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# On a Method of Determining the Mechanism of an Enzymatic Reaction the Kinetics of Which is Known

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In 1903 Henri in his thesis for the doctorate [1] concluded from measurements on the enzymatic inversion of saccharose and some related reactions that an enzyme may combine with the »substrate« or with the reaction products. The occurrence of such combinations will have a definite influence on the kinetics of the reaction, which may therefore be used to prove their existence.

Ten years later Michaelis and Menten published a renowned paper [2] in which they drew attention to Henri's work and made an extensive series of experiments on the same reaction, avoiding some sources of error which had escaped Henri's attention. The effect is now known as the Michaelis effect, and the constant relating to the formation of the compound between enzyme and substrate is usually called the Michaelis constant $k_m$, which symbol dates from Henri's paper.

Although the connection with the work of Henri and Michaelis may not be obvious, the trend of the following is to extend the interpretation of the kinetics of enzymatic reactions on the basis given by these authors. In its main features the method will be the same as that which has been used for many years by many different authors, namely the method of stationarity. It will be given in about the same form as that used in two papers in *Handbuch der Katalyse* [3] with amendments which the application to the special case of an enzymatic reaction has made natural.

In the following we shall treat only one enzymatic reaction, but it is hoped that the treatment may serve as an example of the application of some very simple principles on other reactions and thus be helpful in the elucidation of the mechanisms of other types of enzymatic reaction.

The reaction in question is the well known transamination reaction, *e. g.*

glutamic acid + pyruvic acid $\rightleftharpoons$ keto-glutaric acid + alanine

which proceeds at ordinary temperature only in the presence of a certain enzyme, transaminase. This reaction and a few analogous reactions have recently been experimentally investigated by Sv. Darling, M.Sc., at the Biochemical Institute of the University of Aarhus. The present author has taken no part at all in the experimental investigation, but he has discussed the results and their utilization for unveiling the reaction mechanism with Darling, who has kindly permitted the use of some of the results of the investigation in this paper.

The reaction is obviously of the type:

$$A + B \rightleftharpoons C + G$$

In the following we shall use four facts which appear from Darling's investigation, *viz.*:

1) The equilibrium constant is $(3/2)^2$ at all temperatures from 20° C to about 70° C, *i. e.* $\Delta H$ for the reaction is zero or rather experimentally not discernible from zero.

2) Starting with equivalent concentrations $a$ and $b$ of A and B, respectively, the reaction follows approximately the unimolecular law, the constant, of course, being proportional to the total amount of enzyme.

3) Under the same condition the reciprocal velocity constant increases linearly with $a$, but is not proportional to $a$, *i. e.* a graph with $1/k$ and $a$ as coordinates will be a straight line which does not pass through the origin.

4) When one experiment is started with different values of $a$ and $b$ ($c_A = a$, $c_B = b$), and another with exchange of the values ($c_A = b$, $c_B = a$), it appears that the course of the reaction in the two cases is the same or very nearly the same, *i. e.* we may say that the course is symmetrical in $a$ and $b$.

We now attempt to find by trial and error a mechanism which is in harmony with the kinetics.

Beginning with the simplest possibility, we may assume that the reaction is:

$$E + A + B \rightleftharpoons C + G + E$$

where E denotes the enzyme.

From this assumption we conclude that the reaction is bimolecular with respect to A and B, which certainly is not the case. Therefore this possibility is ruled out.

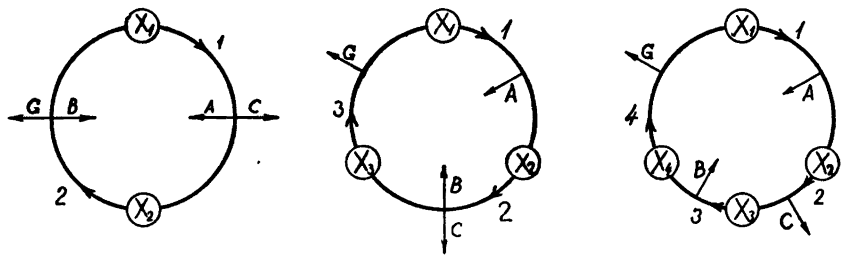



*Fig. 1. Geometrical representations of the sequences pp. 495, 499, 500.*

Next we assume that the reaction is:

$$\begin{array}{lcll} A + X_1 & \rightleftharpoons & X_2 + C & (\pm 1) \\ B + X_2 & \rightleftharpoons & X_1 + G & (\pm 2) \end{array}$$

Here $X_1$ and $X_2$ are symbols for two different forms of the enzyme. If their corresponding concentrations $x_1$ and $x_2$ are added, we get, of course, the total enzyme concentration, which will be denoted $E$.

