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Keywords: Arylesterase, Paraoxonase, Activation parameters, Integrated Michaelis equation, Product inhibition

Paper published in:

***Biochimie* 97, 72-77 (2014)**

DOI: 10.1016/j.biochi.2013.09.22

Abstract

The influence of temperature upon the hydrolysis of phenyl acetate, catalysed by purified human serum arylesterase/paraoxonase (E. C. 3.1.8.1), was studied in the temperature range 10°C – 40°C by spectrophotometry in TRIS buffer, pH 8.0, using both initial rate analysis and progress curve analysis. The kinetic parameters (catalytic constant k_{cat} ; Michaelis constant K_m ; product inhibition constant K_p) were determined by nonlinear regression. All parameters increased with temperature, but the ratios k_{cat}/K_m and K_p/K_m remained practically constant. Binding of both substrate and reaction product (phenol) was exothermic. A negative entropic term accounted for about 50% of the enthalpy change for both the binding and catalytic steps. Thermodynamic analysis suggested that: (1) the rate-limiting step is the nucleophilic attack of the carbonyl group of the substrate by a water molecule, (2) the active site is preorganized with no induced fit, (3) the enzyme-bound calcium plays an important role in stabilizing both the substrate and the transition state. The practical implications of these results are discussed.

1. Introduction

Paraoxonase, also known as arylesterase (EC 3.1.8.1) is a calcium-containing enzyme linked to the high density lipoproteins (HDL). It can hydrolyze a wide range of substrates, including lactones, aromatic esters such as phenyl acetate, or phosphate esters such as paraoxon, the active metabolite of the insecticide parathion. This enzyme was first studied for its ability to detoxify organophosphorus pesticides, but since has been ascribed a protective role against several pathologies [1]. Structural studies have allowed to propose a mechanism of action [2, 3], but the thermodynamics of the hydrolytic reaction remain poorly understood, in spite of the previous studies devoted to the hydrolysis of phenyl acetate catalyzed by sodium acetate [4] or the hydrolysis of nitrophenyl esters by serum albumin, a nonspecific catalyst [5]. It was therefore interesting to study the influence of temperature on the kinetics of the enzyme, in order to investigate the thermodynamic basis of its specificity.

2. Theory

We assume that the reaction follows the simple mechanism:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{\text{cat}}} E + P \tag{1}$$

where E, S, ES and P denote the enzyme, substrate, enzyme-substrate complex and reaction product, respectively. k_1 and k_{-1} are the association and dissociation rate constants, and k_{cat} is the catalytic constant. The Michaelis constant is defined by:

$$K_{\text{m}} = \frac{k_{-1} + k_{\text{cat}}}{k_1} \tag{2}$$

If $k_{\text{cat}} << k_{-1}$, $K_{\text{m}} \approx k_{-1} / k_1$ represents the dissociation constant of the enzyme-substrate complex.

The initial reaction rate v_0 is linked to the initial substrate concentration $[S]_0$ by the Michaelis equation:

$$v_0 = \frac{V_{\text{max}}[S]_0}{K_{\text{m}} + [S]_0} \tag{3}$$

where:

$$V_{\text{max}} = k_{\text{cat}} e_{\text{tot}} \tag{4}$$

is the maximal velocity and e_{tot} the total enzyme concentration.

The association equilibrium constant ($1 / K_{\text{m}}$) and the catalytic rate constant (k_{cat}) depend on the absolute temperature T by means of the classical equations:

$$\ln \frac{1}{K_\mathrm{m}} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{5}$$

$$\ln \frac{k_\mathrm{cat}}{T} = \ln \frac{k_\mathrm{B}}{h} - \frac{\Delta H^\ddagger}{RT} + \frac{\Delta S^\ddagger}{R} \tag{6}$$

where ΔH° and ΔS° denote the binding enthalpy and entropy, $\Delta H^\ddagger$ and $\Delta S^\ddagger$ denote the activation enthalpy and entropy, k_B is Boltzmann's constant, h is Planck's constant and R is the gas constant.

