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A MECHANISTIC MODEL FOR BUTYRYLCHOLINESTERASE

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Summary

A plausible mechanism of action of horse serum butyrylcholinesterase is proposed. It includes substrate activation at the level of deacylation. The rate constant for the acylation of the enzyme appears to be much greater than the rate constant for the deacylation, at low substate concentrations. At higher substrate concentrations the rate constants become more similar. No interaction between the four subunits in binding of inhibitors or in the catalysis was observed. There is one esteratic and one anionic site per subunit apparent from labelling studies with [^{32}P]diisopropylfluorophosphate and binding studies with *N*-methylacridine. Although the tetrametric form of the enzyme appears to be the native one, the monomeric and several other aggregated and dissociated states are catalytically active.

Introduction

The introduction of new substrates and of new techniques for measuring the activity of butyrylcholinesterase (EC 3.1.1.8), which is present in human and horse blood plasma, has revealed kinetics which cannot be described by the Michaelis-Menten equation. This deviation from a simple kinetic model can be explained by the existence of multiple enzyme forms, by a polymer form of the enzyme with interacting catalytic sites, or by an enzyme that is activated by its substrate but in which interacting subunits are not involved.

The steady-state kinetics of horse plasma butyrylcholinesterase were discussed recently in mathematical terms [1]. A (mathematical) model formulated in terms of homotropic cooperative regulations of the enzyme by

Abbreviations: Astra 1397, 10-(α-diethylaminopropionyl)-phenothiazine; Nbs_2, 5,5′-dithiobis(2-nitrobenzoic acid).

the substrate was proposed. This model has been further evaluated in the present investigation using a purified enzyme preparation. The kinetic studies have been extended by testing the effect of certain esterase inhibitors and by studying the stoichiometry of ligand binding to the enzyme. The mechanism of action of butyrylcholinesterase proposed previously has been revised. A mathematical model for the enzyme inhibition is discussed and a mechanistic model of catalysis is proposed.

Materials and Methods

Enzyme, substrate and inhibitors. A partially purified butyrylcholinesterase preparation from horse serum was purchased from Nutritional Biochemicals Corporation (Cleveland, OH, U.S.A.). Butyrylthiocholine iodide and choline iodide were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The triophenyl esters were from Polysciences (Warrington, PA, U.S.A.). Astra 1397 was a gift from Prof. R. Dahlbom (formerly at AB Astra, Södertälje, Sweden). Acridinium chloride was purchased from Kebo-Grave Labcenter, (Spånga, Sweden). Diisopropyl[^{32}P]phosphorofluoridate (96.6 Ci/mol) was obtained from the Radiochemical Centre, Amersham, U.K. *N*-Methyl-α-naphthylcarbamate (carbaryl) was from Union Carbide. All other chemicals were standard commercial products, and solutions were prepared using double-deionized water.

Assay of esterase activity. The enzymatic activity was determined at 30°C by a method first described by Ellman et al. [2] based on measuring the increase in absorbance at 412 nm on either an Aminco DW-2 spectrophotometer or a Beckman 25 spectrophotometer. Unless otherwise stated, the reaction mixture consisted of 3 ml 50 mM Tris-HCl buffer (pH 7.4), containing 0.25 mM 5,5′-dithiobis(2-nitrobenzoic acid) (Nbs_2), 0.02 ml substrate solution and 0.05 ml enzyme solution. It has recently been demonstrated [3] that Nbs_2 does not influence the activity and the kinetics of the enzyme under these conditions.

In one experiment the effect of temperature on the catalysis was studied using a modified technique. A 50 mM sodium phosphate buffer (pH 7.4) in which the enzyme and Nbs_2 were added, was incubated for at least 30 min at the relevant temperature. The temperature was monitored regularly with a thermometer in the cuvette and did not vary more that ±0.1°C.

Purification of cholinesterase. Two different methods for obtaining purified horse serum butyrylcholinesterase were used. In Method I, developed in our laboratory [4], a partially purified commercial preparation of butyrylcholinesterase was subjected to further purification in three steps using (1) DEAE A-25-Sephadex, (2) Sephadex G-200, and (3) hydroxyapatite.

Method II is a modification of a procedure described by Main et al. [5]. Fresh whole blood from a horse was defibrinated and the serum was subsequently separated from the erythrocytes by centrifugation (at 3000 × *g*) in the usual manner. The first step of the purification procedure was precipitation with $(NH_4)_2SO_4$ followed by ion-exchange chromatography on a QAE-Sephadex A-50 column [5]. The purification was completed using two gel filtrations on a Sephadex G-200 column (5 × 90 cm), using 50 mM Tris-HCl buffer (pH 7.4) as eluant.

Analytical disc electrophoresis. Disc electrophoresis was carried out on acrylamide gels using the technique described by Bryan [6]. Protein was stained with Coomassie Brilliant Blue G [7] and glycosides by the periodic acid-Schiff method [8]. Esterase activity was detected using α-naphthyl butyrate or α-naphthyl propionate as substrate, followed by an azo coupling technique using Echtblausalz B in 10 mM Tris-HCl buffer (pH 8.0). Esterase was also detected by incubating the gel in 1 mM thiophenyl butyrate containing 1 mg/ml nitro blue tetrazolium.

