We investigated the target sites of three inhibitory monoclonal antibodies on *Electrophorus* acetylcholinesterase (AChE). Previous studies showed that Elec-403 and Elec-410 are directed to overlapping but distinct epitopes in the peripheral site, at the entrance of the catalytic gorge, whereas Elec-408 binds to a different region. Using *Electrophorus/rat* AChE chimeras, we identified surface residues that differed between sensitive and insensitive AChEs: the replacement of a single *Electrophorus* residue by its rat homolog was able to abolish binding and inhibition, for each antibody. Reciprocally, binding and inhibition by Elec-403 and by Elec-410 could be referred to rat AChE by the reverse mutation. Elec-410 appears to bind to one side of the active gorge, whereas Elec-403 covers its opening, explaining why the AChE-Elec-410 complex reacts faster than the AChE-Elec-403 or AChE-fasciculin complexes with two active site inhibitors, m-(N,N,N-trimethylammonio)trifluoro-acetophenone and echothiophate. Elec-408 binds to the region of the putative “back door,” distant from the peripheral site, and does not interfere with the access of inhibitors to the active site. The binding of an antibody to this novel regulatory site may inhibit the enzyme by blocking the back door or by inducing a conformational distortion within the active site.

The catalytic mechanism of acetylcholinesterase (AChE) is of considerable theoretical and practical interest. Anti-cholinesterase inhibitors are used as pesticides; as therapeutic agents in glaucoma, myasthenia gravis, and Alzheimer’s disease; and, unfortunately, as nerve gases in chemical warfare. Apart from its vital role in cholinergic transmission, AChE offers a considerable challenge for the understanding of its catalytic efficiency. This enzyme hydrolyzes acetylcholine and similar esters at a very high rate, approaching the upper limit allowed by diffusion of the substrate (1, 2). However, the three-dimensional structure of AChE, as determined by x-ray crystallography, revealed that its active site can apparently be reached only through a deep and narrow “catalytic gorge” (3). This organization would predict that the entrance of a substrate molecule into the active site and the exit of products might create a traffic limitation to the catalytic turnover rate. On the basis of molecular dynamics, it has therefore been proposed that products could leave the active site through a “back door,” transitorily opened by concerted movements within the protein (4, 5). However, it has not yet been possible to directly demonstrate the reality of such movements (6).

Inhibitors of AChE act on two target sites on the enzyme, the active site and the peripheral site. Inhibitors directed to the active site prevent the binding of a substrate molecule, or its hydrolysis, either by occupying the site with a high affinity (edrophonium and tacrine) or by reacting irreversibly with the catalytic serine (organophosphates and carbamates). The peripheral site consists of a less well defined area, located at the entrance of the catalytic gorge. Inhibitors that bind to this site include small molecules, such as propidium, curare, gallamine, and peptide toxins from Mamba venoms, the fasciculins (7, 8). Bis-quaternary inhibitors, e.g. decamethonium and BW284C51, simultaneously bind to the active and peripheral sites, thus occupying the entire catalytic gorge. The mechanism by which peripheral site inhibitors block the catalytic activity of AChE is currently the subject of a vivid debate. Inhibition can be explained either by steric blockade of the catalytic gorge entrance (9) or by an allosteric mechanism (10). The three-dimensional structures of AChE-fasciculin complexes show that the entrance of the gorge is blocked by a loop of the toxin, but it is more difficult to imagine that small molecules act in this way (11, 12). It has been proposed that occupancy of the peripheral site induces a movement of the Ω loop (67-95), allosterically modifying the orientation of a tryptophan residue, Trp-84, which serves as the choline binding site (13, 14). According to this view, the binding of a substrate molecule at the peripheral site might explain excess substrate inhibition, a characteristic phenomenon of AChE kinetics (16, 17).

New insights on these questions can be provided by analysis of AChE inhibition by antibodies. A number of inhibitory antibodies have been obtained against AChEs from rabbit, fetal bovine serum, and human. Some of these antibodies behave as competitive inhibitors and either prevent or slow down the reaction of the enzyme by organophosphate inhibitors (18–20). Other antibodies act as noncompetitive inhibitors, presumably through an allosteric mechanism (21). However, the target sites of these antibodies on the enzyme have not been defined. We attempted to approach this question in the case of inhibitory monoclonal antibodies directed against *Electrophorus*.
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AChE. Three distinct monoclonal antibodies, binding to distinct epitopes, have been analyzed for their specificity toward AChE of different species and for their interference with the binding of conventional inhibitors (22). Two inhibitory antibodies, Elec-403 and Elec-410, were mutually exclusive and competitive with peripheral site inhibitors, indicating that they bind near the entrance of the gorge. The third inhibitory antibody, Elec-408, showed no competition with peripheral site ligands or with the other two antibodies, and its effects were additive. Thus, Elec-403 and Elec-410 appear to bind to distinct but overlapping epitopes at the opening of the catalytic gorge, whereas Elec-408 may define a novel regulatory site on the enzyme.

