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Flow microcalorimetric study of enzyme reactions Application to arylesterase from human serum

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Abstract

The enzymatic hydrolysis of phenyl acetate, catalysed by arylesterase/paraoxonase (EC 3.1.8.1) was studied at 37 °C in Tris buffer, pH 8, by spectrophotometry and flow microcalorimetry, using an enzyme purified from human serum. After correction for buffer protonation and product ionization, the hydrolysis reaction was found to be slightly endothermic, with $\Delta H = 8.2\,\mathrm{kJ\,mol^{-1}}$. Microcalorimetric data were analysed with the integrated Michaelis equation to give the kinetic parameters of the enzyme: Michaelis constant $K_m = 2.4\,\mathrm{mM}$, catalytic constant $k_{cat} = 2.4 \times 10^3\,\mathrm{s^{-1}}$, bimolecular rate constant $k_s = 1.0 \times 10^6\,\mathrm{M^{-1}\,s^{-1}}$. These results were in agreement with the spectrophotometric method. This study confirms the usefulness of microcalorimetry in the field of enzyme kinetics.
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Keywords: Arylesterase; Paraoxonase; Microcalorimetry; Integrated Michaelis equation

1. Introduction

Arylesterase (EC 3.1.8.1, also known as paraoxonase) is an enzyme present in the blood of mammals where it is associated with high density lipoproteins (HDL). This enzyme is known mainly for its ability to hydrolyse toxic organophosphorus esters like paraoxon (diethyl-4-nitrophenyl phosphate), the active metabolite of the insecticide parathion. Other synthetic esters such as phenyl acetate are also hydrolysed with a high catalytic efficiency. The physiological substrate remains unknown, in spite of the growing body of evidence suggesting the involvement of the enzyme in the pathogenesis of atherosclerosis [1,2]. The enzyme activity is usually determined by spectrophotometry at 270 nm, using phenyl acetate as substrate. During our studies with various inhibitors of this enzyme [3–5], we experienced some limitations of the spectrophotometric technique, due to the high absorption of some inhibitors. We therefore selected microcalorimetry as a suitable alternative technique, since it does not display such limitations. The purposes of the present paper are: (a) to summarize the theoretical aspects of the microcalorimetric method, taking advantage of the recent mathematical developments concerning the integrated Michaelis equation [6–8]; (b) to validate the microcalorimetric study of arylesterase by comparison with the standard spectrophotometric method.

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2. Theory

We assume that the reaction follows the simple mechanism:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \overset{k_{\mathrm{cat}}}{\rightarrow} \mathrm{E} + \mathrm{P} \quad (1)$$

where E, S, ES and P denote the enzyme, substrate, enzyme–substrate complex and reaction product, respectively, and k_{cat} is the catalytic constant. The Michaelis constant is defined by:

$$K_{\mathrm{m}} = \frac{k_{-1} + k_{\mathrm{cat}}}{k_1} \quad (2)$$

The reaction rate at time t is given by the Michaelis equation:

$$v(t) = \frac{\mathrm{d}p(t)}{\mathrm{d}t} = k_{\mathrm{cat}} e_0 \frac{s_0 - p(t)}{K_{\mathrm{m}} + s_0 - p(t)} \quad (3)$$

where $p(t)$ denotes the product concentration at time t, e_0 the total enzyme concentration, and s_0 the initial substrate concentration. The maximum velocity of the reaction is $V_{\max} = k_{\mathrm{cat}} e_0$.

It has been shown [6,7] that the solution of this differential equation may be expressed in terms of Lambert's W-function:

$$p(t) = s_0 - K_{\mathrm{m}}\, W\left[\frac{s_0}{K_{\mathrm{m}}} \exp\left(\frac{s_0}{K_{\mathrm{m}}} - k_{\mathrm{s}} e_0 t\right)\right] \quad (4)$$

where $k_{\mathrm{s}} = k_{\mathrm{cat}}/K_{\mathrm{m}}$ is the bimolecular rate constant, i.e. the apparent rate constant for the overall reaction $\mathrm{E} + \mathrm{S} \rightarrow \mathrm{ES}$.

