

# Butyrylcholinesterase: Inhibition by Arsenite, Fluoride, and Other Ligands, Cooperativity in Binding

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## SUMMARY

Arsenite is a quasi-irreversible inhibitor of human serum butyrylcholinesterase with a dissociation constant of 0.129 mM at pH 7.4, 25°, 0.067 M phosphate,  $\mu = 0.17$  M. The inhibition process is second order with a rate constant of  $340 \text{ M}^{-1} \text{ min}^{-1}$ . The first order rate of dissociation,  $0.044 \text{ min}^{-1}$ , is unaffected by fluoride but is decreased by substrate. The binding of arsenite and fluoride, as determined by the effect of fluoride on the apparent arsenite-enzyme dissociation constant, is highly anticooperative and may be mutually exclusive. The fluoride-enzyme dissociation constant determined from these experiments is 0.90 mM. The binding of a number of other substances, such as dibucaine, is markedly anticooperative with arsenite binding. The binding of some of these substances is positively cooperative with fluoride binding. The effect can be large; procainamide binds 17 times more strongly in the presence of fluoride. Similarly, the mutual binding of benzoylcholine as substrate and fluoride is cooperative, 30-fold, butyrylthiocholine and fluoride, 21-fold, propionylthiocholine and fluoride, 8.3-fold, and acetylthiocholine and fluoride, only 1.8-fold.

## INTRODUCTION

Butyrylcholinesterase (EC 3.1.1.8) is a cholinesterase that hydrolyzes butyrylcholine > acetylcholine > benzoylcholine (1, 2). By contrast, acetylcholinesterase, which has a well defined function in the transmission of nerve impulses, barely hydrolyzes butyrylcholine (1, 3). Although the function of butyrylcholinesterase is not known, it is found in significant quantities in muscle and nervous tissue and in large quantities in human and other blood sera (3, 4), the enzyme from serum is a tetramer with a molecular weight of 340,000 (5-8).

Butyrylcholinesterase (9) and acetylcholinesterase (9-11) are inhibited by arsenite and also by fluoride (12-14). The inhibition of these two enzymes by arsenite and fluoride is remarkable because neither enzyme contains the structural features normally involved in the binding of these substances.

It turns out that arsenite dissociates only very slowly from acetylcholinesterase (10), and in the present work we found that arsenite also dissociates only very slowly from butyrylcholinesterase. Thus, it is appropriate to describe arsenite as a quasi-irreversible inhibitor of cholinesterase. This property makes it possible to react arsenite with butyrylcholinesterase in the absence of substrate and then measure the extent of reaction by assaying the inhibited enzyme with any convenient sub-

strate, such as acetylthiocholine. The association of arsenite with acetylcholinesterase to form inhibited enzyme is a relatively slow second order reaction. This reaction is itself inhibited by fluoride, and it turns out that the binding of arsenite and fluoride are highly anticooperative and may even be mutually exclusive (11).

The inhibition by arsenite is quite surprising because cholinesterase contains no lipoic acid or, at least in the case of the enzyme from electric eel, any free sulfhydryl groups (9, 15, 16). The enzyme activity is not sensitive to sulfhydryl reagents. The molecular basis for binding arsenite by acetylcholinesterase has recently been uncovered; it is the formation of an arsenite diester with two tyrosine residues (17). Thus, the dissociation constant is the equilibrium constant for the hydrolysis of the arsenite enzyme.

Inhibition of cholinesterase by fluoride is also surprising because cholinesterase contains no intrinsic metal ion nor does it need any metal ion for activity. The molecular basis for fluoride binding is not known nor is the effect of fluoride on the different kinetic steps known with certainty. For example, it is not clear whether fluoride inhibits deacetylation (13, 18).

It often is difficult to interpret the effect of reversible inhibitors on the hydrolysis of substrates because hydrolysis is a complicated process consisting of a number of steps. This is especially true for butyrylcholinesterase, since it does not follow Michaelis-Menten kinetics (19), but is complicated by substrate activation (20, 21) and

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substrate inhibition. It is easier to interpret the effect of a ligand on arsenite inhibition because this process is a simple equilibrium. It is easy to determine if there is any binding interaction between arsenite and a second ligand and, if there is, to evaluate the dissociation constant of the ligand from the free enzyme using this effect.

Moreover, any interaction between the binding of this ligand and the binding of fluoride by the free enzyme can be evaluated by observing the combined effect on the binding of arsenite. The amount of arsenite-enzyme, which is inactive, was measured by extensively diluting the enzyme solution in the assay; fluoride and the other ligands dissociate rapidly from the enzyme but the arsenite-enzyme dissociates (hydrolyzes) only slowly. Thus, arsenite inhibition not only is interesting in itself, but also can be used as a tool for measuring the binding properties of other ligands. No study of the kinetics of arsenite inhibition of butyrylcholinesterase or its nature of binding has yet been made.

