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Monoclonal anti-idiotypic antibodies as functional internal images of enzyme active sites: Production of a catalytic antibody with a cholinesterase activity

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ABSTRACT **Monoclonal antibody 9A8 was selected by immunizing mice with AE-2, a monoclonal antibody directed against the active site of acetylcholinesterase. In accordance with the idiotypic network theory, monoclonal anti-idiotypic antibody 9A8 displayed internal-image properties of the original immunogen, the acetylcholinesterase active site. Hydrolysis of acetylthiocholine and related esters of thiocholine by 9A8 follows saturation kinetics and kinetic parameters were determined. The hydrolytic activity is characterized by a lowered k_{cat} value (81 s^{-1}) and an increased K_m value (0.6 mM) when compared with the original enzyme. However, the rate acceleration ($k_{cat}/k_{uncat} = 4.15 \times 10^8$) remains higher than for the esterase activities usually described for catalytic antibodies directed against transition-state analogs. The 9A8 activity exhibits a relaxation of specificity toward both substrates and inhibitors. This specificity does not correspond to a known enzymatic activity. The anti-idiotypic approach should be valuable for producing different structural and functional copies of the same enzyme active site. This should allow further insights into structure–activity relationships. Furthermore, use of chemically modified enzymes as immunogens may result in anti-idiotypic antibodies with catalytic activities not found in the native enzymes.**

Several examples of catalysis by monoclonal antibodies have been demonstrated since pioneer reports published in 1986 (1–3). A common basis for the principles and methods to elicit catalytic antibodies (abzymes) is to produce haptens that mimic transition-state features in catalytic reactions. Since the introduction of the network theory of immune regulation (4), the properties of anti-idiotypic antibodies have been investigated widely. The combining site of an anti-idiotypic antibody may display structural features which may be the "internal image" of the original antigen. However the remarkable property of idiotypic mimicry has not been exploited to develop catalytic anti-idiotypic antibodies which would mimic the structure of an enzyme active site.

The monoclonal antibody (mAb) AE-2 was produced by Fambrough *et al.* (5) against human erythrocyte acetylcholinesterase (AcChoE) (acetylcholine acetylhydrolase; EC 3.1.1.7). mAb AE-2 has been shown to inhibit the enzyme activity (13) and, although the epitope has not been completely characterized, several reports (13, 22, 23) strongly suggest that it covers at least in part the anionic subsite of the AcChoE active site. On one hand, active-site-directed specific reversible inhibitors of AcChoE [BW 284-C-51, edrophonium, and 2-pyridine aldoxime methiodide (2-PAM)] compete with AE-2 for binding to AcChoE (13, 22). On the other hand, Doctor *et al.* (6) have shown that synthetic peptides containing amino acid sequence from positions 53–84 of the amino terminus of fetal bovine serum AcChoE compete with the enzyme for AE-2. This sequence is a part of the anionic subsite of the active site (7).

We have previously shown that when mAb AE-2 is used as antigen, it is possible to obtain an AcChoE-like activity in a polyclonal IgG preparation from rabbit serum (8). This activity differs from that of AcChoE in its catalytic constants but showed a specificity toward substrates and inhibitors close to that exhibited by AcChoE. In the present study, we have examined the catalytic activity of a monoclonal anti-idiotypic antibody obtained against mAb AE-2.

MATERIALS AND METHODS

Unless otherwise stated, all chemicals were reagent grade, obtained from Sigma.

Preparation of $F(ab')_2$ Fragment of mAb AE-2. The HB73 cell line (American Type Culture Collection) secretes the IgG AE-2 (5). mAb AE-2 was purified from mouse ascites fluid on an Affi-Gel protein A column (Bio-Rad), as detailed by the manufacturer's instructions. The $F(ab')_2$ fragment of mAb AE-2 was prepared by enzymatic digestion with pepsin in 20 mM sodium acetate (pH 4.5). The reaction was stopped by neutralization with 1 M Tris buffer (pH 7.5). The mixture was then centrifuged for 10 min at 800 × *g*. The supernatant was dialyzed against 0.15 M NaCl/0.01 M phosphate (pH 7.2) and applied to a protein A column. The $F(ab')_2$ fragment was eluted in the void volume.

