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INTERACTIONS BETWEEN ACETYLCHOLINESTERASE AND TETRA-*N*-ALKYLAMMONIUM IONS

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Abstract—A study of the mechanism of interaction of acetylcholinesterase with some simple tetra-*N*-alkyammonium ions has been made. Kinetic schemes have been proposed which are consistent with the experimental results observed in the enzyme–tetra-*N*-alkylammonium system in the presence of substrate and in the presence of an organophosphorus inhibitor.

Some years ago it was postulated that the products of hydrolysis in acyl hydrolase systems were formed in two consecutive reactions, with an acylated enzyme intermediate being formed from the enzyme–substrate (Michaelis) complex [1, 2]. The hydrolysis of acetylcholine by acetylcholinesterase (AChE) is one such system. It has been previously shown that the rate of substrate hydrolysis in such a system may be modified by reversible inhibitors by two mechanisms; complex formation between the free enzyme and inhibitor, thereby reducing the concn of Michaelis complex, or complex formation between the acylated enzyme and inhibitor, thereby modifying the rate of de-acylation [3]. A third alternative, complex formation between the Michaelis complex and inhibitor, has been shown not to occur [3]. Reversible inhibitors, or modifiers, of the AChE–substrate system generally contain a quaternary nitrogen atom [3].

In order to gain a better understanding of the mechanisms by which quaternary nitrogen compounds interact with AChE, an investigation of the effects upon substrate hydrolysis of a series of simple tetra-*N*-alkylammonium salts (R_4N^+) has been carried out, where R = methyl (Me), ethyl (Et), *n*-propyl (Pr^n), *n*-butyl (Bu^n), and *n*-pentyl ($Pent^n$). A mechanistic description of the system has been developed which is kinetically rigorous and somewhat simpler than that previously proposed [3].

A kinetic analysis of the system where two different modifiers are present has been proposed and shown to apply to experimentally measured data. A limited number of experiments have also been performed on the AChE–modifier–organophosphorus (irreversible) inhibitor system in order to demonstrate that the AChE–modifier interactions proposed earlier still apply under these conditions.

EXPERIMENTAL

Materials

AChE from bovine erythrocytes (EC 3.1.1.7) was obtained from Sigma Chemicals. Acetylcholine iodide (AChI) was obtained from BDH Biochemicals. $Me_4N^+I^-$, $Et_4N^+I^-$, $Pr^n_4N^+I^-$ and $Bu^n_4N^+I^-$ were obtained from BDH Chemicals and were recrystallised from ethanol/water or ethyl acetate/ethanol before use. $Pent^n_4N^+Br^-$ was prepared at CDE (Porton, U.K.) [4].

AChE activities were measured titrimetrically using a Radiometer automatic titrator (pH meter 26, titrator 11, titrigraph SBR 2c, syringe burette SBU 1a) fitted with twin syringes containing NaOH (0.01 N) and AChI (0.01 M).

Methods

Single-modifier experiments. To a solution of NaCl (0.1 M, 10 ml) containing AChE (1 unit*) in a radio meter cell at 37° and at pH 7.4 was added AChI solution. The surface of the solution was bathed with N_2 to prevent CO_2 uptake. Five concns of AChI were used, ranging from 4.0×10^{-4} to 0.6×10^{-4} M. The rate of AChI hydrolysis was monitored on the radiometer by pH-statting at 7.4, and was constant because the AChI concn was maintained by means of the twin syringes. Hydrolysis rates at five different AChI concns were then measured at six different concns of tetra-*N*-alkylammonium ions. Eadie plots [5] of the rate of substrate hydrolysis (v) against v/[S] (where [S] = substrate concn) were constructed for each different modifier concn. They yielded gradients of $-G$ (defined later in this report) and intercepts of V_m.

Figs 1 and 2 show examples of these plots, and Table 1 shows the results obtained.

Mixed-modifier experiments. These experiments were performed and the results were treated as before, except that two different modifiers were present in solution. The results are shown in Table 2.