Lambert's function is the reciprocal of the function $x\,\mathrm{e}^x$. That is, if $y = W(x)$, then $x = y\,\mathrm{e}^y$. Lambert's function is defined for $x \geq -1/\mathrm{e}$. When $-1/\mathrm{e} < x < 0$, the function has two values; the value $W(x) > -1$ defines the 'principal branch', which is the one used in enzyme kinetics [7].

The reaction may be studied by measuring the product concentration $p(\tau)$ formed during a fixed incubation time τ. In these conditions, Eq. (4) becomes:

$$p(\tau) = s_0 - K_{\mathrm{m}}\, W\left[\frac{s_0}{K_{\mathrm{m}}} \exp\left(\frac{s_0}{K_{\mathrm{m}}} - k_{\mathrm{s}} e_0 \tau\right)\right] \quad (5)$$

Experiments may be performed by varying the substrate concentration s_0 at a constant enzyme concentration e_0, or by varying e_0 at constant s_0. For simulation purposes, it is useful to define dimensionless variables:

$$x = \frac{s_0}{K_{\mathrm{m}}}, \qquad y = k_{\mathrm{s}} e_0 \tau = \frac{V_{\max}\tau}{K_{\mathrm{m}}}, \qquad z = \frac{p(\tau)}{K_{\mathrm{m}}} \quad (6)$$

so that Eq. (5) may be rewritten as:

$$z = x - W(x\,\mathrm{e}^{x-y}) \quad (7)$$

This equation is valid for any enzyme following Michaelis–Menten kinetics, provided that the assay is performed after a fixed incubation time. Some examples of simulated curves, $z = f(x)$ or $z = f(y)$, are depicted on Fig. 1. The main characteristics of such curves are gathered in Table 1.

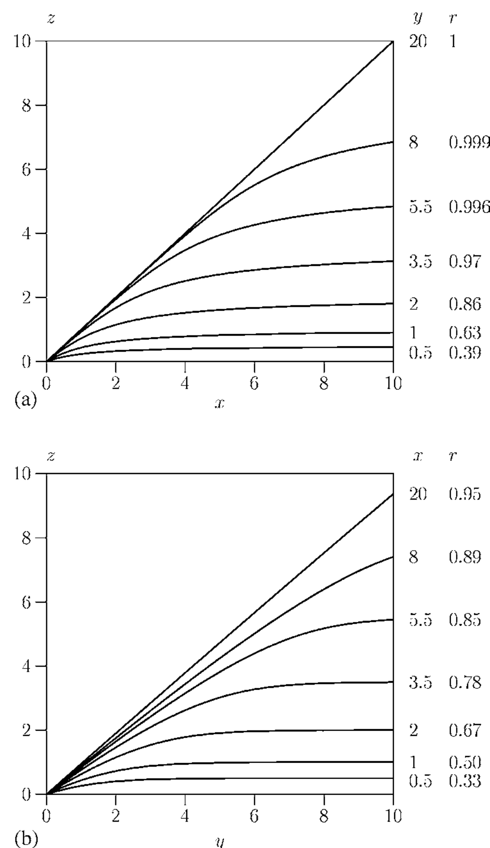

Fig. 1. Plot of the integrated Michaelis equation (Eq. (7)) in the reduced coordinate system (Eq. (6)): (a) product concentration vs. initial substrate concentration and (b) product concentration vs. total enzyme activity. Concentrations are given in K_{m} units. r is the initial slope of the curve.

The initial part of the curve becomes more linear as the value of the constant concentration increases. The slope r of this linear part may be approximated by the slope of the tangent at the origin. In the case of constant enzyme concentration, it is equal to the proportion of substrate transformed during the incubation time, in the condition of a pseudo-first order reaction. Thus, the maximal slope is equal to 1 (see Fig. 1). In the case of constant substrate concentration, the slope is $x/(1+x) = s_0/(K_{\mathrm{m}} + s_0)$ which is always < 1.

Each curve has an horizontal asymptote, conditioned by the maximal amount of substrate which can be transformed under the experimental conditions used.