Fluorometric assay. An Aminco-Bowman spectrofluorometer with a xenon lamp and a ratio photomultiplier was used in studies of the binding of *N*-methylacridinium chloride. 3-ml quartz cuvettes were used, with a minimum volume of 1 ml.

[^{32}P]DFP-Diisopropylfluorophosphate labelling of the enzyme. Different amounts of [^{32}P]diisopropylfluorophosphate were added to an enzyme solution. After about 10 min, 50 μl incubation solution was transferred to a dry filter paper (Munktell No. 5) impregnated with trichloroacetic acid and the spot was dried. The filter paper was then washed extensively on a Büchner funnel with 5% trichloroacetic acid and dried. Immediately before the incubation solution was applied to the filter paper, a 2-μl aliquot was withdrawn and assayed for esterase activity.

Liquid scintillation counting of [^{32}P]diisopropylfluorophosphate-enzyme. The spots on the dried filter paper were cut out and placed flat on the bottom of scintillation vials, which are subsequently filled with 5 ml scintillation solution. The scintillation solution consisted of 5 g PPO and 0.25 g POPOP in 1 l toluene. The disintegration of ^{32}P was measured in a Packard m/2420 Tri-Carb LS5.

Preparation of N-methylacridinium chloride. N-methylacridinium chloride was prepared according to Mooser et al. [9] and the melting point was found to be 183°C (cf. Ref.10).

Hydrolysis of methylcarbamyl enzyme. The methylcarbamylated butyrylcholinesterase was prepared by incubating methylnaphthylcarbamate at a concentrationof $5 \cdot 10^{-6}$ M with butyrylcholinesterase (2 mg/ml of the commercial product) in 50 mM Tris-HCl buffer, pH 7.4, at 30°C for 2 h. Hydrolysis was started by diluting the solution 1000 times with buffer solution containing the reversible cholinesterase inhibitor. After different incubation times, the esterase activity was determined with the Ellman method, using 2.9 ml incubation solution, 0.08 ml 10 mM Nbs_2 and 0.02 ml 0.15 M butyrylthiocholine iodide. When 50 mM choline iodide or 50 mM butyrylcholine iodide were used as the reversible inhibitors, the free and the carbamylated enzyme were separated from unreacted methylnaphthylcarbamate on a Sephadex G-25 superfine column (2 × 20 cm). The top protein fraction was then diluted ten times with buffer solution containing the reversible inhibitor. When butyrylcholine iodide was used, all details were the same except that the buffer concentration was 0.5 M.

Results

Evaluation of the purification

The final elution peak from the Sephadex G-200 column (Method II) was

symmetrical and the specific activity was the same in several fractions. Indeed, the specific activities of the enzyme pools from the two different purification methods differed only by about 10%. The purity of the enzyme was further tested by the analytical polyacrylamide gel electrophoretic procedure described by Bryan [6], using gels of different acrylamide concentrations. When the gels were stained for protein or esterase activity, one main protein and esterase component was detected. About 90—95% of the proteinwas localized in this band. There were two other proteins separated on the gels which also had esterase activity. A Ferguson plot was constructed to determine whether these estereases were related. Fig. 1 shows that the three proteins with activity against α-naphthyl propionate (where A was the dominating protein) were different polymeric forms of the same enzyme, as shown by the common point of intersection. The molecular weights were estimated by the method of Bryan [6] to be approx. 400 000, approx. 200 000 and approx. 100 000, respectively. These molecular weights correspond well with those reported by several authors for the tetramer, dimer and monomer of butyrylcholinenterase. This multiplicity is probably caused by the electrophoretic procedure; otherwise, the different enzyme forms should have been detected during the gel filtrations.

Titration of butyrylcholinesterase with [^{32}P]diisopropylfluorophosphate

The molar concentration of the enzyme was calculated from an absorption at 280 nm of 1.52 *A* units/mg protein per ml and a molecular weight of 317 000 [11]. The enzyme preparation used hydrolyzed 470 μmol of butyrylthiocholine per min and mg of protein in a 50 mM phosphate buffer, pH 8.0, at 25°C.