It seemed important to study arsenite and fluoride inhibition of butyrylcholinesterase because it is surprising that these substances should be inhibitors of this enzyme. Moreover, this enzyme is plentiful in serum and has interesting and unexplained kinetic properties. Also, a comparison with acetylcholinesterase might reveal phenomena that are more difficult to detect and explain with either enzyme alone. It is also interesting that butyrylcholinesterase is much more easily inhibited by fluoride than is acetylcholinesterase. A few preliminary experiments with butyrylcholinesterase revealed the unexpected fact that the binding of fluoride and butyrylthiocholine is highly cooperative. In fact, it turns out that the much greater sensitivity to inhibition by fluoride of butyrylcholinesterase as compared to acetylcholinesterase arises not from stronger binding of fluoride by the free enzyme, but from a remarkable degree of cooperativity in the binding of fluoride and several substrates.

Finally, there are genetic variants of butyrylcholinesterase that are important to detect in patients who will undergo surgery, because these variants do not hydrolyze the muscle relaxant succinylcholine (22-24). These variants are also less readily inhibited by fluoride and dibucaine (22, 23).

## MATERIALS AND METHODS

**Enzyme activity.** Enzyme activity was measured in 67 mM phosphate, pH 7.4, 25° (standard buffer), using a number of substrates (25). Benzoylcholine hydrolysis was measured as the decrease in absorbance at 240 nm. Hydrolysis of the thiocholine substrates was measured as the increase in absorbance at 412 nm with 0.3 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (26).

**Enzyme.** Butyrylcholinesterase was purified from human serum essentially as described by Lockridge and La Du (6). The dibucaine and fluoride numbers (22, 23) indicated that our enzyme was the usual type, not a genetic variant. The specific activity was 90 units/mg using benzoylcholine as substrate.

**Determination of dissociation constants.** Butyrylcholinesterase (0.5 M) and arsenite were allowed to react and come to equilibrium in standard buffer with no substrate present. Seven concentrations of arsenite, A, in the range 0.05-0.80 mM were used. Five  $\mu$ l were then added to a cuvette containing 3 ml of buffer with 1 mM acetylthiocholine as substrate and assayed for less than 1 min. In the presence of this substrate, the dissociation of the arsenite-enzyme is less than 1%/min

so that the increase in enzyme activity that occurred during the assay period (<0.5% of the inhibited enzyme) could be neglected. The substrate is used not to influence the equilibrium but only to measure the amount of free enzyme.

The data were plotted according to

$$\frac{1-f}{f} = \frac{A}{K_A} \quad (1)$$

where  $f$  is the fractional activity, to yield a straight line with slope  $K_A^{-1}$ , where  $K_A$  is the dissociation constant of arsenite-enzyme.

The dissociation constant,  $K_B$ , for ligand B and free enzyme and its cooperativity factor with arsenite,  $\alpha$ , were determined by adding different concentrations of the ligand and using Eq. 1 to calculate the apparent dissociation constant of arsenite,  $K_{A(B)}$ , for each concentration of B. The data were plotted according to

$$\left(1 - \frac{K_A}{K_{A(B)}}\right)^{-1} = (1-\alpha)^{-1} + (1-\alpha)^{-1} \frac{K_B}{B} \quad (2)$$

(If  $\alpha = 1$ , A and B bind independently; if  $\alpha = 0$ , A and B cannot bind simultaneously; if  $\alpha < 1$ , A and B bind anticooperatively; if  $\alpha > 1$ , A and B bind cooperatively.  $\alpha$  is the ratio of  $K_A$  to the dissociation constant of A from the ternary complex  $E \cdot A \cdot B$  and also the ratio of  $K_B$  to the dissociation constant of B from the ternary complex. If B does not bind to either E or  $E \cdot A$ , it will appear that  $\alpha = 1$ .) It is not necessary to keep the concentration of arsenite constant in these experiments. Greater accuracy can be obtained by increasing the concentration of arsenite as the concentration of fluoride is increased so that  $f$  lies between 0.4 and 0.6.