We recently cloned and expressed Electrophorus AChE, so that it became possible to explore the target sites of these antibodies by site-directed mutagenesis (23). In this work, we took advantage of the species specificity of these antibodies. As a first step, we created chimeras between Electrophorus AChE and rat AChE, which is not recognized by the antibodies. An analysis of their inhibition with the antibodies allowed us to define regions that are necessary for antibody binding. As a second step, we designed point mutations that abolished the inhibition of the Electrophorus enzyme by the three antibodies. We thus confirmed that Elec-403 and Elec-410 are targeted to overlapping but not identical parts of the peripheral site, and we showed that Elec-408 binds to a novel regulatory site of AChE, near the putative back door.

**MATERIALS AND METHODS**

**Reagents**—Reagents for biochemistry were purchased from Prolabo (Paris, France) or from Sigma. Products, enzymes and kits for molecular biology were from Ambion, Bioslabs, Invitrogen, Macherey-Nagel, American Pharmacia Biotech, Promega, and United States Biochemical Corp. Oligonucleotides were synthesized by Eurobio (Paris, France).

**Construction of Chimeras**—Standard methods were used to construct the fusion proteins (24). Site-directed mutagenesis was performed according to the method of Kunkel (25) in pCDNA3 (Invitrogen). Mutagenic oligonucleotides were used as primers for unmodified T7 DNA polymerase form II (New England Biolabs, Ozyme, France) on single strands that were produced according to Blonder and Thillet (26).

A stop codon was introduced immediately after the catalytic domain, replacing the first residue of the WAT domain (27), in the cDNAs encoding rat and Electrophorus AChEs, which is not recognized by the antibodies. An analysis of their inhibition with the antibodies allowed us to define regions that are necessary for antibody binding. As a second step, we designed point mutations that abolished the inhibition of the Electrophorus enzyme by the three antibodies. We thus confirmed that Elec-403 and Elec-410 are targeted to overlapping but not identical parts of the peripheral site, and we showed that Elec-408 binds to a novel regulatory site of AChE, near the putative back door.

**Catalytic Parameters**—For the determination of kinetic parameters, nonpurified, secreted AChE was diluted to 0.5 Ellman units (EU)/mL in 1 mM phosphate buffer, pH 7.4, 0.1% bovine serum albumin. One EU corresponds to an increase in absorbance of 1 per min, with a path length of 1 cm. For the determination of the values of $K_m$ and $V_{max}$ assays were performed at 25 °C, 0.5 mM phosphate buffer, pH 7.4, 5.5'-dithiobis(nitrobenzoic acid), 0.1% bovine serum albumin, and a concentration of acetylthiocholine iodide ranging from 0.05 to 60 mM (15 dilutions in duplicate). $K_m$ and $V_{max}$ were defined by the Haldane equation, fitted with the Kaleidagraph software, as described previously (29). Catalytic turnover values ($k_{cat}$) were determined by titrating the active sites of AChE with an irreversible inhibitor O-ethyl S-(2-diisopropylamino-ethyl)methylphosphonothioate (30). Briefly, 100 μL of the enzymatic sample (containing 0.5 EU/mL) were incubated with 100 μL of the dilution of O-ethyl S-(2-diisopropylamino-ethyl)-methylphosphonothioate (11 dilutions in duplicate ranging from 0.05 to 0.5 mM) for 18 h at 4 °C. The residual activity was measured by adding 100 μL of 3-fold concentrated Ellman’s reagent at 20-s intervals, over a period of 3 min. The values given in Table I correspond to the ratios of $k_{cat}$ for each enzyme to that of Electrophorus AChE.

**Inhibition of AChE by Monoclonal Antibodies or Fasciculin**—A 90-μL aliquot of enzyme (0.2 EU/mL) was incubated with 90 μL of Fab' solutions (11 dilutions, ranging from 2.10^-13 to 0.2 mM) or with 90 μL of fasciculin-2 solutions (11 dilutions ranging from 10^-14 to 10^-12 M) in duplicate to ensure equilibrium conditions. After equilibration to room temperature, the residual activity was measured with 20 μL of 10-fold concentrated Ellman’s reagent.

