BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 May; 18 (3): 284-286

Monoclonal antibody 3F3 against conformational epitope of *Torpedo* acetylcholinesterase[1]

FU Feng-Hua[2], WANG Yu-Xia, LI Feng-Zhen, XIN Yan-Bin[3], SUN Man-Ji[4]
(*Institute of Pharmacology and Toxicology*; [3]*Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing 100850, China*)

KEY WORDS *Torpedo*; acetylcholinesterase; epitopes; monoclonal antibodies; soman

AIM: To study the type of epitope of native *Torpedo* acetylcholinesterase (AChE) directed by its monoclonal antibody (McAb) 3F3.
METHODS: Enzyme-linked immunosorbent assay (ELISA) was used for the assay of the reaction between antigen and antibody. **RESULTS**: McAb 3F3 immunoreacted well with the native AChE, but not with the reduced- and alkylated-AChE (RA-AChE) at all. Soman did not interfere the binding of 3F3 with AChE molecule. The synthesized 24-peptide containing the active serine residue of the AChE active center did not react with McAb 3F3. **CONCLUSION**: 3F3 is a monoclonal antibody against the conformational epitope of *Torpedo* AChE active center, but dose not occupy the active serine residue of the enzyme.

Acetylcholinesterase (acetylcholine acetylhydrolase, EC 3.1.1.7, AChE) catalyses the hydrolysis of the neurotransmitter acetylcholine and thereof maintains the normal function of the cholinergic nerves. There are two subsites at the active center of AChE: an anionic subsite encompassed by hydrophobic domains and an esteristic subsite with an active serine and its charge relay system. Monoclonal antibodies (McAb) are recognized as sophisticated probes in the protein study. McAb 3F3 against AChE from Torpediniforms *Torpedo torpedo* was reported to bind the active center and inhibit the enzyme activity[1]. In this paper, we examined the type of the epitope directed by McAb 3F3.

MATERIALS AND METHODS

Native AChE was prepared[2] from the electric organ of Tropediniforms *Torpedo torpedo* with a specific activity of 6 MU · g^{-1}[3]. RA-AChE denoted the AChE reduced by dithiothreitol and then alkylated by iodoacetamide[4]. Soman-AChE signified the AChE in 0.1 % NaN_3-phosphate buffer 50 mmol·L^{-1} (pH 8.0)-1,2,2-trimethylpropyl methyl phosphonofluoridate 1 μmol·L^{-1} (1:500, vol/vol) treated at 37 ℃ for 30 min. *o*-Phenylenediamine dihydrochloride (OPD) was purchased from Merck Co. Horseradish peroxidase-labeled goat anti-mouse IgG was obtained from Institute of Microbiology and Epidemiology. Pas peptide (NH_2-Thr-Val-Thr-Ile-Phe-Gly-Glu-Ser*-Ala-Gly-Gly-Ala-Ser-Val-Gly-Met-His-Ile-Leu-Ser-Pro-Gly-Ser-Arg-OH), 193-216 amino acid residues of AChE containing the active serine* of the active center, was a gift from Dr Keliang LIU. Anti-AChE McAb[5] were purified from ascites fluids produced in BALB/c mice injected with hybridoma cells[1]. All other chemicals and reagents used were of AR. Polyvinyl chloride microplate was a product of Tianjin Plexity Factory.

ELISA[6] was used throughout the antigen-antibody reactions. Antigens (native AChE, RA-AChE or soman-AChE) were coated onto microplate. McAb against native AChE was used as the first antibody. To find whether 3F3 combines with the active serine, competitive ELISA was carried out likewise as ELISA with the modification of incubating the first antibody (McAb 3F3) with Pas peptide at room temperature for 1 h and succesively at 4 ℃ for 24 h beforehand. The mouse non-specific IgG (2.5 g·L^{-1}) or casein (1 g·L^{-1}) and RA-AChE (1 g·L^{-1}) 1:200 diluted with coating buffer were adopted respectively in the negative and positive controls. Samples with absorbance (A) at 492 nm higher than two-fold of the negative control were judged as a positive reaction.

RESULTS

Immunoreactivity between anti-native AChE monoclonal antibodies and native AChE, RA-AChE, or soman-AChE The native AChE reacted well with all McAb as expected. However, the

[1] Project supported by the National Natural Science Foundation of China, № 38970211.
[2] Now in *Department of Immunology, Binzhou Medical College, Binzhou 256603, China*.
[4] Correspondence to Prof SUN Man-Ji.
Received 1996-02-12 Accepted 1996-12-02

nfold RA-AChE reacted with most of the McAb xcept 3F3. McAb 3F3 was the only one being ble to immunoreact with the native but not the nfolded AChE (Tab 1).

b 1. Immunoreactivity between anti-native AChE McAb nd native AChE or reduced-alkylated AChE. n = 3 xperiments of ELISA. $\bar{x} \pm s$. Negative control, mouse nspecific IgG 2.5 g·L^{-1}, *0.017 ± 0.010.