If both $[S]_0$ and T are varied simultaneously, a global equation can be obtained by bringing eqs. (5) and (6) into eq. (3):

$$v_0 = \frac{k_\mathrm{B}}{h} \cdot e_\mathrm{tot} \cdot [S]_0 \cdot T \cdot \frac{\exp(-a/T + b)}{\exp(c/T - d) + [S]_0} \tag{7}$$

with $a = (\Delta H^\ddagger / R)$, $b = (\Delta S^\ddagger / R)$, $c = (\Delta H^\circ / R)$, $d = (\Delta S^\circ / R)$

In addition, we assume that the reaction product P can bind to the enzyme with a dissociation constant K_p:

$$E + P \underset{K_\mathrm{p}}{\rightleftharpoons} EP \tag{8}$$

K_p can be determined by recording the complete time course of the reaction, assuming that the Michaelis equation (3) is valid at any time t:

$$v = -\frac{\mathrm{d}[S]}{\mathrm{d}t} = \frac{V'_\mathrm{max}[S]}{K'_\mathrm{m} + [S]} \tag{9}$$

where K'_m and V'_max are the apparent kinetic parameters:

$$K'_\mathrm{m} = K_\mathrm{m} \frac{K_\mathrm{p} + [S]_0}{K_\mathrm{p} - K_\mathrm{m}} \tag{10}$$

$$V'_{\mathrm{max}} = V_{\mathrm{max}} \frac{K_{\mathrm{p}}}{K_{\mathrm{p}} - K_{\mathrm{m}}} \tag{11}$$

According to previous studies [6-8], the analytical solution of the differential equation (9) is the integrated Michaelis-Menten equation:

$$[P] = [S]_0 - K'_{\mathrm{m}} \cdot \mathrm{W}\left\{ \frac{[S]_0}{K'_{\mathrm{m}}} \exp\left(\frac{[S]_0 - V'_{\mathrm{max}} t}{K'_{\mathrm{m}}} \right) \right\} \tag{12}$$

where $[P]$ denotes the product concentration at time t. W is Lambert's function, which is such that, if $w = \mathrm{W}(x)$, then $x = w \exp(w)$.

So, if K_{m} and V_{max} are known, K_{p} can be estimated by fitting eq. (12) to the whole progress curve. Alternatively, K_{p} can be determined by recording the initial rate v_0 in the presence of added product and using the classical equation for competitive inhibition:

$$v_0 = \frac{V_{\mathrm{max}}[S]_0}{K_{\mathrm{m}}(1 + [P]_0/K_{\mathrm{p}}) + [S]_0} \tag{13}$$

where $[P]_0$ denotes the concentration of the added product at time 0. The binding enthalpy and entropy for the product P are estimated from eq. (5) by using K_{p} instead of K_{m}

3. Material and methods

3.1. Enzyme

Human serum 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 [9]. 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.* [10], 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 [11]. Sera from subjects having the same homozygous phenotype A were pooled, and the enzyme was purified by the method of Gan *et al.* [12]. Details of the purification procedure have been published elsewhere [13]. A molecular weight of 40 kDa for the proteic part of the glycoproteic enzyme was used to compute enzyme concentrations [14]. The enzyme and substrate solutions were prepared in TRIS buffer (9 mM) containing 1 mM $CaCl_2$ as activator. The pH of the buffer was adjusted to 8.0 at the desired temperature by NaOH titration in a thermostatted cell.

3.2. Kinetic studies

The hydrolysis of phenyl acetate was followed by spectrophotometry as described previously [13]. At each temperature (10 °C to 40 °C), a set of progress curves was recorded for different initial substrate concentrations (0.1 to 10 mM) by measuring the absorbance of the reaction product (phenol) as a function of time. For each curve, the initial rate v_0 was computed from the extinction coefficient of phenol, determined at the same temperature. The kinetic parameters V_{max} and K_m were estimated from the variation of the initial rate v_0 with substrate concentration $[S]_0$, according to eq. (3). The catalytic constant k_{cat} was computed from eq. (4), using a final enzyme concentration of 8.5 nM. Enthalpy and entropy for substrate binding, as well as activation enthalpy and entropy, were estimated from the linear least squares fit of eqs. (5) and (6) to the K_m and k_{cat} data, and were further refined by fitting eq. (7) to the whole data set of initial rates (65 points), using nonlinear regression.

The product inhibition constant K_p was determined by recording an additional progress curve at each temperature, with an initial substrate concentration equal to about two times the Michaelis constant at the same temperature. The integrated Michaelis equation (12) was fitted to

each curve by nonlinear regression. The apparent K'_m value was used to compute the product inhibition constant K_p from eq. (10). Enthalpy and entropy for the binding of the reaction product phenol were determined by fitting eq. (5) with K_p instead of K_m.