The cooperativity factor,  $\beta$ , for the binding of B and fluoride, F, to the free enzyme was determined by the further addition of different concentrations of F and using Eq. 1 to calculate the apparent dissociation constant of arsenite,  $K_{A(B,F)}$ , for each concentration of F. The data were plotted according to

$$\frac{K_{A(B,F)}}{K_{A(B)}} = 1 + \frac{1 + \beta \frac{B}{K_B}}{1 + \frac{B}{K_B}} \cdot \frac{F}{K_F} \quad (3)$$

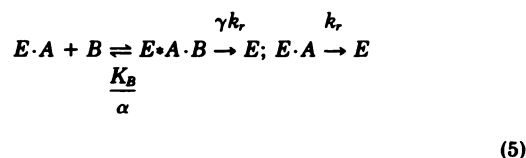
( $K_B$  and  $K_F$  were known from the application of Eq. 2. This equation applies when  $\alpha$  for F is near zero, as we found.  $\beta$  is the ratio of  $K_B$  to the dissociation constant for B from  $E \cdot F \cdot B$  and the ratio of  $K_F$  to the dissociation constant of F from  $E \cdot F \cdot B$ .)

Concentrated enzyme 0.5  $\mu$ M was used and the equilibrium solution was diluted  $\times 1000$  for measuring the free enzyme, so that neither B nor F affected the assay. Acetylthiocholine, 1 mM, was used as substrate.

**Determination of rate constants: Dissociation of arsenite-enzyme.** Enzyme that had been inhibited ~80% with arsenite was extensively diluted with standard buffer with or without added ligands and assayed at various times by further dilution in the assay medium. The first order rate constant was calculated from

$$\ln \frac{(1-f)}{(1-f_0)} = -k_r t \quad (4)$$

where  $f_0$  is the initial fractional enzyme activity. If ligand B is added in different concentrations the scheme yields



$$\left(1 - \frac{k_r(B)}{k_r}\right)^{-1} = (1-\gamma)^{-1} + (1-\gamma)^{-1} \frac{K_B}{\alpha} \cdot \frac{1}{B}$$

where  $k_{r(B)}$  is the apparent first order rate constant determined from Eq. 4 and measures the effect of bound ligand on the rate of dissociation of arsenite from the enzyme.

**Formation of arsenite-enzyme.** Three concentrations of arsenite were added to a concentrated solution of enzyme in standard buffer and a sample was assayed at various times. The second order rate constant was determined using the pseudo-first order equation for a reversible reaction.

$$(1 - f_{eq}) \ln \frac{(f - f_{eq})}{(1 - f_{eq})} = -k_i A t \quad (6)$$

using concentrations of  $A$  for which  $f_{eq}$ , the fractional enzyme activity at equilibrium, was small.

## RESULTS

**Arsenite inhibition.** The inhibition of butyrylcholinesterase by arsenite is a second order reaction with the rate constant,  $k_i = 340 \text{ M}^{-1} \text{ min}^{-1}$ . The first order constant for the dissociation (hydrolysis) of the arsenite enzyme is  $k_r = 0.044 \text{ min}^{-1}$ . These two constants yield the equilibrium (dissociation) constant,  $K_A = 0.129 \text{ mM}$ . The dissociation constant was also determined by measuring  $f$  at equilibrium and plotting  $(1 - f)/f$  versus arsenite for seven concentrations in the range 0.04–0.8 mM to yield  $K_A = 0.131 \text{ mM}$  (Fig. 1). The points fell precisely on a straight line with no indication that a higher power of arsenite might be involved or that more than one constant might be involved. Thus, it appears that the binding of one arsenite per subunit is sufficient to completely inhibit the enzyme. Also the subunits of the tetramer appear to behave independently and identically, at least with respect to arsenite binding.

Although 1 mM fluoride inhibited the formation of arsenite-enzyme, even 0.05 M (phosphate reduced to keep  $\mu = 0.17 \text{ M}$ ) had no effect on the rate of dissociation. We studied the effect of fluoride on the equilibrium between

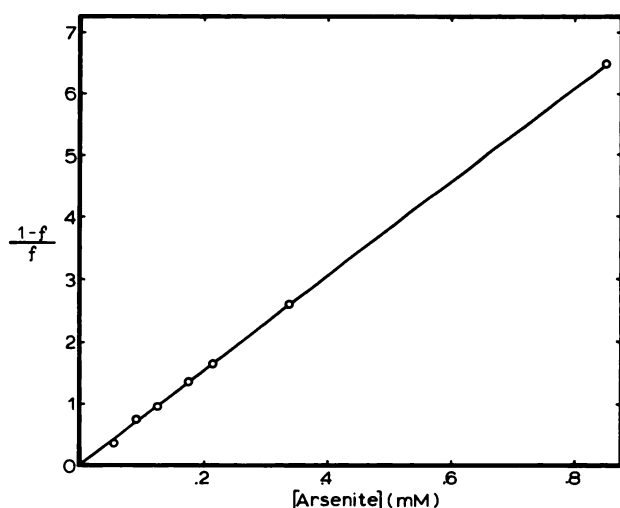


FIG. 1. The equilibrium between butyrylcholinesterase and arsenite according to Eq. 1

$f$  is the fraction of active enzyme and  $1 - f$  is the fraction of inactive enzyme (arsenite-enzyme). The slope is  $K_A^{-1}$ .  $K_A = 0.131 \text{ mM}$ . Concentrated enzyme was incubated with arsenite in standard buffer and allowed to come to equilibrium. Samples were then extensively diluted with standard buffer and enzyme activity was measured with 1 mM acetylthiocholine.

