Analogue and Digital Representations of Enzyme Kinetics*

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In the considerable time interval between the establishment of chemical equations for enzyme action by Michaelis and Menten (1), and their extension by Briggs and Haldane (2), to the present time, there has been no satisfactory mathematical approach to a detailed comparison of the over-all or intermediate kinetics with those of the chemical equations. In spite of other efforts to make approximate solutions (3, 4) we showed some time ago that the mechanical differential analyzer (5) and more recently the electronic analogue computer (6) could be used in the solution of these problems. However, both the mechanical differential analyzer and the electronic analogue computer have definite limitations in terms of the number of components of an enzyme sequence that can be represented. In order that multi-enzyme systems might be adequately studied (7-11), we began, in 1953, to develop a program for a digital computer that would allow the representation of 40 simultaneous chemical reactions.

In the preceding paper (11) the method of use of the digital computer program and the types of representation have been described in detail. It is the purpose of this paper to establish criteria for the accuracy of the digital computer program for a simple enzyme system by comparing it with solutions previously obtained from the mechanical differential analyzer.

**Chemical Equations**

The chemical equations are:

\[ E + S \overset{k_1}{\rightarrow} ES \]

\[ ES \overset{k_3}{\rightarrow} E + P \]

The enzyme concentration is 1.0 and this is its maximal value obtained by Dr. J. G. Brainerd of the Moore School of Electrical Engineering, University of Pennsylvania. The digital computer can indicate only a one-way reaction and thus the reverse reaction equation must be represented separately.

The velocity constants for the reactions and the enzyme concentration are set equal to 1. The substrate concentration is varied, although the particular solution of Fig. 1 is taken for a substrate concentration of 8. The units of concentration may be molar (M), in which case the time scale is in seconds. If they are micromolar (e.g., \(1 \times 10^{-5} \text{ M}\)) and the reaction velocity constant \(k_1\) is \(1 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}\), then the time scale is again in seconds. Thus, the time scale is inversely proportional to the value of \(k_1\); i.e., if \(k_1 = 10^6 \text{ M}^{-1} \text{ sec}^{-1}\) or if \(e = 1 \times 10^{-5} \text{ M}\), the time scale must be divided by 10.

With the digital computer, the differential equations are written by the program itself, and they need not be presented here. The values of the velocity constants are unity as stated above. The enzyme concentration is 1.0 and this is its maximal value in the graph of Fig. 1. The concentration of substrate is 8.0 and this is the value of its maximum on the graph. The maximum for ENS is 1 and that for P is 8. The stoichiometries are set at 1.

The accuracy of the mechanical differential analyzer as used probably exceeds 0.1%. The accuracy used in the digital computer solution was set at 0.1%. In both cases, the effective accuracy is limited by the data output. For the differential analyzer, a mechanical plotter was used in which an accuracy of three significant figures is available. With the digital computer, numerical data are accurate to at least four significant figures are available. The high speed printer which gives the graphical output of Fig. 1 plots 100 points on the ordinate and either 100 or 200 points on the abscissa, ensuring an accuracy of two significant figures. Rate data from both graphical procedures is less accurate, however, and it is apparent that numerical values for reaction rates should be obtained directly from the digital computer and not from the graphs of concentration versus time.

The latter are, however, quite satisfactory for preliminary tests of various complex reaction mechanisms as discussed in the preceding communication (11).

**RESULTS**

The plan of both experimental and theoretical solutions for the Briggs-Haldane modifications (2) of the Michaelis equations is to demonstrate a "cycle" of enzyme-substrate activity in which the substrate-free enzyme is treated with a sufficient excess of substrate that the intermediate compound reaches a

* This research has been supported in part by a grant from the Office of Naval Research. The differential analyzer data were obtained by Dr. J. G. Brainerd of the Moore School of Electrical Engineering, University of Pennsylvania. The digital computer solutions were obtained at the University of Pennsylvania Computer Center.

1 These particular data were obtained with a modified differential equation solver developed by one of us (P. S.).
FIG. 1. Analogue (top) and digital computer solutions of the one-enzyme system:

\[ E + S \xrightleftharpoons[k_1]{k_2} ES \xrightarrow{k_3} E + P \]

for \( k_1 = k_2 = k_3 = 1 \), \([E] = 1\), and \( x_0 = 8\). The concentrations of \( ES \), \( S \), and \( P \) are plotted at the top by the graphical output of the mechanical differential analyzer and on the bottom by the high speed printer of the digital computer, Univac I. In the latter, the symbol \( C \) represents \( ES \). The time scale of the analogue solution is in seconds and of the digital solution in tenths of seconds (DC-10).
maximal concentration somewhat below the saturation value. If the enzyme concentration is sufficiently small, the intermediate will have a readily measurable life-time and its decomposition into free enzyme can also be observed. This curve (see Fig. 1) has definite characteristics:

(a) if it rises towards a saturation value, then the rate of its rise must considerably exceed its rate of decomposition;

(b) the maximal value of the concentration of the intermediate is reached very early in the cycle;

(c) for these conditions, the steady state exists only for a small fraction of the total duration of the cycle.

