MATCH
Communications in Mathematical and in Computer Chemistry

MATCH Commun. Math. Comput. Chem. **70** (2013) 63-72

ISSN 0340 - 6253

'Die Kinetik der Invertinwirkung' of L. Michaelis and M.L. Menten Revisited After 100 Years: Closed-Form Solutions of Genuine Invertase-Reaction Dynamics

Marko Goličnik

Institute of Biochemistry, Faculty of Medicine, University of Ljubljana, Vrazov trg 2, 1000 Ljubljana, Slovenia, e-mail: marko.golicnik@mf.uni-lj.si

(Received October 31, 2012)

ABSTRACT

Michaelis and Menten found the direct mathematical analysis of invertase-catalyzed hydrolysis of sucrose (where the products formed simultaneously isomerize) unrealistic 100 years ago, and hence, they avoided this problem by proper adaptation of their experiment. However, they assumed that highly complex mathematical functions might generate the time-courses of all of the reactants of this pioneering enzyme-catalyzed reaction; however, this is a problem that has not been solved since its original publication in 1913. Their classic work on invertase-reaction dynamics was recently revisited anew using ^{1}H NMR spectroscopy, although despite Michaelis-Menten's vision of the possibility of mathematical solutions to describe the authentic (i.e. uninterrupted) invertase-reaction system, no-one has faced this challenge over this last century. Hence, the closed-form solutions of genuine invertase-reaction dynamics are now presented in this report.

1. INTRODUCTION

Michaelis and Menten are nowadays regarded as the founders of enzyme kinetics, and their 100-years-old work still attracts much attention even in these modern times. Thus, the complete translation of their now classic paper on 'Die Kinetic der Invertinwirkung' [1] was published recently as current topic in the *Biochemistry* journal by Johnson and Goody [2], and their pioneering enzyme-catalyzed reaction has been experimentally revisited anew, by Exnowitz et al [3], although ^{1}H NMR spectroscopy was used to follow the cleavage of sucrose by invertase, instead of optical polarimetry.

The experimental practice of following enzyme kinetics of a reaction over an extended period of time and try to explain observations in terms of solutions of integrated rate equations was used by Henri [4], even 10 years before Michaelis and Menten. Indeed, back in 1913, the main contribution of Michaelis and Menten to the progressive development of enzymology was paradoxically their popularization of Henri's rate equation, and of course, the experimental improvement of his work. Their recognition that monitoring the progress of the inversion reaction by direct continuous (but unresponsive) polarimetric measurements leads to falsification of the true rate of inversion is very important. Indeed, the polarization angle of the investigated reaction solution is superimposed on the changes in the polarization of all of the optically active reactants (i.e. sucrose, glucose and fructose) where the freshly formed products (i.e. α-glucopyranose and β-fructofuranose) slowly mutarotate to other isomeric forms until equilibrium is reached. Michaelis and Menten avoided this problem by an appropriate adaptation of their experiment. However, they assumed that highly complex mathematical functions can generate theoretical time-courses for all of the reactants, which would allow them all to be fitted to then-unattainable continuous multiresponse experimental data.

NMR spectroscopy is the technique that allows direct determination of the stereochemistry of all of the reactants, and the quantification of each isomer can also be carried out by integrating the NMR spectra. This multiresponse experimental approach was recently applied to the now centenary-old invertase-reaction system [3], although the closed-form solutions of this system, the existence of which was also divined by Michaelis and Menten, has still to be derived. Thus, the main purpose of this report is to puzzle out this 100-year-old prediction of Michaelis and Menten.