This (closed) sequence may also be represented geometrically by means of diagram I in Fig. 1. This diagram is intended to mean that by reaction (+ 1) A disappears (from the world outside the circle), while C appears, and similarly for reaction (+ 2). A reaction in the opposite direction is symbolized by the same diagram only with all arrows reverted.

To describe the reaction quantitatively we say that *e. g.* $X_1$ has a certain probability per unit time $\bar{\omega}_1$ * to react according to reaction (+ 1) and the reaction probability $\bar{\omega}_{-2}$ to react according to (—2) *etc.*, the $\bar{\omega}$'s being either constants or constants multiplied by one or (rarely) two concentrations. In our scheme $\bar{\omega}_1 = k_1 a$ or $\bar{\omega}_1 = k_1(a—x)$ if $a$ is the concentration of A at $t = 0$, and $x$ is the amount which has reacted at time $t$.

Now it is well known to-day that with time in seconds the constants occurring in these expressions are either very large, *i. e.* about $10^{13}$ for reactions of the unimolecular type, abt. $10^{11}$ for reactions of the bimolecular type or the same large numbers multiplied by an exponential of the form $e^{-A/T}$, which for reactions with measurable velocities is a very small fraction of 1, *e. g.* $10^{-11}$ or less.

From this it follows that if sums like $\bar{\omega}_1 + \bar{\omega}_{-1}$ occur in our expressions, and we know that there is a difference in energy-level for the two systems between

* $\bar{\omega}$ should be read as the greek letter pi. The correct type, (*e. g.* Guggenheim and Fowler, *Statistical thermodynamics*, 1939) was not available.

which the reaction takes place, we may, with an accuracy usually greater by far than the accuracy of our experiments, omit either one or the other member of the sum. On the other hand, when the two systems are on the same level, we may according to present views assume that both are large, *i. e.* that they do not contain the exponential factor.

In the case considered here we have the extra simplification that $\Delta H$ of the reaction is zero, which means that the effect of the activation-energies disappears in the equilibrium expression. From these considerations it follows that we may ascribe a meaning to the orientation of the diagram. As it stands it is intended to mean that the reaction probabilities of the »upward» reactions (— 1) and (+ 2) are immensely small as compared to those of the »downward» reactions (+ 1) and (— 2). If we had placed $x_1$ and $x_2$ on the same level in the diagram, this would mean that all four probabilities are large, but this again would mean that the reaction would be immeasurably fast unless the enzyme concentration is practically nil. In the following we shall not consider this case.

We shall now proceed to discuss the partition of the enzyme on the two states: $X_1$ and $X_2$. This partition will, of course, depend on the momentary values of the concentrations of A, B, C, and G, but besides this it may depend explicitly on time. To find this dependence on time of $x_1$ and $x_2$, we treat the problem tentatively as if the reaction-probabilities were constant in time. Of course, this assumption is not strictly true, but they may vary so slowly with time that their dependence on time is of no consequence.

The mathematical treatment of a problem of this type is well known [4].

Denoting differentiation with respect to time with a dot, we obviously get:

$$-\dot{x}_1 = x_1\ (\bar{\omega}_1 + \bar{\omega}_{-2}) - x_2\ (\bar{\omega}_2 + \bar{\omega}_{-1})$$
$$-\dot{x}_2 = -x_1\ (\bar{\omega}_1 + \bar{\omega}_{-2}) + x_2\ (\bar{\omega}_2 + \bar{\omega}_{-1})$$

Putting $-\dot{x}_1 = \lambda\ x_1$, $-\dot{x}_2 = \lambda\ x_2$, we get the characteristic equation:

$$\begin{vmatrix} \bar{\omega}_1 + \bar{\omega}_{-2} - \lambda & -(\bar{\omega}_2 + \bar{\omega}_{-1}) \\ -(\bar{\omega}_1 + \bar{\omega}_{-2}) & \bar{\omega}_2 + \bar{\omega}_{-1} - \lambda \end{vmatrix} = 0$$

the roots of which are $\lambda_0 = 0$, $\lambda_1 = \bar{\omega}_1 + \bar{\omega}_{-2} + \bar{\omega}_2 + \bar{\omega}_{-1}$.