The curves obtained at the lowest values of the constant concentration may be approximated by a 'limiting curve':

- in the case of constant enzyme concentration, it is the classical Michaelis–Menten equation, since, at low enzyme

Table 1
Graphical properties of the integrated Michaelis equation (Eq. (7)) in the reduced coordinate system (Eq. (6))

Curve	Initial slope	Horizontal asymptote	Limiting curve
$z = f(x)$	$r = 1 - \mathrm{e}^{-y}$	y	$xy/(1+x)$ (when $y \rightarrow 0$)
$z = f(y)$	$r = x/(1+x)$	x	$x(1 - \mathrm{e}^{-y})$ (when $x \rightarrow 0$)

concentration, the quotient $p(\tau)/\tau$ approximates the initial rate of the reaction:

$$\frac{p(\tau)}{\tau} \approx v_0 = \frac{k_{\text{cat}} e_0 s_0}{K_\text{m} + s_0} \tag{8}$$

- in the case of constant substrate, it is the pseudo-first order approximation:

$$p(\tau) \approx s_0 \left[1 - \exp\left(-k_\text{s} e_0 \tau\right)\right] \tag{9}$$

It is assumed that the assay technique is such that the instrument response q is proportional to the product concentration:

$$q = H\, p(\tau) \tag{10}$$

where H is a proportionality factor. For instance, in flow microcalorimetry, q is the heat evolved per unit volume and H is the apparent reaction enthalpy.

If H is known, it is possible to compute the product concentration $p(\tau)$ from the observed signal q for every experiment. The kinetic parameters K_m and k_s may then be determined by fitting the appropriate rate equation (either the integrated Michaelis equation (5) or its approximate forms (8) and (9)) to the experimental data, using a nonlinear regression procedure which minimizes the sum of squared residuals:

$$\Phi = \sum_{i=1}^{n} (p_i - \hat{p}_i)^2 \tag{11}$$

where n denotes the number of points, p_i the observed value $p(\tau)$ and $\hat{p}_i$ the theoretical value computed from the rate equation.

Among the many non-linear minimization procedures available, we have chosen Marquardt's method, which is a modified Newton-Raphson method ensuring a high convergence rate. This method requires the partial derivatives of the fitted function with respect to its parameters. In case of Eq. (5), one obtains:

$$\frac{\partial p}{\partial K_\text{m}} = \frac{p}{K_\text{m}} \frac{L}{1+L}, \qquad \frac{\partial p}{\partial k_\text{s}} = K_\text{m} e_0 \tau \frac{L}{1+L} \tag{12}$$

$$L = W\left[\frac{s_0}{K_\text{m}} \exp\left(\frac{s_0}{K_\text{m}} - k_\text{s} e_0 \tau\right)\right] \tag{13}$$

3. Materials and methods

3.1. Enzyme

Arylesterase has two isozymes A and B which differ by the aminoacid at position 192: glutamine (Q) in the A isozyme and arginine (R) in the B isozyme [1]. There are therefore three phenotypes: A, AB and B (respective genotypes QQ, QR and RR). These isozymes differ by their hydrolytic activity towards paraoxon (diethyl-4-nitrophenyl-phosphate), the B isozyme being the most active. This property allowed the determination of the phenotype of each subject, according to the method of Eckerson et al. [9], by computing the ratio of the hydrolysis rates of paraoxon, in the presence of 1 M NaCl, and phenyl acetate. We used this method to phenotype sera from healthy volunteers [10]. Sera from subjects having the same homozygous phenotype A were pooled, and the enzyme was purified by the method of Gan et al. [11]. Details of the purification procedure have been published elsewhere [5]. A molecular weight of 40 kDa for the proteic part of the glycoproteic enzyme was used to compute enzyme concentrations [12]. The enzyme and substrate solutions were prepared in Tris buffer (9 mM, pH 8 at 37 °C) containing 1 mM $CaCl_2$ as activator. A preliminary spectrophotometric study yielded the kinetic parameters listed in Table 4.