2. THEORY AND CLOSED-FORM SOLUTIONS

Michaelis and Menten studied the enzyme invertase, which irreversibly catalyzes the hydrolysis of sucrose (S) to α-glucopyranose (αG) and β-fructofuranose (F_f). This is illustrated in Scheme 1, where product inhibition can be neglected when the substrate concentration is low enough. However, they also realized that this catalyzed reaction based on an enzyme that eponymously inverts the optical

rotation of its reaction mixture is not the unique reason for this phenomenon, as both of its products also slowly isomerize on the time-scale of the enzyme-catalyzed reaction.

$$\mathbf{E + S} \overset{K_S}{\rightleftharpoons} \mathbf{ES} \xrightarrow{k_{cat}} \mathbf{E + \alpha G + F_f}$$

$$\alpha\mathbf{G} \underset{k_{\beta\alpha}}{\overset{k_{\alpha\beta}}{\rightleftharpoons}} \beta\mathbf{G}$$

$$\mathbf{F_f} \underset{k_{pf}}{\overset{k_{fp}}{\rightleftharpoons}} \mathbf{F_p}$$

Scheme 1

By assuming ES to be in equilibrium with E and S (and also based on earlier insights by Henri [4]), Michaelis and Menten described the kinetics of this enzyme-catalyzed reaction according to the now-familiar hyperbolic v *versus* [S] relationship given in the traditionally known Michaelis-Menten equation:

$$v = \frac{d[S]}{dt} = -\frac{V[S]}{K_S + [S]} \tag{1}$$

where V is the limiting rate ($V = k_{cat}[E]_0$), and K_S is the dissociation constant of the Michaelis ES complex. Henri, unfortunately, is not usually credited with the formulation of this equation, although some name Eq. (1) also as the Henri-Michaelis-Menten equation [5,6].

The closed-form solution of Eq. (1) is expressed in terms of the Lambert W function [7,8], as:

$$[S] = K_S W\left(\frac{[S]_0}{K_S} e^{\left(\frac{[S]_0 - Vt}{K_S}\right)}\right) = K_S W(\xi) \tag{2}$$

where the time-dependent argument of W is marked as variable ξ in:

$$\xi = \frac{[S]_0}{K_S} e^{\left(\frac{[S]_0 - Vt}{K_S}\right)} \tag{3}$$

It can be seen that Eqs. (1) and (2) are accurate when the criterion for total enzyme concentration $[E]_0 << ([S]_0 + K_S)$ guarantees the validity of the standard quasi-steady-state approximation (sQSSA) [6], when consequently K_S stands for the Michaelis constant, instead of the dissociation constant. In the case of most *in-vitro* assays, the latter criterion is satisfied easily. However, the sQSSA condition breaks down under *in-vivo* conditions, where the intracellular concentrations of enzymes are usually not significantly lower than the magnitude of their substrates. Therefore, Berberan-Santos [6]

recently extended the Michaelis-Menten kinetics for higher enzyme concentrations and derived the following equation, Eq. (4), for the time-course of substrate depletion:

$$[S] = K_s W(\xi) - [E]_0 \frac{W(\xi)}{1+W(\xi)} \tag{4}$$

This is valid when $[E]_0$ is lower than $[S]_0+K_S$ by just one order of magnitude [6]. However, biochemists usually analyze enzyme kinetics indeed within the sQSSA framework, and Eq. (4) reduces to Eq. (2) under such conditions. Hence, all further expressions and discussions here refer only to the sQSSA (i.e. consequently, to Eqs. (1) and (2)).

As both of the cleavage products (αG and F_f) convert slowly to their secondary isomers, i.e. β-glucopyranose (βG) and β-fructopyranose (F_p), until these reach equilibrium, the isomerization rate equations are given as:

$$\frac{d[\beta G]}{dt} = k_{\alpha\beta}[\alpha G] - k_{\beta\alpha}[\beta G] \tag{5}$$

$$\frac{d[F_p]}{dt} = k_{fp}[F_f] - k_{pf}[F_p] \tag{6}$$

As taking the mass balance into account is reasonable for $[S]_0>>[E]_0$, Eq. (2) can be reformulated as follows:

$$[\alpha G] + [\beta G] = [F_p] + [F_f] = [S]_0 - K_S W(\xi) \tag{7}$$

Adequately, differential Eqs. (5) and (6) can be transformed into the first-order nonhomogeneous linear differential equations, as:

$$\frac{d[\beta G]}{dt} + (k_{\alpha\beta} + k_{\beta\alpha})[\beta G] = k_{\alpha\beta}([S]_0 - K_S W(\xi)) \tag{8}$$

$$\frac{d[F_p]}{dt} + (k_{fp} + k_{pf})[F_p] = k_{fp}([S]_0 - K_S W(\xi)) \tag{9}$$