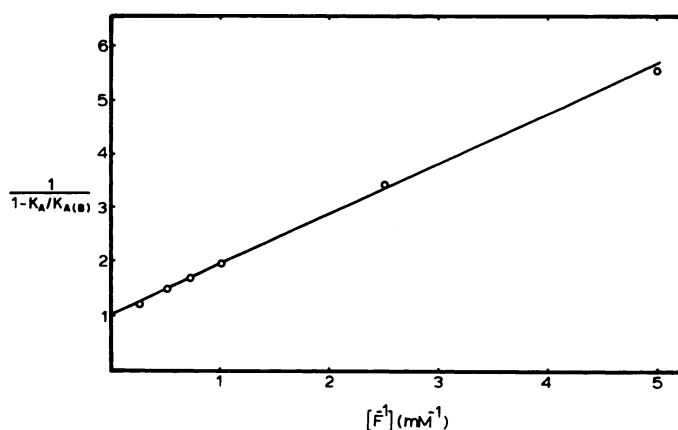


FIG. 2. The equilibrium between butyrylcholinesterase, arsenite, and fluoride according to Eq. 2

$\alpha \approx 0$ ,  $K_F = 0.9 \text{ mM}$ . The concentration of arsenite was adjusted to keep  $f$  between 0.4 and 0.6 for accuracy. The solution was diluted  $\times 1000$  in the assay. Acetylthiocholine was used as substrate because it is only slightly cooperative in binding with fluoride.

butyrylcholinesterase and arsenite using Eq. 2 (Fig. 2). The apparent dissociation constant increased with increasing concentrations of fluoride, which indicates that fluoride binds more strongly to the free enzyme than to the arsenite enzyme. Since the intercept is very close to 1.0,  $\alpha$  must be very small, perhaps zero. Thus, fluoride and arsenite binding is highly anticooperative and may be mutually exclusive. This result confirms the observation that fluoride inhibits the rate of formation of arsenite-enzyme but has no effect on its rate of dissociation. The value of  $K_F$  from Fig. 1 is 0.90 mM.

The compound 2-PAM<sup>1</sup> has a marked effect on arsenite inhibition; at 0.1 mM concentration, the  $k_i$  is increased over 5-fold to  $1830 \text{ M}^{-1} \text{ min}^{-1}$  and  $k_r$  is increased over 3-fold to  $0.145 \text{ min}^{-1}$ . We investigated the effect of 2-PAM on the equilibrium between arsenite and the enzyme using Eq. 2, which showed that there was considerable cooperativity in the binding of 2-PAM and arsenite ( $\alpha = 2.6$ ) (Table 1). This result was confirmed by applying Eq. 5 to measurements of the dissociation rate of arsenite-enzyme in different concentrations of 2-PAM. The value of  $\gamma$  was 13 and of  $\alpha$  was 3.0. 2-PAM also enhances the rates of association and dissociation of arsenite and acetylcholinesterase (10).

All other ligands that were studied were anticooperative in binding with arsenite to varying degree (Table 1), and all decreased the rate of formation of arsenite-enzyme. Procainamide at 0.1 mM, for example, decreased the apparent second order inhibition rate constant for arsenite to  $23 \text{ M}^{-1} \text{ min}^{-1}$  and decreased the dissociation rate constant to  $0.0075 \text{ min}^{-1}$ . But we did not investigate the effect on rates in detail. Acetylthiocholine at 1 mM, the substrate used in these assays, reduced the dissociation rate constant to  $0.008 \text{ min}^{-1}$ .

**Cooperativity in the binding of fluoride and other ligands.** This question was investigated by observing the combined effect of fluoride and other ligands on the apparent equilibrium constant for the dissociation of arsenite in accordance with Eq. 3. In all cases except

<sup>1</sup> The abbreviation used is: 2-PAM, pyridine-2-aldoxime methiodide.

TABLE 1

The dissociation constant of various inhibitors and the cooperativity factors,  $\alpha$  and  $\beta$ , for their interaction with arsenite and fluoride

The dissociation constants and  $\alpha$  and  $\beta$  values were determined by the compounds' effect on arsenite inhibition, with the exception of the substrates. For substrates, the value given is the  $K_m$ .  $\alpha$ , cooperativity with arsenite;  $\beta$ , cooperativity with fluoride.