**Inhibition of AChE by Chemical Inhibitors**—A 100-μL aliquot of enzyme (0.5 EU/mL) was incubated with 100 μL of inhibition solution (15 dilutions, ranging from 10^-9 to 10^-3 M) for propidium, from 3.10^-10 to 10^-5 M for edrophonium, and from 3.10^-11 to 10^-8 M for BW288C51) in duplicate for 2 h at 4 °C. The residual activity was measured with 100 μL of 3-fold concentrated Ellman’s reagent by monitoring A_{414 nm} at 20-s intervals, over a period of 3 min.

**Kinetics of Inhibition with m-(N,N,N-Trimethylammonium)trifluoroacetophenone (TMTFA) and Echotoxidep**—The purified tetrameric form of Electrophorus AChE (obtained by treatment of asymmetric forms with trypsin, as described previously (31)) and its complexes, obtained in the presence of an excess of each inhibitory monoclonal antibody or of fasciculin were diluted in 20 mM Tris-HCl, pH 7.4, 0.1% bovine serum albumin, to approximately the same level of activity (1–2 EU/mL). Aliquots (160 μL) in triplicate, were incubated with 20 μL of buffer or with 20 μL of TMTFA or echotoxidep (final concentration, 10^-6 M) for various times (from 5 min to 1 h). We then added 20 μL of 10-fold concentrated Ellman’s reagent, and monitored the remaining activity for 3 min. The plots represent the residual activity as a function of incubation time with the inhibitor and were fitted by a bi-exponential function, from which we obtained pseudo-first-order constants ($k_{cat}$).

**Binding Assays**—The test was performed in microtiter plates (96-well deep-well Immunononc monoclonal plates) coated with anti-mouse IgG antibodies, as described previously (31). 200 μL of the dilutions of Fab' (11 dilutions ranging from 2.10^-13 to 2.10^-8 M) in duplicate, were incubated with 90 μL of monoclonal or equivalent fragments (Fab') of the three monoclonal antibodies Elec-403, Elec-408, and Elec-410 were incubated for 3–4 h at room temperature. After extensive washing, 200 μL of AChE (0.1 EU/mL) were incubated overnight with immobilized Fab' in each well at 4 °C. The plates were then washed, prior to addition of 200 μL of Ellman’s reagent to each well.

**RESULTS**

**Inhibition of Electrophorus AChE by Elec-403, Elec-408 and Elec-410: Do the Antibodies Block Access to the Active Site?**—In the presence of an excess of inhibitory antibody, Electrophorus AChE still shows a residual activity: 1% with Elec-403, 7% with Elec-410, and 30% with Elec-408 (22). This reflects an intrinsic activity of the AChE-antibody complexes, as shown, for example, by the fact that it could be further reduced by addition of a second, compatible inhibitory antibody (22). The presence of a measurable residual activity with antibody indicates that the substrate, acetylthiocholine, is still able to reach the active site in the complex, but this access may be severely restricted. In order to obtain an indication about a possible blockade of entry into the active site by the monoclonal antibodies, we analyzed the effect of two active site inhibitors on the residual activity of AChE-antibody complexes. We used echotoxidep, a positively

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charged organophosphate inhibitor containing a choline moiety, and TMTFA, an analog of the tetrahedral intermediate, possessing a very high affinity for the active site (32, 33).

Fig. 1 shows the kinetics of AChE inhibition with echothiophate and TMTFA. We used comparable activities of AChE alone, and of its complexes with a noninhibitory antibody, Elec-106, with each of the three inhibitory antibodies and with fasciculin (−−−), at saturating concentrations. At the indicated times, residual activity was measured by determining the initial rate of hydrolysis of ATC. From the nonlinear curve, we calculated the pseudo-first-order rate constant, $k_{\text{obs}}$. The effect of fasciculin on inhibition by echothiophate and TMTFA was determined in a different experiment, by comparison with free AChE and the Elec-403-AChE complex.

 echoing the rates of inhibition by both echothiophate and TMTFA. In this case, the curves could be fitted with two exponentials, one of which showed the same rate as the free enzyme: this probably corresponds to a small proportion of enzyme that was not bound by the antibody and represents a relatively high proportion of residual activity, because of the very low activity of the AChE-Elec-403 complex. The slowly reacting component, representing the AChE-Elec-403 complex, shows a rate of 0.0003 min$^{-1}$ with TMTFA (0.02 min$^{-1}$ for the free enzyme), and 0.001 min$^{-1}$ with echothiophate (0.015 min$^{-1}$ for the free enzyme). The differences observed between the Elec-403, Elec-408, and Elec-410 antibodies show that they do not interfere with AChE in the same way, in agreement with the fact that they bind to distinct epitopes.