Organophosphorus inhibitions. AChE solution (10 units/ml in 0.1 M NaCl and 5×10^{-3} M phosphate buffer at pH 7.4—0.5 ml) at 37° was made 10^{-4} M with respect to *O*-ethyl *S*-*n*-propyl methyl phosphonothiolate. Aliquots of 0.08 ml were removed at various times and diluted into 0.1 M NaCl solution (10 ml), and the remaining AChE activity was assayed by making the solution 5×10^{-4} M with AChI and pH-statting at 7.4 on the radiometer: continuing inhibition by the organophosphate was

* 1 unit will hydrolyse 1 μmole of acetylcholine to choline and acetate per minute at pH 8 and 37°.

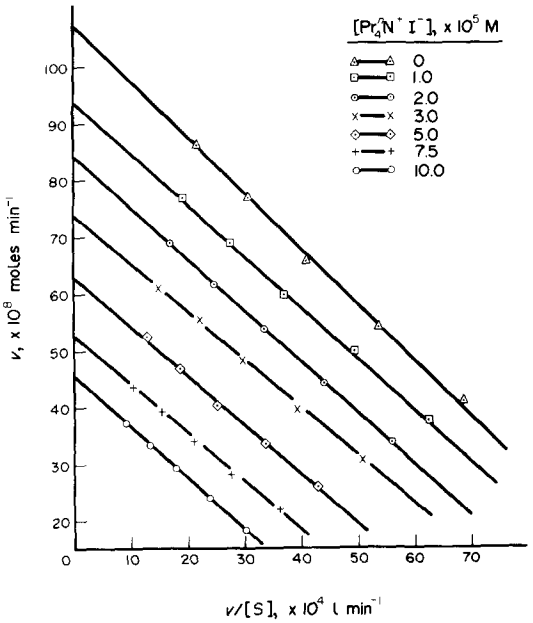


Fig. 1. Plots of v against $v/[S]$ at different concns of AChI and $Pr_4^nN^+I^-$.

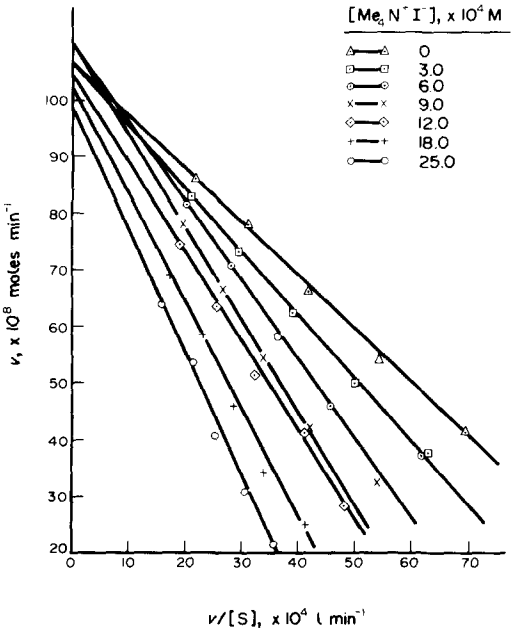


Fig. 2. Plots of v against $v/[S]$ at different concns of AChI and $Me_4N^+I^-$.

insignificant following this degree of dilution. The inhibition was repeated at different modifier concns. Apparent first-order rate coefficients were calculated as shown in the kinetic analysis. The results are shown in Table 3, and are the means of at least two experiments.

Kinetic analysis

Single modifier. The system to be analysed is shown below; E is free enzyme, S is substrate, and M is the reversible inhibitor or modifier. ES and EM are the complexes formed between the enzyme and substrate and the enzyme and modifier respectively. EA is acylated enzyme and EAM the complex formed from EA and M.

$$\begin{array}{ccc} E + S \overset{k_1}{\rightleftharpoons} ES \overset{k_2}{\rightarrow} EA & \overset{k_3}{\rightarrow} & E \\ + & & + \\ M & & M \\ \downarrow\uparrow K_4 & & \downarrow\uparrow K_5 \\ EM & & EAM \overset{k_6}{\rightarrow} EM \end{array}$$

$$K_1 = \frac{[ES]}{[E][S]}; \; K_4 = \frac{[EM]}{[E][M]}; \; K_5 = \frac{[EAM]}{[EA][M]}$$

$$[E_0] = [E] + [ES] + [EA] + [EM] + [EAM] \quad (1)$$

where $[E_0]$ represents the initial enzyme concn.