3.2. Calorimetry

The flow microcalorimeter (LKB 10700-1) was operated at 37 °C. It was used in the mixing mode. The enzyme and substrate solutions were perfused by a Gilson two-channel peristaltic pump at a total flow rate of 6.5 μL/s. The incubation time τ was equal to the residence time in the microcalorimetric cell, determined experimentally as described in Section 4.2. The signal generated by the thermopiles was amplified by a Keithley 150-B microvoltmeter and digitized by a Gilson 506-C analog-digital converter. The digitized signal was sent to a PC microcomputer by means of a RS-232 interface. The signal was recorded until a steady state was reached. The ordinate of the maximum was used to compute the thermal output of the calorimeter, which was then divided by the total flow rate to give the heat evolved per unit volume (q in μJ/μL). Electrical calibration was performed with the instrument's built-in resistor.

3.3. Apparent hydrolysis enthalpy

The apparent hydrolysis enthalpy of phenyl acetate was measured at a substrate concentration of 1 mM (after dilution in the calorimetric cell), using whole serum as enzyme source. The amount of substrate hydrolysed during the residence time τ was estimated by spectrophotometry using substrate and enzyme concentrations identical to the ones used in the calorimetric experiment. The apparent enthalpy was obtained from Eq. (10).

3.4. Titration experiments

In order to compute the hydrolysis enthalpy of phenyl acetate, it was necessary to perform a series of titration experiments, so that the ionization enthalpies of the reaction products could be estimated.

Each experiment consisted of mixing aqueous solutions of the titrated compound (e.g. phenol), at various concentrations, with an aqueous solution of titrant (e.g. NaOH). The concentration of the latter was kept constant and was always in excess (after dilution in the calorimetric cell) over the high-

Table 2
Experimental thermodynamic data at 37 °C

	Reaction	ΔH (kJ mol^{-1})	pK_a
(1)	$AcOH + Tris \rightarrow AcO^- + TrisH^+$	-48.1 ± 0.2	
(2)	$Tris + H^+ \rightarrow TrisH^+$	-45.0 ± 0.3	
(3)	$PhOH + OH^- \rightarrow PhO^- + H_2O$	-27.1 ± 0.2	
(4)	$H^+ + OH^- \rightarrow H_2O$	-54.7 ± 0.3	
(5)	$AcOH \rightarrow AcO^- + H^+$	-3.0 ± 0.5[a]	
(6)	$PhOH \rightarrow PhO^- + H^+$	$+27.6 \pm 0.5$[b]	9.81 ± 0.01[c]

[a] Computed from reactions (1) and (2).
[b] Computed from reactions (3) and (4).
[c] By spectrophotometry.

est concentration of the titrated compound. The heats of dilution of the reactants were found negligible in our conditions.

Titration enthalpies were estimated from the slope of the regression lines relating the heat q evolved per unit volume to the concentration C of the titrated compound, such that $q = \Delta H\, C$. For each experiment, 5–10 concentrations were used. The resulting values appear in Table 2 and are checked against available literature data in Table 3.

In addition, the pK_a of phenol was determined at 37 °C by spectrophotometry in carbonate buffers, using a method previously described [21]

3.5. Kinetic studies

Two series of experiments were performed. In the first series, the substrate concentration was kept at 0.5 mM while the enzyme concentration varied from 25 to 100 nM (all concentrations are given after mixing in the calorimetric cell). In the second series, the enzyme concentration was kept at 25 nM while the substrate concentration varied from 0.25 to 5 mM.

3.6. Treatment of data

All computations were performed on a PC computer, using our own programs, written in Pascal. Two of these programs are freely available on the authors' website: (1) the WINREG program, which consists of a Windows executable file with an Excel-like interface, is distributed in french version only; (2) the REGNLIN program, available as source code for the Borland Delphi 6.0 compiler, has a much simpler interface but uses exactly the same mathematical algorithms than WINREG. The procedure used to compute Lambert's function was a Pascal translation of a Fortran program written by Barry et al. [8].

Table 3
Comparison of the enthalpy values found in this work with literature values

Reaction	This work	Literature	Reference
$Tris + H^+ \rightarrow TrisH^+$	−45.0	−45.4[a]	[14]
$AcOH \rightarrow AcO^- + H^+$	−3.0	−3.5[b]	[15]
$PhOH \rightarrow PhO^- + H^+$	+27.6	+24.6 ($I = 0.2$ M)[b]	[16]
		+21.3 ($I = 0.1$ M)[b]	[17]
$H^+ + OH^- \rightarrow H_2O$	−54.7	−54.7 at 35 °C	[18]
		−53.6[a]	[19]
		−53.3[a]	[20]

Enthalpies are in kJ mol^{-1}. The temperature is 37 °C, unless otherwise indicated.
[a] Interpolated from ΔH vs. temperature data.
[b] Interpolated from pK_a vs. temperature data.

