Molecular simulation of a single-chain antibody against AChE to explore molecular basis of inhibitory effect of 3F3 McAb on enzyme activity[1]

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KEY WORDS computer simulation; acetylcholinesterase; gene expression; monoclonal antibodies

ABSTRACT

AIM: To explore the molecular basis of the inhibitory effect of 3F3, a monoclonal antibody against acetylcholinesterase (AChE), by computer-aided molecular simulation. **METHODS:** The single-chain 3F3 antibody (Sc3F3) was designed by joining V_H and V_L *via* a flexible linker $(Gly_4Ser)_3$. The amino acid sequence of the recombinant Sc3F3 was then subjected to computer-aided molecular modeling, and docking with the antigen molecule AChE to mimic the immunoactive interaction in a three-dimensional fashion. **RESULTS:** The modeled structure of Sc3F3 manifested the common features of a classical antibody. Both V_H and V_L were composed of two β-sheets and connecting loops. The docking profile of the action between Sc3F3 with AChE demonstrated the formation of a stable structure. The van der Waals force played an important role suggesting that the complex was formed mainly *via* hydrophobic interactions between Sc3F3 and AChE molecules. **CONCLUSION:** The spatial structure of the complex of Sc3F3 and AChE showed that Sc3F3 overlaid the entrance of the active center gorge of AChE blocking the access of substrate.

INTRODUCTION

Monoclonal antibodies produced from hybridoma cells with high affinity and specificity are used as the sophisticated tools in topological study of the surface structure of their matched antigen[1]. Monoclonal antibodies against acetylcholinesterase (AChE, EC 3.1.1.7) can be used as the probes in exploration of the catalysis mechanism of AChE[2] . The inhibitory monoclonal antibody 3F3[3] (IgG1)[4] against *Torpediniformes narcine timlei (Torpedo)* electric organ AChE was found to immunoreact well with the native AChE, but not with the reduced- and alkylated AChE at all[5]. The synthesized authentic 24-peptide (NH_2-TVTIFGES*AGGA-SVGMHILSPGSA-OH) containing the active serine residue of the AChE active center did not react with 3F3[5]. It implies that 3F3 must bind on the conformational epitope of the enzyme, the binding site of 3F3 on the AChE molecule is important to the function of the active center. Thus we prompted to explore the molecular basis of inhibitory effect of 3F3 on *Torpedo* AChE by molecular modeling. Since antibody behaves its binding activity with antigen directly *via* the complementary determining regions (CDR), we created a single-chain antibody (ScFv) with smaller molecular weight

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using 3F3 as prototype by genetic engineering and molecular modeling[6]. Secondly, the recombinant single-chain antibody of 3F3 (Sc3F3) produced by genetic engineering was sequenced for confirmation of the correctness of Sc3F3 designed. Then the sequence of Sc3F3 was subjected to computer-aided three-dimensional modeling.

It is well known that among similar proteins the three-dimensional structures are more conservative than their primary structures. So long as 50 % homology exists in the amino acid sequence, the space deviations of α-carbon atoms in the main chain would be less than 0.3 nm with a root mean square bias of 0.1 nm. Replacement of amino acid residues often happen at the turns on the surface of the protein, it has little influence on the structure of the main backbone of the protein molecule, especially the hydrophobic core[7]. Thus it is feasible to predict the three-dimensional structure of a protein with reference to sterically defined proteins with high sequence homology. In construction of the Sc3F3, variable regions from the heavy and light chains (V_H, V_L) of 3F3 were linked together with a 15-peptide[6]. The interactions between V_H and V_L only influence their relative sterical positions and the conformations of several amino acid residues intervening in the contact of the two chains, but there is little impact on the integral chain structure. Hence it is tenable to model V_H and V_L separately, then connects them by computer-aided modeling.

MATERIALS AND METHODS

Cells and reagents 3F3 hybridoma cells, 3F3 antibody, and the purified *Torpedo* AChE were from our own laboratory. TRIZOL reagent total RNA isolation reagent, PGEM-T easy vector, Wizard^R Plus Minipreps DNA Purification System, Taq DNA polymerase, DNA molecular marker, and restriction enzymes were purchased from Promega Co. Oligonucleotide primers and marker were from Pharmacia Biotech Co. *E coli* JM109 and the horseradish peroxidase-taged sheep-anti-mouse IgG were from Hua-Mei Co.

