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An Addendum to the Henri-Michaelis Mechanism

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It is shown that in an enzymic reaction obeying the Henri-Michaelis law the existence of an inactive form with low energy content of the enzyme-substrate complex must exist besides the active, energy-rich form.

The Henri-Michaelis mechanism [1-3] can be written in the form

$$A + X_1 \rightleftharpoons X_2 \quad (\pm 1)$$
$$X_2 \rightarrow X_1 + B \quad (2)$$

where the stoichiometric equation of the overall reaction is A = B.

As pointed out by Haldane and Briggs [4], Michaelis in his original treatment of the reaction makes the error of assuming equilibrium as regard to (±1) which is obviously untrue, if the rate of reaction is not zero.

Application of the steady-state method yields, with s for steady state rate, X_1 for $[X_1]$, x_2 for $[X_2]$ and w_i for the probability of reaction i in unit time:

$$x_1/s = 1/w_1 + w_{-1}/w_1w_2 \quad (3)$$
$$x_2/s = 1/w_2 \quad (4)$$

or as $x_1 + x_2$ = total enzyme concentration = E,

$$E/s = (1 + w_{-1}/w_2)/w_1 + 1/w_2 \quad (5)$$

According to the mechanism $w_1 = k_1(a—x)$; $w_{-1} = k_{-1}$; $w_2 = k_2$, where $a—x = [A]$ at time t.

$$E/s = 1/k_2 + (1+k_{-1}/k_2)/k_1(a—x) \quad (6)$$

which of course agrees exactly with Haldane and Briggs' result.

Replacing $1/k_2$ by A_0 and $(1+k_{-1}/k_2)/k_1$ by A_1 and $1/s$ by dt/dx we get by integration

$$Et = A_0x + A_1 \ln a/(a—x) \quad (7)$$

where $A_1/A_0 = K_m$, the Michaelis constant. (The symbol K_m is due to Henri). Obviously

$$(k_2 + k_{-1})/k_1 = K_m \quad (8)$$

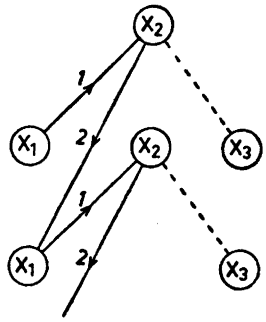

Fig. 1.

A number of enzymic reactions but by no means all yield experimentally an expression of the type (7). (It is convenient to denote the function on the right hand side of (7) by the name a chronometric integral.) Therefore a A_0 and A_1 must in these cases be of the same order of magnitude. This however is impossible if the reaction has an activation-energy. In that case the diagram of the reaction must be qualitatively as in Fig. 1.

The encircled symbols represent the "states" of the enzyme. The system proceeds along the path indicated by arrows. Vertical distances indicate qualitatively energy differences. Particularly the vertical distance between two consecutive X_1's indicates the loss of (free) energy during the reaction. Now from a well known theorem a reaction proceeding from a lower to a higher energy level has an activation energy while its reverse has none.

Further unimolecular and bimolecular rate-constants are known to be, within a few powers of ten, $10^{12} \times \exp(-Q/RT)$ sec^{-1} and $10^{11}\exp(-Q/RT)$ litre $mole^{-1}$ sec^{-1}, respectively. Therefore $1 + k_{-1}/k_2 \simeq 1$ and $k_1 a \simeq 10^9 \exp(-Q/RT)$ if $a \simeq 10^{-2}$ mole/litre. Consequently $A_1/a \simeq 10^{-9} \times \exp(Q/RT)$ sec and $A_0 \simeq 10^{-12}$ sec, that is A_0 is disappearingly small as compared to A_1/a or the reaction must be expected to be purely of the first order. If in the diagram X_2 were placed below the lower X_1 the result would be a reaction of the zeroth order.

To make $A_0 a$ and A_1 comparable it is necessary to add a reaction of the type indicated in Fig. 1 by dotted lines

$$X_2 \rightleftharpoons X_3 \qquad (\pm 3)$$

whose steady state rate is zero. (± 3) means that we assume the existence of an isomer X_3 of X_2 with a low energy-content. We now have

$$x_1 + x_2 + x_3 = E \tag{9}$$

and $x_3 = K_3 x_2$, where K_3 is large ($\simeq \exp Q_3/RT$)

$$x_1 + x_2 (1 + K_3) = E \tag{10}$$

Using (3) and (4) we thus get instead of (5)

$$E/s = (1 + w_{-1}/w_2)/w_1 + (1+K_3)/w_2 \qquad (11)$$

or

$$A_0 = (1 + K_3)/k_2;\ A_1 = (1 + k_{-1}/k_2)/k_1 \qquad (12)$$

where evidently K_3/k_2 may very well be of the same order of magnitude as $1/k_1a$.

This extension of the original picture is new but it has probably existed implicitly in the minds of the originators of the theory. It means simply that the greater part of the enzyme-substrate complex does exist in appreciable amounts in the solution but that only a minute fraction of it is active in reaction (2).

It should be added, that Huennekens [5] (p. 606) discusses a mechanism in which three forms of the enzyme-substrate complex are assumed. But these are connected in series, while in our case one of the two forms is branched off from the main closed sequence. The two assumptions (a reaction in series *versus* a branched sequence) lead to quite different kinetic expressions and only the latter one to a natural explanation of the actual occurrence of reactions of the Michaelis-type.

Gibson [6] assumes four different forms, two with high energy content and two with low but a discussion even of this rather complicated case along the lines indicated above and in former papers [3,7] by the present author again shows that K_m/a in cases where the rate depends strongly on temperature will be either very large in which case the reaction is of the first order, or very small in which case the reaction is of the zero'th order. Incidentally, the present author completely agrees with Gibson in his statement, that activation energies calculated from rate-data for reactions whose mechanism has not been unravelled have no physical significance, compare for example Ref.[8].

Thus again in contrast to the assumption that energetically different forms of the enzyme-substrate complex are inserted in series, the assumption that one (or several) of the forms is branched off from the main closed sequence leads to a natural explanation of the frequent occurrence of reactions obeying the Michaelis law.

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