The general solution becomes:

$$x_1 = E\ (\bar{\omega}_2 + \bar{\omega}_{-1})/\lambda_1 + A\ \exp\ (-\lambda_1 t)$$
$$x_2 = E\ (\bar{\omega}_1 + \bar{\omega}_{-2})/\lambda_1 - A\ \exp\ (-\lambda_1 t)$$

where $A$ is a constant which can be determined when, and only when we know the partition of the enzyme on the two states at time zero. In most cases this knowledge is difficult or impossible to obtain, but fortunately it is unnecessary, for when we remember the orientation of the diagram, $\lambda_1$ can to all intents and purposes be put equal to $\bar{\omega}_1 + \bar{\omega}_{-2}$ which are both very large. If for instance the concentrations applied in the experiment are of the order of magnitude $10^{-3}$ molar, $\bar{\omega}_1 + \bar{\omega}_{-2}$ will be something like $10^8$ reciprocal seconds, which means that the exponentials above have practically disappeared at the same moment the reaction is started, *i. e.* the partition on the two forms is stationary practically from the start.

This being so, we may safely apply the method of stationarity to calculate the reaction velocity $s$:

$$s = x_1\bar{\omega}_1 - x_2\bar{\omega}_{-1}$$
$$s = x_2\bar{\omega}_2 - x_1\bar{\omega}_{-2}$$

the solution of which may be written:

$$Lx_1/s = \bar{\omega}_2 + \bar{\omega}_{-1} \qquad L = \bar{\omega}_1\bar{\omega}_2 - \bar{\omega}_{-1}\bar{\omega}_{-2}$$
$$Lx_2/s = \bar{\omega}_1 + \bar{\omega}_{-2}$$

or, as $x_1 + x_2 = E$

$$LE/s = M$$
$$M = \bar{\omega}_2 + \bar{\omega}_{-1} + \bar{\omega}_1 + \bar{\omega}_{-2}$$

If we express the concentration at time $t$ of A by $a - x$, where $a$ is the value at $t = 0$, and similarly for the other participants then obviously $s = \mathrm{d}x/\mathrm{d}t$, and the equation is a differential equation for the determination of $x$ as a function of $t$, or what is more convenient for the determination of $t$ as a function of $x$.

The integration is fairly easy, but unnecessary as we see at a glance that the expression is hopelessly unsymmetrical in $a$ and $b$. For if the orientation of the diagram is as given, $\bar{\omega}_2$ and $\bar{\omega}_{-1}$ disappear from $M$ so that $M = k_1(a - x) + k_2(g + x)$, whereas if it is turned upside down $\bar{\omega}_1$ and $\bar{\omega}_{-2}$ disappear with a similar consequence. As $L$ is always symmetrical in $a$ and $b$, this proves our case.

The next step is to assume a sequence containing three partial reactions as represented by diagram II or by the sequence:

$$\begin{array}{lcll} A + X_1 & \rightleftharpoons & X_2 & (\pm 1) \\ B + X_2 & \rightleftharpoons & X_3 + C & (\pm 2) \\ X_3 & \rightleftharpoons & X_1 + G & (\pm 3) \end{array}$$

As in the former case we start with an investigation of the dependence on time of the partition of the enzyme on the three states. The characteristic equation becomes of the third degree and has the roots $\lambda_0 = 0$, $\lambda_1$ and $\lambda_2$. $\lambda_1$ and $\lambda_2$ may contain an imaginary part, but it can easily be seen that their real part must be positive and large so that again the members containing the exponentials exp $(-\lambda_1 t)$ and exp $(-\lambda_2 t)$ disappear in practically no time.