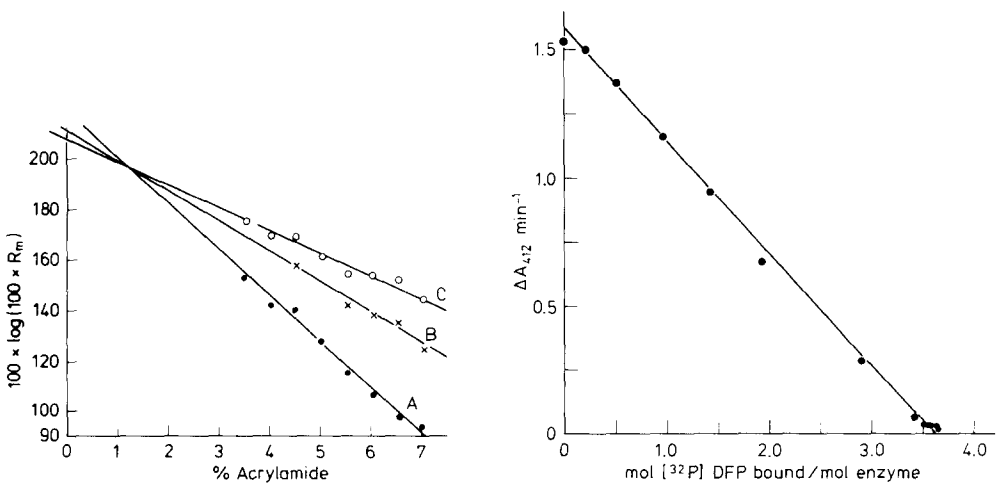

Fig. 1. The logarithm of the mobilities of the different esterases relative to that of bromophenol blue plotted against the percentage of acrylamide. The mobility increases in the order from A to C. The esterase activity was localized with α-naphthylproprionate.

Fig. 2. Titration of butyrylcholinesterase (Method II) with [^{32}P]diisopropylfluorophosphate. The [^{32}P]-diisopropylfluorophosphate concentration was varied within the range of 0—10 μM. The enzyme concentration was 0.33 μM in a total incubation volume of 0.11 ml. Esterase activity was determined with butyrylthiocholine.

Fig. 2 illustrates the change in the esterase activity as a function of bound molecules of [^{32}P]diisopropylfluorophosphate per enzyme molecule. The linearity suggests that all of the phosphorylated sites have the same effect on the esterase activity and that a phosphorylation of such a site does no influence the enzymatic hydrolysis of butyrylthiocholine by a different part of the enzyme. The intercept on the abscissa was calculated by linear regression and found to be 3.86 ± 0.20 mol bound diisopropylfluorophosphate/mol enzyme. Thus, there are most probably four catalytic sites on one enzyme molecule. Previously, Main et al. reported that two diisopropylfluorophosphate molecules were bound by each molecule of the commercially available enzyme [12] and three or possibly four diisopropylfluorophosphate molecules were bound by an enzyme preparation purified essentially as the enzyme generally used in this study [5]. No difference was found in the present study when the partly purified commercial enzyme, further purified by Method I, was used instead.

Stoichiometry and affinity of ligand binding

Titration of butyrylcholinesterase with *N*-methylacridinium ion is illustrated by graphs of $1/[F_b]$ vs. $1/[F_f]$ in Fig. 3, where $[F_b]$ and $[F_f]$ are the bound and free concentrations, respectively, of the fluorescent inhibitor. A similar experiment has already been performed [13]. However, in the previous study an enzyme preparation with only two bound diisopropylfluorophosphate molecules per tetramere was used.

The procedure used to interpret the data was the same as that reported by Mooser et al. [9]. The fluorescence at 490 nm, using excitation with light of 358 nm, was totally quenched when the fluorescent inhibitor was bound to the enzyme, as has previously been reported for both acetylcholinesterase and butyrylcholinesterase [9,13]. The reciprocal value of the intercept on the ordinate yields the enzyme binding site normality. The ratio of this normality and the active site concentration (assumed to be equal to 4 times the enzyme concentration, as revealed by the diisopropylfluorophosphate titration) was equal to 0.96 ± 0.05. This means that there is one binding site for the quaternary compound for every catalytic site, i.e., one anionic site per catalytic site.

The dissociation constant of the enzyme-inhibitor complex was $4.4 \cdot 10^{-8} \pm 0.7 \cdot 10^{-8}$ M. The same stoichiometry and almost the same dissociation constant were obtained when the enzyme was first treated with diisopropylfluorophosphate. Thus, there is no cooperative binding of the inhibitor and the binding is not influenced by phosphorylation of a site.

A mathematical model for butyrylcholinesterase

A mathematical model for the butyrylcholinesterase from horse serum, based on a steady-state kinetics analysis using different substrates, was recently suggested from this laboratory [1]. This model

$$v = \frac{V_1[A] + V_2[A]^2}{1 + K_1[A] + K_2[A]^2} \quad (1)$$

offered the best description of the kinetics of the hydrolysis of butyrylthiocholine and thiophenylacetate. The model (Eqn. 1) is supported by the Hill plots presented in a recent paper by the present authors [4].

