A Note on the Differential Equation of Simple Enzyme Kinetics

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The classical model of enzyme action[2] assumes that enzyme, E (in total concentration, E_0), reacts with substrate, S (in total concentration, S_0), to form "complex," ES, which then breaks down irreversibly to regenerate E and produce products, P, *i.e.*

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{ES}$$

$$\mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \qquad (1)$$

Letting $[\mathrm{P}] = p(t)$ and $[\mathrm{S}] = s(t)$, we may write the exact differential equation for p in system (1) as

$$\frac{p''}{k_1 S_0 p'_m} = \left(1 - \frac{p'}{p'_s}\right) - \left(1 - \frac{p'}{p'_m}\right)\left(\frac{p}{S_0} + \frac{p'}{p'_m}\frac{E_0}{S_0}\right) \qquad (2)$$

where $p'_m = k_2 E_0$ is the maximum possible ("saturation") rate of production of P, $p'_s \equiv \{\bar{K}S_0/(1 + \bar{K}S_0)\}\, p'_m$, and $\bar{K} \equiv k_1/(k_{-1} + k_2)$.

Material balance requires that

$$s + p + (p'/p'_m)E_0 = S_0 \qquad (3)$$

where it will be recognized that $[\mathrm{ES}] = (p'/p'_m)E_0$. It is evident that both p'_m and p'_s are both "natural" constants of the system, and it will also be observed that $\bar{K}$ and p'_m (hence p'_s) are usually considered to be obtainable from conventional enzyme kinetic experiments. The object of this note is to sketch approximately the theoretical time course of p and p', and to examine the conditions under which these approximations can be fitted to data for the purpose of inferring kinetic constants, *e.g.*, k_1, k_{-1}, etc.

If at $t = 0$, $p = 0$ and $s = S_0$, it is evident from physical considerations that $p'(0) = 0$ and that, eventually, $p \to S_0$, $s \to 0$, and p' is again 0. p' must therefore have attained a maximum value, p'_i, at some value of t. At this maximum, $p'' = 0$, so from eq. 2 we have

$$\left(1 - \frac{p'_i}{p'_s}\right) = \left(1 - \frac{p'_i}{p'_m}\right)\left(\frac{p_i}{S_0} + \frac{p'_i}{p'_m}\frac{E_0}{S_0}\right) \qquad (4)$$

The r.h.s. of equation 4 is positive, so it follows that p' may equal, but may never exceed, p'_s. The only case in which $p'_i \to p'_s$ is when $p'_s \to p'_m$, *i.e.*, $\bar{K}S_0 \to \infty$; in all other cases p'_i falls short of p'_s.

During the "early phase" of the reaction p and p' are both small, and it is therefore permissible to neglect their products and squares in eq. 2, which then becomes

$$\frac{p''}{k_1 S_0 p'_m} = 1 - \left(\frac{1}{p'_s} + \frac{1}{p'_m}\frac{E_0}{S_0}\right)p' - \frac{p}{S_0} \qquad (5)$$

The solution of eq. 5 is

$$p/S_0 - 1 = \frac{m_2 e^{m_1 t} - m_1 e^{m_2 t}}{m_1 - m_2} \qquad (6)$$

where m_1 and m_2 are given by

$$\frac{-(1/\bar{K} + E_0 + S_0) \pm \{(1/\bar{K} + E_0 + S_0)^2 - 4p'_m/k_1\}^{1/2}}{2/k_1} \qquad (7)$$

Experimental measurement of these two decay constants provides a basis for estimating individual velocity constants. For example, the sum of m_1 and m_2 is $-k_1$ multiplied by a readily measured factor, *viz.*, $1/\bar{K} + E_0 + S_0$. In the special case that E_0 is negligible relative to either $1/\bar{K}$ or S_0 in expression 7, then one m goes to zero (exponential term degenerates to a constant), and the other m approaches $-k_1 S_0 p'_m/p'_s$. This latter case has already been treated by Gutfreund and Roughton,[3] although their rationale has not yet been published. The logical consistency of this or any other approximation (see below) can be checked by ascertaining that throughout the relevant time interval, the neglected terms, as estimated from the approximate expression, do indeed remain negligibly small.

As we have noted already, there is only one maximum in p', *i.e.*, only one instant at which $p'' = 0$; however, under many conditions p'' may remain small (and $(p''/p'_m)E_0$ negligible relative to s' or p' in the differential of eq. 3) for a considerable time before and after the point of inflection. The "steady state approximation" consists in assuming that for the range in question p'' is small enough so that it may be neglected in eq. 2. The resulting equation is awkwardly integrable to give $p(t)$, or easily integrable if one transforms to the variable, s

$$(1/\bar{K}) \ln s + s = -p'_m t + C_1 \qquad (8)$$

where C_1 is a constant of integration which must be evaluated for the range of approximation. Equation 8 is directly usable if s is measured. If p is measured one must further assume that $(p'/p'_m)E_0$ is negligible relative to either s or p (in equation 3) in order to obtain a usable equation

$$(1/\bar{K}) \ln (S_0 - p) - p = -p'_m t + C_2 \qquad (9)$$

where C_2 is also a constant of integration to be evaluated for the range of approximation. Equations 8 and 9 can be compared with experimental data to check the validity of the assumptions which they contain, even though such comparisons do not yield information other than that presumed to be obtainable in conventional enzyme kinetics. Indeed, if in equation 9 one assumes p/S_0 small relative to unity, and therefore retains only the linear term in expanding the logarithm, the result can be put in the form

$$p = p'_s t + \text{Constant} \qquad (10)$$

which is the traditional equation used in enzyme kinetic studies, wherein the measured rate (somewhat loosely called the "initial rate") is supposed to be p'_s.