If the rate of acid production $= v$ then $v = k_3[EA] + k_6[EAM]$, i.e.:

$$v = [EA](k_3 + k_6K_5[M]). \quad (2)$$

Application of steady-state theory to [EA] and substitution into equations (1) and (2) leads to:

$$v = \frac{[E_0]k_2K_1[S](k_3 + k_6K_5[M])}{(1 + K_1[S] + K_4[M])(k_3 + k_6K_5[M]) + k_2K_1[S](1 + K_5[M])}.$$

This can be rearranged to the following equation:

$$v = \frac{-v}{[S]}G + V_m \quad (3)$$

where

$$G = \frac{(1 + K_4[M])(k_3 + k_6K_5[M])}{K_1(k_2 + k_3) + (K_1K_5[M])(k_2 + k_6)} \quad (4)$$

and

$$V_m = \frac{[E_0]k_2(k_3 + k_6K_5[M])}{(k_2 + k_3) + K_5[M](k_2 + k_6)}. \quad (5)$$

Dividing equation (4) by equation (5) leads to:

$$\frac{G}{V_m} = \frac{1 + K_4[M]}{[E_0]k_2K_1}$$

Table 1. V_m and G values resulting from Eadie plots at different modifier concns

[Me$_4$N$^+$] (×10^4 M)	V_m (×10^8 moles min^{-1})	G (×10^4 M)	[Et$_4$N$^+$] (×10^3 M)	V_m (×10^8 moles min^{-1})	G (×10^4 M)	[Pr$_4^n$N$^+$] (×10^5 M)	V_m (×10^8 moles min^{-1})	G (×10^4 M)	[Bu$_4^n$N$^+$] (×10^5 M)	V_m (×10^8 moles min^{-1})	G (×10^4 M)	[Pent$_4^n$N$^+$] (×10^5 M)	V_m (×10^8 moles min^{-1})	G (×10^4 M)
0	107	0.95	0	106	0.91	0	108.0	0.98	0	104.0	0.91	0	108.0	0.98
3.0	106	1.12	0.5	108	1.58	1	93.7	0.90	0.5	92.7	0.88	3.0	97.6	1.12
6.0	110	1.40	1.0	117	2.36	2	84.2	0.90	1.0	83.9	0.91	5.0	91.3	1.14
9.0	110	1.60	1.5	114	3.10	3	73.7	0.85	2.0	73.7	1.10	8.0	82.2	1.18
12.0	104	1.58	2.0	116	3.71	5	63.0	0.86	3.0	58.9	0.89	12.0	82.0	1.52
18.0	102	1.90	3.0	106	4.50	7.5	52.9	0.88	4.0	50.9	0.87	15.0	74.2	1.59
25.0	98.2	2.12	5.0	96.3	6.18	10	42.8	0.87	6.0	41.7	0.93	20.0	71.3	1.72

Table 2. V_m and G values resulting from Eadie plots at different mixed-modifier concns