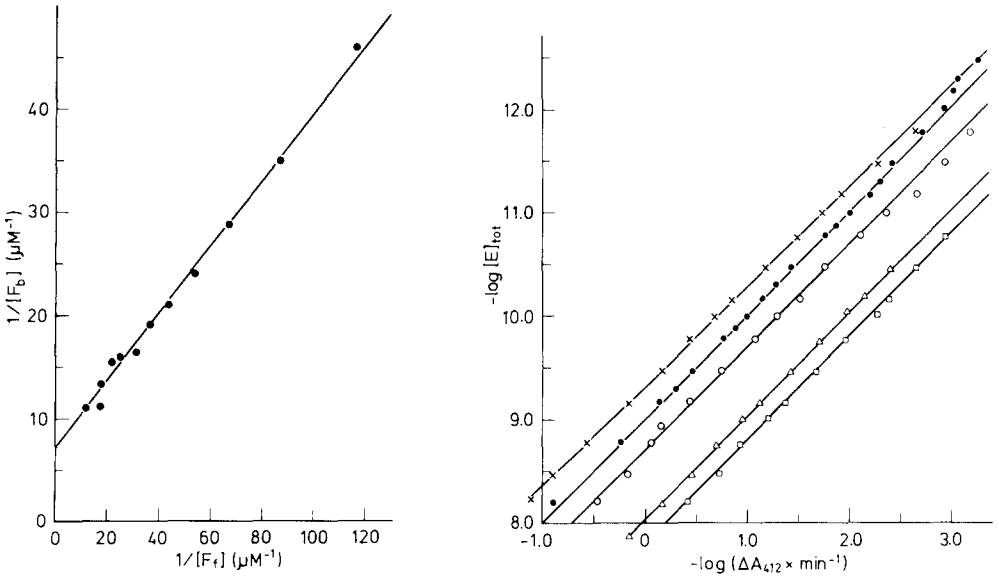

Fig. 3. *N*-Methylacridinium binding to the purified cholinesterase (Method II). The fluorometric measurements were made in 10 mM Tris-HCl buffer, pH 7.4, at 25°C. The enzyme normality was $1.4 \cdot 10^{-7}$ M.

Fig. 4. Evidence for a steady state kinetic behaviour of the cholinesterase. Purified enzyme (Method II) was used and the substrate concentrations were as follows (mM): butyrylthiocholine, X, 10; •, 1.0; ○, 0.10 and □, 0.010; △, thiophenyl acetate, 0.50. The correlation coefficients were greater than 0.998, and the slopes 1.0 ± 0.05.

In order to determine whether catalysis proceeds at steady-state [14] the enzyme concentration was varied more than 1000-fold (Fig. 4). The lines obtained have a slope of 1.0 for all substrate concentrations used. The conclusion drawn is that the enzyme and substrate are at a steady-state, independent of the substrate and enzyme concentrations, when substrate/enzyme lies within the range of $1 \cdot 10^{12}$—$1 \cdot 10^{3}$.

The effect of certain inhibitors on cholinesterase activity

For the purpose of determining a probable mechanistic model for butyrylcholinesterase, the inhibitory effects of choline, Astra 1397 and *N*-methylacridinium chloride were studied. It is apparent from the Cornish-Bowden plots (Figs. 5a, b and c) that a linear relationship was obtained. The effect of choline iodide was investigated with choline concentrations as high as 0.5 M, and the linearity of the plot was still maintained.

With Astra 1397 the results were similar tho those obtained with choline. In this case the plots were linear up to a concentration of at least 100 μM. Astra 1397 has been described as a useful selective inhibitor of blood plasma cholinesterase that does not affect acetylcholinesterase [15,16].

N-Methylacridinium, the potent cholinesterase inhibitor used in the fluorimetric titration of the anionic site, also gave a linear Cornish-Bowden plot. The inhibition constants can be approximated by extrapolating the linear parts of the Lineweaver-Burk plots. The competitive inhibition constant was $3.4 \cdot 10^{-8} \pm 0.7 \cdot 10^{-8}$ M and the non-competitive inhibition constant was $5.0 \cdot 10^{-8} \pm 1.1 \cdot$

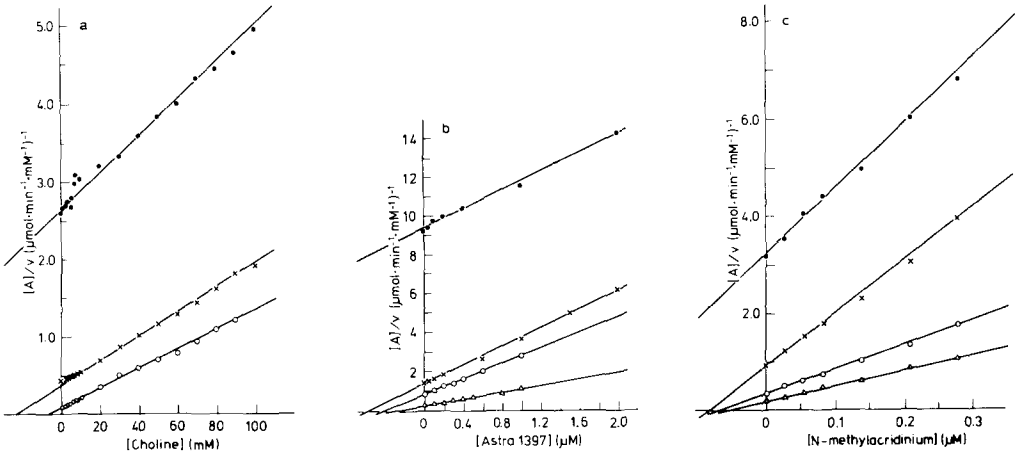

Fig. 5. Inhibition of butyrylcholine esterase activity by: (a) choline iodide. The enzyme used was from the DEAE-Sephadex A-25 pool (Method I). Substrate concentrations (mM): •, 10; X, 1.0; ○, 0.10. (b) Astra 1397. The enzyme was the same as in a. Substrate concentrations (mM): •, 10; X, 1.0; ○, 0.50; and △, 0.10. (c) *N*-Methylacridinium chloride. Purified enzyme (Method II) was used. The buffer was 10 mM Tris-HCl, pH 7.4, and the temperature 25°C. Substrate concentrations (mM): •, 1.0; X, 0.20; ○, 0.040; and △, 0.010 mM.