Design of single-chain antibody Sc3F3 The single-chain antibody Sc3F3 was designed[6] in our laboratory by joining the variable regions of the heavy chain (V_H , 119 amino acid residues, 2-120 from N- to C-terminus) and the light chain (V_L ,107 residues, 136-242) *via* a flexible linker peptide $(Gly_4Ser)_3$.

Heavy chain:
2-120
AQVKLQESGPELEKPGASVKISCKASGYSFTGYYIHWV
KQSHVKSLEWIGRINPYNGATRYNQNFKDKANLAVD
KSSRTAYMELHSLTSEDSAVYYCALSTMITDFDYWGP
GATVTVSS
Light chain:
136-242
DIELTQSPAIMSASPGERVTMTCSASSSIRYIYWYQQK
PGSSPRLLIYDTSNVAPGVPFRFSGKGKGTSYSLTINRMEA
EDAATYYCQEWSGYPYTFGGGXKXEXKR
Linker:
121-135
GGGGSGGGGSGGGGS

Modeling of V_H and V_L of Sc3F3 V_H and V_L were modeled separately. Then a linker sequence of 15 residues between them was put to make a complete sequence of Sc3F3. The linker peptide consisted of 12 glycines and 3 inserting serines, thereof increased the flexibility and hydrophilicity of Sc3F3. Since the linker peptide did not keep a stable structure, the contribution of linker peptide in molecular modeling was not considered.

V_H and V_L sequences were sent to the Swiss-Model server (http://www.expasy.ch/swissmod/SWISS-MODEL.html) for analysis (FASTA, BLAST, *etc*). The sorted out proteins with >35 % homology to the V_H and V_L sequences were adopted as modeling templates. The three-dimensional structures were modeled using ProMed software[8-10] , and then optimized respectively under the module Discover[11] in InsightII 98.0 software package from MSI Co on SGI O2 workstation. Firstly, all of the heavy atoms were fixed, 1000 steps of calculation in the consistent-valence forcefield were carried out by the steepest descent method, followed by optimization using the conjugated gradient method. To overcome the regional potential barrier releasing all the atoms, molecular dynamics simulation was performed at 300 K for 20 ps (1 ps=10^{-12} s). The conformation was collected consecutively at 1-ps intervals, then optimized as above-mentioned to the smallest gradient 8.36 kJ· Å^{1}· mol^{-1}, and the lowest-energy conformation was chosen as the ultimate model.

Assembly of the integral structure of Sc3F3 The amino acid sequences of V_H and V_L were aligned with reference to a structurely identified antibody 1 plg[12] to determine the structure conservative regions (SCR). Then the backbone atoms were superimposed respectively on 1-plg for their relative steric positions, thus

the preliminary structure of Sc3F3 was obtained. The final structure was acquired by optimization, and tested for residue compatibility with Profile-3D program[13].

Calculation of solvent accessible surface of Sc3F3 amino acid residues The solvent accessible surfaces were calculated and displayed using programs of Delphi and Insight II (Biosgm, Inc, San Diego, CA). The solvent accessible surface area of each residue was calculated using an implement of Lee and Richards algorithm[15].

Three-dimensional structure optimization of *Torpedo* AChE The crystal structure of *Torpedo* AChE (2ACE, in PDB) has been reported in 1991[16]. However, the data missed 5 amino acid residues (485-489) and some side chain atoms. We sent the sequence of *Torpedo* AChE to Swiss-Model server to model the whole sequence again restricting 2ACE as template. The structure sent back from the server was optimized. The conformation with the lowest energy was reckoned to be the ultimate model.