During the terminal phase of reaction one may again linearize equation 2 by appealing to the fact that both $p' \to 0$ and $(S_0 - p) \to 0$. Straightaway this permits one to drop the term containing p'^2 as a factor, and obtain

$$p'' = k_1 p'_m (S_0 - p) - k_1\{1/\bar{K} + E_0 + (S_0 - p)\}p' \qquad (11)$$

Equation 11 is still not integrable unless or until it can also be assumed that $(S_0 - p)$ has become negligible relative to either $1/\bar{K}$ or E_0. From this point on the equation is a second-order equation with constant coefficients. Its solution contains

(1) In accordance with Navy regulations, it is noted that the opinions expressed in this article are those of the authors and do not necessarily reflect the views of the Navy Department or the Naval Service at large.

(2) L. Michaelis and M. L. Menten, *Biochem. Z.*, **49**, 333 (1913).

(3) Cited by F. J. W. Roughton in *Disc. Faraday Soc.*, **17**, 116 (1954).

two exponential terms whose decay constants are given by

$$\frac{-\{1/\bar{K} + E_0\} \pm \{(1/\bar{K} + E_0)^2 - 4p'_m/k_1\}^{1/2}}{2/k_1} \quad (12)$$

In certain favorable cases these transients could also be employed in deducing velocity constants. For example, if k_{-1} were very large compared to $(k_2 + k_1E_0)$, one decay constant would tend to zero, and the other to $-k_{-1}$, while if k_2 were very large compared to $(k_{-1} + k_1E_0)$, one decay constant would tend to $-k_1E_0$, and the other to $-k_2$.

With modern, high speed computing devices it is quite feasible to find by trial the unique set of values for the parameters needed to reproduce a given experimental concentration time curve for any component of the reaction system. This is easily done with an analog computer where the programming is direct and simple. However, the usefulness of the procedure may be limited in situations where there are wide differences in order of magnitude between the various parameters, *e.g.*, E_0 and S_0.

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Indicators for the Paper Chromatography of Lipids[1]

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The inclusion reaction has received considerable attention in recent years. One of the basic principles resulting from research in this field is that shape rather than chemical character of a molecule determines whether or not it can be included by a certain host. The complexing reaction can be carried out in minute amounts such as are used in practical paper chromatography. This is easily demonstrated by spraying a filter paper bearing a spot of octadecane with α-cyclodextrin and subsequently exposing it to iodine vapors. The area covered with hydrocarbon remains white, while the remainder of the paper turns bluish purple. This occurs because of different reactivities of the free and of the complexed α-dextrin. Free α-dextrin reacts with iodine to form a deeply colored inclusion complex very similar to that resulting from starch with iodine. In fact, the two reactions are closely related[2] and the same phenomenon can be observed with starch on a spotted paper. Comparative examination proved α-dextrin to be the preferable indicator in actual chromatograms. When the α-dextrin is already occupied by a guest molecule, in the above case the octadecane, it is no longer available for the iodine reaction which produces color. Accordingly, the spot of hydrocarbon stays white. This holds for other compounds having low chemical reactivity besides hydrocarbons; for example, esters, ethers or other types of molecules that react with α-dextrin. Long chain fatty acids, alcohols and esters fall into this category. Their chemical characteristics are not pronounced so that it is difficult to find indicators of a sensitivity adequate for use in paper chromatograms. Although various suggestions have been made to solve these difficulties,[3] paper chromatography does not hold its due place among analytical tools in the lipid field.

α-Dextrin can be used successfully on chromatograms of fatty alcohols, acids, methyl or ethyl esters and monoglycerides. Diglycerides and triglycerides do not respond to this indicator method, since they do not react readily with α-dextrin. However, they can be detected by splitting them into their components after chromatographic separation. The necessary hydrolysis is carried out *in situ* enzymatically by means of commercial pancreatin. In this way it is possible to make visible the separated di- and triglycerides on the paper.

Two other indicators were found to be applicable to certain types of lipids. Iodine vapors have been used for detecting a variety of substances[4] but not for higher fatty acids. In the lipid series we found it specific for spotting unsaturated components. For instance, oleic acid and its derivatives appear as brown spots on chromatograms when exposed to iodine vapors. The limitation of the method is given by the fact that symmetrical oleodipalmitin cannot be located unambiguously in actual chromatograms. A more specific reagent is lead tetraacetate which is applicable to the chromatography of monoglycerides. The reagent hydrolyzes in air to form brown lead dioxide; with α-monoglycerides it undergoes the normal glycol splitting reaction and yields colorless lead compounds.[5] Consequently, monoglycerides appear as white spots on a brown background in chromatograms sprayed with lead tetraacetate solution. For the detection of di- and triglycerides it can be used after splitting them by the above-mentioned enzymatic hydrolysis.

Complexing is the most universal method and is certainly applicable to many other compounds besides the lipids.

Table I shows R_f values obtained by chromatographing model mixtures and individual lipids for identification. Different solvent systems were used in ascending technique with silicone impregnated paper, *i.e.*, a reversed phase chromatography.

Whatman No. I paper was modified according to procedures reported in the literature. We found impregnation with silicone[6] to be the most versatile preparation for our purpose. Since silicone is not washed out by the developing solvents as hydrocarbons are from impregnated papers, the same paper can be used for reversed phase chromatography in the second dimension. Uniform coating is easily

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