An additional experiment to determine K_p was carried out at 25 °C by measuring the initial reaction rates at several substrate concentrations (0.25 to 4 mM) in the presence of added phenol (0.2 to 0.6 mM). The phenol concentration was limited to avoid an excessive increase of the initial absorbance. The results were analysed according to eq. (13).

4. Results

4.1. Initial rate analysis

The initial rates (v_0) of phenyl acetate hydrolysis are plotted in Fig. 1 as a function of initial substrate concentration ($[S]_0$) at several temperatures from 10 °C to 40 °C. Inspection of the graph revealed that both V_{max} and K_m increased with temperature (K_m is the substrate concentration corresponding to V_{max} / 2) but the initial slope of the curves (equal to V_{max} / K_m) was almost independent of temperature. These conclusions were confirmed by the quantitative analysis (Table 1). The kinetics was always michaelian; the precision of the non-linear least squares

estimation was approximately 4% for k_{cat} and 6% for K_m. There was a systematic increase in both k_{cat} and K_m with temperature, but the ratio k_{cat} / K_m (which represents the bimolecular rate constant k_s for the reaction E + S $\rightarrow$ E + P) remained approximately constant: k_s = (6.4 ± 0.7) × 10^5 M^{-1} s^{-1}. This parameter is linked to the pseudo-first order rate constant k_{obs} at low substrate concentration ($[S]_0$ << K_m) by the relationship: k_{obs} = V_{max} / K_m = k_s e_{tot}

Fig. 2 shows the variations of the kinetic parameters with temperature, together with the linear fit of eqs. (5) and (6). Of special interest is the fact that ln (1 / K_m) varies linearly with (1/T), indicating that K_m depends on two rate constants only. According to eq. (2), there are two hypotheses: the first one, $K_m \approx k_{-1} / k_1$, considers K_m as the dissociation constant of the enzyme-substrate complex; the second one, $K_m \approx k_{cat} / k_1$, would mean that the ES complex is at steady state but not at equilibrium, a phenomenon already observed with cholinesterases [15]. In agreement with previous studies, we have privileged the first hypothesis.

Enthalpies and entropies determined by linear regression were used as initial estimates for fitting the global equation (7) to the whole data set.

According to the residual standard deviation, the precision of the initial rates was 0.36 μM s^{-1}. The final values of the thermodynamic parameters are displayed in Table 1. The results were used to estimate the K_m value at 10°C since it could not be determined with a sufficient precision from the Michaelis-Menten fit at this temperature. As expected from the increase of K_m with temperature, the binding reaction was strongly exothermic, but this effect was partly compensated by an important loss of entropy, such that the term $T\Delta S°$ accounted for about 50% of $\Delta H°$, resulting in a relatively low affinity of the enzyme for its substrate ($\Delta G° \approx$ -17 to -15 kJ mol^{-1} over the temperature range).

The activation enthalpy was close to the binding enthalpy in absolute value but with opposite sign: this was in agreement with the quasi-constancy of k_s over the temperature range. The formation of the transition state was also characterized by an important loss of entropy which contributed to the relatively high value of the activation free energy ($\Delta G^{H} \approx$ 55 to 57 kJ mol^{-1} over the temperature range).

4.2. Progress curve analysis

Fig. 3 shows the fit of the integrated Michaelis equation (12) to the set of progress curves recorded at several temperatures, with $[S]_0 \approx 2\ K_m$. The

product inhibition constant K_p was computed from the apparent Michaelis constant K'_m. An additional experiment to determine K_p was carried out at 25°C by measuring the initial rate of hydrolysis in the presence of added phenol. The result (K_p = 1.67 ± 0.32 mM) was in agreement with the value of 1.99 mM obtained by the progress curve method. However, the initial rate method suffered from the important rise of the initial absorbance due to the added phenol. For this reason, we consider the progress curve method as more reliable.