**Chimeras of Electrophorus/Rat AChEs**—In order to obtain information about the target sites of the three inhibitory antibodies, we prepared Electrophorus/rat chimeric constructs, by introducing restriction sites in the coding sequences of the two enzymes, without modifying the encoded peptide sequences. Chimeras El/165/Rt, El/266/Rt and El/337/Rt contain increasingly longer N-terminal regions of Electrophorus AChE, fused with the complementary rat peptide regions, beyond residues 165, 266, and 337. We introduced a stop codon at the end of the catalytic domain so that these constructs produced soluble AChE monomers (34, 35). The distribution of Electrophorus and rat regions on the surface of the catalytic subunit is schematically illustrated in Fig. 2. The three chimeras were active, and their catalytic parameters, as well as their sensitivity to classical inhibitors, show that they do not markedly differ from the parent natural AChEs (Table I).

We analyzed the sensitivity of the Electrophorus, rat and chimeric enzymes to monovalent fragments F(ab)$^\prime$ obtained from the three inhibitory monoclonal antibodies and to fasciculin (Fig. 3). The chimeras were inhibited by fasciculin, in agreement with the fact that both parent enzymes are sensitive to this toxin (Fig. 3A). However, it is surprising that the El/337/Rt chimera, which contains the largest Electrophorus peptide segment, is more sensitive to inhibition by fasciculin than the rat enzyme.

Elec-410, which also binds to the peripheral site, was found to inhibit the three chimeras, El/165/Rt, El/266/Rt, and El/337/Rt (Fig. 3B). This showed that the N-terminal fragment preceding position 165, which constitutes the only distinct Electrophorus contribution in chimera El/165/Rt, must contain residues that are sufficient for the binding of Elec-410. Elec-410 differs from Elec-403 because it inhibits Bungarus AChE, although less strongly than Electrophorus AChE (22). It has no effect on Torpedo or mammalian AChEs. We thus looked for residues in the N-terminal segment that would be exposed, involved in the contact with fasciculin and common to Electrophorus and Bungarus AChE sequences, but different from the other AChE sequences. These criteria identified Ser-74 (replaced by Leu in rat AChE) as a possible player in this interaction. In fact, mutation S74L abolished inhibition by both Elec-403 and Elec-410 (Table I). It increased 5-fold the affinity of the enzyme for fasciculin but did not affect inhibition by Elec-408.

In the case of Elec-403, which is directed to the peripheral site, chimeras El/165/Rt and El/266/Rt showed no inhibition and did not bind the antibody (Fig. 3C). Chimera El/337/Rt was inhibited by Elec-403 and in fact showed a 100-fold higher affinity than Electrophorus AChE. There was essentially no residual activity in the presence of an excess of antibody. The fact that chimera El/337/Rt is sensitive to Elec-403, whereas chimera El/266/Rt is not, suggests that the segment by which they differ, located between positions 266 and 337, participates...
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and 507–514, it was only slightly reduced after replacement of peptide 484–491 (INVDGSIDSRR in Electrophorus replaced by the shorter rat peptide DPRDKSP), and was totally abolished after replacement of peptide 453–467 (EKRLNYTLEERLSR to DPSNYTVEERFAQ). We then showed that point mutations of a single residue within this peptide, L460V or E463R, also abolished inhibition by Elec-408. In contrast, inhibition by Elec-403, Elec-410, or fasciculin was not affected by these mutations. The distance of these residues from the peripheral site is too large to be covered by an antibody, in agreement with the previous conclusion that Elec-408 acts at a distinct site.

Residues 460 and 463 are close to the putative back door, which was proposed to allow the exit of reaction products by the opening of a passage between residues Val-129, Gly-441, and Trp-84. It is therefore possible that Elec-408 acts by interfering with this back door mechanism. In order to examine whether the back door residues might be directly involved in its binding, we mutated valine Val-129 into a leucine, expecting that the presence of a larger side chain at this position might affect the binding of the antibody and/or the catalytic turnover rate of the enzyme. The kinetic constants of the mutant enzyme are given in Table I: its $K_m$ value was increased about 2-fold, and the IC$_{50}$ for edrophonium was increased more than 4-fold, indicating a conformational perturbation in the active site. However, inhibition by Elec-403, Elec-408, or Elec-410 was not affected by this mutation.

