the conservation equations (6), this behavior of [C] corresponds to an initial decrease of $[A]_t$ and $[B]_t$ until the minimum is reached and then to monotonic increase toward asymptotic values $[A]_{t=0}$ and $[B]_{t=0}$ (Figure 1a, progress curve [A]-st; the progress curve for [B] is identical to [A]-st except it that is shifted along the *y*-axes).

In contrast, the SV model for the considered reactions yields classical behavior for $[C]_t$, *i.e.*, when $[C]_{t=0} = 0$ there is a monotonic increase of [C] toward the equilibrium value (see Figure 1a, progress curve [C]-sv) and monotonic decrease of [A], and [B] toward corresponding equilibrium values (Figure 1a, progress curve [A]-sv).

Case 2. $[C]_{t=0} > 0$ and $[C]'_{t=0} < 0$ (initially negative slope).

The Schnell-Turner model predicts monotonic decrease of $[C]_{t=0}$ toward zero concentration (Figure 1b) and a corresponding increase of [A] and [B] to their initial values. The Savageau-Voit approach also predicts a decrease of $[C]_t$, yet not toward zero but toward an equilibrium value $[C]_e$ (Figure 1b). Similarly, [A] and [B] increase toward the corresponding equilibrium values.

While these two models generally yield different time profiles for concentrations, within a certain limited time period they may yield progress curves that are almost indistinguishable. This is shown in Figures 2 and 3. Using the Schnell-Turner model we have generated 200 data points for $[C]_t$ (assuming $[C]_{t=0} = 0$) from $t = 0$ to $t = t_m$, the time when the curve reaches a maximum. Then we

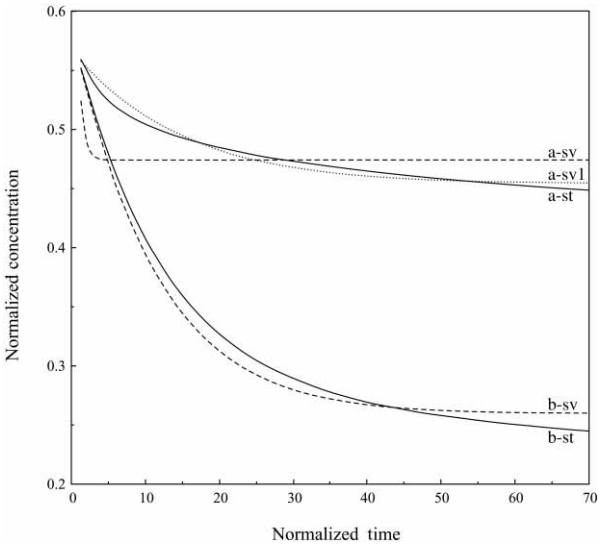

Figure 3. Progress curves for normalized concentration of reaction (7) for ST model and for SV model with parameters adjusted to best coincide (in the least square sense) with the ST progress curves. For model equations see the Figure 2. The initial concentrations were $Y(1) = Z(1) = 0.56$. Curve a-st (ST model) is defined by parameters $\tilde{k}_1 = 2.7$, $R = 0.8$, $\delta = 0.5$, $h = 0.2$. Curve a-sv corresponds to SV model with best fit parameters $\tilde{\kappa}_1 = 1.3846$, $\alpha = \beta = 1$. Curve a-sv1 corresponds to SV model fitted with respect to four parameters: $\tilde{k}_1 = 0.0694$, $\alpha = 1.285$, $\beta = 1.004$, $\delta = 0.021$. Curve b-st is defined by parameters $\tilde{k}_1 = 0.08$, $R = 0.7$, $\delta = 0.05$, $h = 0.2$. Curve b-sv corresponds to SV model with best fit parameters $\tilde{\kappa}_1 = 0.0397$, $\alpha = \beta = 1$.

fitted these points (using least-squares fit) by the function for [C] obtained from the SV model with κ_1, α, β being free parameters. It appears convenient to use normalized concentration and time (see the caption of Figure 2). The resulting best fit progress curves are shown in Figure 2 for three different parameter sets a, b, c. The two models would be difficult to discriminate in the time interval $[0, t_m]$ for parameter sets a and b. This is not the case for parameter set c where there is considerable disagreement as well as unrealistically large parameters (see caption of Figure 2). However, if we allow fitting with respect to k_{-1} as well, the two models yield hardly distinguishable progress curves in the interval $[0, t_m]$. It has to be noted that such agreement is obtained at the expense of unrealistically large parameter β. For times longer than t_m, all SV curves diverge from ST curves.