Product inhibition occurred at each temperature and the inhibition constant K_p was always higher than K_m. Note that the case $K_p < K_m$ can be easily detected by the progress curve method since it will result in negative values of the apparent constants K'_m and V'_{max} (eqs. 10-11). Moreover, both K_p and K_m increased with temperature, but their ratio remained practically constant (1.44 ± 0.10). It is interesting to notice that the pseudo-first order rate constant is not affected by product inhibition, since from eqs. 10-11 we obtain:

$$\frac{V'_{max}}{K'_m} = \frac{V_{max}}{K_m} \cdot \frac{K_p}{K_p + [S]_0} = k_{obs} \cdot \frac{K_p}{K_p + [S]_0} \qquad (14)$$

So, if $[S]_0 << K_m < K_p$, $V'_{max} / K'_m \approx k_{obs}$ and is practically independent of temperature. This finding comforts the idea that the

measurement of k_{obs} may be more useful than the initial rate assay to determine the activity of arylesterase [16].

The binding enthalpies and entropies for the reaction product (phenol) were computed as described previously and the results are shown in Table 1. The values were close to those obtained for the substrate, but the resulting free energies were slightly lower in absolute values ($\Delta G° \approx$ -16 to -14 kJ mol^{-1} over the temperature range), in agreement with the relationship $K_p > K_m$. Also, the very close values of the two binding enthalpies was in agreement with the fact that K_p / K_m was practically independent of temperature.

5. Discussion

5.1. Enzyme purity and stability

Arylesterase/paraoxonase (ARE) is a component of the high density lipoproteins (HDL). When prepared by the method of Gan [12], it is obtained together with another protein named HPBP (Human Phosphate Binding Protein). This protein is essential to maintain the active site conformations of the enzyme. It also plays a role in stabilizing the enzyme and promotes the formation of hetero-oligomers, mainly a heterotetramer (2 ARE + 2 HPBP) [17, 18]. It has been shown by differential scanning calorimetry [17] that the ARE/HPBP complex is

stable for temperatures up to about 50°C, which exceeds the temperature range used in this work. So, we did not have to correct our results for the thermal inactivation of the enzyme.

5.2. Mechanism of action of arylesterase

The mechanism, deduced from structural studies of recombinant enzymes [2, 3], is depicted in Fig. 4. It consists of a nucleophilic attack of the ester carbon by a water molecule, with a histidine as the proton acceptor. This leads to the formation of a tetrahedral oxyanionic intermediate which decomposes into the reaction products. So, the simple mechanism of eq. (1) must be expanded as:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} ET \overset{k_3}{\rightarrow} E + P_1 + P_2 \qquad (15)$$

where ET denotes the complex between the enzyme and the tetrahedral intermediate, P_1 and P_2 the reaction products (here: acetic acid and phenol).

For this mechanism, $K_m = [(k_{-1} + k_2) / k_1] \bullet f$ and $k_{cat} = k_2 \bullet f$ where $f = k_3 / (k_2 + k_3)$ [19]. However, the linear nature of the plots of ln $(1 / K_m)$ and ln (k_{cat} / T) *vs.* $(1 / T)$, as shown in Fig. 2, suggests that K_m and k_{cat} represent either a single rate constant or a ratio of rate constants. We can easily explain this result by assuming that the nucleophilic attack is the

rate-limiting step, while the decomposition of the unstable tetrahedral intermediate is faster. So, if $k_2 << k_3$ and $k_2 << k_{-1}$, $K_m \approx k_{-1} / k_1$ represents the equilibrium constant for the dissociation of the ES complex, and $k_{cat} \approx k_2$ represents the rate constant for the formation of the ET complex.

5.3. Binding of substrate and reaction product

Structural studies suggest that the methyl group and the aromatic ring of phenyl acetate interact with hydrophobic sites of the enzyme (Fig. 4). Additional binding occurs between the calcium ion and the carbonyl group of the substrate. We can therefore expect that phenol, which lacks the methyl group, will have a reduced affinity for the enzyme. This is confirmed by our observations (see Results). Moreover, the weak difference between the affinities of the two compounds is in agreement with the very close values of their octanol-water partition coefficients: log P = 1.49 for phenyl acetate and 1.46 for phenol, as estimated by Leo's method [20]. However, the thermodynamics of enzyme binding differs widely from a simple octanol-water transfer. For phenol, this transfer is slightly exothermic ($\Delta H° \approx$ -8 kJ mol^{-1}) with an important rise in entropy ($\Delta S° \approx$ +18 J mol^{-1} K^{-1}) [21]. On the other hand, the enzyme binding is about 4 times more exothermic with an important loss in entropy. The

increased exothermicity can be attributed to the calcium complexation and to the formation of hydrogen bonds. The entropy loss can be attributed to the inclusion of the ligand into a highly organized network of intermolecular bonds involving calcium and several amino acids (Fig. 4).