The conditions of stationarity become:

$$s = x_1\bar{\omega}_1 - x_2\bar{\omega}_{-1}$$
$$s = x_2\bar{\omega}_2 - x_3\bar{\omega}_{-2}$$
$$s = x_3\bar{\omega}_3 - x_1\bar{\omega}_{-3}$$

The solution is:

$$Lx_1/s = \bar{\omega}_2\bar{\omega}_3 + \bar{\omega}_{-1}\bar{\omega}_3 + \bar{\omega}_{-1}\bar{\omega}_{-2}$$
$$Lx_2/s = \bar{\omega}_3\bar{\omega}_1 + \bar{\omega}_{-2}\bar{\omega}_1 + \bar{\omega}_{-2}\bar{\omega}_{-3}$$
$$Lx_3/s = \bar{\omega}_1\bar{\omega}_2 + \bar{\omega}_{-3}\bar{\omega}_2 + \bar{\omega}_{-3}\bar{\omega}_{-1}$$

$$L = \bar{\omega}_1\bar{\omega}_2\bar{\omega}_3 + \bar{\omega}_{-1}\bar{\omega}_{-2}\bar{\omega}_{-3}$$

and consequently:

$$LE/s = M$$

where $M$ is the sum of the members in the 9-membered »partition matrix»:

$$\begin{Bmatrix} x_1 \\ x_2 \\ x_3 \end{Bmatrix} \qquad \left\{ \begin{array}{ccc} \bar{\omega}_2\bar{\omega}_3 & \bar{\omega}_{-1}\bar{\omega}_3 & \bar{\omega}_{-1}\bar{\omega}_{-2} \\ \bar{\omega}_3\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_{-3} \\ \bar{\omega}_1\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_{-1} \end{array} \right\}$$

the form of which is easy to remember (start with $\bar{\omega}_2$). It is called the partition matrix because the sum of the members in each line is proportional to the quantity of the form of the enzyme which is indicated in the three-membered matrix to the left of $M$.

As the total amount of enzyme is known (in principle) and constant in time in each experiment, the amounts of the three forms at any time can be easily calculated by means of this matrix when the different constants have been determined.

For the following it will be convenient to write down a matrix of the same form (9 members) containing only the simultaneous concentrations of the four substances A, B, C, and G appearing as factors in the expressions with omission of the constants, the respective concentrations being named $a$, $b$, $c$, and $g$.

In the case considered this matrix (the $c$-matrix) becomes:

$$\left\{\begin{array}{ccc} b & 1 & c \\ a & \boxed{ca} & \boxed{cg} \\ \boxed{ab} & \boxed{gb} & g \end{array}\right\}$$

When the diagram is orientated as shown in II, the members outside the frames disappear as compared to those inside.

It appears on consideration of the $c$-matrix that in this case $M$ may be symmetrical in $a$ and $b$, namely if $c = g$, and $k_{-2}k_1 = k_{-3}k_2$.

Further inspection of the six different possibilities of orientation:

$$\left|\begin{array}{cc} & x_1 \\ x_3 & x_2 \end{array}\right| \left|\begin{array}{cc} x_2 & x_3 \\ & x_1 \end{array}\right| \left|\begin{array}{cc} & x_2 \\ x_1 & x_3 \end{array}\right| \left|\begin{array}{cc} x_3 & x_1 \\ & x_2 \end{array}\right| \left|\begin{array}{cc} & x_3 \\ x_2 & x_1 \end{array}\right| \left|\begin{array}{cc} x_1 & x_2 \\ & x_3 \end{array}\right|$$

will show that this is also the only orientation of the diagram which can lead to the desired symmetry in $a$ and $b$.

For comparison with the experiments we shall introduce the $\bar{\omega}$-values which follow from the sequence assumed:

$\bar{\omega}_1 = k_1\ (a—x)$; $\bar{\omega}_{-1} = k_{-1}$; $\bar{\omega}_2 = k_2\ (b—x)$; $\bar{\omega}_{-2} = k_{-2}\ (c + x)$;
$\bar{\omega}_3 = k_3$; $\bar{\omega}_{-3} = k_{-3}\ (g + x)$; and furthermore: $1/s = \mathrm{d}t/\mathrm{d}x$

Now most of Darling's experiments were made with $a = b$; $c = g = 0$. On these assumptions integration of the differertial equation leads to the expression:

$$2qk_3Et = -a\left(\frac{p+q}{1+q}\right)^2 \ln\,(1-\alpha\,(1+q)) + a\left(\frac{p-q}{1-q}\right)^2 \ln\,(1-\alpha\,(1-q)) + 2a\alpha q\frac{(1-p)^2}{1-q^2}$$

where:

$$p = k_{-2}/k_2 = k_{-3}/k_1,\ q^2 = k_{-1}k_{-2}k_{-3}/k_1k_2k_3 \text{ and } \alpha = x/a.$$