Compound	Dissociation constant with butyrylcholinesterase $\mu M$	$\alpha$	$\beta$
Choline	1200	0.50	0.85
<i>N,N'</i> -Diethylethylenediamine- <i>N</i> -acetamide	1200	0.55	1.4
Acetylthiocholine	62		1.8 <sup>a</sup>
Methyltriethylammonium iodide	1100	0.54	1.7
<i>N,N'</i> -Diethylethylenediamine- <i>N</i> -butyramide	180	0.41	5.5
Propionylthiocholine	29		8.3 <sup>a</sup>
2-Pyridiniumaldoxime methiodide	1300	2.6	9.6
Dibucaine	1.3	0.21	13
Procainamide	6.8	0.20	17
Butyrylthiocholine	23		21 <sup>a</sup>
Benzoylcholine	6.8		30 <sup>a</sup>
Fluoride	900	0	

<sup>a</sup> $\beta$  determined from the concentration of fluoride that inhibits the hydrolysis of the compound 50% at the substrate concentration that shows the greatest inhibition.

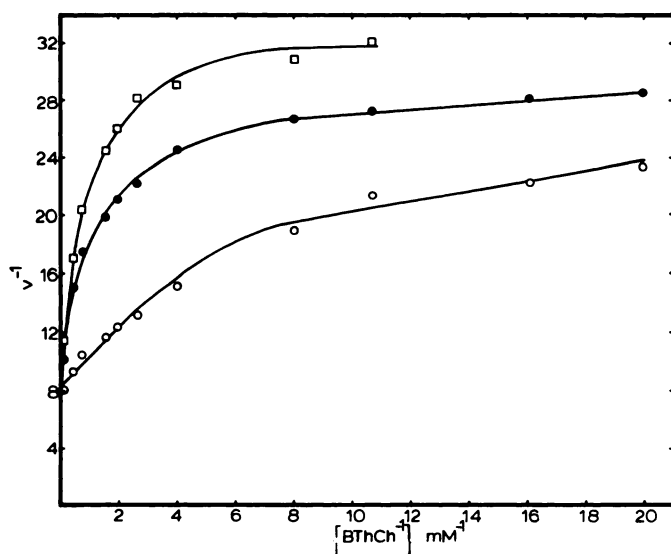


FIG. 3. Lineweaver-Burk plot, butyrylcholinesterase (BThCh) as substrate

Enzyme concentration:  $\sim 0.1$  nM; no F (O);  $31 \mu M$  F (●);  $62 \mu M$  F (□). Note substrate activation. The velocity is in relative units.

with choline, there was a small to large degree of cooperativity,  $\beta > 1$  (Table 1). In the series of *N*-acyl-*N,N'*-diethylethylenediamine derivatives, there is a marked increase in cooperativity as the acyl group changes from acetyl to butyryl to *p*-aminobenzoyl, an increase that is paralleled in the thiocholine (choline) substrate series.

**Cooperativity in the binding of fluoride and substrates.** Concentrations of fluoride that are far less than  $0.90$  mM, the  $K_F$  for fluoride and the free enzyme, are sufficient to inhibit the hydrolysis of all the substrates except acetylthiocholine. Thus, it is immediately apparent that there is substantial cooperativity in the binding of fluoride and substrates (Figs. 3–7).

We were able to discount the possibility that fluoride binds to two sites, one that is detected by its effect on arsenite inhibition and the other by its effect on substrate

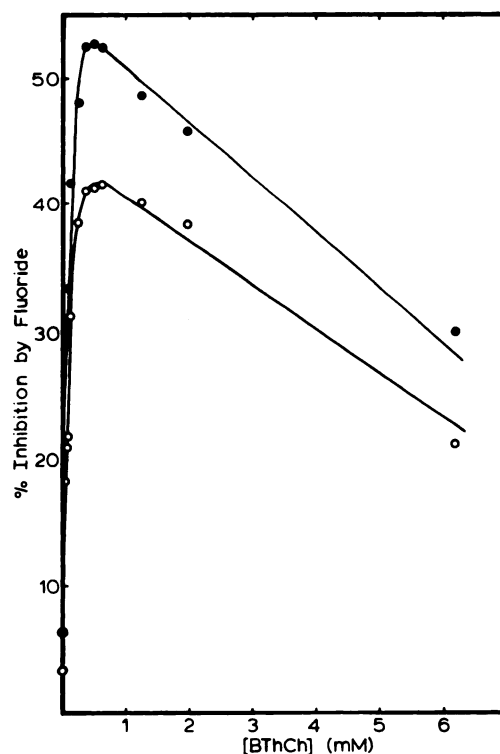


FIG. 4. Fluoride inhibition as a function of butyrylthiocholine (BThCh) concentration