It has been suggested that the combination of an unfavourable entropy change compensated by a favourable enthalpic term is characteristic of an 'induced fit' process [22]. But this phenomenon is also accompanied by an important change in heat capacity ($\Delta C_p° > 4$ kJ mol^{-1} K^{-1}) due to the 'ordering of flexible ligand binding sites'. In our case, the linearity of the plots in Fig. 2 indicates a negligible heat capacity change. Indeed, an attempt to fit an improved version of eq. (5), incorporating a $\Delta C_p°$ term, resulted in statistically non-significant parameters. This result suggests that both the substrate and reaction product bind to a pre-existing conformer of the enzyme, in agreement with the conclusions of Ben-David *et al.* [2]. According to these authors, each class of arylesterase substrates (carboxylic esters, phosphate esters, lactones…) interact with a specific conformer of the enzyme.

Lorentz *et al.* [23, 24] reported K_m values at 25°C and 30°C for the hydrolysis of phenyl- and thiophenyl acetate by whole human serum. From their results we estimated $\Delta H° \approx$ -40 and -16 kJ mol^{-1}, $\Delta S° \approx$ -77 and

-0.6 J mol^{-1} K^{-1} for phenyl- and thiophenyl acetate, respectively. Although the precision of these estimates is poor (since there are only two temperatures), and the enzyme is not purified, the values for phenyl acetate compare well with our results.

Cléry-Barraud *et al*. [18] studied the influence of hydrostatic pressure upon the hydrolysis of several substrates catalysed by arylesterase, with or without HPBP. The results allowed to determine the volume variation $\Delta V°$ for the binding step. For phenyl acetate, in the presence of HPBP, a slight decrease in volume ($\Delta V° \approx$ -2.7 mL mol^{-1}) was noticed for pressures up to 150 MPa. This seems to be the consequence of the many intermolecular bonds which occur during the binding of the substrate.

5.4. Catalysis by arylesterase

The rate-limiting step for the hydrolysis of phenyl acetate, catalysed by arylesterase, is the nucleophilic attack of the ester carbon by water. The activation enthalpy of 37.4 kJ mol^{-1} corresponds to an Arrhenius activation energy $E_a \approx$ 40 kJ mol^{-1} (since $E_a = \Delta H^{\ddagger} + RT$ and the mean temperature in our assay is 298 K). This also corresponds to a Q_{10} of 1.69 (Q_{10} is the factor by which k_{cat} increases when the temperature is raised by 10 K, from 298 K to 308 K).

Table 2 compares our findings with some literature values. The following comments apply:

(1) The activation energy for arylesterase is about half the value for the uncatalysed reaction. So, the enzyme is very efficient in its function of stabilizing the transition state. It has been suggested that this stabilization is due to the 'electrostatic preorganization of the enzyme active site' [25]. Obviously, the calcium ion contributes an important part in this process. Also, the existence of a preorganized site is in agreement with our conclusions regarding the binding step.

(2) Lorentz *et al*. [23, 24] studied the hydrolysis of phenyl- and thiophenyl acetate by whole human serum. Comparison with our results is difficult since the assay methods differ widely. However, the higher activation energy of thiophenyl acetate supports the conclusion that the formation of the tetrahedral intermediate is the rate-limiting step: if the decomposition of the intermediate was limiting, one could expect a lower activation energy for the thioester, since thiophenol is a better leaving group (lower pK_a) than phenol.

(3) Sakurai *et al*. [5] studied the hydrolysis of 4-nitrophenyl acetate by serum albumin, a non-specific catalyst. Later, Lockridge *et al*. [26] showed that the hydrolysis of nitrophenyl esters by albumin is due mainly to the acetylation of 82 amino acids, only one of them (Tyr-411)

being deacetylated, but at a very low rate (k_3 ≈ 0,0002 min^{-1}). So, it is likely that the activation energies reported by Sakurai *et al*. refer to the global acetylation step. With arylesterase, the k_{cat} for 4-nitrophenyl acetate is only about 2% of its value for phenyl acetate [11]. This would increase the activation energy by about 10 kJ mol^{-1}, a value insufficient to explain the observed difference in the activation enthalpies. So, arylesterase is much more efficient than serum albumin, which seems to be due to the presence of a calcium ion.