On comparison of this formula with the actual experiments, it appears that the first member in the sum on the right hand side is the leading one, *i. e.* the reaction follows with good approximation the unimolecular law (for a reversible reaction). Further it appears from the formula that the reciprocal constant

of the reaction increases with $a$, which is qualitatively in agreement with the experiments. The formula, however, disagrees with the experiments as it shows that the reciprocal constant should be proportional to $a$, while as a matter of fact the experiments show that it increases linearly with $a$, the straight line connecting the empirical points in the $(a, 1/k)$-plot not passing through the origin.

We must, therefore, also discard this mechanism and proceed to investigate the consequences of the sequence:

$$\begin{array}{rcll} A + X_1 & \rightleftharpoons & X_2 & (\pm 1) \\ X_2 & \rightleftharpoons & X_3 + C & (\pm 2) \\ B + X_3 & \rightleftharpoons & X_4 & (\pm 3) \\ X_4 & \rightleftharpoons & X_1 + G & (\pm 4) \end{array}$$

which is represented by diagram III.

The investigation follows the same lines as in the former cases, that is we start with the investigation of the dependence on time of the partition of the enzyme on the different states, and having proved that the partition must become stationary in a time which is negligible compared to the time in which the over-all reaction has proceeded perceptibly, we write down the expression:

$$LE/s = M$$

which is a differential equation connecting the degree of reaction $x$ with time.

In this case it is a little complicated to estimate the values of the roots in the characteristic equation which determine the time for attainment of stationary conditions, simply because the equation is of the third (fourth) degree. If the equation is written:

$$a_0\lambda^4 - a_1\lambda^3 + a_2\lambda^2 - a_3\lambda + a_4 = 0$$

it is at once seen that $a_0 = 1$ and $a_4 = 0$, so that $\lambda_0 = 0$ is as always one of the roots.

The other constants are positive. We find $a_3 = M$, *i. e.* $a_3$ equals the sum $M$ of the 16 members in the below partition matrix (p. 503).

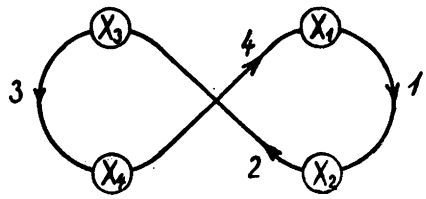
Furthermore $a_1$ equals the sum of the eight reaction probabilities. $a_2$ is a sum of 20 products of the form $\bar{\omega}_i\bar{\omega}_j$ $(i \neq \pm j)$:

$$a_2 = \sum \begin{Vmatrix} \bar{\omega}_2\bar{\omega}_3 & \bar{\omega}_{-1}\bar{\omega}_3 & \bar{\omega}_{-1}\bar{\omega}_{-2} \\ \bar{\omega}_3\bar{\omega}_4 & \bar{\omega}_{-2}\bar{\omega}_4 & \bar{\omega}_{-2}\bar{\omega}_{-3} \\ \bar{\omega}_4\bar{\omega}_1 & \bar{\omega}_{-3}\bar{\omega}_1 & \bar{\omega}_{-3}\bar{\omega}_{-4} \\ \bar{\omega}_1\bar{\omega}_2 & \bar{\omega}_{-4}\bar{\omega}_2 & \bar{\omega}_{-4}\bar{\omega}_{-1} \end{Vmatrix} + \sum \begin{Vmatrix} (\bar{\omega}_1 + \bar{\omega}_{-4}) & (\bar{\omega}_3 + \bar{\omega}_{-2}) \\ (\bar{\omega}_2 + \bar{\omega}_{-1}) & (\bar{\omega}_4 + \bar{\omega}_{-3}) \end{Vmatrix}$$

Now if the diagram is as indicated, *i. e.* if we assume that $\bar{\omega}_{-1}$ and $\bar{\omega}_4$, and only they contain the exponential factor, we get for $a_1$ a sum of 6 quantities $\bar{\omega}$, for $a_2$ a sum of 11 products $\bar{\omega}\bar{\omega}$, and for $a_3$ a sum of 6 products $\bar{\omega}\bar{\omega}\bar{\omega}$, none of which contains the exponential factor exp $(-A/T)$.