The general solutions of Eqs. (8) and (9) are represented in the form of integrals as:

$$[\beta G] = e^{-\int (k_{\alpha\beta}+k_{\beta\alpha})dt} \left(\int k_{\alpha\beta} e^{\int (k_{\alpha\beta}+k_{\beta\alpha})dt} ([S]_0 - K_S W(\xi))dt + C \right) \tag{10}$$

$$[F_p] = e^{-\int (k_{fp}+k_{pf})dt} \left(\int k_{fp} e^{\int (k_{fp}+k_{pf})dt} ([S]_0 - K_S W(\xi))dt + C \right) \tag{11}$$

As Eqs. (10) and (11) differ only in the isomerization rate constants, only the derivation for [βG] is presented here. Hence, from Eq. (10) we obtain:

$$[\beta G] = C\,\mathrm{e}^{-(k_{\alpha\beta}+k_{\beta\alpha})t} + \frac{k_{\alpha\beta}[S]_0}{(k_{\alpha\beta}+k_{\beta\alpha})} - k_{\alpha\beta}K_S\,\mathrm{e}^{-(k_{\alpha\beta}+k_{\beta\alpha})t}\int \mathrm{e}^{(k_{\alpha\beta}+k_{\beta\alpha})t}\,W(\xi)\,dt \tag{12}$$

Eq. (3) can be used for retransformation of the integral *I* in Eq. (12) in terms of the variable ξ, as:

$$t = \frac{[S]_0}{V} - \frac{K_S}{V}\ln\left(\frac{K_S}{[S]_0}\xi\right) \Rightarrow dt = -\frac{K_S}{V}\frac{d\xi}{\xi} \tag{13}$$

and thus we find:

$$I = \int \mathrm{e}^{(k_{\alpha\beta}+k_{\beta\alpha})t}\,W(\xi(t))dt = -\frac{K_S}{V}\mathrm{e}^{(k_{\alpha\beta}+k_{\beta\alpha})\frac{[S]_0}{V}}\left(\frac{K_S}{[S]_0}\right)^{-\frac{K_S}{V}(k_{\alpha\beta}+k_{\beta\alpha})}\int \xi^{-\frac{K_S}{V}(k_{\alpha\beta}+k_{\beta\alpha})-1}W(\xi)d\xi =$$

$$= -\frac{K_S}{V}\mathrm{e}^{(k_{\alpha\beta}+k_{\beta\alpha})\frac{[S]_0}{V}}\left(\frac{K_S}{[S]_0}\right)^{-\alpha+1}\int \xi^{-\alpha}W(\xi)d\xi \tag{14}$$

where $\alpha = K_S(k_{\alpha\beta} + k_{\beta\alpha})/V + 1$.

After solving the integral in Eq. (14) the solution for integral *I* is:

$$I = \frac{K_S}{V}\mathrm{e}^{(k_{\alpha\beta}+k_{\beta\alpha})\frac{[S]_0}{V}}\left(\frac{K_S}{[S]_0}\right)^{-\alpha+1} \times$$

$$\times\left(\frac{\mathrm{e}^{\alpha W(\xi)}\,\xi^{-\alpha}\left((\alpha-1)W(\xi)\right)^{\alpha}\left(\Gamma\left(3-\alpha,(\alpha-1)W(\xi)\right)+(\alpha-1)\Gamma\left(2-\alpha,(\alpha-1)W(\xi)\right)\right)}{(\alpha-1)^3}\right) \tag{15}$$

where Γ stands for the incomplete gamma function [9].