4. Results

4.1. Hydrolysis enthalpy of phenyl acetate

The enthalpy ΔH for the reaction:

$$PhOAc + H_2O \rightarrow PhOH + AcOH \tag{14}$$

was computed from the apparent enthalpy ΔH_{app}, the ionization enthalpies of phenol and acetic acid (respectively ΔH_1 and ΔH_2), the protonation enthalpy of Tris (ΔH_3) and the fraction of phenol ionized (α), according to the equation:

$$\Delta H = \Delta H_{app} - \alpha\,\Delta H_1 - \Delta H_2 - (1+\alpha)\Delta H_3 \tag{15}$$

since acetic acid was totally ionized in our conditions.

By using the equation:

$$\alpha = \frac{1}{1 + 10^{pK_a - pH}} \tag{16}$$

together with the results of Table 2, one obtains the hydrolysis enthalpy $\Delta H = 8.2 \pm 1.2$ kJ mol^{-1}(Table 4). The reaction is slightly endothermic, in agreement with previous results for other aromatic esters [22].

4.2. Arylesterase kinetics

The kinetic parameters of arylesterase were estimated from the data obtained at several enzyme or substrate concentrations, using the following procedure:

(1) For each experiment, the product concentration $p(\tau)$ was determined from Eq. (10), where H was equated to the observed hydrolysis enthalpy ΔH_{app}.
(2) The pseudo-first-order rate equation (Eq. (9)) was used to estimate k_s from the data obtained at low substrate concentration ($s_0 = 0.5$ mM) and variable enzyme concentration. A preliminary estimate was found by linear regression applied to the linearized form of Eq. (9):

$$\ln\left[1 - \frac{p(\tau)}{s_0}\right] = -k_{obs} e_0 \tag{17}$$

where k_{obs} is the pseudo-first order rate constant: $k_{obs} = k_s \tau$.

This preliminary estimate was then refined by nonlinear regression applied to Eq. (9), giving $k_{obs} = (61.1 \pm 1.6) \times 10^6\ M^{-1}$. This value was combined with the spectrophotometric result $k_s = (1.03 \pm 0.04) \times 10^6\ M^{-1}\ s^{-1}$ (Table 4), in order to compute the residence time $\tau = k_{obs}/k_s = (59.3 \pm 3.3)s$. Using the flow rate f = 6.5 μL/s it was then possible to estimate the effective volume of the calorimetric cell: $V = f\tau = (385 \pm$

Table 4
Thermodynamic and kinetic parameters of human serum arylesterase at pH 8 and 37 °C

Parameter	Microcalorimetry	Spectrophotometry
ΔH_{app} (kJ mol^{-1})[a]	-40.2 ± 0.3	
ΔH (kJ mol^{-1})[b]	8.2 ± 1.2	
K_m (mM)	2.44 ± 0.26	2.23 ± 0.06
k_s (M^{-1} s^{-1})	$(1.02 \pm 0.03) \times 10^6$	$(1.03 \pm 0.04) \times 10^6$
k_{cat} (s^{-1})	$(2.44 \pm 0.33) \times 10^3$	$(2.30 \pm 0.03) \times 10^3$

[a] Observed reaction enthalpy in Tris buffer.
[b] Hydrolysis enthalpy of phenyl acetate, corrected for product ionization and buffer protonation.

21) μL, in excellent agreement with the value of 396 μL obtained experimentally by Johnson and Biltonen [13] with a totally different technique.