$[Bu_4^nN^+]$ ($\times 10^5$ M)	$[Me_4N^+]$ ($\times 10^4$ M)	V_m ($\times 10^8$ moles min^{-1})	G ($\times 10^4$ M)	$[Bu_4^nN^+]$ ($\times 10^5$ M)	$[Et_4N^+]$ ($\times 10^3$ M)	V_m ($\times 10^8$ moles min^{-1})	G ($\times 10^4$ M)	$[Bu_4^nN^+]$ ($\times 10^5$ M)	$[Pr_4^nN^+]$ ($\times 10^5$ M)	V_m ($\times 10^8$ moles min^{-1})	G ($\times 10^4$ M)
0	0	99.0	0.99	0	0	97.4	1.09	0	0	94.2	0.98
0.25	1.0	92.4	1.10	0.25	0.25	93.1	1.40	0.25	0.5	82.0	1.00
0.5	2.0	87.0	1.19	0.5	0.5	84.2	1.45	0.5	1.0	73.9	1.01
1.0	4.0	76.0	1.21	1.0	1.0	88.5	2.47	1.0	2.0	61.0	1.00
1.5	6.0	72.5	1.54	1.5	1.5	82.0	2.78	1.5	3.0	52.2	1.00
2.0	8.0	64.3	1.51	2.0	2.0	86.0	3.78	2.0	4.0	45.0	0.96
3.0	12.0	55.2	1.56	3.0	3.0	83.1	5.30	3.0	6.0	37.1	1.09

Table 3. Comparison of observed and calculated k_i values in the presence of modifiers

Modifier	Concn (M)	K_4[M]	k_i (obs.) ($\times 10^3$ sec^{-1})	k_i (calc.) ($\times 10^3$ sec^{-1})
—	Control	—	3.30 ± 0.15 (N = 4)	—
Me_4N^+	1.79×10^{-3}	1	1.50 ± 0.05 (N = 2)	1.65
	7.14×10^{-3}	4	0.61 ± 0.03 (N = 2)	0.66
$Bu_4^nN^+$	0.37×10^{-4}	1	1.83 ± 0.05 (N = 2)	1.65
	1.48×10^{-4}	4	0.84 ± 0.03 (N = 2)	0.66
SAD 128	0.45×10^{-4}	1	1.74 ± 0.06 (N = 2)	1.65
	1.82×10^{-4}	4	0.84 ± 0.02 (N = 2)	0.66

N = number of experiments.

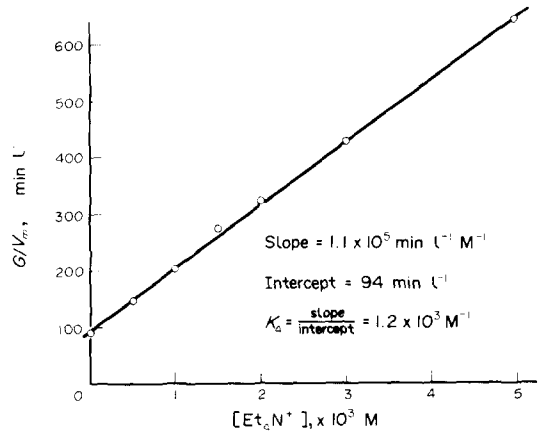


Fig. 3. Plot of G/V_m against $[Et_4N^+]$.

so that a plot of G/V_m against [M] will give a line of gradient $K_4/([E_0]k_2K_1)$, and an intercept (on the ordinate) of $1/([E_0]k_2K_1)$. Hence K_4 can be calculated. Fig. 3 shows a typical example of this plot.

When [M] = 0, $V_m = V_0$, i.e.:

$$V_0 = \frac{[E_0]k_2k_3}{k_2 + k_3}. \tag{6}$$

Dividing equation (5) by equation (6) gives:

$$\frac{V_m}{V_0} = V_r = \frac{1 + \frac{k_6}{k_3}K_5[M]}{1 + K_5[M]\frac{(k_2 + k_6)}{(k_2 + k_3)}}.$$

Rearrangement gives:

$$\frac{1 - V_r}{[M]} = V_rK_5\frac{(k_2 + k_6)}{(k_2 + k_3)} - \frac{k_6}{k_3}K_5.$$

Thus a plot of $(1 - V_r)/[M]$ against V_r will give a line of gradient $K_5\,[k_2 + k_6)/(k_2 + k_3)]$ and an intercept (on the abscissa) of $(k_6/k_3)[(k_2 + k_3)/(k_2 + k_6)]$. Fig. 4 shows a typical example of this plot, and Table 4 lists the various parameters for this series of modifiers. Plots of $(1 - V_r)/[M]$ against V_r were not possible for Me_4N^+ and Et_4N^+ because V_m was constant.