Figure 3 shows examples when $[C]_{t=0} > 0$ and $[C]'_{t=0} < 0$. As above the SV model was fitted to ST model data, assuming κ_1, α, β as free parameters. Obviously, within given time range the two progress curves are visibly different, especially when comparing the curves a-sv and a-st. Progress curve a-sv1 is obtained by allowing k_{-1} to be a free parameter as well. The two curves are much closer. However, it should be stressed that eventually SV progress curves will diverge from the corresponding ST curves, since the latter tend to zero, while SV progress curves achieve non-zero equilibrium values.

MICHAELIS-MENTEN REACTION

Michaelis-Menten enzymatic mechanism

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} E + P \tag{15}$$

is often considered as a prototype for enzyme catalyzed reactions. Here we discuss some kinetic aspects of this reaction in heterogeneous crowded media, which were not considered by Schnell and Turner.[5] The related equations in the ST model are

$$d[E]/dt = -k(t)[E][S] + (k_{-1} + k_2)[C], \tag{16}$$

$$d[S]/dt = -k(t)[E][S] + k_{-1}[C], \tag{17}$$

$$d[C]/dt = k(t)[E][S] - (k_{-1} + k_2)[C], \tag{18}$$

$$d[P]/dt = k_2[C], \tag{19}$$

where $k(t)$ is given by (1). With the usual initial conditions

$$[C]_{t=0} = [P]_{t=0} = 0, \quad [E]_{t=0} = [E_0], \quad [S]_{t=0} = [S_0], \tag{20}$$

the conservation equations are

$$[E] + [C] = [E_0], \quad [S] + [C] + [P] = [S_0]. \tag{21}$$

($[E_0]$ is the total or starting amount of enzyme; $[S_0]$ that of substrate.)

The system of equations (16–19) with the given initial conditions has an unique positive solution with an unique stationary point, which is uniformly asymptotically stable.[22] Equilibrium concentrations (stationary point) are obtained as solutions of equations (16–19) when all derivatives are zero and should be valid for all t. It is easy to verify that the equilibrium concentrations are $[E]_e = [E_0]$, $[S]_e = [C]_e = 0$, $[P]_e = [S_0]$, the same as for the reaction in ideal media, where $k(t) = k_0$ is a constant. Thus, in regard to equilibrium, the ST model does not deviate from the standard result, as it is in the case of bimolecular binding (7).

SV model for (15) yields equations similar to equations (16–19) with $k(t)[E][S]$ term replaced by $\kappa_1[E]^\beta[S]^\alpha$ (see Refs. 5, 20). Equilibrium concentrations remain the same, and therefore ST and SV model are in agreement with respect to the equilibrium.

This is not the case for pseudo-steady state conditions, usually achieved when the initial substrate concentration $[S_0]$ far exceeds the initial enzyme concentration $[E_0]$. Well-known Michaelis-Menten formula for reaction velocity $v = d[P]/dt$ in pseudo steady-state condition $d[C]/dt|_{t=t'} = 0$ can be obtained by solving the system of equations (*cf.* Eq. 21)

$$k(t')[E]_{t'}[S]_{t'} - (k_{-1} + k_2)[C]_{t'} = 0,$$
$$[E]_{t'} + [C]_{t'} = [E_0] \tag{22}$$