For all chimeras and point mutants, we analyzed the binding of the three inhibitory monoclonal antibodies by an enzyme-linked immunosorbent assay (not shown) (31). In all cases, we found a complete correlation between binding and inhibition.

Is It Possible to Create a Binding Site for an Inhibitory Antibody by Mutation of Rat AChE?—The preceding results show that it is possible to suppress the binding of a monoclonal antibody by replacement of a single residue, explaining their species specificity. Conversely, we wondered whether it would be possible to create a binding site by mutagenesis of rat AChE. Antibody Elec-410 appeared to be a good candidate, because it reacts with Bungarus AChE, in addition to Electrophorus AChE, showing a broader specificity than Elec-403 and Elec-408. Mutation S74L abolished the interaction of Electrophorus AChE with Elec-410, and according to our three-dimensional model, the surface residues surrounding this position in the peripheral site are conserved between the two species. We therefore made the reverse mutation in rat AChE, L74S. The rat L74S mutant was clearly bound by Elec-410 in the enzyme-linked immunosorbent assay (not shown) and was inhibited by this antibody above $10^{-7}$ M (Fig. 3C).

Although it appeared more difficult, we also attempted to render the rat AChE sensitive to Elec-403, by replacing three residues by their Electrophorus counterparts (L74S, H277Q, and H280L). The resulting enzyme was active and was inhibited by Elec-403. The degree of inhibition, as a function of antibody concentration, was identical to that of Electrophorus AChE (Fig. 3B).

DISCUSSION

This investigation was initiated mainly as an attempt to define the target sites of the inhibitory antibodies Elec-403, Elec-408, and Elec-410. We studied chimeras between the sensitive Electrophorus and the insensitive rat enzymes.

Catalytically active chimeras showed only moderate differences with the parent enzymes in their $K_m$, $K_{cat}$, and $k_{cat}$ parameters and in their sensitivity to reversible inhibitors directed against the active site (edrophonium), the peripheral site (propidium), or both (BW284C51), as shown in Table I. The observed variations confirm that multiple regions of the enzyme contribute to its catalytic properties and to its interaction...
with inhibitors. An analysis of the sensitivity of the chimeras to the inhibitory antibodies confirmed unambiguously that Elec-403, Elec-408, and Elec-410 are directed to distinct sites. Assuming that the $F(ab)'_9$ antibodies interact only with exposed residues, in a contact zone that does not extend over more than 6 residues across, we could delineate their recognition sites. This analysis led us to consistent results, despite paradoxical effects: for example, chimera El/165/Rt showed a considerably higher affinity for Elec-403 than did Electrophorus AChE.

In the case of Elec-403 and Elec-410, which were known to interact with the peripheral site (22), a comparison between the sequences of the chimeras and those of sensitive and insensitive enzymes identified a few residues that might be specifically involved in the target sites. In the case of Elec-408, a series of microchimeras, involving peptide segments of about 15 residues, allowed us to identify the region of binding. These predictions were entirely borne out by site directed mutation of a few residues: in each case, the replacement of a single Electrophorus residue by its rat counterpart was able to totally abolish the binding of the antibody and consequently its inhibitory effect. Thus, Ser-74 and Leu-280 of Electrophorus AChE are in the contact zone of Elec-403, Ser-74 is also in the contact zone of Elec-410, and Glu-463 is in the contact zone of Elec-408.

Such dramatic effects are not due to the fact that we usually replaced a small residue with a larger one, because the replacement by a smaller residue was equally effective in some cases (L-460V abolished the binding of Elec-408). In the chimeras, as well as in the point mutants, we found that a loss of inhibition by the monoclonal antibodies was always paralleled by a loss of binding. Therefore, we never obtained mutants that would