Simultaneous measurements of the utilization of substrate and the formation of product also have characteristic features. The trace for the disappearance of substrate has a characteristic "jump" decrease of concentration which corresponds roughly in magnitude to the amount of substrate immediately bound to the enzyme. Thereafter, the substrate curve tends toward a zero-order reaction. It should be noted, however, that the curve is sharply inflected at the time the maximal concentration of the intermediate is reached and it is rather difficult to measure accurately the rate of disappearance of substrate at that time. On the other hand, it is interesting to note that later deviations from the zero-order reaction kinetics are hard to observe even though the trace for the intermediate compound clearly shows the system to be no longer in a steady state. The formation of product has a special characteristic that no product can be formed before the intermediate is formed and there is an induction period in product formation.

Quantitative Comparison of Differential Analyzer and Digital Computer Results—Fig. 1 compares differential analyzer and digital computer representation of the cyclic response of the intermediate and the various phases of the substrate and product curves on graphs which are plotted to approximately the same scale. The kinetics of the digital computer solution are hard to measure even slightly. These values are equal for this case where $k_3 = 1$. As is to be expected, there is less accuracy in measuring the rates than in measuring the concentrations; discrepancies of 6% are observed.

An over-all check on the shape of the solution has been found from the simple relationship:

$$k_3 = \frac{x_0}{p_m tf_{\text{off}}}$$

where $x_0$ is the initial value of substrate concentration and $t_{\text{off}}$ is the interval between $p = 0$ and the time when $p_m$ has fallen to half its value. Here the data agree to an accuracy of 10%. It should be noted that the accuracy of the agreement is about the same for the digital and the analog computer solutions.

The numerical data give a more accurate test of Equation 8 than was available previously. The accuracy is particularly good for $x_0 = 8$; at smaller values a 10 to 15% deviation is observed. This is discussed by Higgins (12) where suitable correction terms are formulated for these conditions.

### Table I

Comparison of solutions of Briggs-Haldane modification of Michaelis-Menten equations

<table>
<thead>
<tr>
<th>Substrate concentration</th>
<th>$p_m$ measured value</th>
<th>$p_m = \frac{1}{1 + \frac{x}{x_m}}$</th>
<th>$\dot{p}_m = \dot{p}_m$</th>
<th>$k_3 = \frac{x_0}{p_m tf_{\text{off}}} = 1.0$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 4 8</td>
<td>1 2 4 8</td>
<td>1 2 4 8</td>
<td>1 2 4 8</td>
</tr>
<tr>
<td>Analogue computer</td>
<td>0.235 0.410 0.602 0.772</td>
<td>0.25 0.41 0.60 0.78</td>
<td>0.23 0.34 0.54 0.70</td>
<td>1.2 1.1 1.0 0.99</td>
</tr>
<tr>
<td>Differential analyzer</td>
<td>0.235 0.41 0.60 0.77</td>
<td>0.25 0.39 0.61 0.79</td>
<td>0.23 0.36 0.55 0.68</td>
<td>1.3 1.1 1.1 1.0</td>
</tr>
<tr>
<td>Digital computer</td>
<td>0.234 0.401 0.602 0.773</td>
<td>0.234 0.401 0.604 0.773</td>
<td>0.234 0.401 0.602 0.773</td>
<td>1.15 1.10 1.11 0.997</td>
</tr>
<tr>
<td>Graphical output</td>
<td>0.234 0.401 0.602 0.773</td>
<td>0.234 0.401 0.604 0.773</td>
<td>0.234 0.401 0.602 0.773</td>
<td>1.15 1.10 1.11 0.997</td>
</tr>
<tr>
<td>Numerical data</td>
<td>0.234 0.401 0.602 0.773</td>
<td>0.234 0.401 0.604 0.773</td>
<td>0.234 0.401 0.602 0.773</td>
<td>1.15 1.10 1.11 0.997</td>
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SUMMARY AND CONCLUSIONS

An accuracy test for the digital computer solution of the one enzyme Briggs-Haldane modification of the Michaelis-Menten equation has been critically examined by a number of criteria. It is found that graphical output data show that the concentrations are accurate to 1% and rates to about 6% of the full scale. The numerical data on concentrations and rates are accurate to 0.1%. Since the same logical procedures are used for the solution of more complicated problems with the digital computer, it may be concluded that a similar accuracy will be obtained.

REFERENCES

Analogue and Digital Representations of Enzyme Kinetics
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