Immunization, Hybridoma Production, and Cloning. Biozzi mice, a strain selected for its high immunological responsiveness (Institut Curie, Paris), were immunized intraperitoneally with 0.15 mg of purified $F(ab')_2$ fragment in complete Freund's adjuvant (total volume, 500 μl). At 2-week intervals, the animals were given three similar booster injections of AE-2 $F(ab')_2$ in incomplete Freund's adjuvant. The serum taken at each step was tested for the anti-idiotypic antibody activity by ELISA.

Three days after the final booster injection, the spleen cells from immunized mice were fused with Sp2/0 myeloma cells in a 2:1 ratio with 50% (wt/vol) polyethylene glycol 4000. After fusion, the cells were distributed in 96-well culture dishes and grown in RPMI 1640 medium (Flow Laboratories) with 20% (vol/vol) fetal bovine serum supplemented with hypoxanthine and azaserine on days 2–10, and with 10% fetal bovine serum supplemented with hypoxanthine on days 11–15.

Selected clones were expanded, and cells producing monoclonal anti-idiotypic antibody were obtained by single-cell cloning by limiting dilution. Ascites fluid was obtained after

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Abbreviations: AcChoE, acetylcholinesterase; BtChoE, butyrylcholinesterase; mAb, monoclonal antibody; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); 2-PAM, 2-pyridine aldoxime methiodide; DFP, diisopropyl fluorophosphate.

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injection of hybridoma cells into the intraperitoneal cavity of a BALB/c mouse that had been primed with 0.5 ml of pristane. Ascites fluid was harvested 1–2 weeks after injection.

Screening of Anti-AE-2 Antibodies. Culture supernatants were tested for the presence of mouse antibody directed against the $F(ab')_2$ fragment of AE-2. Microtiter wells were incubated overnight with 100 μl of $F(ab')_2$ fragment at 2.5 μg/ml in 50 mM carbonate buffer (pH 9.6). The plate was then blocked by adding 0.1 ml of 1% (wt/vol) gelatin in 150 mM NaCl/20 mM phosphate, pH 7.4 (phosphate-buffered saline, PBS) and then washed three times with 0.1% Tween 20 in PBS. Supernatants were added and incubated at 37°C for 2 hr. After three washes, 100 μl of goat anti-mouse IgG, IgM horseradish peroxidase conjugate (1:2000 dilution in PBS) was added in each well. After a 1-hr incubation at 37°C, the plate was washed three times with 0.1% Tween 20 in PBS and the peroxidase substrate [1 mM 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) and 0.03% H_2O_2] was added. A_{405} of each well was measured after incubation at room temperature for 30 min. mAbs 7B10 (IgG) and 83D4 (IgM), raised against human breast cancer tissues, were used as negative controls.

Purification of Monoclonal Anti-AE-2 Antibodies. Ascites fluid from mice injected with cloned hybridoma cells was fractioned by FPLC gel filtration on an HR 10/30 column (Pharmacia), followed by ion-exchange chromatography on a Mono Q HR 5/5 column (Pharmacia).

Catalytic Activity and Inhibition Experiments. The cholinesterase activity of mAb 9A8 was measured either on solid phase or in solution by the method of Ellman *et al.* (9). Usually, various concentrations of acetylthiocholine iodide were added in the presence of 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), and the formation of thionitrobenzoate was followed at 405 nm. Spectrophotometric assay at 400 nm was also used to follow *p*-nitrophenyl acetate hydrolysis (10). All the experiments were carried out in 10 mM phosphate buffer at pH 7.4.

Inhibition of the catalytic activity of the anti-idiotypic antibody by mAb AE-2 was carried out in the same way as described above, by using samples (170 μg/ml) incubated with various dilutions of mAb AE-2 for 2 hr at 37°C with shaking.

Measurements of irreversible inhibition by diisopropyl fluorophosphate (DFP) and echothiopate iodide [*O,O*-diethyl *S*-(2-trimethylammoniumethyl)phosphorothioate iodide; Promedica, Paris] were done by adding 1 mM acetylthiocholine after increasing times of incubation with the inhibitor. BW 284-C-51 [1,5-bis(4-allyldimethylammoniumphenyl)pentan-3-one] and ethopropazine [10-(2-diethylaminopropyl)-phenothiazine hydrochloride], which are reversible active-site-directed inhibitors, and gallamine triethiodide or propidium (from Calbiochem), which are peripheral-site-directed inhibitors, were added to the catalyst just before addition of the substrate.

