SHORT COMMUNICATIONS

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Analytical method for the estimation of enzyme kinetic parameters

The existing graphical methods[1] for the determination of the Michaelis constant, K_m, and the maximal rate, V, require experimental estimations of the rate of enzyme reactions, v, at the broad range of substrate concentrations, $[S]$, beginning with the smallest ones up to at least twice the K_m values. While studying the cholinesterase hydrolysis of indophenyl acetates which are hardly soluble in water these methods have proved to be unreliable in this case, and a new method of computation has been suggested by us[2].

From the Michaelis–Menten equation

$$v = \frac{V\,[S]}{K_m + [S]} \tag{1}$$

it derives that

$$\left(\frac{dv}{d[S]}\right)_{[S]=0} = \frac{V}{K_m} \tag{2}$$

According to Eqn. 2, the tangent to the experimental curve in coordinates v against $[S]$ at $[S] = 0$ forms an angle α, of which the cotangent is equal to K_m/V (see Fig. 1). Introducing value K_m/V into the modified Eqn. 1

$$\frac{1}{V} = \frac{1}{v} - \frac{K_m}{V}\cdot\frac{1}{[S]} \tag{3}$$

we compute the value V for all experimental values v at corresponding $[S]$'s. We determine the value K_m out of Eqn. 2, taking the mean value of V.

The aim of the present paper is to improve this method, making it universal and more precise.

It is impossible to draw exactly the tangent to the curve, and the received value $(K_m/V)_{exp}$ will always differ from the true one $(K_m/V)_t$ to the value $\Delta\,(K_m/V)$:

$$(K_m/V)_t = (K_m/V)_{exp} \pm \Delta\,(K_m/V) \tag{4}$$

Due to this fact the values V computed with the help of Eqn. 3 change regulary with the increase of $[S]$ (see Table I, 3rd column). One can estimate the systematic error $\Delta\,(K_m/V)$ with the help of the algebraic method.

For any two values, $[S]_1$ and $[S]_2$, we have

$$\frac{1}{V_1} = \frac{1}{v_1} - \left(\frac{K_m}{V}\right)_{exp}\cdot\frac{1}{[S]_1} \tag{5}$$

$$\frac{1}{V_2} = \frac{1}{v_2} - \left(\frac{K_m}{V}\right)_{exp}\cdot\frac{1}{[S]_2} \tag{6}$$

TABLE I

THE RESULTS OF THE COMPUTATION OF VALUES K_m AND V FOR THE CHOLINESTERASE HYDROLYSIS OF DICHLOROINDOPHENYL ACETATE

0.05 M phosphate buffer (pH 8.0); temp., 25°; $[E]_0 = 2 \cdot 10^{-11}$ M; Ctg$\alpha = K_m/V = 0.023$ from Fig. 1.

$(S) \times 10^4$ (M)	$v \times 10^3$ (enzyme units/sec)	$V \times 10^3$ from $(K_m/V)_{exp}$ (enzyme units/sec)	$\Delta(K_m/V) \times 10^3$	$V \times 10^3$ from $(K_m/V)_t$ (enzyme units/sec)	$K_m \times 10^4$ (M)
0.2	0.77	3.0	3.30	6.80	1.56
0.6	1.90	5.2	3.22	7.00	1.62
1.0	2.70	5.9	3.25	7.15	1.64
1.5	3.30	6.0	3.05	6.85	1.57
2.0	3.85	6.2	3.11	6.90	1.58

Substracting Eqn. 6 from Eqn. 5 we get

$$\Delta\left(\frac{1}{V}\right) = \frac{1}{v_1} - \frac{1}{v_2} - \left(\frac{K_m}{V}\right)_{exp} \cdot \left(\frac{1}{[S]_1} - \frac{1}{[S]_2}\right) \tag{7}$$

The error of the mean values of v_1 and v_2 being negligible, according to Eqn. 3 we find

$$\frac{1}{v_1} - \frac{1}{v_2} = \left(\frac{K_m}{V}\right)_t \cdot \left(\frac{1}{[S]_1} - \frac{1}{[S]_2}\right) \tag{8}$$

Introducing Eqn. 8 into Eqn. 7 we get

$$\Delta\left(\frac{1}{V}\right) = \Delta\left(\frac{K_m}{V}\right) \cdot \left(\frac{1}{[S]_1} - \frac{1}{[S]_2}\right) \tag{9}$$

The mean values $\Delta(K_m/V)$ may be computed from Eqn. 9 using the possible combinations of the experimental values $[S]$ and v. According to the value $\Delta(K_m/V)$ (see Table I, 4th column), we find $(K_m/V)_t$ with the help of Eqn. 4. Knowing the value $(K_m/V)_t$, one can compute the values K_m and V with the help of Eqn. 3 (see Table I, 5th and 6th column).

The computed values K_m and V for cholinesterase hydrolysis of 2,6-dichloro-

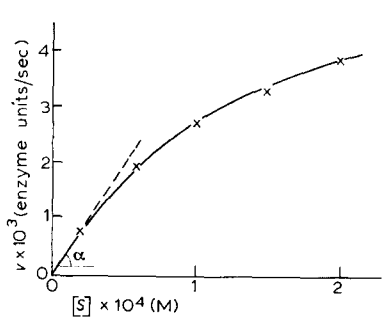

Fig. 1. The dependence of the hydrolysis rate (v) of dichloroindophenyl acetate under the action of serum cholinesterase upon the dichloroindophenyl acetate concentration ($[S]$); 0.05 M phosphate buffer (pH 8.0); temp., 25°.

TABLE II

KINETIC PARAMETERS OF THE CHOLINESTERASE HYDROLYSIS OF 2,6-DICHLOROINDOPHENYL ACETATE AND ACETYLCHOLINE

Experimental conditions see text.

Substrate	*Method*	*Serum cholinesterase*		*Bovine acetylcholinesterase*	
		$K_m \times 10^4$ *(M)*	$V \times 10^6$ *(moles/min)*	$K_m \times 10^4$ *(M)*	$V \times 10^7$ *(moles/min)*
2,6-Dichloroindophenyl acetate	Analytical	1.6	18	1.8	9.6
	Graphical[5]	12	1.2	1.9	8.2
Acetylcholine	Analytical	11	1.0	2.1	8.0

indophenyl acetate are given in Table II. As enzymes the preparations of horse serum cholinesterase (EC 3.1.1.8) (Mechnikov Institute, Moscow) and bovine erythrocyte acetylcholinesterase (EC 3.1.1.7)[3] are used. The hydrolysis of 2,6-dichloroindophenyl acetate was measured with the help of the differential photometrical method[2] in 0.05 M phosphate buffer (pH 8.0) at 20° at the range of $[S]$ $4 \cdot 10^{-5}$–$1.5 \cdot 10^{-4}$ M. Besides this, the hydrolysis of acetylcholine under the action of these enzymes was studied. The rate of the cholinesterase hydrolysis of acetylcholine was measured using the continuous potentiometric titration[4] in 0.007 M phosphate buffer (pH 7.5) at 25°. The values K_m and V were computed graphically[5] in the range of $[S]$ $1 \cdot 10^{-4}$–$2 \cdot 10^{-3}$ M and with the help of the described analytical method in the much narrower range of $[S]$ $1 \cdot 10^{-4}$–$3 \cdot 10^{-4}$ M. These values given in Table II have shown a good coincidence.

This method may be used in all cases when use of high substrate concentrations is impossible, for example, when the substrates are hardly soluble or when the inhibition by high substrate concentrations takes place.

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