10^{-8} M. Both these constants, which do not differ significantly, are very similar to the dissociation constant for the inhibitor-enzyme complex.

The feature of the inhibition which is revealed by these plots (Figs. 5a, b and c) is that at high substrate concentrations this inhibition is of a competitive nature and at low concentrations it is non-competitive, with an intermediate region of mixed competitive-non-competitive inhibition.

The complete rate equation is then

$$v = \frac{V_1[A] + V_2[A]^2}{1 + K_1[A] + K_2[A]^2 + (K_3 + K_4[A])[I]} \quad (2)$$

which is an extension of Eqn. 1.

The effect of inhibitors on esterase activity with thiophenyl esters as substrates

In order to determine whether there was any difference in the catalysis and binding of choline esters and other esters, a study analogous to that discussed in the previous section was carried out with thiophenyl esters as substrates.

The effect of choline on the hydrolysis of the acetate (Fig. 6a) was small and resulted in non-linear Dixon plots. Non-linearity was also observed when Astra 1397 was used as inhibitor. From Fig. 6b it is evident that the effect with thiophenyl acetate was significantly different from that found with butyrylthiocholine as substrate, even at extremely high inhibitor concentrations. The shape of the plots indicates that he inhibitor terms also appear in the numerator.

The efffect of choline with thiophenyl butyrate as substrate was essentially the same as with the acetate, although a small activation at high substrate and low inhibitor concentrations was observed. The inhibitor binding site and substrate binding site do not seem to overlap sufficiently to give rise to steric hindrance effects.

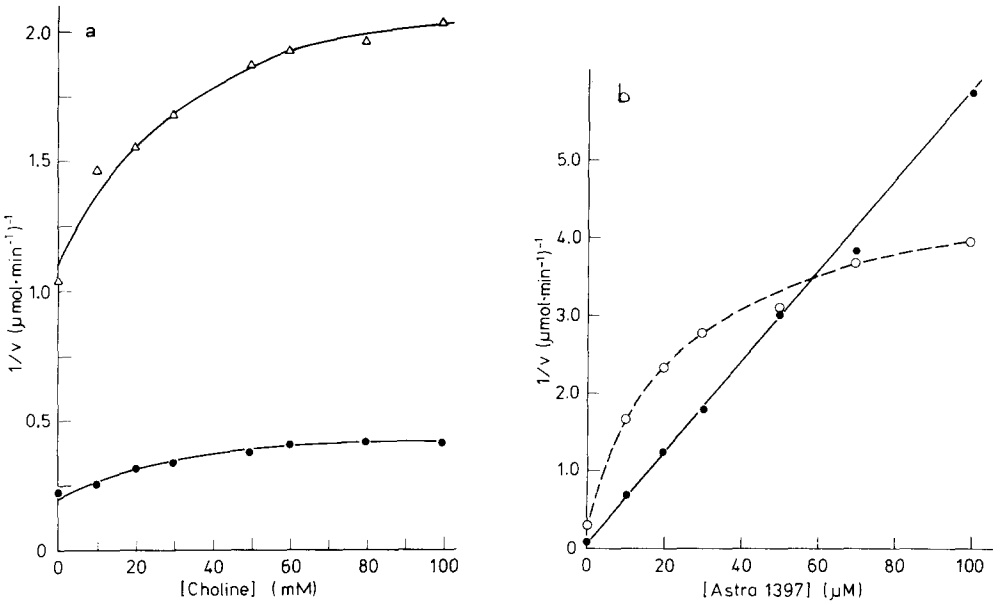

Fig. 6. a. Dixon plots with choline iodide as inhibitor and thiophenyl acetate as substrate. Thiophenyl acetate concentrations were 5 mM (●) and 0.5 mM (△). The enzyme used was the starting material from Method I. b. Dixon plots showing the difference between butyrylthiocholine and thiophenyl acetate as substrates. Substrate concentrations: 1 mM butyrylthiocholine iodide (●); 5 mM thiophenyl acetate (○).

Reactivation of carbamoylated enzyme

In order to determine whether certain cholinesterase inhibitors affect decarbamoylation of the carbamoylated butyrylcholinesterase, the enzyme was inhibited to 80% with methylnaphthylcarbamate and thereafter the recovery of the activity was studied (Fig. 7). Blanks containing non-carbamoylated enzyme and the respective modifier were used throughout the experiment to compensate for a 20% denaturation of the highly diluted enzyme after about 6 h at 30°C. The logarithmic plots of the recovery were linear, suggesting a single carbamoylated species where the rate of reactivation is independent of the degree of carbamoylation.