Mixed modifiers. The system to be analysed is shown below: M_1 and M_2 are different modifiers. Other symbols have been defined previously.

$$\begin{array}{lll} EM_1 & EAM_1 \xrightarrow{K_{6\,(1)}} EM_1 \\ \downuparrows K_{4\,(1)} & \downuparrows K_{5\,(1)} \\ M_1 & M_1 \\ + & + \\ E + S \overset{K_1}{\rightleftharpoons} ES \xrightarrow{k_2} EA \xrightarrow{k_3} E \\ + & + \\ M_2 & M_2 \\ \downuparrows K_{4\,(2)} & \downuparrows K_{5\,(2)} \\ EM_2 & EAM_2 \xrightarrow{k_{6\,(2)}} EM_2 \end{array}$$

K_1 has been defined previously.

$$K_{4\,(1)} = \frac{[EM_1]}{[E][M_1]};\ K_{4\,(2)} = \frac{[EM_2]}{[E][M_2]};$$

$$K_{5\,(1)} = \frac{[EAM_1]}{[EA][M_1]};\ K_{5\,(2)} = \frac{[EAM_2]}{[EA][M_2]}$$

$$[E_0] = [E] + [EM_1] + [EM_2] + [ES] + [EA] + [EAM_1] + [EAM_2] \tag{7}$$

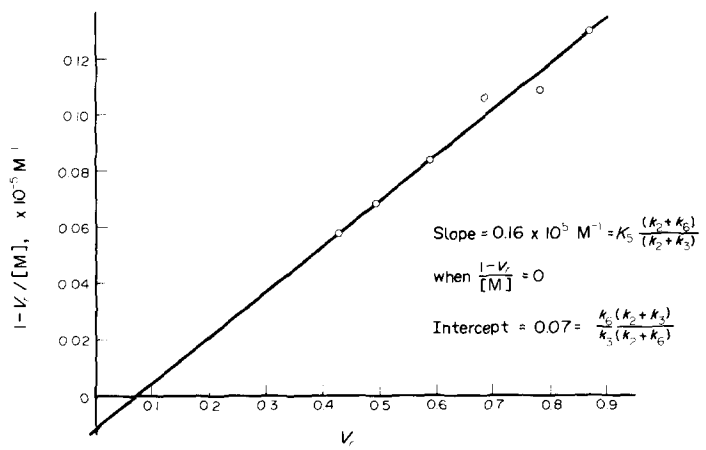


Fig. 4. Plot of $(1 - V_r)/[M]$ against V_r for $Pr^n_4N^-$.

Table 4. Parameters calculated graphically

Parameter	Me_4N^-	Et_4N^-	$Pr^n_4N^-$	$Bu^n_4N^-$	$Pent^n_4N^-$
K_4 (M^{-1})	6.5×10^2	1.2×10^3	1.2×10^4	2.7×10^4	8.5×10^3
$K_5\frac{(k_2 + k_6)}{(k_2 + k_3)}$ (M^{-1})	—	—	1.6×10^4	2.5×10^4	6.8×10^3
$\frac{k_6(k_2 + k_3)}{k_3(k_2 + k_6)}$	—	—	0.07	0	0.41

$$v = k_3[EA] + k_{6(1)}[EAM_1] + k_{6(2)}[EAM_2], \text{ i.e.:}$$

$$v = [EA](k_3 + k_{6(1)}K_{5(1)}[M_1] + k_{6(2)}K_{5(2)}[M_2]). \quad (8)$$

Application of steady-state theory to [EA] and substitution into equations (7) and (8) leads to:

$$v = \frac{k_2[E_0](k_3 + k_{6(1)}K_{5(1)}[M_1] + k_{6(2)}K_{5(2)}[M_2]}{\begin{array}{r}(1 + K_{4(1)}[M_1] + K_{4(2)}[M_2])(k_3 + k_{6(1)}K_{5(1)}[M_1] + k_{6(2)}K_{5(2)}[M_2]) \\ +(1 + K_{5(1)}[M_1] + K_{5(2)}[M_2])k_2 + (k_3 + k_{6(1)}K_{5(1)}[M_1] + k_{6(2)}K_{5(2)}[M_2])\end{array}}{K_1[S]}$$