Docking between Sc3F3 and *Torpedo* AChE The CDR was firstly aimed at the fixed AChE molecule. Then moving Sc3F3 approached to AChE in reasonable contact, and the initial site of docking was determined according to the binding energy calculated. The related amino acid residues of Sc3F3 were released and the whole system was optimized *via* molecular mechanic and dynamic calculations under condition of AChE fixation. Finally, AChE and Sc3F3 were set as the centers respectively, and searched within a radius of 5 Å for the atoms reacting with the modeling structures. The related amino acid residues were further released and optimized to obtain the defined model. The final complex structure had a large solvent accessible buried surfaces between Sc3F3 and *Torpedo* AChE, and the interaction energy was -825.42 kJ/mol.

RESULTS

Detection of CDR of Sc3F3 CDR were identified by multiple sequence comparison[14]. V_H and V_L each contained three CDR and two cysteine residues necessary for the maintenance of the antibody structure (Fig 1). CDR of V_H were estimated at 32-36, 51-66, and 100-108 amino acid residues, while CDR of V_L at 24-34, 46-54, and 88-95 residues.

Structure of the modeled Sc3F3 molecule The modeled structure of Sc3F3 was shown in Fig 1. It had the common features of a classical antibody. Both V_H and V_L were composed of two β-sheets and connecting loops. The two-disulfide bonds located at Cys23-Cys97 in V_H and Cys23-Cys87 in V_L with an average bond-length of 2.0 Åaccording to the standard length of disulfide bond for stabilizing the molecule structure. The total energy of V_H and V_L after optimization came to 5089.15 and 3849.36 kJ, whereas the energy of Sc3F3 (8050.68 kJ) decreased 887.83 kJ (Tab 1) nearly equal to the total interacting energy (-774.14 kJ) between V_H and V_L. It implied that V_H and V_L had a tendency to form a dimer. Nine pairs of hydrogen bonds took part in the formation of the dimer (Tab 2). Those hydrogen bonds played an important role in the stabilization of molecules. All the six loops situated at one side of the dimer to form the CDR. The C-termius of V_H was 35.2 Ådistant from the N-termius of V_L. The linker peptide was 48.8 Åin length residing at the opposite side of CDR and fitting well for linking

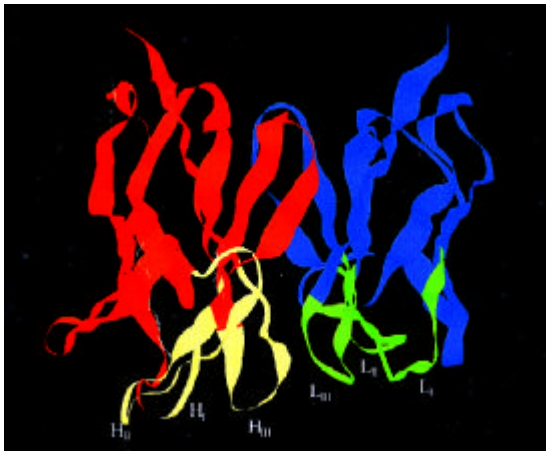

Fig 1. Three-dimensional structure of Sc3F3 constructed by computer homology modeling. The red denoted V_H; the blue denoted V_L; H_I, H_{II}, and H_{III} in yellow color, CDR of V_H; L_I, L_{II}, and L_{III} in green color, CDR of V_L.

Tab 1. The system energy of V_H, V_L, and Sc3F3 (kJ).

	V_L	V_H	Sc3F3
Total energy	3849.17	5089.36	8050.83
Bond energy	1355.40	1475.30	2826.03
Theta energy	1874.57	2362.45	4242.30
Phi energy	470.29	472.07	958.24
Out of plane energy	15.51	23.73	40.46
Nonbond energy	1159.15	1138.38	1680.17
Non-bond repulsion energy	15784.40	17530.47	33805.11
Non-bond dispersion energy	-14625.25	-16392.09	-32124.95
Coulomb energy	-1025.74	-382.57	-1696.59

Tab 2. Hydrogen bonds formed between V_H and V_L in Sc3F3.