It is evident from the plots that N^{10}-methyl-9-aminoacridine and Astra 1397 block decarbamoylation and that choline iodide at a concentration of 50 mM has no effect. The analogous relationship between the carbamoyl and acyl enzymes [17] supports the idea that the strong cholinesterase inhibitors block the deacylation reaction of the enzyme. However, no effect of the substrate butyrylthiocholine on the rate of recovery could be seen with the method used, even at an initial concentration of 50 mM. The rate of reactivation was estimated from Fig. 7 to be 0.0044 min^{-1} and the half-life of the *N*-methylcarbamoylated enzyme is thus 2.5 h at 30°C.

Kinetics of the partially carbamoylated enzyme

Previous reports that butyrylcholinesterase catalyzes the hydrolysis of different ester substrates by a homotropic cooperative mechanism involving a pair of subunits might be tested further if it were possible to obtain enzyme molecules with a single catalytic site. This might be accomplished by partially car-

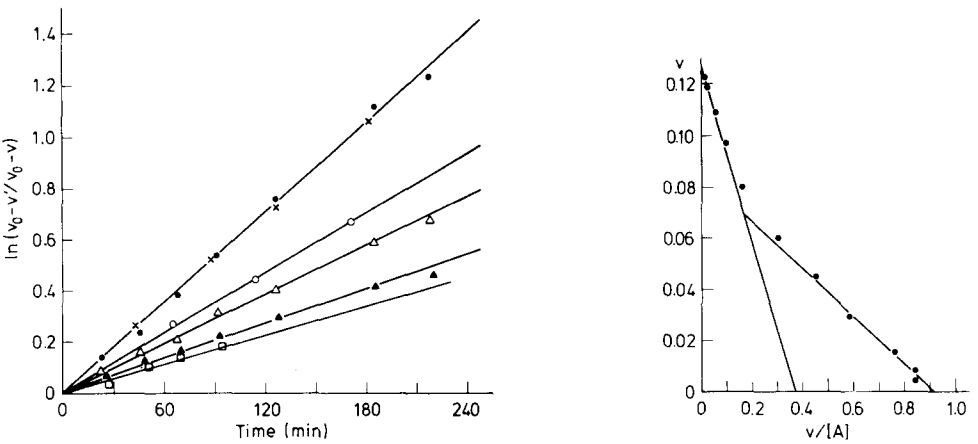

Fig. 7. The reactivation of butyrylcholinesterase inhibited by $5 \cdot 10^{-6}$ M methylnaphthylcarbamate in the presence of various cholinesterase inhibitors. v_0 is the activity of unmodified enzyme, v' is the activity of the inhibited enzyme at $t = 0$ and v is the activity of the recovering enzyme, with butyrylthiocholine as substrate. ●———●, no modifier present; X———X, 50 mM choline iodide; ○———○, 2 μM Astra 1397. The N^{10}-methyl-9-aminoacridine iodide concentrations were (μM): △———△, 1.7; ▲———▲, 5 μM; □———□, 8.3 μM.

Fig. 8. The steady-state kinetic behaviour of butyrylcholinesterase inhibited to 90% by $2 \cdot 10^{-5}$ M methylnaphthylcarbamate. Excess methylnaphthylcarbamate was removed by gel filtration before the experiment. The substrate used was butyrylthiocholine iodide, in the concentrations range 10—0.01 mM. The solid lines represent the limiting slopes at high and low substrate concentrations respectively.

bamoylating the enzyme. Upon incubating the enzyme with $2 \cdot 10^{-5}$ M methylnaphthylcarbamate, the activity decreases exponentially to 10% of the original activity, where it reaches an equilibrium. It is reasonable to believe that carbamates such as methylnaphthylcarbamate carbamoylate all the catalytic sites, as do organophosphates. This means that only about 10% of the residual activity comes from the proposed subunit pairs and the rest from isolated subunits. This would result in a more linear $v/(v/[S])$ plot. The kinetic experiment (Fig. 8) was performed in a 15-min period during which the enzyme was stored on ice. The non-linearity index [18] is 2.6, which is similar to the value of 2.9 found for the free enzyme. This is strong evidence against a mechanism where interaction between the subunits is responsible for the non-hyperbolic substrate dependence.