Finally, we find the solution of Eq. (12) as:

$$[\beta G] = C\,\mathrm{e}^{-(k_{\alpha\beta}+k_{\beta\alpha})t} + \frac{k_{\alpha\beta}[S]_0}{(k_{\alpha\beta}+k_{\beta\alpha})} - \frac{k_{\alpha\beta}K_S^2}{V}\mathrm{e}^{(k_{\alpha\beta}+k_{\beta\alpha})\left(\frac{[S]_0}{V}-t\right)}\left(\frac{K_S}{[S]_0}\right)^{-\alpha+1} \times$$

$$\times\left(\frac{\mathrm{e}^{\alpha W(\xi)}\,\xi^{-\alpha}\left((\alpha-1)W(\xi)\right)^{\alpha}\left(\Gamma\left(3-\alpha,(\alpha-1)W(\xi)\right)+(\alpha-1)\Gamma\left(2-\alpha,(\alpha-1)W(\xi)\right)\right)}{(\alpha-1)^3}\right) \tag{16}$$

At time t = 0 the initial condition for [βG] and the relationship for W(ξ) are [βG] = 0 and $W(\xi) = [S]_0/K_S$ (see Appendix). Hence, the constant C in the final solution of Eq. (16) is defined as:

$$C = -\frac{k_{\alpha\beta}[S]_0}{\left(k_{\alpha\beta}+k_{\beta\alpha}\right)} + \frac{k_{\alpha\beta}K_S^2}{V}\mathrm{e}^{\left(k_{\alpha\beta}+k_{\beta\alpha}\right)\frac{[S]_0}{V}}\left(\frac{K_S}{[S]_0}\right)^{-\alpha+1} \times$$

$$\times(\alpha-1)^{\alpha-3}\left(\Gamma\left(3-\alpha,(\alpha-1)\frac{[S]_0}{K_S}\right)+(\alpha-1)\Gamma\left(2-\alpha,(\alpha-1)\frac{[S]_0}{K_S}\right)\right) \qquad (17)$$

As closed-form solutions for [S] and [βG] are now given by Eqs. (2) and (16), the solution for [αG] can be indirectly achieved from the mass balance of Eq. (7). The similar solutions for $[F_p]$ and $[F_f]$ can be determined by the same equations, although the rate constants $k_{\alpha\beta}$ and $k_{\beta\alpha}$ must be replaced by k_{fp} and k_{pf}, respectively.

3. SIMULATIONS and DISCUSSION

The closed-form solutions for the concentrations of all of the reactants, i.e. [S], [αG], [βG], $[F_p]$ and $[F_f]$, as functions of time were generated by computation using the direct models of Eqs. (2), (7) and (16), in the Wolfram Mathematica 7 computer program. The Lambert W(x) function is built into this technical mathematics software tool under the name of ProductLog, where ProductLog[x] gives the solutions for the principal branch of W(x).

The data for the simulated concentrations of reactants *versus* time are shown as symbols in Figure 1, where the numerical values of the kinetic parameters were assigned from the literature (V = 0.012 mM s^{-1}, K_s = 28.7 mM [3]; $k_{\alpha\beta} = 4.91 \times 10^{-5}$ s^{-1}, $k_{\beta\alpha} = 2.76 \times 10^{-5}$ s^{-1} [10]; $k_{fp} = 2.04 \times 10^{-3}$ s^{-1}, $k_{pf} = 0.91 \times 10^{-3}$ s^{-1} [11]).

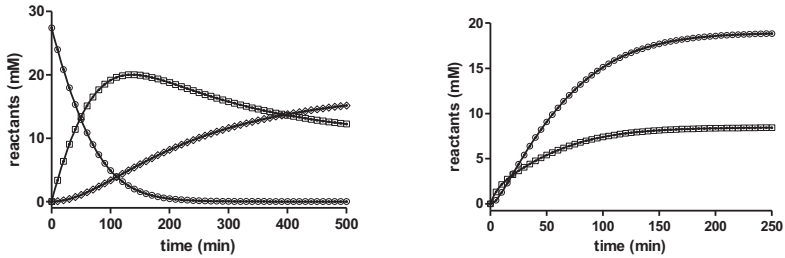

Fig. 1 Time-courses of concentrations for the various reactants in the invertase-catalyzed reaction. Data from the progress curve simulation performed with Eqs. (2), (7) and (16) are shown: left – [S] (circle), [αG] (square), [βG] (diamond); right – $[F_p]$ (circle), $[F_f]$ (square). Lines represent theoretical concentrations obtained using the numerical integration.