Cléry-Barraud *et al*. [18] found an activation volume $\Delta V^{\ddagger} \approx$ +3.3 mL mol^{-1} for the hydrolysis of phenyl acetate by the arylesterase / HPBP complex. An increase in activation volume has been attributed to bond cleavage and charge neutralization, while a decrease has been attributed to bond formation and ionization [27]. It is likely that the breakage of the carbonyl double bond and the subsequent neutralization of the oxygen charge by the calcium ion contribute to the observed increase in activation volume.

5.5. Practical applications

The present work may lead to applications in the following fields:

(1) Arylesterase assays: for the reasons explained in paragraph 4.2, a continuous assay at low substrate concentration may be more useful

than an initial rate assay, for instance if a progressive inhibitor must be studied in the presence of substrate.

(2) Determination of thermodynamic parameters: equation (7) provides a useful alternative to the classical Arrhenius plots.

(3) Ligand optimization: the knowledge of both ΔH and ΔS, and of course the knowledge of the reaction mechanism, helps designing specific ligands for an enzyme [28].

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Table 1. Kinetic and thermodynamic parameters of arylesterase determined by the initial rate method (K_m , k_{cat}) and by the progress curve method (K_p) at different temperatures. Note that K_m and K_p are dissociation constants, while $\Delta H°$ and $\Delta S°$ refer to the corresponding association reactions.

Temperature (°C)	K_m (mM)	K_p (mM)	k_{cat} (s^{-1})
10	0.68 ([a])	0.89	454
15	0.97	1.35	503
20	1.05	1.58	659
25	1.33	1.99	796
30	1.80	2.82	1200
35	2.07	2.75	1541
40	3.03	4.43	1901
$\Delta H°$ (kJ mol^{-1})	-34.2 ± 5.0	-35.9 ± 2.9	
$\Delta S°$ (J $mol^{-1}K^{-1}$)	-60.2 ± 16.5	-68.9 ± 9.9	
ΔH^H(kJ mol^{-1})			+37.4 ± 1.5
ΔS^H(J $mol^{-1}K^{-1}$)			-63.1 ± 5.1

([a]) The K_m value at 10°C was estimated after fitting of eq. (7) and was not used for linear regression (Fig. 2).

Table 2. Comparison with some literature values. The activation enthalpy $\Delta H^{\ddagger}$ and the Arrhenius activation energy E_a are in kJ mol^{-1}. Q_{10} is the factor by which k_{cat} increases when the temperature is raised by 10 K, from 298 K to 308 K. k_{unc} is the rate constant of the uncatalyzed reaction. ARE : human serum arylesterase ; SA : serum albumin.

Catalyst	Substrate	$\Delta H^{\ddagger}$	E_a	Q_{10}	k_{cat} / k_{unc}	Ref.
None	PhOAc		77.4			(a)
ARE (purified)	PhOAc	37.4	40	1.69	2.5×10^8	(b)
ARE (whole serum)	PhOAc PhSAc		26 45.5	1.42 1.81		(c) (c)
SA (various species)	*p*NO_2-PhOAc	61.9 – 66.1			1000 - 2000	(d)

(a) Ref. [4]
(b) This work ; k_{unc} value from ref. [4]
(c) Ref. [23, 24]
(d) Ref. [5]

Fig. 1. Initial rates of phenylacetate hydrolysis, catalysed by purified human serum arylesterase/paraoxonase, *vs* initial substrate concentrations, for several temperatures. The curves correspond to the Michaelis equation and were fitted according to eq. (7).