Data as in Fig. 3. The points at zero substrate concentration are fractions of enzyme with bound fluoride calculated from  $K_F = 0.9$  mM.  $31 \mu M$  F (O);  $62 \mu M$  F (●).

hydrolysis. First there is only a 1.8-fold difference in the concentrations of fluoride needed to inhibit arsenite inhibition and acetylthiocholine hydrolysis. Also, the degree of cooperativity is determined by the acyl group of substrates much the way that the acyl group of the amide inhibitors determines the degree of cooperativity with fluoride as determined by the effect on arsenite inhibition. Procainamide, which strongly resembles the sub-

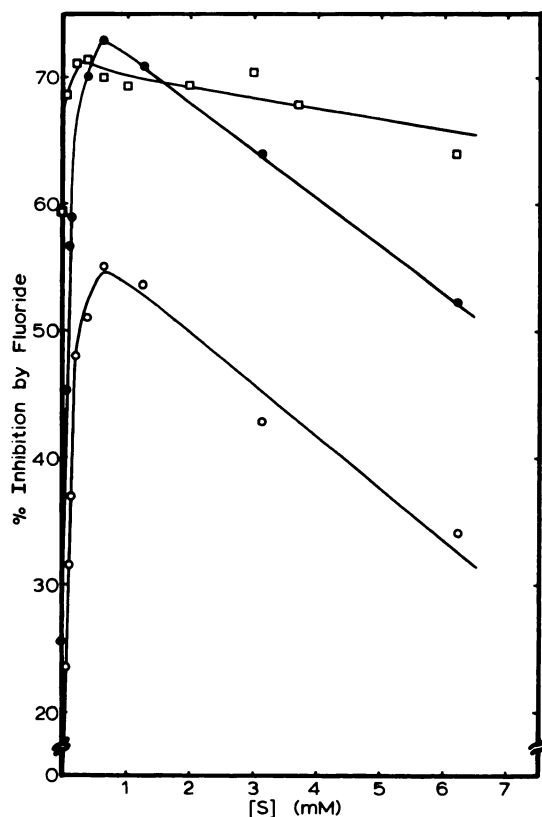


FIG. 5. Fluoride inhibition as a function of propionylthiocholine and acetylthiocholine concentrations

The points at zero substrate concentration are the fractions of enzyme with bound fluoride calculated from  $K_F = 0.90 \mu\text{M}$ . The value, 12%, for propionylthiocholine and 0.311 mM fluoride is not shown. Propionylthiocholine, 0.125 mM fluoride (O); propionylthiocholine, 0.311 mM fluoride (●); acetylthiocholine, 1.25 mM fluoride (□).

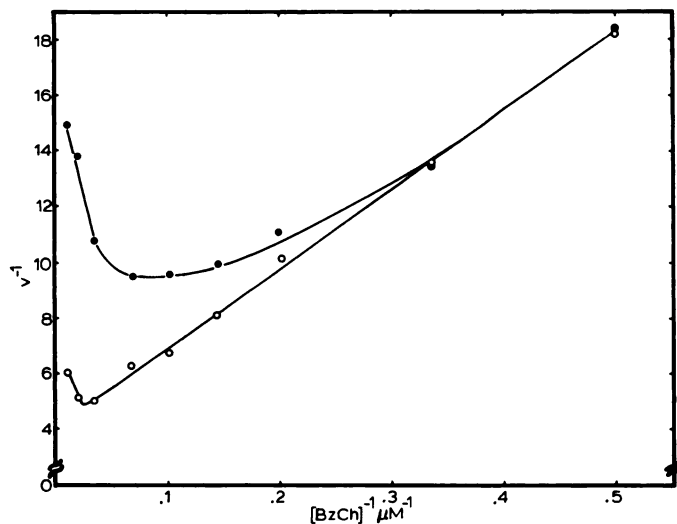


FIG. 6. Lineweaver-Burk plot, benzoylcholine (BzCh) as substrate. No fluoride (O); 50  $\mu\text{M}$  fluoride (●). Enzyme concentration, 0.3 mM. The sharp upturn at high  $S$  with fluoride present cannot be due to simple cooperativity in the binding of fluoride and substrate with the enzyme. The velocity is in relative units.