The lines that are also shown in Figure 1 represent the calculated concentrations, as obtained using the DynaFit computer program [12], which uses numerical integration for solving kinetic problems. The model mechanism and the adequate rate constants were entered into the program input script file. The values of the bimolecular association rate constant and the total enzyme concentration were k_{on} = 10^8 $M^{-1}s^{-1}$ and $[E]_T$ = 1 μM, and the first-order rate constants; i.e. dissociation rate constant k_{off} and turnover number k_{cat}, were adequately determined to satisfy the parameters V and K_s. The model mechanism was translated into the system of ordinary differential equations using DynaFit, with a modified Livermore solver used for ordinary differential equations to compute the progress curves.

The comparison of these results verifies the correctness of the derived closed-form solutions in this report. As expected, the progress curves for the secondary reaction product isomers, i.e. βG and F_p, are sigmoid, because their production follows the original products αG and F_f, although this sigmoidality is also rate dependent. Thus, the latter is more distinctive in the case of glucose (Fig. 1, left) as the rates $k_{\alpha\beta}$ and $k_{\beta\alpha}$ are more than 30-fold lower than k_{fp} and k_{pf}, albeit the equilibrium constants between both of the isomers of the products are similar (≈ 2). This means that the secondary isomers βG and F_p are 2-fold more stable, although there are significant differences between glucose and fructose in the way that their isomers reach equilibrium. The progress curve for F_f (Fig. 1, right) is concave downwards, and it does not reach a maximum, while the progress curve for αG does (Fig. 1, left). The reason is that the conversion rates of F_f to F_p, and *vice versa*, are faster processes than the invertase-catalyzed hydrolysis of sucrose itself (and consequently the formation of F_f). It is just the opposite in the case of glucose. The concentration of αG reaches a maximum because the isomerization to βG is slower than the enzyme-catalyzed reaction, and thus the less stable αG anomer accumulates at first, and then afterwards its concentration slowly descents to the equilibrium value.

The demonstrated multiresponse reaction system was not feasible in the times of Michaelis and Menten, who used a uniresponse polarimetric technique for studying the reaction. However, multiresponse modeling, which considers more of the reactants of a reaction system under study, offers distinct advantages over a uniresponse approach, in that the model equations can be more rigorously constrained. Therefore, model discrimination, verification of the accepted model, and estimation of the model parameters can be performed more precisely in comparison with uniresponse modeling. Michaelis and Menten saw the problem curiously clearly enough on this matter, but unfortunately, the physico-chemical techniques were not ready for such experimental approaches during the early decades of the 20th century.

Michaelis, who commenced his career as a physician, was also exceedingly talented and proficient in applied mathematics [13]. He (and also Michaelis) was well aware that the most elegant specifications of a reaction can be performed when the appropriate integrated rate equations exist as closed-form solutions that describe the dynamics of the model system (e.g. Eq. (2)), even though the numerical integration methods were not adapted for their practical purpose in that pre-computer era. Hence, they were focused on finding the integrated form of the rate equations to account for the entire progress curves of enzyme-catalyzed reactions, although finally they pragmatically adapted the experiments so that the observed data was within the capabilities of the mathematical analysis of their time. They ascertained that the isomerization of products is a trivial process that does not affect the enzyme-catalyzed reaction itself when the reaction is stopped at a specific time, and the response quantity is measured after the isomers equilibrate. Furthermore, the idea occurred to them that the data analysis through differentiation of concentrations into rates is easier than the integration of the rate equation itself. Thus, the characterization of the kinetic behavior of an enzyme in terms of its initial rates at various substrate concentrations was their pioneering and original contribution in terms of the enzymology, although they believed that highly complex mathematical model functions might generate the raw time-course concentrations of the genuine invertase-reaction dynamics.

4. CONCLUSIONS

The (Henri-)Michaelis-Menten equation (see Eq. (1)) has been widely studied since 1913, as it is of fundamental importance in enzyme kinetics for both theoretical and practical reasons [5]. However, still nowadays little is known about its closed-form solution, which was reported for the first time by Beal [14,15] at least seven decades after Michaelis and Menten's work, although, only 15 years ago, Schnell and Mendoza [7] were the first who recognized that this solution can actually be expressed by the Lambert W(x) function, as given in Eq. (2). There are two main reasons why the latter equation has not been recognized and was not widely adopted among the broader life-science community: (i) most of available curve-fitting programs are not set-up to handle equations that involve W(x) function; and (ii) the most common approach to progress-curve analysis these days is to use numerical integration methods and software to fit the differential equations directly. Hence, some people might be doubtful about the applicability of these mathematically more complex solutions that are presented in this report, but I believe that this theoretical problem is worth noting also from (and because of) its historical point of view.