(3) The integrated Michaelis equation (Eq. (5)) was fitted to the results obtained at variable substrate concentration. A linearized form of Eq. (5) was obtained by integration of Eq. (3) with the initial condition $p(0) = 0$:

$$p(t) = K_m \ln\left[1 - \frac{p(t)}{s_0}\right] + k_{cat} e_0 t \tag{18}$$

By setting $t = \tau$ and noticing that $k_s = k_{cat}/K_m$ one obtains:

$$p(\tau) = K_m \left\{\ln\left[1 - \frac{p(\tau)}{s_0}\right] + k_s e_0 \tau\right\} \tag{19}$$

This equation was fitted to the data by linear regression in order to get preliminary estimates of the parameters K_m and k_s. These estimates were further refined by nonlinear regression applied to Eq. (5). The final estimate of K_m is given in Table 4. It is also in agreement with the spectrophotometric result, but its precision is lower. Since the substrate solution underwent a twofold dilution in the microcalorimetric experiment, it was not possible to study substrate concentrations beyond about $2K_m$.

The catalytic constant k_{cat} was computed as $k_s K_m$. Of course, the microcalorimetric value suffers from the lower precision of K_m (Table 4).

Knowing the kinetic parameters k_s and K_m, it was possible to compute the reduced variables (Eq. (6)). The fitted curves were then plotted in the reduced coordinate system (Fig. 2) so that they could be compared with the theoretical curves in Fig. 1.

As predicted by theory, the curve obtained at a single enzyme concentration and several substrate concentrations (Fig. 2a) displayed an initial linear part, extending to $x \approx 0.5$ (i. e. $s_0 \approx K_m/2$) in our conditions. The slope of this linear part corresponds to the fraction of substrate hydrolysed in pseudo-first order conditions. The existence of a linear curve would be an advantage if microcalorimetry was used as an assay method to determine the substrate concentration.

The curve obtained at a single substrate concentration $s_0 \approx K_m/5$ and several enzyme concentrations (Fig. 2b) was very well described by the pseudo-first order approximation (Eq. (9)). This curve corresponds to the 'limiting curve' obtained at low substrate concentration (Fig. 1b).

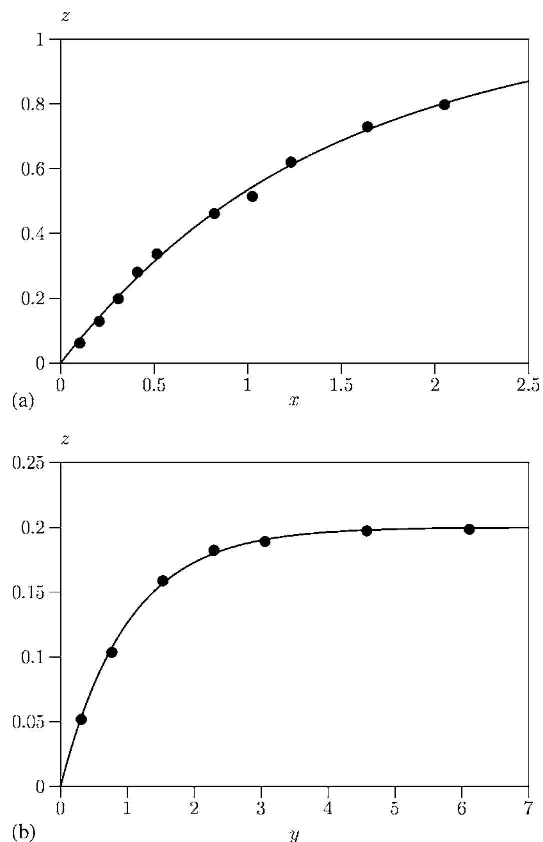

Fig. 2. Comparison of theoretical curves and experimental points, for the flow microcalorimetric study of human arylesterase. The thermodynamic and kinetic parameters (ΔH, K_m, k_s) were determined by nonlinear regression, and were used to compute the reduced coordinates (Eq. (6)): (a) product concentration vs. initial substrate concentration, (b) product concentration vs. total enzyme activity, at low substrate concentration ($s_0 \approx K_m/5$).

5. Discussion

5.1. Microcalorimetric study of enzyme reactions

The application of microcalorimetry to the study of enzyme kinetics requires the determination of the reaction enthalpy, as well as the effective volume (or 'thermal volume') of the microcalorimetric cell. In this work, the volume was estimated by comparing the pseudo first-order rate constants obtained by microcalorimetry and spectrophotometry. The estimated volume was found consistent with a previous experimental determination involving a quite different procedure [13]. Of course, this approach assumes that the enzyme reaction can be studied by microcalorimetry and spectrophotometry in similar conditions. Obviously, this cannot be the case will all enzymes, but once the effective volume is determined its value may be used with other enzymes, as long as the calorimetric conditions are comparable.