Reactivation of irreversible organophosphate inactivation by 2-PAM was tested after mAb catalytic activity was inhibited >95% by incubation with 1 μM echothiopate from 1 hr. Phosphorylated mAb was incubated with 1 mM 2-PAM for 45 min in 10 mM phosphate buffer (pH 7.4), diluted 10-fold to stop the reaction, and then dialyzed against 1000 volumes of the buffer for 20 hr with three changes. Controls were subjected to the same treatment, either with a nonphosphorylated mAb or without reactivator.

Chemical modification of histidine residues by diethyl pyrocarbonate was performed by following a procedure described for AcChoE (11). mAb (175 μg) and diethyl pyrocarbonate (2.5 mM) in 500 μl of 10 mM phosphate buffer (pH 7.4) were incubated at 4°C for 20 min. The mixture was then diluted 10-fold to stop the reaction, and the excess reagent was removed by dialysis as described for the reactivation studies. Protection experiments were performed by adding 1 mM BW 284-C-51 to the incubation medium, before addition of the protein-modifying reagent.

RESULTS

Immunization with $F(ab')_2$ Fragment of mAb AE-2. The $F(ab')_2$ fragment of the murine mAb AE-2 was prepared by pepsin treatment, purified by protein A chromatography, and characterized by SDS/polyacrylamide gel electrophoresis. We have checked that the $F(ab')_2$ fragment inhibits human erythrocyte AcChoE activity with the same efficiency as the whole mAb AE-2 molecule. The $F(ab')_2$ fragment binds AcChoE either in solution or on the surface of the microtiter plate with an affinity comparable to that of the whole antibody molecule. To maximize the probability of inducing anti-idiotypic antibodies and of detecting them in the hybridoma system, $F(ab')_2$ fragment was used both for immunization and in the screening of anti-idiotypic production in mice by ELISA. Mice were thus immunized with $F(ab')_2$ fragment and given booster injections at 2-week intervals. Six weeks after the first injection, the mice were bled and the serum was tested for anti-idiotypic activity by using the $F(ab')_2$-based ELISA. As shown in Fig. 1, anti-AE-2 antibodies were detected.

Production of Monoclonal Anti-Idiotypic Antibodies. Three days after the final booster injection spleen cells were fused with Sp2/0 nonsecreting myeloma cells and the hybridoma cells were screened for anti-idiotypic antibodies by ELISA. The production of abzymes differs from other efforts to obtain mAbs because of the need for a double selection, by immunological and catalytic activity tests, to identify the immunoglobulins of interest. Only 1 of 600 wells (1F5) screened was positive for the two tests. Stable subclones were established from 1F5 by limiting dilution and their anti-idiotypic activity was evaluated by testing the ability of secreted immunoglobulin to bind to microwells coated with $F(ab')_2$ fragment of mAb AE-2 (Fig. 2). The appearance of cholinesterase activity in the culture medium of 1F5 and of its subclones was tested by incubating the supernatant culture medium in microtiter plates to which goat anti-mouse immunoglobulins had been adsorbed; after the plates were washed 2 mM acetylthiocholine and 1 mM DTNB were added. The catalytic activity exhibited by 1F5 and by one subclone, 9A8, differed significantly from that obtained with 6G3, a subclone negative for anti-idiotypic activity, and from the various controls (Fig. 3). We checked that the measured catalytic

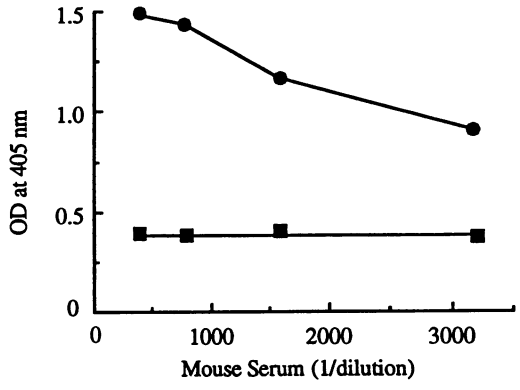

FIG. 1. Titration of AE-2-immunized mouse serum. Serial dilutions of mouse serum, obtained before immunization (■) and after the final booster injection (●), were incubated at 37°C for 2 hr in wells coated with $F(ab')_2$ fragment of mAb AE-2 and containing 0.1 ml of PBS with 0.005% Tween 20. Bound antibodies were determined by ELISA.