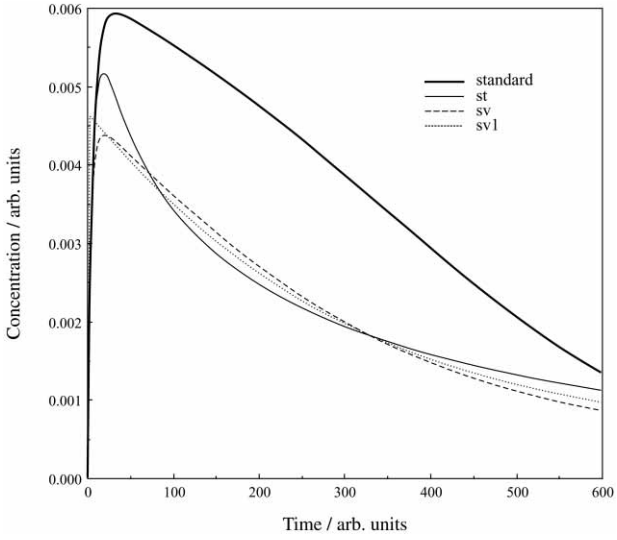

Figure 4. Progress curves for concentration of intermediate C in Michaelis-Menten reaction (15). Curve labeled as »standard« corresponds to reaction in ideal conditions with values of kinetic parameters as in the paper of Schnell and Turner:[5] $[E_0] = 0.01$, $[S_0] = 0.1$, $k_1 = 1$, $k_{-1} = 0.02$, $k_2 = 0.04$. Curve labeled »st« mimics the corresponding curve in Figure 6 of the same paper. The parameters are the same as for the standard curve, except $k_1 = 3$ and we chose $\tau = 7$, $h = 0.45$. Best fit of »st« curve by SV model (labeled by sv) yielded the following values for free parameters $\kappa_1 = 6.38 \times 10^6$, $\alpha = 2.696$, $\beta = 4.180$. When the fit is performed with respect to 5 parameters (curve »sv1«) the following values were obtained: $\kappa_1 = 6.017 \times 10^3$, $\alpha = 1$, $\beta = 2.783$, $k_{-1} = 9.94$, $k_2 = 0.04$.

with respect to $[C]_{t'}$:

$$v = \frac{V_m [S]_{t'}}{K_M + [S]_{t'}}, \quad V_m = k_2 [E_0],$$

$$K_M = (t' + \tau)^h \frac{k_{-1} + k_2}{k_1}. \tag{23}$$

Thus, the same form is obtained as in the standard Michaelis-Menten equation,[23] except that the Michaelis constant is modified by the correction factor $t' + \tau^h$. The maximal reaction velocity V_m remains the same as in the standard Michaelis-Menten equation.

SV model yields rather different formula for v in pseudo-steady state conditions, as discussed in detail by Savageu.[20] Indeed, he obtained the following nonlinear equation for $x = v/V_m$:

$$([S]_{t'} / K)^\alpha = x(1 - x)^{-\beta} \tag{24}$$

where $K_f = [(k_{-1} + k_2)/\kappa_1]^{1/\alpha} (V_m/k_2)^\lambda$, $\lambda = (1 - \alpha)/\beta$ is the »fractal Michaelis constant«. Thus, the dependence of reaction velocity on substrate concentration in pseudo-steady-state conditions, most often measured by biochemists, represent a possibility to discriminate between ST and SV models. Since it may be somewhat difficult to determine whether the pseudo-steady state conditions are met, a better possibility might be to compare the progress curves (Figure 4). This was done by Schnell and Turner[5] who found that SV model does not adequately fit data obtained by lattice gas automata simulation, while their model fits the data well. Thus, the two models are predicting progress curves which differ sufficiently even when the model parameters are adjusted for the best agreement. It should be noted that Schnell and Turner apparently tried only to adjust powers α and β of SV model (ranging between 1 and 10) by systematically working through ten thousand combinations. We have tried to fit simultaneously progress curves for [C] and [P] obtained by ST model (Figure 4, curve »st«) with corresponding functions of the SV model, using the powerful Simplex Induction Hybrid minimizer.[24] We assumed that κ_1, α and β are free parameters. Namely, since the two models originate from different assumptions for the association step, it could be reasonable to assume that κ_1 is different from k_1. As one can see from Figure 4 the fit (curve denoted by »sv«) also does not lead to agreement between the two models. We went one step further and tried the fit with respect to all five parameters κ_1, α, β, k_{-1}, k_2, but the agreement can be considered only marginally better (curve denoted by »sv1«). On the other hand, we found that for the product concentration [P], the two models agree well, even when only three parameters were left free (data not shown). This example confirms that for Michaelis-Menten reaction ST model and SV model would give quantitatively different predictions at least for the progress curve of the intermediate complex.