Donor[1]	Acceptor	Distance (Å)	Angle (º)
H40:HE21	L86:OH	2.38	133.55
H40:HE22	L37:OE1	2.32	137.55
L86:HH	H44:O	1.70	159.38
H45:HG	L99:O	1.75	165.78
L93:HH	H61:O	1.73	159.40
L95:HH	H103:O	1.81	151.24
L35:HH	H105:OD2	1.81	158.33
H105:HD2	L88:OE1	1.59	170.99
L42:HG	H111:O	2.08	134.31

[1]Amino acids were numbered separately for V_H and V_L

of V_H and V_L without interfering the structures of CDR. Profile-3D compatibility test of Sc3F3 showed a score of 107, higher than the anticipated value 102. All the 3D-1D averaged scores of the residues were above the base line (Fig 2), suggesting that the residues were all compatible in a reasonable fashion.

In the profile-3D test of V_H and V_L monomers, the scores of some amino acid residues were less than zero (Fig 3); it further indicated that the hydrophobic amino acid residues originally exposed outside the molecular surface had been buried inside during formation of the heterodimer. Thus, the modeled Sc3F3 manifested itself a rational integral frame. The solvent-accessible surface areas of amino acid residues in CDR were calculated (Tab 3).

Molecular basis of inhibitory effect of Sc3F3 on acetylcholinesterase activity The optimized model of *Torpedo* AChE was imposed upon the prototype 2ACE, deviations of the space shift (RMS) of all the atoms were 1.70 Å, indicating that the optimized AChE structure was quite similar to that of the crystal

Tab 3. Solvent accessibility of amino acid residues in Sc3F3 complementary determining regions.

	CDR1		CDR2		CDR3	
Heavy chain	GLY_32	42.3671	ARG_51	6.0803	SER_100	0.0000
	TYR_33	39.6309	ILE_52	10.6134	THR_101	30.3471
	TYR_34	24.7437	ASN_53	9.5089	MET_102	138.3842
	ILE_35	0.3859	PRO_54	3.8691	ILE_103	78.8349
	HIS_36	9.7512	TYR_55	144.5701	THR_104	47.3171
			ASN_56	110.1383	ASP_105	10.1426
			GLY_57	44.0734	PHE_106	68.2508
			ALA_58	33.0665	ASP_107	42.4652
			THR_59	57.9069	TYR_108	65.7571
			ARG_60	69.9235		
			TYR_61	35.5650		
			ASN_62	12.9359		
			GLN 63	165.7094		
			ASN_64	98.9747		
			PHE_65	9.2064		
			LYS_66	125.3784		
Light chain	SER_24	38.2647	LEU_46	15.2716	GLN_88	4.9312
	ALA_25	10.3442	ILE_47	0.0000	GLU_89	0.0000
	SER_26	72.1942	TYR_48	90.2434	TRP_90	50.2106
	SER_27	68.2129	ASP_49	36.3973	SER_91	25.1128
	SER_28	92.1655	THR_50	29.5429	GLY_92	35.2516
	ILE_29	1.0396	SER_51	49.8146	TYR_93	80.9178
	ARG_30	184.4778	ASN_52	60.1866	PRO_94	22.7706
	TYR_31	53.3214	VAL_53	60.6449	TYR_95	1.8178
	ILE_32	0.0000	ALA_54	5.8875		
	TYR_33	2.1227				
	TRP_34	0.6546				