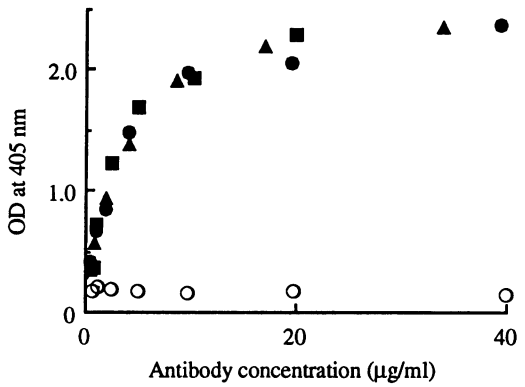

FIG. 2. Binding of the monoclonal anti-idiotypic antibody 9A8 (subclone of 1F5) to F(ab')$_2$ fragment of AE-2. Serial dilutions of three different preparations of purified 9A8 at 0.2 mg/ml (■), 0.34 mg/ml (▲), and 0.79 mg/ml (●) were incubated in wells coated with F(ab')$_2$ fragment of AE-2. Binding was assayed by ELISA using goat anti-mouse IgG, IgM peroxidase conjugate. mAb 7B10 was used as negative control (○).

activity varied linearly with the amount of antibody bound to anti-mouse immunoglobulins.

The isotype of the mAb was determined to be IgM. To further characterize mAb 9A8, cells were injected into mice to generate ascites. Monoclonal IgM 9A8 was then purified from ascites, after precipitation in distilled water, by FPLC gel filtration followed by ion-exchange FPLC on Mono Q.

Catalytic Properties of mAb 9A8. The catalytic activity of mAb 9A8 was measured in solution with acetylthiocholine at 0.1–10 mM. In contrast to AcChoE, which usually exhibits an excess-substrate inhibition above 1 mM substrate, the hydrolysis of acetylthiocholine by mAb 9A8 follows characteristic Michaelis–Menten kinetics which saturate at high substrate concentration (Fig. 4*A*). The Lineweaver–Burk plot of the data does not deviate from linearity (Fig. 4*B*), and the maximum velocity varies linearly with the antibody concentration. The kinetic constants derived from experimental results in phosphate buffer (pH 7.4) with mAb 9A8, human erythrocyte AcChoE, which is the immunogen used to induce the production of mAb AE-2, and fetal bovine serum AcChoE, which is present in the serum added to the culture medium, are summarized in Table 1.

The AcChoE activity is characterized by its specificity for the acetic ester of choline. To check the specificity of the

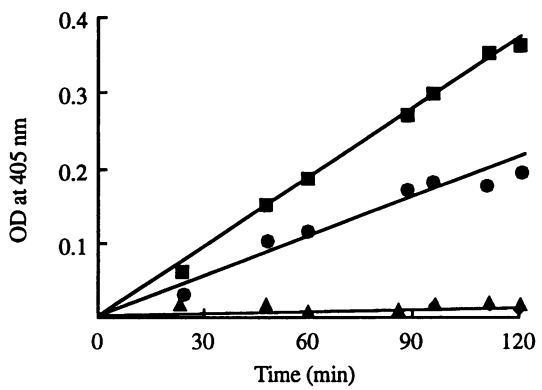

FIG. 3. Catalytic AcChoE activity of mAb 9A8. Purified 1F5 (■) and 9A8 (●) antibodies were incubated in wells coated with anti-mouse immunoglobulins. After washing, the catalytic activity of bound antibodies was revealed by adding 100 μl of 150 mM NaCl/10 mM phosphate, pH 7.4, containing 2 mM acetylthiocholine and 1 mM DTNB. An unrelated monoclonal IgM (83D4) and a subclone of 1F5 negative for anti-idiotypic activity (6G3) were used as negative controls (▲).