This can be rearranged to the following equation:

$$v = \frac{-v}{[S]}G + V_m \quad (9)$$

where

$$G = \frac{(1 + K_{4(1)}[M_1] + K_{4(2)}[M_2])\,(k_3 + k_{6(1)}K_{5(1)}[M_1] + k_{6(2)}K_{5(2)}[M_2])}{K_1\left[k_2(1 + K_{5(1)}[M_1] + K_{5(2)}[M_2]) + k_3 + k_{6(1)}K_{5(1)}[M_1] + k_{6(2)}K_{5(2)}[M_2]\right]} \quad (10)$$

and

$$V_m = \frac{k_2[E_0](k_3 + k_{6(1)}K_{5(1)}[M_1] + k_{6(2)}K_{5(2)}[M_2])}{k_2(1 + K_{5(1)}[M_1] + K_{5(2)}[M_2]) + k_3 + k_{6(1)}K_{5(2)}[M_1] + k_{6(2)}K_{5(2)}[M_2]}. \quad (11)$$

Dividing equation (10) by equation (11) leads to:

$$\frac{G}{V_m} = \frac{1 + K_{4(1)}[M_1] + K_{4(2)}[M_2]}{[E_0]k_2K_1}$$

$$= \frac{1}{[E_0]k_2K_1} + \frac{[M_1]}{[E_0]k_2K_1}(K_{4(1)} + K_{4(2)} \cdot C)$$

where $C = [M_2]/[M_1]$, so that a plot of G/V_m against $[M_1]$ will give a gradient of $[(K_{4(1)} + K_{4(2)} \cdot C)]/([E_0]k_2K_1)$ and an intercept (on the ordinate) of $1/([E_0]k_2K_1)$.

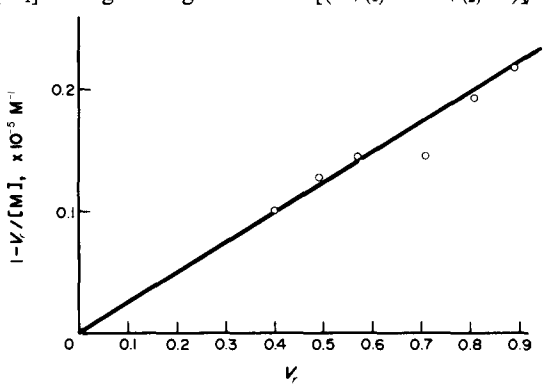


Fig. 5. Plot of $(1 - V_r)/[M]$ against V_r for $Bu^n_4N^+$.

Hence $(K_{4(1)} + K_{4(2)} \cdot C)$ can be calculated and compared with the K_4 values obtained from the single-modifier experiments. A comparison of these values is shown in Table 5.

V_r is calculated as in the single-modifier analysis, i.e.:

$$V_r = \frac{1 + \frac{k_{6(1)}}{k_3}K_{5(1)}[M_1] + \frac{k_{6(2)}}{k_3}K_{5(2)}[M_2]}{1 + K_{5(1)}[M_1]\left(\frac{k_2 + K_{5(1)}}{k_2 + k_3}\right) + K_{5(2)}[M_2]\left(\frac{k_2 + k_{6(2)}}{k_2 + k_3}\right)}. \quad (12)$$

Rearrangement of equation (12) gives:

$$\frac{1 - V_r}{[M_1]} = V_r\left[K_{5(1)}\left(\frac{k_2 + k_{6(1)}}{k_2 + k_3}\right) + C \cdot K_{5(2)}\left(\frac{k_2 + k_{6(2)}}{k_2 + k_3}\right)\right] - \left(\frac{k_{6(1)}}{k_3}K_{5(1)} + C \cdot \frac{k_{6(2)}}{k_3}K_{5(2)}\right)$$