Monoclonal antibody	Absorbance at 492 nm (ELISA) Native AChE	RA-AChE
*3F3	0.79 ± 0.08	0.016 ± 0.012
1C8	0.240 ± 0.015	0.35 ± 0.04
2C4	0.42 ± 0.14	0.33 ± 0.06
1E9	0.242 ± 0.011	0.19 ± 0.04
2E6	0.56 ± 0.21	0.230 ± 0.027
1F9	0.34 ± 0.19	0.20 ± 0.03
2F10	0.85 ± 0.17	0.207 ± 0.024
2F3	0.282 ± 0.029	0.26 ± 0.04
2F6	0.61 ± 0.13	0.34 ± 0.05
2G7	0.52 ± 0.11	0.35 ± 0.06
2G8	0.28 ± 0.07	0.27 ± 0.03
1H8	0.42 ± 0.18	0.31 ± 0.04
1H11	0.45 ± 0.19	0.202 ± 0.020
2H5	0.74 ± 0.03	0.33 ± 0.05
Negative control	0.048 ± 0.015	0.025 ± 0.012

Immunoreactivity experiment showed that oman had no influence on the binding of 3F3 with AChE (Tab 2).

b 2. Antigenicity of AChE treated with soman. n = 3 xperiments of ELISA. $\bar{x} \pm s$. $^{a}P > 0.05$ *vs* native AChE. egative control, mouse nonspecific IgG 2.5 g·L^{-1}.

Antigen	Absorbance at 492 nm (ELISA) 3F3	Negative control
Native AChE	0.36 ± 0.09	0.006 ± 0.006
Soman-AChE	0.35 ± 0.04[a]	0.003 ± 0.003

Immunoreactivity between Pas peptide and cAb 3F3 In the competitive ELISA, while the irst antibody 3F3 was pre-incubated with Pas eptide, $A_{492\ nm}$ was not lower than the positive ontrol. Pas peptide did not affect the mmunoreactivity between native AChE and McAb F3 in various dilutions in ascites fluids. Pas did ot immunoreact with McAb 3F3 at all (Tab 3).

Tab 3. Competitive ELISA of Pas peptide. n = 3 experiments of ELISA. $\bar{x} \pm s$. Negative control, casein 1 g·L^{-1}.

	McAb 3F3 dilution	Absorbance at 492 nm (ELISA) Pas	Positive control
Native AChE	1:1×10^3	1.86 ± 0.06	1.641 ± 0.014
	1:1×10^4	0.453 ± 0.023	0.429 ± 0.025
	1:5×10^4	0.200 ± 0.015	0.174 ± 0.005
	1:1×10^5	0.162 ± 0.015	0.139 ± 0.006
	1:5×10^5	0.120 ± 0.011	0.109 ± 0.003
Negative control	1:1×10^3	0.014 ± 0.011	

DISCUSSION

AChE became unfold RA-AChE after it had been reduced and alkylated. 3F3 reacted well with native AChE but not RA-AChE. It suggests that the epitope directed by 3F3 is a discontinuous (conformational) one. Pas peptide containing active serine of AChE active center did not react with 3F3, whereas the soman-AChE reacted well with 3F3. These results implies that the active serine and the flanking amino acid residues deeply residing in the narrow gorge of the active center are not essential for the epitope in combining with its specific McAb 3F3. In summary, 3F3 is a McAb against the conformational epitope of *Torpedo* AChE active center, but dose not occupy the active serine residue of the enzyme.

REFERENCES

1 Wang JB, Li FZ, Fu FH, Sun MJ, Yan GZ, Xin YB, *et al*. Effects of purified anti-acetylcholinesterase monoclonal antibodies on the enzyme activity. Chin J Pharmacol Toxicol 1989; **3**: 225 - 9.

2 Sun MC, Gao TD, Xing ZY, Zhang H, Zhou TC. Preparative affinity chromatography of acetylcholinesterase from electric organs of Torpediniforms *nacline timelei*. Chin Biochem J 1985; **1** (2): 47 - 52.

3 Ellman GL, Courtney DK, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; **7**: 88 - 95

4 Liao J, Sun MC. Continuous antigenic determinants of acetylcholinesterase. Chin J Pharmacol Toxicol 1990; **4**: 44 - 8.

5 Xin YB, Yan GZ, Xu PF, Li FZ, Zhang H, Wang JB, *et al*. The production of anti-acetylcholinesterase monoclonal antibodies. Chin J Pharmacol Toxicol 1989; **3**: 68 - 72.

6 Doctor BP, Camp S, Gentry MK, Taylor SS, Taylor P. Antigenic and structural differences in the catalytic subunits of the molecular forms of acetylcholinesterase. Proc Natl Acad Sci USA 1983; **80**: 5767 - 71.