This shows that the three roots are all large, so that the exponentials exp $(-\lambda t)$ will disappear practically instantaneously.

If, however, the diagram is assumed to be IV in Fig. 2.




IV

*Fig. 2. Geometrical representation of the sequence p. 500, second form.*

$x_2$ and $x_4$ are obviously confined to two »valleys» between two »hills», and it must be expected that the transition from $x_2$ to $x_4$ and *vice versa* is »slow». This appears, too, when we try to solve the characteristic equation. In this case $\bar{\omega}_{-1}$, $\bar{\omega}_2$, $\bar{\omega}_{-3}$, and $\bar{\omega}_4$ all contain the exponential term exp $(-A/T)$. This has the following consequences:

Of $a_1$ a sum of 4 $\bar{\omega}$'s remains: $\bar{\omega}_1 + \bar{\omega}_{-4} + \bar{\omega}_3 + \bar{\omega}_{-2}$,
and of $a_2$ only the product: $(\bar{\omega}_1 + \bar{\omega}_{-4})\ (\bar{\omega}_3 + \bar{\omega}_{-2})$ remains. None of these members contains the exponential term. In $a_3$, however, the exponential term is retained, as in the lines corresponding to $x_1$ and $x_3$ in the partition matrix it appears in the second power, while in the $x_2$ and $x_4$ lines it appears in the first power. This corresponds to the fact that we have assumed $x_2$ and $x_4$ to be situated in »valleys», while $x_1$ and $x_3$ have been placed on the top of the »hills».

What is left of $a_3$ is a sum of eight members all containing one exponential term, four in the $x_2$-line and four in the $x_4$-line of $(M)$. It is, therefore, obvious that in this case two roots are large, and one is small. As the equation becomes:

$$\lambda^3 - (\bar{\omega}_1 + \bar{\omega}_{-4} + \bar{\omega}_3 + \bar{\omega}_{-2})\lambda^2 + (\bar{\omega}_1 + \bar{\omega}_{-4})\ (\bar{\omega}_3 + \bar{\omega}_{-2})\lambda - M = 0$$

the two large roots must be very nearly:

$$\lambda_2 = \bar{\omega}_1 + \bar{\omega}_{-4} \quad ; \quad \lambda_3 = \bar{\omega}_3 + \bar{\omega}_{-2}$$

while the third is determined (approximately) by neglecting the first two members of the equation and solving for $\lambda$.

What we now need is only an estimate of the small root $\lambda_1$. For this purpose we consider the situation at the start of an experiment, where the concentrations $c$ and $g$ are zero. Under these conditions the $\bar{\omega}$'s of the »negative» reactions (—2) and (—4) disappear, and we get (compare the expression for $M$ p. 503):

$$\bar{\omega}_1\bar{\omega}_3\lambda - \bar{\omega}_1\bar{\omega}_3\ (\bar{\omega}_2 + \bar{\omega}_4) = 0$$

*i. e.*:

$$\lambda_1 = \bar{\omega}_2 + \bar{\omega}_4 = k_2 + k_4$$

Under the same conditions we get for $s$ from $LE/s = M$:

$$E\bar{\omega}_1\bar{\omega}_2\bar{\omega}_3\bar{\omega}_4 = s\bar{\omega}_1\bar{\omega}_3(\bar{\omega}_2 + \bar{\omega}_4)$$

*i. e.*:

$$s = E\,\frac{\bar{\omega}_2\bar{\omega}_4}{\bar{\omega}_2 + \bar{\omega}_4} = E\,\frac{k_2k_4}{k_2 + k_4}$$

an expression for the velocity of the over-all reaction. From this we get the decay-constant, which is to be compared with $\lambda_1$ by dividing by $a$, the initial concentration of $A$:

$$\frac{s}{a} = \frac{E}{a}\,\frac{k_2k_4}{k_2 + k_4}$$

If $k_2 = k_4 = k$, we find $\lambda_1 = 2k$; $s/a = \frac{1}{2}k\ E/a$. As $E/a$ is in most experiments a very small fraction of 1, it appears that even if the absolute value of $\lambda_1$ is small, it will be large as compared to the »decay-constant» of the over-all reaction, and this knowledge is sufficient for our purpose. In the case that $k_2$ and $k_4$ are different, for instance $k_2 \gg k_4$, the ratio between $\lambda_1$ and $s/a$ becomes still larger, as in that case $\lambda_1 = k_2$ and $s/a = k_4\ E/a$. Thus we have proved that even in this rather disadvantageous case it must be assumed that the partition of the enzyme on the four different states becomes stationary a relatively very short time after the start of the experiment.