### TABLE I

| Inhibitor | $K_m$ (mM) | $K_i$ (mM) | Relative $k_{cat}$ | Propidium | Edrophonium | BW284C51 | Elec-403 | Elec-408 | Elec-410 | Fasciculin |
|-----------|------------|------------|-------------------|-----------|-------------|---------|---------|---------|---------|------------|
| Electrophorus AChE | 125 ± 5 | 11 ± 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Rat AChE | 70 ± 10 | 15 ± 2 | 0.39 ± 0.05 | 5 | 0.17 | 0.67 | 0 | 0 | 0 | 12 |
| El/165/Rt | 110 ± 10 | 32 ± 2 | 0.49 ± 0.03 | 1 | 0.24 | 0.20 | 0 | 0 | 0 | 0.2 |
| El/266/Rt | 265 ± 15 | 39 ± 4 | 0.63 ± 0.03 | 0.38 | 0.23 | 0.11 | 0 | 0 | 0 | 0.2 |
| El/337/Rt | 190 ± 5 | 22 ± 2 | 0.80 ± 0.03 | 1 | 0.18 | 0.62 | 195 | 0 | 0 | 0.6 |
| S75/74L | 120 ± 15 | 11 ± 2 | 1.05 ± 0.04 | 0.42 | 0.42 | 1.67 | 0 | 0.7 | 0 | 5 |
| V131/129L | 270 ± 15 | 21 ± 1 | 1.15 ± 0.01 | 0.67 | 0.23 | 0.17 | 1.1 | 1.3 | 1 | 1.4 |
| L282 (280)H | 135 ± 10 | 12 ± 1 | 1.21 ± 0.02 | 0.71 | 1 | 1 | 0 | 1.1 | 0.3 | 0.5 |
| E494/463R | 125 ± 5 | 12 ± 1 | 0.97 ± 0.03 | 0.62 | 0.67 | 0.83 | 11 | 0 | 1.1 | 1.4 |

**FIG. 3**. Inhibition of AChE by inhibitory monoclonal antibodies and fasciculin. Various concentrations of fasciculin (A) or of monovalent fragments ($F(ab)'_9$) of inhibitory monoclonal antibodies (Elec-403 (B), Elec-410 (C), and Elec-408 (D)) were incubated with AChE for 24 h at 4 °C, before measurement of the residual activity. ○, Electrophorus AChE; ●, rat AChE; ○, mutated L74S rat AChE; □, mutated L74S/H277Q/H280L rat AChE; ■, chimera El/165/Rt; ●, chimera El/266/Rt; and ▲, chimera El/337/Rt.
have become resistant by disruption of an allosteric mechanism.

Because the replacement of a single residue could abolish the binding of an antibody to *Electrophorus* AChE, we investigated whether the reciprocal mutation might create a binding site on the rat AChE. The case of Elec-410 appeared favorable, and indeed, the L74S mutant did bind the antibody, although at relatively high concentrations (above $10^{-7}$ M). We observed an inhibition, directly resulting from the binding of the antibody. In the case of Elec-403, the replacement of three residues in the rat AChE (L74S, H277Q, and H280L) was even more impressive, because the sensitivity of the mutated enzyme was equivalent to that of *Electrophorus* AChE. These experiments conclusively confirm the localization of the binding/inhibition sites.

Figs. 4 and 5 show that the target sites of Elec-403 and Elec-410 are distinct but overlap, because they both include Ser-74 and are located at the entrance of the gorge, in agreement with the fact that these antibodies can be displaced by peripheral site ligands. Elec-410 binds to one side of the open-
ing of the catalytic gorge, whereas Elec-403 spanned this opening, in a manner similar to that of fasciculin (11, 12). This difference may explain the fact that the residual activity of the AChE-Elec-410 complex is about 7-fold higher than that of the AChE-Elec-403 complex, which represents only 1% of the activity of the free enzyme. We analyzed the effect of the antibodies on the access to the active site with the reversible inhibitor TMTFA and the organophosphate inhibitor echortho-phate. The AChE-Elec-408 complex was inactivated as rapidly as the free enzyme by both inhibitors, in agreement with the fact that Elec-408 does not interfere with the entrance of the catalytic gorge. The Elec-410 complex showed the same rate of inactivation as the free enzyme with echortho-phate but a reduced rate of binding of TMTFA. The Elec-403 complex, like the AChE-fasciculin complex, showed a very dramatically reduced rate of inactivation with both compounds. These inhibition rates showed that Elec-403 blocks the access to the active site as efficiently as fasciculin, and much more than Elec-410.

Elec-408 clearly acts at a different site, because it does not interfere with peripheral site ligands. This characteristic is shared by two inhibitory monoclonal antibodies that have been reported in the literature to act through an allosteric mechanism: 13D8 (21) and C1B7 (36), which have been produced as efficiently as fasciculin, and much more than Elec-410.

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Elec-408 clearly acts at a different site, because it does not interfere with peripheral site ligands. This characteristic is shared by two inhibitory monoclonal antibodies that have been reported in the literature to act through an allosteric mechanism: 13D8 (21) and C1B7 (36), which have been produced as efficiently as fasciculin, and much more than Elec-410.

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