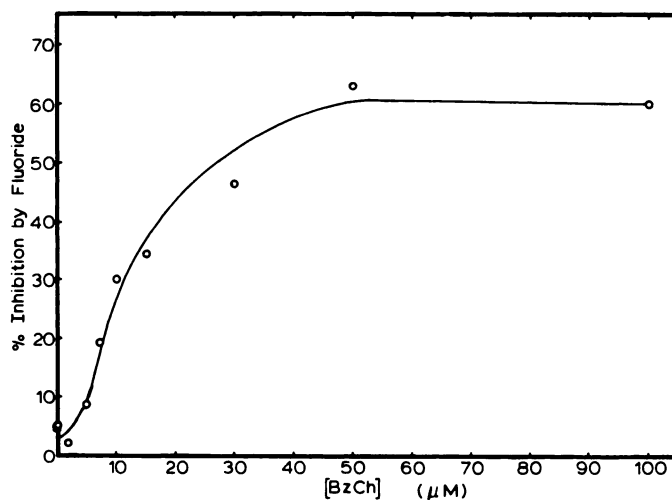


FIG. 7. Fluoride inhibition as a function of benzoylcholine (BzCh) concentration

The point at zero substrate concentration is the fraction of enzyme with bound fluoride calculated from  $K_F = 0.90 \text{ mM}$ . Data as in Fig. 6.

strate, seems to bind at only one site, the active site, for it has the same dissociation constant determined from the competitive inhibition of benzoylcholine hydrolysis ( $7.5 \mu\text{M}$ ) as determined from the effect on arsenite inhibition. Thus, since procainamide is cooperative with fluoride binding, it makes good sense that benzoylcholine should be cooperative with fluoride binding and there is no reason to invent a second fluoride-binding site. Indeed, as mentioned in the Introduction, even one fluoride-binding site is surprising.

Finally, we measured fluoride and procainamide cooperativity in inhibiting acetylthiocholine hydrolysis using a method described by Yonetani and Theorell (27). The substrate concentration, 0.2 mM, was well below the concentration where substrate activation occurs. We found  $\beta = 19$  in agreement with  $\beta = 17$  determined from the arsenite equilibrium.

The cooperativity in the binding of fluoride and substrates was confirmed by the observation that the per cent inhibition increased as the substrate concentration was increased. In the case of propionylthiocholine and butyrylthiocholine and to a much lesser extent with acetylthiocholine, the per cent inhibition ultimately declined as the concentration of substrate pushed far into the range of substrate activation. On the low substrate side of maximum inhibition with 0.06 mM butyrylthiocholine, the inhibition with respect to varying fluoride concentration was normal, i.e., the same *apparent*  $K_F = 0.120 \text{ mM}$  was obtained with four fluoride concentrations in the range 0.03–0.2 mM, calculated from  $f = (1 + F/K_F)^{-1}$ .

Fluoride inhibition of benzoylcholine hydrolysis in our range of substrate concentrations,  $<0.1 \text{ mM}$  (limited by the optical density), may be qualitatively different from other substrates because fluoride introduces strong substrate inhibition (Figs. 6 and 7).

## DISCUSSION

Although there are distinct quantitative differences, butyrylcholinesterase from human serum behaves very

similarly in its reactions with arsenite as acetylcholinesterase from electric eel. Both are inhibited in a second order reaction and both arsenite-enzymes regain activity slowly on dilution. The two tyrosine residues that are esterified in the reaction with arsenite and their spacial relationship must be similar in both enzymes. The structural features that give rise to a change in conformation when 2-PAM is bound must also be similar since both enzymes react with arsenite much more rapidly and both arsenite enzymes dissociate much more rapidly in the presence of 2-PAM. Fluoride binding and the extreme anticooperativity of fluoride and arsenite binding seem to involve fundamental and special structural features of both enzymes, for these are striking phenomena that are not related to the enzyme mechanism per se and are not found with other serine esterases.

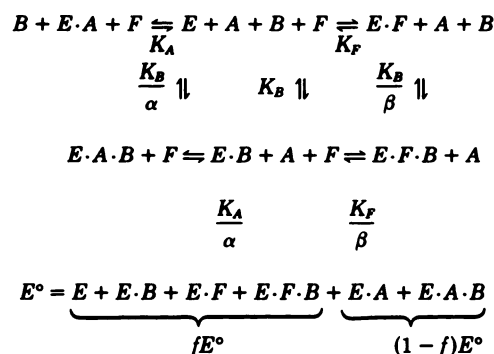
Although the arsenite enzyme is catalytically inactive, it still binds substrates as judged by the fact that the arsenite-enzyme binds a number of substances that strongly resemble the substrates. Also, we found that the arsenite-enzyme regained activity more slowly in the presence of substrate. However, we judge that the arsenite-enzyme is not acylated by the bound substrate because the arsenite-enzyme of acetylcholinesterase is not carbamylated (10) by the potent carbamylating inhibitor *N*-methyl-7-dimethylcarbamoylquinolinium iodide (28, 29).

The arsenite equilibrium emerged in this study as a means of measuring the binding of other ligands and their mutual interactions. The arsenite method has the advantages that the measurements are easily made and the results are readily interpreted because an equilibrium rather than a rate process is being studied.