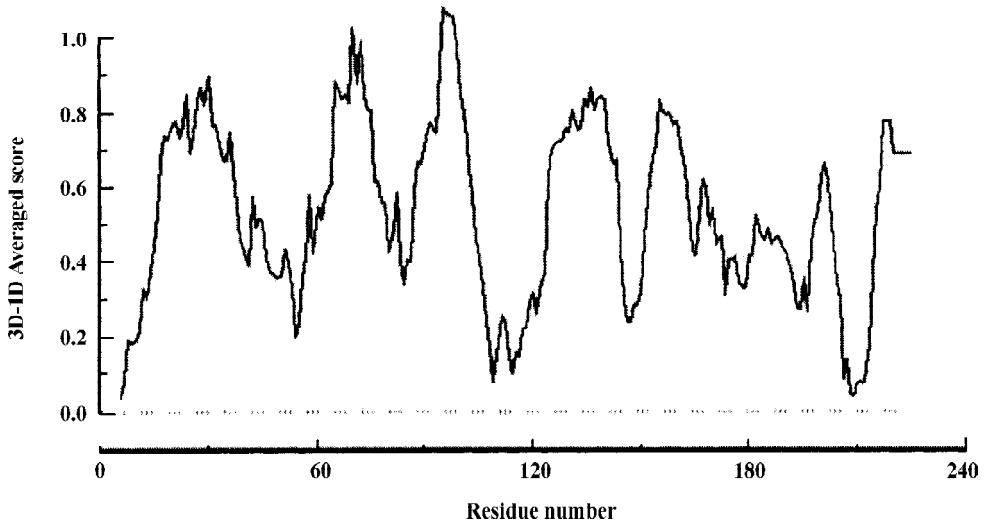

Fig 2. Profile-3D rational evaluation of Sc3F3. Score>0, residues are compatible.

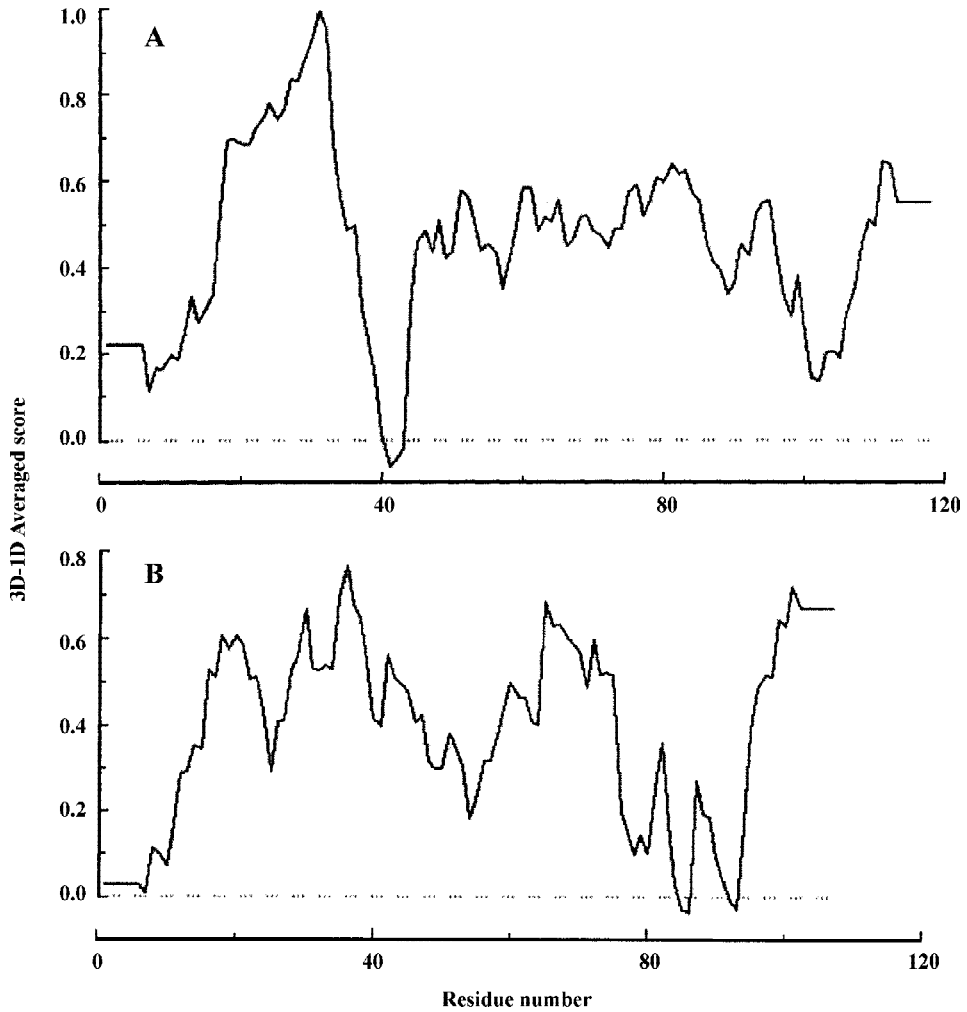

Fig 3. Profile-3D rational evaluation of V_H and V_L. Score<0, residues are incompatible. A: Heavy chain; B: Light chain.

structure. It declared that the modeling AChE was reliable. Besides, the optimized AChE consisted of all the atoms in the molecule, much more complete than 2ACE. This model was selected as the antigen for next

step in molecular docking.