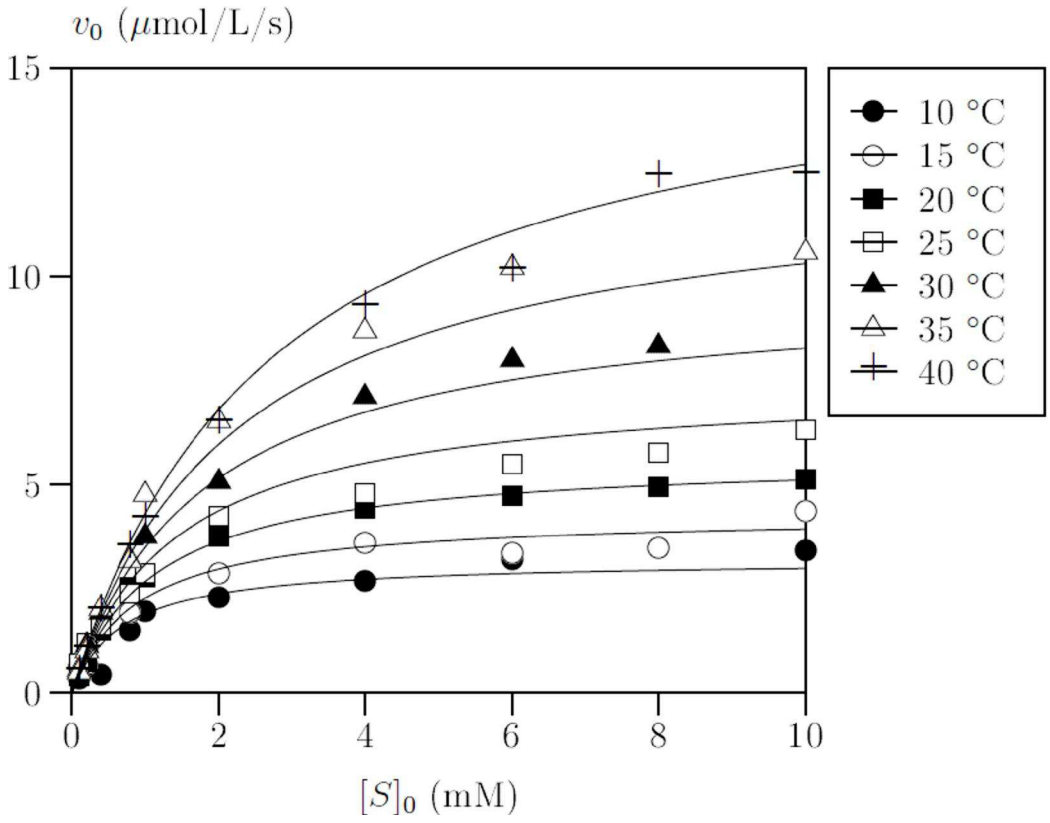

Fig. 2. Variations of the kinetic parameters (Michaelis constant K_m ; product inhibition constant K_p ; catalytic constant k_{cat}) with temperature. The lines were fitted according to eq. (5-6). Upper panel: equilibrium dissociation constant $K = K_m$ (↙) or K_p (VIII). Lower panel: catalytic constant.