Temperature dependence of the enzyme hydrolysis

The maximal velocity (V) of the hydrolysis of butyrylthiocholine iodide was obtained from the limiting slope of the Woolf-Hanes plot $([S]/v)/[S]$, at high substrate concentrations. Another parameter that might give information on the kinetics at low substrate concentrations is the 'maximal velocity' (V') extrapolated from the double reciprocal plot $(1/v)/(1/[S])$. The kinetics were studied at seven different temperatures and the results are presented as Arrhenius plots in Fig. 9. When ln V was plotted against the reciprocal temperature, a curve which is concave downwards was obtained, suggesting that V is not dependent on a single rate constant. It is, however, impossible for a two-enzyme system to yield this type of behaviour. When V' was plotted in the

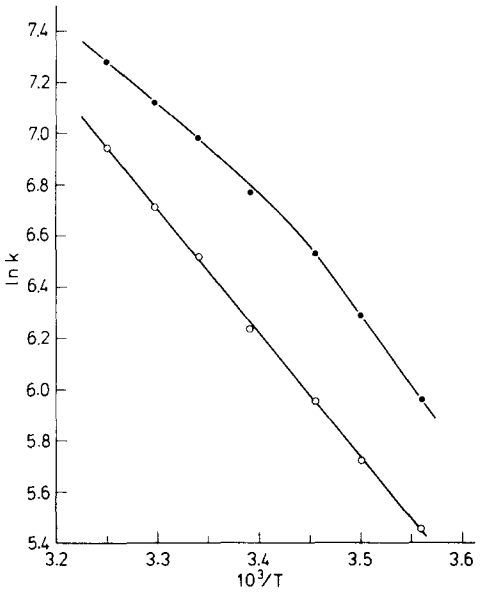

Fig. 9. Arrhenius plots for butyrylcholinesterase with butyrylthiocholine as substrate. The upper graph (●) represents the case with $k = V[E]_0$ and the lower one with $k = V'[E]_0$ where $[E]_0$ is the total enzyme concentration.

same manner, a linear plot was obtained. This means that V' is dependent either on a single rate constant or on a product of rate constants and/or inverted rate constants.

Discussion

Since Hardegg and Schaeffer [19,20] first showed that the kinetics of butyrylcholinesterase do not follow the Michaelis-Menten equation, several authors have confirmed this deviation and proposed different explanations. One of these is that the substrate is acted on by more than one enzyme each following the Michaelis-Menten kinetics [21]. The deviation from standard kinetics was shown in a previous paper [4] to persist during purification. It will be remembered that Main et al. [12] recently reported an alteration of the kinetics during purification, which could not be confirmed in the present study. However, Main et al. did not determine whether the activity measured originated from different enzyme forms.

Data obtained with some non-choline esters (e.g., thiophenylacetate) and more recently also with butyrylthiocholine as substrate at high salt concentrations [18] showed a type of kinetics which could not be explained as the resultant of two Michaelis-Menten equations. The shape of the Arrhenius plot is also not consistent with a two-enzyme model. We therefore conclude that the kinetics should be interpreted in terms of a single enzyme.

Artefacts due to impurities in either substrate or enzyme can be rejected, since different types of substrate were used and no sigmoid kinetics were observed with thiocholine esters as substrates.

If the substrate acts as a modifier, there must be a modifier site that has no catalytic activity; but the results presented above suggest that every substrate (inhibitor) binding site is also catalytically active. This situation probably also results in non-linear inhibition.

A plausible mechanism should involve an acyl intermediate, as has previously been proposed for both acetylcholinesterase [22,23] and butyrylcholinesterase [1,24]. The formation of such an intermediate will be assumed in the following discussion of the mechanism.

The kinetic data were previously interpreted in terms of a homotropic cooperative mechanism on the basis of the results then available on the stoichiometry of ligand binding to butyrylcholinesterase and the fact that substrate inhibition was observed only at very high concentrations of butyrylthiocholine iodide and thiophenylbutyrate. This inhibition was then supposed to be mechanistically similar to the acetylcholine inhibition of acetylcholinesterase. The 'substrate activation' observed with butyrylcholinesterase at intermediate substrate concentrations was thought not to originate from binding of an extra substrate molecule to the anionic site of the acylated subunit.

A cooperative mechanism was preferred and found to be consistent with the data available at that time. This hypothesis was tested in the present study in two main ways. First, a titration of the enzyme by diisopropylfluorophosphate was performed (Fig. 2). This experiment revealed that a phosphorylated catalytic site on the enzyme has no effect on the rate of butyrylthiocholine hydrolysis, which would have been expected from the analogy with an acylated enzyme.

The second test of the cooperative hypothesis was carried out by following the kinetics with a partially carbamoylated enzyme (Fig. 8). The results of that experiment do not support a cooperative mechanism. However, they do support a mechanism where all events in the catalytic process are isolated to the individual subunits. A very plausible mechanism of this kind would be analogous to substrate inhibition of acetylcholinesterase, i.e., an enzyme-substrate intermediate consisting of an acylated enzyme subunit and a substrate molecule bound at either the anionic site (butyrylthiocholine) or at the hydrophobic regions surrounding that site (thiophenyl esters). A probable mechanism is presented in Fig. 10, in which the enzyme-inhibitor complexes are included. The constant $a > 1$. No EAI or FAI complex can exist, at least not when butyrylthiocholine is used as substrate, because of the apparent competition between substrate and inhibition as revealed by Eqn. 1. No complex of the structure EII can exist because of the linear Cornish-Bowden plots. A path between FI and EI does not exist when the inhibitor is an acridine derivative or Astra 1397. Thus, they block deacylation of the enzyme. When choline iodide is the inhibitor, there might exist a path between FI and EI, probably with a rate constante equal to k_3. This conclusion is supported by the decarbamoylation study (Fig. 7) and by the fact that choline has no effect on deacetylation of acetylcholinesterase. The question whether the acylation or the deacylation is the rate determining step has been investigated for acetylcholinesterase [25]. A circumstance that makes the interpretation more difficult in the case with butyrylcholinesterase is that one can not find two substrates with an identical deacylation complex because that complex consists of