Table 5. Comparison of observed parameters from mixed-modifier experiments with those calculated from the single-modifier experiments

Modifier mixture and ratio	$K_{4(1)} + C \cdot K_{4(2)}$ (M^{-1})*		V_r†		Gradient†	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
$Bu^n_4N^+$:Me_4N^+ $C = 40$	6.5×10^4	5.3×10^4	0.16	0.19	3.6×10^4	3.1×10^4
$Bu^n_4N^+$:Et_4N^+ $C = 100$	1.6×10^5	1.5×10^5	0.73	0.74	10.0×10^4	9.8×10^4
$Bu^n_4N^+$:$Pr^n_4N^+$ $C = 2$	5.7×10^4	5.1×10^4	0.06	0.04	5.8×10^4	5.7×10^4

* Observed values are from plots of G/V_m against $[M_1]$.
† Observed values are from plots of $(1 - V_r)/[M_1]$ against V_r.

so that a plot of $(1 - V_r)/[M_1]$ against V_r will give a gradient of $K_{5\,(1)}[(k_2 + k_{6\,(1)})/(k_2 + k_3)] + C \cdot K_{5\,(2)}[(k_2 + k_{6\,(2)})/(k_2 + k_3)]$ and an intercept (on the abscissa) of $(k_{6\,(1)}/k_3)K_{5\,(1)} + C \cdot (k_{6\,(2)}/k_3)K_{5\,(2)}$. Hence these values can be calculated and compared with the values obtained from the single-modifier experiments (see Table 4).

Organophosphorus inhibitions. The system to be analysed is shown below; E and M are enzyme and modifier respectively, I is the organophosphorus inhibitor, and EI is phosphonylated enzyme.

$$\begin{array}{ccc} E + I & \xrightarrow{k_i} & EI \\ + & & \\ M & & \\ K_4 \downarrow\uparrow & & \\ EM & & \end{array}$$

K_4 has been defined previously, and k_i is the rate coefficient of inhibition of E by I. Complex formation between E and I, analogous to that between E and S, does occur, but is kinetically unimportant at the inhibitor concn used. It has been shown [6] that for systems of this type:

$$\ln \frac{[E_0] - [EI]}{[E_0]} = \frac{k_i[I]}{(1 + K_4[M])} .t.$$

$[I] \gg [E_0]$, and is considered to remain constant. Plots of $\ln ([E_0] - [EI])/[E_0]$ against t produced a line of gradient $-k_i/(1 + K_4[M])$. Values of [M] were chosen (from the single-modifier results) that would reduce k_i by either a factor of 2 or a factor of 5. Three modifiers, Me_4N^+, $Bu_4^nN^+$ and SAD 128,* were treated in this way.

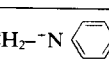
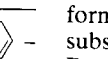
DISCUSSION

The kinetic schemes developed for single and mixed modifiers have been shown to apply to the experimentally observed results. Furthermore, the parameters obtained from the analysis of the single-modifier experiments have been shown to be in agreement with those obtained from the analysis of the mixed-modifier experiments and those from the analysis of the AChE–modifier–organophosphorus inhibition system.

Reversible enzyme inhibitors can modify the rate of substrate hydrolysis by forming an unreactive complex with free enzyme, or by forming a complex with acylated enzyme which does not deacylate, or does so at a slower rate than uncomplexed acylated enzyme [3]. Examination of Table 4 shows that plots of $(1 - V_r)/[M]$ against V_r were not possible for the modifiers Me_4N^+ and Et_4N^+. This was because V_m values were constant (Table 1) over the experimental concns used, and indicates that either $K_5 = 0$, or that $k_6 = k_3$. It is not possible to unambiguously determine which possibility is correct, but since $K_4 \neq 0$, K_5 is unlikely to be zero and therefore $k_3 = k_6$ is the most likely explanation. These findings indicate that Me_4N^+ and Et_4N^+ inhibit the hydrolysis of substrate purely by competing with substrate for the active site on the free enzyme, and differ from previous findings [7], which showed that de-acylation occurred at slightly reduced rates with these two modifiers. Values of V_r could be calculated for the other three modifiers ($Pr_3^nN^+$, $Bu_4^nN^+$ and $Pent_4^nN^+$) in the homologous series, indicating that inhibition of substrate hydrolysis occurs both because the rate of de-acylation is reduced, and because the concn of free enzyme is reduced due to complex formation (K_4 values were also able to be calculated). Examination of the K_4 values (Table 4) of these latter three compounds shows that they have a greater affinity for free AChE than either Me_4N^+ or Et_4N^+. Table 4 shows that the parameter $(k_6/k_3)/[(k_2 + k_3)/(k_2 + k_6)]$ is zero for Bu_4N^+, i.e. the graph (Fig. 5) for this compound gives a line which intersects the origin, indicating that $k_6 = 0$, i.e. no de-acylation of the acylated enzyme–modifier complex occurs. Previous workers found that this complex de-acylated very slowly [7].