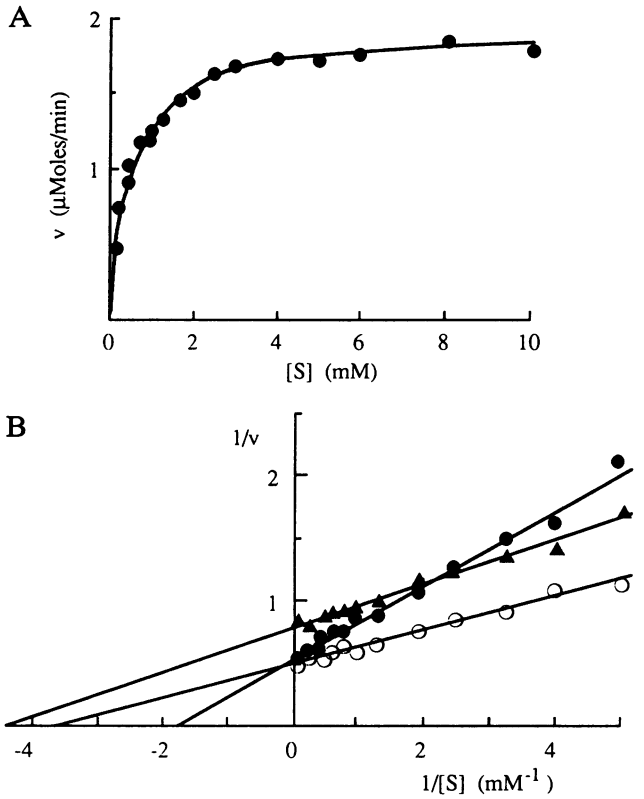

FIG. 4. Kinetics of substrate hydrolysis catalyzed by mAb 9A8 at pH 7.4. (*A*) Acetylthiocholine hydrolysis by mAb 9A8 as a function of substrate concentration from 0.1 to 10 mM. Enzyme activities were determined in solution by the Ellman method. (*B*) Lineweaver–Burk plot of the hydrolysis of acetyl- (●), propionyl- (○), and butyrylthiocholine (▲). In all cases, the assay mixture contained IgM at 35 μg/ml.

reaction catalyzed by mAb 9A8, three substrates, acetyl-, propionyl-, and butyrylthiocholine, were used. The results obtained with mAb (Fig. 4*B*), human erythrocyte AcChoE, and butyrylcholinesterase (BtChoE) (acylcholine acylhydrolase, EC 3.1.1.8), which is the other enzyme catalyzing the hydrolysis of choline esters in mammals, are shown in Table 2.

AcChoE hydrolyzes esters other than choline esters. For example, the hydrolysis of the neutral ester *p*-nitrophenyl acetate is slowly catalyzed by AcChoE. We found that mAb 9A8 catalyzed the hydrolysis of *p*-nitrophenyl acetate (Table 2), following Michaelis–Menten kinetics with a k_{cat} value of 37 s^{-1} and a K_m value of 1.05 mM.

Table 1. Kinetic constants for the hydrolysis of acetylthiocholine by mAb 9A8, human erythrocyte (HE) AcChoE and fetal bovine serum (FBS) AcChoE

Catalyst	k_{cat}, s^{-1}	K_m, mM	K_i,* mM	k_{cat}/K_m × 10^{-5}, $M^{-1}s^{-1}$	k_{cat}/k_{uncat},† × 10^{-8}
mAb 9A8	81	0.60	n.i.	1.35	4.15
HE AcChoE	8000	0.13	22	615	410
FBS AcChoE	5800	0.11	18	527	297

k_{cat} values were determined assuming a molecular weight of 90,000 per active site for monoclonal IgM 9A8 and 75,000 per active site for AcChoE.

*The K_i value is the inhibition constant for excess substrate. n.i., Not inhibited.

†The rate acceleration (k_{cat}/k_{uncat}) was calculated by using a value of 1.95 10^{-7} s^{-1} for the uncatalytic constant of hydrolysis of acetylthiocholine in our experimental conditions.

Table 2. Specificity of hydrolysis of various choline esters and of a neutral ester by mAb 9A8, human erythrocyte (HE) AcChoE, and BtChoE

	Relative rate of hydrolysis			
	Thiocholine esters			Neutral ester (*p*-nitrophenyl acetate)
Substrate	Acetyl	Propionyl	Butyryl	
mAb 9A8	1	1.06	0.67	0.46
HE AcChoE	1	0.45	0.04	0.08
BtChoE	1	2.1	5.6	0.02

Inhibitor Studies of the Reaction Catalyzed by mAb 9A8. The residual cholinesterase activity of mAb 9A8 (170 μg/ml) was measured after 2 hr of incubation with various dilutions of mAb AE-2. More than 50% inhibition of the catalytic activity was observed when AcChoE from human erythrocyte was incubated with mAb AE-2. This percentage was higher in the case of mAb 9A8; ≈70% inhibition was observed when AE-2 at 125 μg/ml was incubated with 9A8 at 170 μg/ml. Many specific inhibitors of AcChoE are known. Among them, organophosphates irreversibly block the enzyme by phosphorylation of the active-site serine, whereas many quaternary ammonium compounds are specific reversible inhibitors directed against the anionic subsite of the active site. In addition to the two subsites of its catalytic center, AcChoE possesses an additional anionic site of regulation, at a locus topographically distinct from the active site. The selective binding of various quaternary ligands, such as gallamine or propidium, to this latter site inhibits the enzymatic activity (12).