The method described in this paper also relies on the assumption that the instrument response is proportional to the product concentration (Eq. (10)). Johnson and Biltonen have shown that the validity of this hypothesis depends on the existence of a thermal equilibrium between the exiting solution and the body of the calorimeter cell [13]. From the physical characteristics of the LKB instrument the authors found that this requirement is achieved within experimental conditions comparable to those of our study.

The present investigation used the mixing cell of the calorimeter. Some previous studies were performed by Beezer and O'Neill et al. with the flow-through cell of the instrument [23–27]. These authors described procedures for measuring the effective volume (which was found to be dependent on the flow rate) and for determining the thermodynamic and kinetic parameters of enzyme reactions, with special reference to the urea/urease [23,27] and butyrylcholine/cholinesterase [24] systems. In this work, this method could not be used, due to the relatively high enzyme activity necessary to obtain a measurable signal: since the method requires that the reaction is initiated outside the calorimeter, the substrate would have been completely hydrolysed before reaching the cell. It will be interesting in the future to look for enzyme systems which could be studied by the two techniques.

5.2. Integrated Michaelis equation

The microcalorimetric analysis of enzyme reactions often requires the fitting of the integrated Michaelis equation. In previous works, this fitting was performed mostly by linear regression applied to a linearized form of the equation [28–32]. There exist several linearization schemes, and their respective merits have been discussed [28]. However, many curve-fitting studies have shown that nonlinear regression gives more reliable parameters. In such methods, linearization is used only to get the initial estimates of the parameters, which are then refined by a nonlinear optimization algorithm such as Marquardt's method.

We have developed a computer program which performs the nonlinear least squares fit of the integrated Michaelis equation. The independent variable may be the reaction time, the initial substrate concentration or the total enzyme concentration. The program is not limited to calorimetry but can be applied to a wide variety of analytical techniques, for instance chromatographic or radio-isotopic. In line with the recent mathematical research concerning the integrated Michaelis equation [6,7], the program uses Lambert's function to obtain an analytical expression of the solution. In the past, the integration of the Michaelis equation has been done numerically. The precision of numerical integration is limited, and it may vary considerably over the integration range. On the other hand, the algorithms used to compute Lambert's function [8] ensure that the maximal precision compatible with the floating point type of the computer (19–20 significant digits in our case) is always reached. Moreover, Lambert's function is already implemented in most popular mathematical programs such as Maple, so that users may easily perform their own simulations, with the additional advantage of the dimensionless coordinate system developed in this work. It is hoped that these mathematical improvements will expand the application range of the integrated Michaelis equation.

5.3. Kinetic parameters of arylesterase

The results show that flow microcalorimetry could be a useful method for kinetic studies of arylesterase, especially when the spectrophotometric method cannot be applied, for instance in inhibition studies involving inhibitors which absorb at the same wavelength than the reaction product. The kinetic parameters determined by microcalorimetry are in excellent agreement with the spectrophotometric values, although the Michaelis constant is slightly less precise, due to the limited solubility of the substrate.

Very few kinetic studies have been done with arylesterase, and most of them were performed at 25 °C. In this work, a temperature of 37 °C was selected as more physiologically significant.

The K_m value reported here at 37 °C (2.4 mM) can be compared with previous results (1.16 mM at 25 °C, 1.51 mM at 30 °C [33]) and with our own estimate at 25 °C (1 mM, by spectrophotometry). By grouping these values together, an apparent binding enthalpy of −47 kJ mol^{-1} can be computed. This value is difficult to interpret since K_m is a complex constant which depends on several rate constants (Eq. (2)). However, it suggests that an exothermic step takes part in the formation of the enzyme–substrate complex.