Although both enzymes are inhibited by fluoride and bind fluoride with comparable strength, the marked cooperativity in the binding of fluoride and substrates with butyrylcholinesterase makes this enzyme more sensitive to fluoride and makes it appear that it binds fluoride more strongly. The extent of cooperativity of fluoride and substrate binding depends strongly on the acyl group, a dependence that is also observed with the nonsubstrate *N*-acyl derivatives of *N*,*N*'-diethylethylenediamine; butyryl, propionyl, and benzoyl derivatives are highly cooperative but acetyl derivatives are only slightly cooperative.

Although there is no previous report of marked cooperativity in the binding of fluoride and other ligands, there are interesting cases of cooperative binding between two ligands with acetylcholinesterase; the binding of 2-PAM is highly cooperative with the binding of  $\alpha$ -naphthol and  $\alpha$ -naphthol acetate (30). There is also an interesting report that Flaxedil increases the potency of fluoride as a nucleophile in reactivating organophosphate-inhibited acetylcholinesterase (31).

The electrostatic attraction between fluoride and cationic ammonium ions might be offered as a simple explanation for cooperativity in binding but the situation must be more complicated because there is very little cooperativity with substituted amines. Moreover, it is the nature of the acyl group attached to cationic amines that largely determines the degree of cooperativity.



Aside from differences in substrate specificity, these two cholinesterases differ in two unusual ways. Acetylcholinesterase shows substrate inhibition whereas butyrylcholinesterase shows both substrate activation and inhibition. Although only slight cooperativity in the binding of fluoride and one or two ligands has been noted with acetylcholinesterase (18), this phenomenon is a prominent and remarkable feature of butyrylcholinesterase.

There are also a number of unusual features that are common to both enzymes but not observed with other serine esterases. These features are arsenite inhibition of a non-sulfhydryl-enzyme, fluoride inhibition of a non-metallo-enzyme, and the kinetic response to 2-PAM in the reaction with arsenite. These features suggest a common ancestral gene. It is interesting that these features should have been retained in both enzymes over the course of evolution, especially since there is no known function of serum butyrylcholinesterase that might have militated against mutations.

#### APPENDIX

*Derivation of equations.* The equations for the combined effect of fluoride *F* and a ligand *B* on the equilibrium between arsenite *A* and the enzyme *E* was derived from the scheme above, where  $E^\circ$  is the total enzyme and *f* is the fractional enzyme activity after extensive dilution. *F* and *B* dissociate rapidly on dilution but *A* as a quasi-irreversible inhibitor dissociates very slowly. The apparent dissociation constant for *A* when *B* and *F* are present is

$$K_{A(B,F)} = \frac{f \cdot A}{1-f} = K_A \frac{\left(1 + \frac{B}{K_B} + \frac{F}{K_F} + \beta \frac{B}{K_B} \frac{F}{K_F}\right)}{\left(1 + \frac{\alpha B}{K_B}\right)}$$

when  $F = 0$

$$K_{A(B)} = K_A \frac{1 + \frac{B}{K_B}}{1 + \frac{\alpha B}{K_B}}$$

which can be put in the linear form, as in the text equation,

$$\left(1 - \frac{K_A}{K_{A(B)}}\right)^{-1} = (1 - \alpha)^{-1} + (1 - \alpha)^{-1} \frac{K_B}{B} \quad (2)$$

( $B$  can be  $F$ ) from which  $\alpha$  and  $K_B$  may be determined. Also, as in the text equation,

$$\frac{K_{A(B,F)}}{K_{A(B)}} = 1 + \frac{1 + \frac{\beta B}{K_B} \cdot F}{1 + \frac{B}{K_B}} \quad (3)$$

from which  $\beta$  may be determined since  $K_B$  and  $K_F$  are known from Eq. 2. For fluoride,  $\alpha$  is very small; therefore, enzyme complexes containing both  $F$  and  $A$  were ignored in the derivation of Eq. 3. Eq. 2 can be used for  $F$  (instead of  $B$ ); then enzyme complexes of  $F$  and  $A$  are not ignored and  $\alpha$  and  $K_F$  can be determined.

If  $\alpha = 1$  the binding of  $B$  is independent of  $A$

< 1 the binding of  $B$  is anticooperative with  $A$

= 0 the binding of  $B$  is mutually exclusive with  $A$

> 1 the binding of  $B$  is cooperative with  $A$

If  $\beta = 1$  the binding of  $B$  is independent of  $F$

< 1 the binding of  $B$  is anticooperative with  $F$

= 0 the binding of  $B$  is mutually exclusive with  $F$

> 1 the binding of  $B$  is cooperative with  $F$ .

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