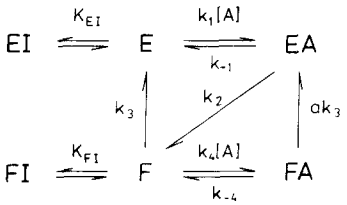

Fig. 10. A mechanistic model of butyrylcholinesterase. E, free enzyme; EA, enzyme-substrate complex; F, acylated enzyme intermediate; FA, acylated enzyme-substrate complex; EI, enzyme-inhibitor complex; FI, acylated enzyme-inhibitor complex.

acylated enzyme plus a substrate molecule (FA), but because of the non-linear Arrhenius plot (Fig. 9) with $(\ln V)/(1/T)$ suggesting $k_2 \approx ak_3$, where

$$V = \frac{ak_2k_3}{k_2 + ak_3}.$$

In the case with $(\ln V')/(1/T)$ that plot turns out to be linear, although the expression of V' is more complex. However, it is expected that $a > 1$, because of the substrate activation observed, when $k_2 \gg k_3$. This could result in a linear Arrhenius plot. It seems reasonable that $k_2 \gtrsim k_3$ at high substrate concentrations and that $k_2 \gg k_3$ at low concentrations of the substrate butyrylthiocholine.

The mechanism presented in this paper finds further support in the fact that Main et al. [26] recently showed that a butyrylcholinesterase from rabbit serum consisting of active momomers had non-hyperbolic kinetics.

Acknowledgements

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References

1 Augustinsson, K.-B., Bartfai, T. and Mannervik, B. (1974) Biochem. J. 141, 825—834
2 Ellman, G.L., Courtney, K.D., Andres, V., Jr. and Featherstone, R.H. (1961) Biochem. Pharmacol. 7, 88—95
3 Augustinsson, K.-B. and Eriksson, H. (1974) Biochem. J. 139, 123—127
4 Augustinsson, K.-B. and Eriksson, H. (1975) Croat. Chem. Acta 47, 277—285
5 Main, A.R., Soucie, W.G., Buxton, I.L. and Arinc, E. (1974) Biochem. J. 143, 733—744
6 Bryan, J.K. (1977) Anal Biochem. 78, 513—519
7 Diezel, W., Kopperschläger, G. and Hofmann, E. (1972) Anal. Biochem. 48, 617—620
9 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606—2616
9 Mooser, G., Schulman, H. and Sigman, D.S. (1972) Biochemistry 11, 1595—1602
10 Adrien, A. (1966) The Acridines, 2nd edn., p. 341, St. Martin's Press, New York
11 Teng, T.-L., Harpst, J.A., Lee, J.C., Zinn, A. and Carlson, D.M. (1976) Arch. Biochem. Biophys. 176, 71—81
12 Main, A.R., Tarkan, E., Aull, J.L. and Soucie, W.G. (1972) J. Biol. Chem. 247, 566—571
13 Chan, L.M., Himel, C.M. and Main, A.R. (1974) Biochemistry 13, 86—90
14 Wong, J.T.-F. (1975) Kinetics of Enzyme Mechanisms, pp. 11—13, Academic Press, New York
15 Augustinsson, K.-B. (1955) Scand. J. Clin. Lab. Invest. 7, 284—290
16 Augustinsson, K.-B., Eriksson, H. and Faijersson, Y. (1978) Clin. Chim. Acta 89, 239—252
17 Wilson, I.B. and Alexander, J. (1962) J. Biol. Chem. 237, 1323—1326
18 Eriksson, H. and Augustinsson, K.-B. (1977) Acta Chem. Scand. B 31, 912—913

19 Hardegg, W. and Schaeffer, H. (1952) Arch. Ges. Physiol. 255, 136—153
20 Hardegg, W. (1958) Ph.D. Dissertation, University of Heidelberg, Institute of Physiology
21 Heilbronn, E. (1958) Acta Chem. Scand. 12, 1879—1880
22 Wilson, I.B., Bergmann, F. and Nachmansohn, D. (1950) J. Biol. Chem. 186, 781—790
23 Krupka, R.M. and Laidler, K.J. (1961) J Am. Chem. Soc. 83, 1445—1460
24 Balcom, J.K. and Fitch, W.M. (1970) J. Biol. Chem. 245, 1637—1647
25 Wilson, I.B. and Cabib, E. (1956) J. Am. Chem. Soc. 78, 202—207
26 Main, A.R., McKnelly, S.C. and Burgess-Miller, S.K. (1977) Biochem. J. 167, 367—376