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References

[1] B. Mackness, P.N. Durrington, M.I. Mackness, Gen. Pharmacol. 31 (1998) 329.
[2] P.N. Durrington, B. Mackness, M.I. Mackness, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 473.
[3] J. Debord, T. Dantoine, J.C. Bollinger, M.H. Abraham, B. Verneuil, L. Merle, Chem. Biol. Interact. 113 (1998) 105.
[4] J. Debord, J. C. Bollinger, M. H. Abraham, L. Merle, T. Dantoine, Internet J. Chem. 4 (2002).
[5] J. Debord, J.C. Bollinger, L. Merle, T. Dantoine, J. Inorg. Biochem. 94 (2003) 1.
[6] S. Schnell, C. Mendoza, J. Theor. Biol. 187 (1997) 207.
[7] C.T. Goudar, J.R. Sonnad, R.G. Duggleby, Biochim. Biophys. Acta 1429 (1999) 377.
[8] D. A. Barry, S. J. Barry, P. J. Culligan-Hensley, ACM Trans. Math. Software 21 (1995) 172 (Program available at http://www.netlib.org/toms/743).

[9] H.W. Eckerson, C.M. Wyte, B.N. La Du, Am. J. Hum. Genet. 35 (1983) 1126.
[10] T. Dantoine, J. Debord, J.P. Charmes, L. Merle, P. Marquet, G. Lachâtre, C. Leroux-Robert, J. Am. Soc. Nephrol. 9 (1998) 2082.
[11] K.N. Gan, A. Smolen, H.W. Eckerson, B.N. La Du, Drug Metab. Dispos. 19 (1991) 100.
[12] R.C. Sorenson, S.L. Primo-Parmo, C.L. Kuo, S. Adkins, O. Lockridge, B.N. La Du, Proc. Natl. Acad. Sci. U.S.A. 92 (1995) 7187.
[13] R.E. Johnson, R.L. Biltonen, J. Am. Chem. Soc. 97 (1975) 2349.
[14] J.O. Hill, G. Ojelund, I. Wadsö, J. Chem. Thermodyn. 1 (1969) 111.
[15] J. Halle, R. Bates, J. Solution Chem. 4 (1975) 1033.
[16] T. Kiss, B. Toth, Talanta 29 (1982) 539.
[17] J. Bunting, D. Stefanidis, J. Am. Chem. Soc. 112 (1990) 779.
[18] V. Vasilev, L. Shekhanova, Zh. Neorg. Khim. 19 (1974) 2969.
[19] H. Ots, Acta Chem. Scand. 26 (1972) 3810.
[20] J. Christensen, G. Kimball et al., Thermochim. Acta 4 (1972) 141.
[21] J. Debord, J.L. Fraysse, B. Pénicaut, Analusis 16 (1988) 519.
[22] R.N. Goldberg, Y.B. Tewari, J. Phys. Chem. Ref. Data 23 (1994) 1035.
[23] A.E. Beezer, H. Tyrrell, T. Steenson, Protides of the Biological Fluids, Pergamon Press, 1972, p. 563.
[24] A.E. Beezer, C. Stubbs, Talanta 20 (1973) 27.
[25] M.A.A. O'Neill, A.E. Beezer, C. Labetoulle, L. Nicolaides, J.C. Mitchell, J.A. Orchard, J.A. Connor, R.B. Kemp, D. Olomolaiye, Thermochim. Acta 399 (2003) 63.
[26] M.A.A. O'Neill, A.E. Beezer, G.J. Vine, R.B. Kemp, D. Olomolaiye, P.L.O. Volpe, D. Oliveira, Thermochim. Acta 413 (2004) 193.
[27] M.A.A. O'Neill, A.E. Beezer, J.C. Mitchell, J.A. Orchard, J.A. Connor, Thermochim. Acta 417 (2004) 187.
[28] A. Juszkiewicz, M. Kot, W. Zaborska, Thermochim. Acta 320 (1998) 45.
[29] M. Kot, W. Zaborska, A. Juszkiewicz, Thermochim. Acta 354 (2000) 63.
[30] M.J. Todd, J. Gomez, Anal. Biochem. 296 (2001) 179.
[31] M. Stodeman, F.P. Schwartz, Anal. Biochem. 308 (2002) 285.
[32] M. Stodeman, F.P. Schwartz, Anal. Biochem. 321 (2003) 1.
[33] K. Lorentz, W. Wirtz, T. Weiss, Clin. Chim. Acta 308 (2001) 69.