In order to obtain any more information from the calculated parameters certain assumptions have to be made. Previous workers [2] have shown that, in the hydrolysis of acetylcholine by electric eel AChE, the rate coefficient of breakdown of the Michaelis complex is between 10^2 and 10^3 times greater than that of the de-acylation of acylated enzyme, i.e. that $k_2 \gg k_3$. If this is also the case for bovine erythrocyte AChE, the parameters (Table 4) derived from the gradients and intercepts when $(1 - V_r)/[M])$ is plotted against V_r can be simplified to K_5 and the ratio k_6/k_3 respectively. Consequently it can be seen that the ratio k_6/k_3 is very small for the modifier $Pr_3^nN^+$, indicating that de-acylation of the acylated enzyme–modifier complex (EAM) is slow compared to that of the free acylated enzyme (EA). The ratio k_6/k_3 is 0.41 for $Pent_4^nN^+$, indicating that the rate of de-acylation of EAM is significant compared to that of EA. The simplified parameter leading to K_5 indicates that the three modifiers have similar affinities for free AChE and acylated AChE, and that the values of K_5 are 3–4 times higher than those found by previous workers [7].

Conventional theories of the reversible inhibition of enzymes in the presence of substrate state that compounds which effect only K_m values (obtained from the slopes in Eadie plots) are termed competitive inhibitors, and those which effect only V_m values (obtained from the intercepts in Eadie plots) are termed non-competitive inhibitors [8]. Examination of Table 1 shows that the values of G (obtained from the slopes in Eadie plots) for the modifiers $Pr_3^nN^+$ and $Bu_4^nN^+$ are constant, and so the two modifiers can conventionally be termed non-competitive inhibitors. However, examination of Table 4 shows that K_4 values are able to be calculated for these two modifiers, indicating that they also form a complex with free AChE, in competition with substrate. It has also been shown that the modifier $Bu_4^nN^+$ competes with an organophosphorus inhibitor for the active site on AChE (Table 3) thereby reducing the expected rate of phosphonylation, and again indicating that the action of this inhibitor is

* SAD 128 is Me_3C–  N^+–CH_2OCH_2–^-N  –$CMe_3 \cdot 2Cl^-$, and has been used in the treatment of nerve agent poisoning in animals. It has been treated in the same way as other modifiers in the single experiments. The results will be given in more detail in a later publication.

not purely non-competitive. These findings indicate that conventional theories of reversible enzyme inhibition are in need of modification where enzyme–substrate interactions produce covalent enzyme intermediates.

O-Ethyl *S*-*n*-propyl methyl phosphonothiolate, the irreversible inhibitor used to phosphonylate AChE in the presence of various modifiers (Table 3), exists in two optically active forms whose rate coefficients of inhibition of AChE vary by a factor of 15 [9]. Phosphonylation of AChE is therefore caused essentially by the more active enantiomer in this case. From the results shown in Table 3 it is therefore not possible to gain any information on the stereochemical aspects of phosphonylation of AChE in the presence of modifiers, although it is expected that the presence of a modifier will reduce the observed rate of phosphonylation by the same degree irrespective of the configuration of the inhibitor.

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