ACKNOWLEDGEMENTS

This study was supported by the Slovenian Research Agency (grant P1-170).

APPENDIX

W(x) is defined as the inverse of the function that satisfies Eq. (A1):

$$y\mathrm{e}^{y} = \xi \tag{A1}$$

i.e., the solution to Eq. (A1) is expressed by the Lambert W(ξ) function as y = W(ξ), and therefore, Eq. (A1) is more frequently written in the form of Eq. (A2):

$$W\left(\xi\right)\mathrm{e}^{W(\xi)} = \xi \tag{A2}$$

or

$$W\left(\xi\right) + \ln\left(W\left(\xi\right)\right) = \ln\left(\xi\right) \tag{A3}$$

Thus, if ξ is expressed in the form as given in Eq. (3) (for t = 0):

$$\xi = \frac{\left[S\right]_0}{K_s}\mathrm{e}^{\frac{[S]_0}{K_S}} \tag{A4}$$

then the following relationship can be expressed:

$$W\left(\frac{\left[S\right]_0}{K_s}\mathrm{e}^{\frac{[S]_0}{K_S}}\right) = \frac{\left[S\right]_0}{K_s} \tag{A5}$$

REFERENCES

[1] L. Michaelis, M. L. Menten, Die Kinetik der Invertinwirkung, *Biochem. Z.* **49** (1913) 333-369.

[2] K. A. Johnson, R. S. Goody, The original Michaelis constant: translation of the 1913 Michaelis-Menten paper, *Biochemistry* **50** (2011) 8264-8269.

[3] F. Exnowitz, B. Meyer, T. Hackl, NMR for direct determination of K_m and V_{max} of enzyme reactions based on the Lambert W function-analysis of progress curves, *Biochim. Biophys. Acta* **1824** (2012) 443-449.

[4] V. Henri, *Lois Generales de l'Action des Diastases*, Hermann, Paris, 1903.

[5] I. H. Segel, *Enzyme Kinetics*, Wiley, New York, 1993.

[6] M. N. Berberan-Santos, A general treatment of Henri-Michaelis-Menten enzyme kinetics: exact series solution and approximate analytical solutions, *MATCH Commun. Math. Comput. Chem.* **63** (2010) 283-318.

[7] S. Schnell, C. Mendoza, Closed-form solution for time-dependent enzyme kinetics, *J. Theor. Biol.* **187** (1997) 207-212.

[8] M. V. Putz, A. M. Lacrama, V. Ostafe, Full analytic progress curve of enzymic reactions in vitro, *Int. J. Mol. Sci.* **7** (2006) 469-484.

[9] M. Abramowitz, I. A. Stegun, *Handbook of Mathematical Functions*, Dover, New York, 1964.

[10] C. E. Lin, C. J. Yu, C. L. Chen, L. D. Chou, C. Chou, Kinetics of glucose mutarotation assessed by an equal-amplitude paired polarized heterodyne polarimeter, *J. Phys. Chem. A* **114** (2010) 1665-1669.

[11] B. Andersen, F. Grønlund, H. C. Jørgensen, A kinetic study of fructopyranose-fructofuranose interconversion at normal and elevated pressure over an extended pH range, *Acta Chem. Scand. A* **38** (1984) 109-114.

[12] P. Kuzmic, Dynafit – a software package for enzymology, *Methods Enzymol.* **467** (2009) 247-280.

[13] L. Michaelis, *Einführung in die Mathematik*, Springer, Berlin, 1922.

[14] S. L. Beal, On the solution to the Michaelis–Menten equation, *J. Pharmacokinet. Biopharm.* **10** (1982) 109–119.

[15] S. L. Beal, Computation of the explicit solution to the Michaelis–Menten equation, *J. Pharmacokinet. Biopharm.* **11** (1983) 303–319.