DISCUSSION AND CONCLUSION

The Schnell-Turner model is an interesting attempt to describe the kinetics of reactions *in vivo* characterized by macromolecular crowding. It is based on the idea that the association rate is proportional to the number of sites on a fractal visited by a random walker.[10,22] This results in a time-dependent rate coefficient which is defined for all times by the Zipf-Mandelbrot distribution.[5] When applied to bimolecular binding we found that this model leads to somewhat unexpected result, namely, that the equilibrium concentrations of the bound complex is zero, and consequently the association equilibrium constant is zero. The interpretation of this finding leads to the conclusion that after a sufficiently long time all of the reacting molecules are so securely trapped (or separated) in heterogeneous crowded media, that they cannot come close enough to interact. At the same time the molecules of the bound complex are nowhere trapped, and have all eventually dissociated. This could happen if we assume that reacting molecules attach to the surrounding macromolecules in the media, while the bound complex does not attach to those macromolecules. Such a scenario is implausible to happen, and so cannot be assumed for any

kinetic reaction in crowded media. Consequently, this represents a serious drawback for the ST model.

Another attempt to describe the kinetics reactions *in vivo*, by Savageau and Voit,[14–21] is based on the notion that association rate coefficients are proportional to the product of fractional powers of concentrations of reacting molecules. When applied to bimolecular binding the SV model yields non-zero equilibrium concentrations, and consequently non-zero equilibrium constant. The latter may be considered equilibrium constant for binding in ideal media modified by a correction factor for non-ideal conditions, a concept introduced by Minton.[5–9]

We have shown that progress curves for bimolecular binding predicted by the two models could also behave very differently: for certain initial conditions the ST model predicts a progress curve for bound complex that achieves a maximum at a finite time and then decreases monotonously to zero, while the SV model predicts monotonic increase to a nonzero equilibrium value. Both, the equilibrium values predicted by the two models, and the behavior of progress curves are sufficient to discriminate these two models by adequate measurements, or at least by simulations based on gas lattice automaton. The latter comparison was performed by Schnell and Turner, however, for the case of Michaelis-Menten reaction. Their model predicts the same equilibrium concentrations as the SV model and as the standard kinetic model for ideal media. When the progress curves are compared the ST model fits the simulated data much better than does the SV model. This was the main justification for the ST model.[5] We have confirmed the finding that SV and ST models can lead to rather different progress curves in the case of Michaelis-Menten reaction, and therefore these curves can serve for possible discrimination between the two models.

Summarizing our findings, we are faced with a perplexing situation: The ST model is deficient in describing elementary bimolecular binding, yet apparently describes well the Michaelis-Menten reaction; on the other hand the SV model does not show any general deficiency in describing bimolecular binding, yet it does not describe well the Michaelis-Menten reaction. Since the bimolecular binding $A + B \rightleftharpoons C$ is a simpler reaction than the Michaelis-Menten reaction, we would argue that the ST and SV models should be first tested against experimental data for bimolecular binding in crowded media. The simplest efficient test would be to measure the equilibrium concentrations. Then a test against experimental data for progress curves should be performed to find out whether the expressions for rate coefficients in these models can reliably describe the concentration profiles in time.

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SAŽETAK

Reakcijska kinetika u unutarstaničnom okolišu: Dva predložena modela predviđaju kvalitativno različite rezultate

Željko Bajzer, Miljenko Huzak, Kevin Neff i Franklyn G. Prendergast

Nedavno su Schnell i Turner predložili model za rekcijsku kinetiku u okolišu zaposjednutom makromolekulama. U ovom se radu taj model primjenjuje na elementarno bimolekularno vezanje. Nađeno je da model daje vrijednost konstante ravnoteže jednaku nuli što nije uobičajeno. Krivulje napredovanja reakcije su kvalitativno različite od krivulja koje predviđa model od Savageau-a, zasnovan na potencijama koncentracija s razlomljenim eksponentima. U slučaju Michaelis-Menten reakcije ti modeli predviđaju kvalitativno slične krivulje napredovanja reakcije i identične ravnotežne koncentracije. Oba modela se analitički i numerički ispituju a njihove razlike i sličnosti se raspravljaju s obzirom na moguće eksperimentalno vrednovanje tih modela.