The irreversible inhibition of the cholinesterase activity of mAb 9A8 was studied with DFP, a general phosphorylating agent of serine esterases, and with echothiopate, which is specific for AcChoE, by incubating the catalyst with the inhibitor for 10 s to 1 hr before addition of the substrate. The 50% inhibition constants (IC_{50} values) were determined from inhibition curves with various inhibitor concentrations (Table 3). No measurable spontaneous reversibility of the reaction was obtained with phosphorylated mAb 9A8 by dilution in phosphate buffer for 3 hr. However, when mAb phosphorylated by echothiopate was incubated for 45 min with a reactivator compound, 1 mM 2-PAM, 65% of the initial activity was restored.

Two reversible inhibitors of cholinesterases were used, BW 284-C-51 preferentially inhibits AcChoE, while ethopropazine is a specific inhibitor of BtChoE. In both cases the IC_{50} values differ from values obtained with AcChoE and BuChoE. Gallamine and propidium are known as reversible specific inhibitors of AcChoE which exert their action by interacting with a peripheral site, distinct from the active site. Even at high gallamine concentration (1 mM), no inhibition of the cholinesterase activity was obtained with the mAb 9A8 preparation. A similar result was obtained with propidium at up to 0.1 mM.

Inactivation of AcChoE by chemical modification of histidine residues with diethyl pyrocarbonate has been described (11). Incubation of mAb 9A8 with 2.5 mM diethyl pyrocarbonate for 20 min resulted in the total loss of catalytic activity. However, when 1 mM BW 284-C-51 was included in the assay medium during the incubation with diethyl pyrocarbonate, >85% of the initial activity was protected against inactivation.

Table 3. Inhibition of hydrolysis of acetylthiocholine by mAb 9A8, human erythrocyte (HE) AcChoE, and BtChoE

	IC_{50}, μM		
Inhibitor	mAb 9A8	HE AcChoE	BtChoE
Irreversible			
DFP	0.32	0.5	0.01
Echothiopate	0.012	0.01	>1
Reversible			
BW 284-C-51	10	0.05	>100
Ethopropazine	1	>1000	0.05
Peripheral site			
Gallamine	n.i.	21	n.i.

IC_{50} values were determined in the presence of 1 mM substrate from inhibition curves representing three independent experiments. For irreversible inhibition, the inhibitor was incubated for various times with the catalyst before addition of the substrate. n.i., Not inhibited at 1 mM gallamine.