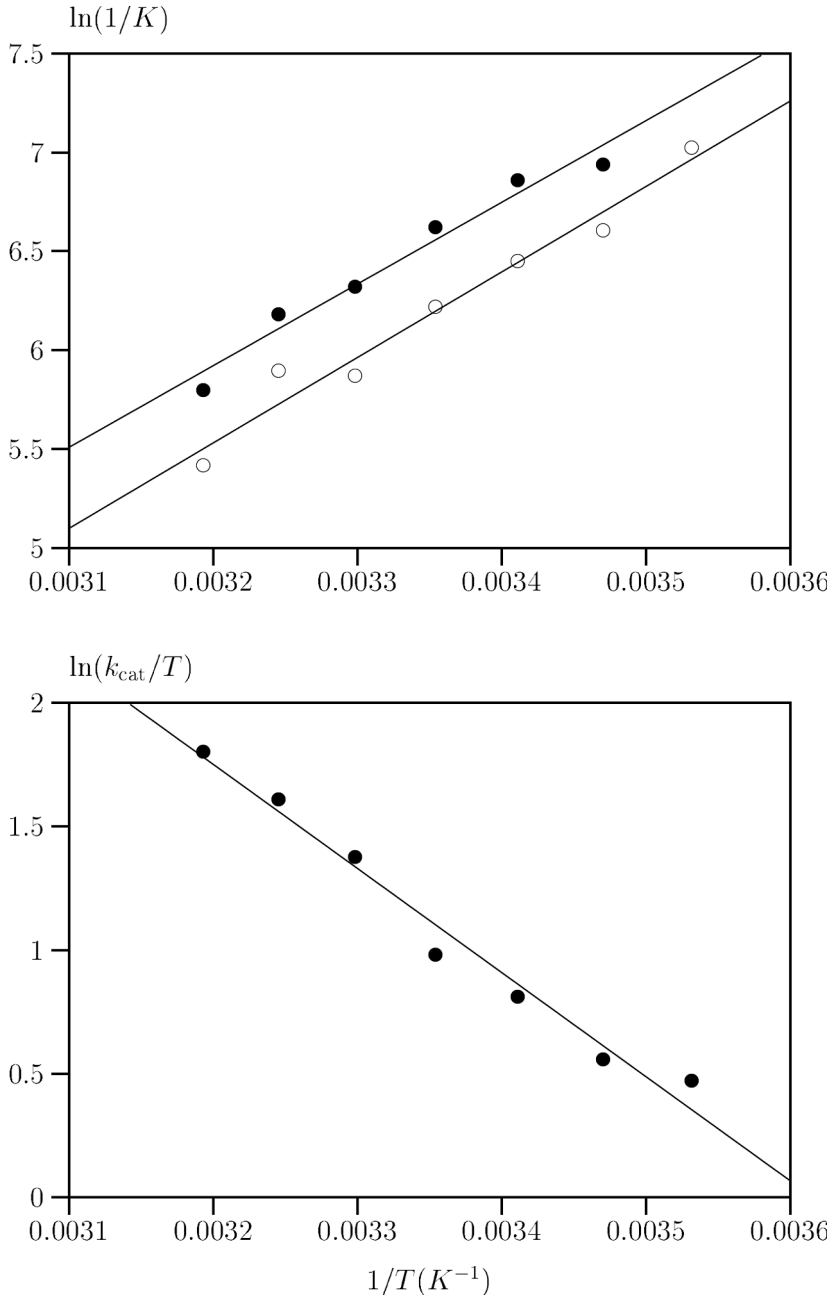

Fig. 3. Progress curves of phenyl acetate hydrolysis at several temperatures. The initial substrate concentration was twice the Michaelis constant at the same temperature. The curves correspond to the integrated Michaelis equation, eq. (12).

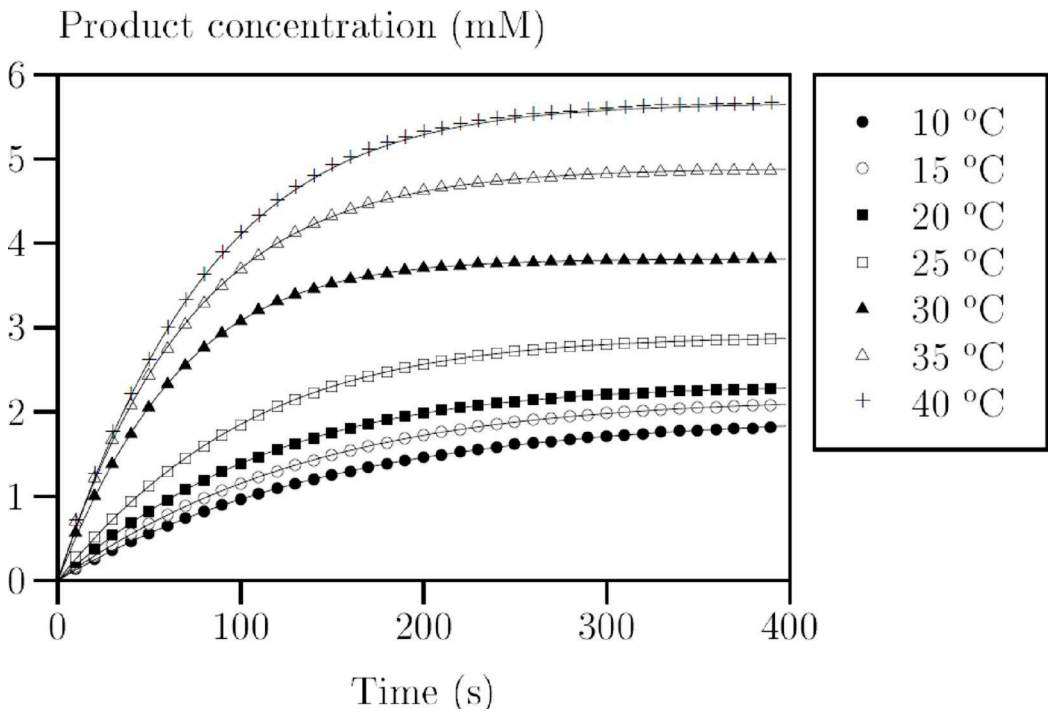

Fig. 4. Mechanism of action of arylesterase/paraoxonase with the substrate phenyl acetate, showing the formation (k_2) and decomposition (k_3) of the tetrahedral intermediate. The hydrophobic areas are plotted in grey.