The rest is easy. We shall use the equation:

$$EL/s = M$$

where

$$L = \bar{\omega}_1\bar{\omega}_2\bar{\omega}_3\bar{\omega}_4 - \bar{\omega}_{-1}\bar{\omega}_{-2}\bar{\omega}_{-3}\bar{\omega}_{-4}$$

and $M$ is the sum of the 16 members in the partition matrix:

$$\begin{Bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \end{Bmatrix} \begin{Bmatrix} \bar{\omega}_2\bar{\omega}_3\bar{\omega}_4 & \bar{\omega}_{-1}\bar{\omega}_3\bar{\omega}_4 & \bar{\omega}_{-1}\bar{\omega}_{-2}\bar{\omega}_4 & \bar{\omega}_{-1}\bar{\omega}_{-2}\bar{\omega}_{-3} \\ \bar{\omega}_3\bar{\omega}_4\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_4\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_{-3}\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_{-3}\bar{\omega}_{-4} \\ \bar{\omega}_4\bar{\omega}_1\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_1\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_{-4}\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_{-4}\bar{\omega}_{-1} \\ \bar{\omega}_1\bar{\omega}_2\bar{\omega}_3 & \bar{\omega}_{-4}\bar{\omega}_2\bar{\omega}_3 & \bar{\omega}_{-4}\bar{\omega}_{-1}\bar{\omega}_3 & \bar{\omega}_{-4}\bar{\omega}_{-1}\bar{\omega}_{-2} \end{Bmatrix}$$

The corresponding $c$-matrix is:

$$\begin{Bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \end{Bmatrix} \begin{Bmatrix} b & b & c & c \\ ba & ca & ca & cg \\ a & a & g & g \\ ab & gb & gb & gc \end{Bmatrix}$$

The members which according to diagram III do not contain exponentials are inside the frames. It is seen that the expression for $s$ cannot be made exactly symmetrical in $a$ and $b$, but the constants may have such values that the dissymmetry is small. As a matter of fact Mr. Darling has found by his experiments that under certain starting conditions a dissymmetry exists.

To use the expression it is integrated as before, but as the aim of this paper is only to discuss the method, and as the author has no part in the experiments, we shall not enlarge on details.

It must, however, be added that by repetition of the three first lines in the partitionmatrix below the matrix, and displacement of the frame by two lines downward, it can be seen that another possibility exists for getting an expression of a similar form. Inspection of the diagram and the corresponding sequence shows, however, that there is no real difference between these two possibilities, as the latter can be arrived at from the former simply by changing the meaning of the symbols.

A more essential addition is that diagram IV discussed above might also lead to kinetics which harmonize with the experimental facts. As mentioned above all the members in the $x_1$ and $x_3$ lines are in this case to be omitted from the partition matrix (for the application in the expression for $s$). Comparison with the $c$-matrix shows that by an appropriate choice of the constants the expression can be made symmetrical in $a$ and $b$ (when $c = g$). It is for the experiments to show whether one or the other mechanism is the right one, the essential thing being that the consequences are sufficiently different to make a decision possible.

So far we have only discussed the analysis of the kinetic results and have completely disregarded the information which may be gained by chemical considerations. The reason for this is that the results become more conclusive when arrived at independently in different ways.

Not to forget completely that we are dealing with a chemical reaction, we may, however, add that from a chemical point of view the mechanism expressed in diagram IV might seem to be more probable than that in diagram III, as (1) and (3) are associations, while (2) and (4) are dissociations.

## SUMMARY

The kinetics of an enzymatic reaction:

$$A + B \rightleftharpoons C + G$$

are discussed by means of the so-called partition-matrix, a matrix from which the stationary partition of the enzyme on its different possible forms and the stationary reaction velocity can be derived.

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