DISCUSSION

Among the monoclonal anti-idiotypic antibodies obtained against mAb AE-2, one of them, IgM 9A8, exhibits cholinesterase activity. After production of 9A8 in ascitic fluids and purification steps, the cholinesterase activity was studied and its kinetic properties were compared with those of the natural enzymes (Table 1). The catalytic properties of an enzyme molecule may be characterized by its first-order rate constant (k_{cat}) and by its Michaelis constant (K_m). The k_{cat} value differs by 2 orders of magnitude from that of AcChoE. The K_m value is 5-fold higher than the value obtained with the natural enzyme. The accurate determination of the k_{cat} value requires the precise determination of active-site concentration. This value, which might thus be underestimated, does not allow one to conclude that the catalytic activity was not due to AcChoE contaminating the 9A8 preparation. Conversely, the fact that the Michaelis constant, an intrinsic property of the catalyst which does not depend on its concentration, largely differs from the values usually measured with AcChoE is indicative of a catalyst different from the AcChoE enzyme. On the other hand, hydrolysis of acetylthiocholine, which is inhibited by excess substrate when the natural enzyme from any source is used, is purely Michaelian when mAb 9A8 is the catalyst. Moreover, it appears that the specificity of catalysis of choline esters or of a neutral ester by mAb 9A8 differs both from that of a true AcChoE and from that of BtChoE (Table 2), the other cholinesterase present in mammals. This specificity looks like a relaxed specificity from AcChoE. These differences in kinetic properties with respect to substrate specificity and kinetic constants do not correspond to a natural cholinesterase activity as described in various tissues and various species. This has been confirmed by the use of specific inhibitors (Table 3). Irreversible inhibition experiments using DFP and echothiopate clearly indicate the role played by a serine residue in the catalytic process. The specificity of 9A8 inhibition by echothiopate and BW 284-C-51, two AcChoE-specific inhibitors, indicates that the affinity of these compounds for the catalyst present in the 9A8 preparation is very close to that of AcChoE. However, the catalytic activity of 9A8 is inhibited by ethopropazine, a BtChoE-specific inhibitor, with an affinity of the same order of magnitude as for the enzyme. With gallamine, a peripheral-site-specific inhibitor of AcChoE, no inhibition of the cholinesterase mAb 9A8 activity can be detected, even at gallamine concentrations up to 1 mM. All these results obtained with cholinesterase inhibitors agree with those obtained for the substrate specificity. The measured activity does not correspond to a true AcChoE when excess-substrate inhibition, substrate specificity, or inhibition by ethopropazine or gallamine are considered. Moreover, this activity cannot be explained by presence of a BtChoE as a contaminant in the 9A8 preparation if we take into account the substrate specificity and inhibition by echothiopate or BW 284-C-51. This conclusion is strengthened by the fact that mAb AE-2 inhibits the mAb 9A8 cholinesterase activity, whereas mAb AE-2 fails to inhibit the activity of BtChoE (13).

The cholinesterase activity measured with mAb 9A8 resembles in many aspects the activity catalyzed by AcChoE, but differs in others. Crystallographic analysis of the AcChoE molecule has confirmed the existence of a catalytic triad (Glu, Ser, His) in its active site (7). Experiments studying irreversible inhibition of mAb 9A8 activity by echothiopate and reactivation by 2-PAM clearly indicate the presence of a serine residue in the active site of the antibody. Moreover, the loss of catalytic activity after chemical modification by diethyl pyrocarbonate, which can be prevented by BW 284-C-51, indicates the presence of an essential histidine residue in the active site. Thus, the esterase activity of the antibody appears to be related to the presence of at least two of the amino acids that constitute the catalytic triad of AcChoE. In contrast, the cholinesterase activity differs from the activity catalyzed by AcChoE, both from a quantitative point of view, with kinetic constants differing largely from those measured with the enzyme, and from a qualitative point of view, as shown by absence of inhibition by excess substrate or relaxed specificity toward substrates and inhibitors. We conclude that the cholinesterase activity present in our purified monoclonal anti-idiotypic antibody preparation is not due to a contaminating agent and results from the structural image of the AcChoE active site on mAb 9A8.

Anti-idiotypic antibodies have been isolated and extensively characterized for studies of ligand–receptor interaction (14–17). The combining site of these anti-idiotypic antibodies not only mimics the original antigen at the level of primary or secondary structure, forming an internal image of the antigen (18), but is also able to exhibit a functional activity which mimics the physiological activity of the antigen (19).

The existence of catalytic human autoantibodies has been reported for a peptidase activity against vasoactive intestinal peptide in people with asthma (20) and for a DNA-nicking activity in the sera of patients with systemic lupus erythematosus (21). In this latter case, the authors suggest that these catalytic autoantibodies are anti-idiotypic antibodies to topoisomerase I, since in some cases the sera of patients have an increased titer of primary antibodies to topoisomerase I.

The possibility of isolating catalytic antibodies by using the properties of the idiotypic network opens routes to study structure–activity relationships of enzymatic activities and to produce new catalytic activities. By our approach, it would be possible to produce different anti-idiotypic antibodies using the same enzyme active site as antigen. These different copies possessing similar structural information but with different "chemical alphabets" would allow one to assess the degree of freedom in the primary sequence to obtain not only the binding of a given molecule, but also a specific catalytic activity.

The specificity of enzymatic activities may be modified by chemical modifications of amino acids. The production of anti-idiotypic antibodies from these chemically modified enzyme active sites would result in protein catalysts bearing new activities but without modified amino acid residues. The information contained in the chemical modification would be integrated into the genetic material of the cells producing these catalytic antibodies.

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