Setting the regions that did not interact with the ligand fixed, and allowing regions interacted with the ligands flexsible would be easy and timesaving in procedure of docking [17]. The initial site was chosen in reference to the mode of action between 3F3 and AChE[4] and the crystal structure of the complex of fasciculin II and AChE[18]. The docking profile was obtained (Fig 4) *via* optimization and dynamic simulation. The total energy of the Sc3F3-AChE complex decreased 836 kJ, demonstrating the formation of a stable structure. The van der Waals force played an important role (Tab 4) suggesting that the complex formed mainly via hydrophobic interactions between Sc3F3 and AChE molecules. Eight pairs of hydrogen bonds were also formed between Sc3F3 and AChE (Tab 5). It could be seen that Sc3F3 overlaid the entrance of the active center gorge of AChE blocking the access of substrate

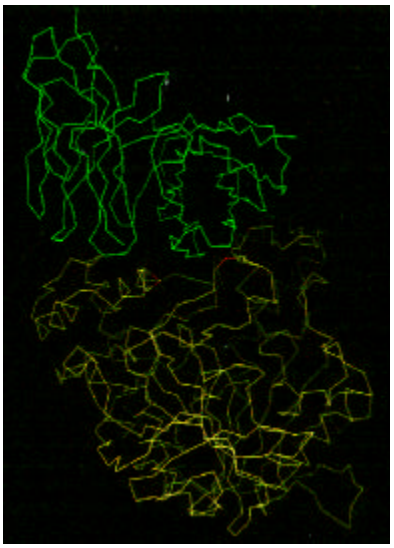

Fig 4. Docking of Sc3F3 and AChE. AChE and Sc3F3 were shown in yellow and green color, respectively. Trp 279 and Tyr 70 were shown in red color.

Tab 4. Energy of the docking complex of Sc3F3 and AChE.

Van der Waals energy =	-646.52 k J
Repulsion energy =	384.66 kJ
Dispersion energy =	-1029.18 kJ
Coulomb energy =	-178.90 kJ
Total energy =	-825.42 kJ

(Fig 4). This constructs the molecular basis of inhibitory effect of Sc3F3 on AChE activity.

Tab 5. Hydrogen bonds formation between Sc3F3 and AChE (B means heavy chain).

Donor[1]	Acceptor	Distance (Å)	Angle (°)
Anti-59B:HN	AChE-70:OH	1.89	167.92
AChE-73:HE2	Anti-66B:NZ	2.14	160.27
AChE-269:HZ1	Anti-91:O	2.48	150.03
Anti-60B:HH22	AChE-273:OE1	2.13	146.69
AChE-273:HE2	Anti-90:NE1	2.16	159.77
Anti-34B:HH	AChE-280:OD1	1.68	169.68
Anti-55B:HH	AChE-285:O	1.71	166.23
AChE-285:HD2	Anti-56B:OD1	1.72	174.67

DISCUSSION

In a previous research, Sc3F3 expressed in *E coli* HB2151 had been purified. The immuno-reactivity test indicated that the purified Sc3F3 could well reacted with AChE inhibiting the AChE activity, though it was much weaker than that of the prototype 3F3 as expected[6]. The computer-aided molecular docking predicted that Sc3F3 would not only keep the binding ability with AChE, but also conserve the inhibitory action on the enzymatic activity *via* steric blockade of the catalytic gorge entrance hampering the access of the substrate. The genetic engineering product Sc3F3 intensively verified that it was the case, the recombinant single-chain antibody Sc3F3 remained both the immunoreactivity and the inhibitory ability towards AChE, thereof well confirmed the modeling anticipation.

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2-120
AQVKLQESGPELEKPGASVKISCKASGYSFTGYYIHWV
KQSHVKSLEWIGRINPYNGATRYNQNFKDKANLAVD
KSSRTAYMELHSLTSEDSAVYYCALSTMITDFDYWGP
GATVTVSS
Light chain:
136-242
DIELTQSPAIMSASPGERVTMTCSASSSIRYIYWYQQK
PGSSPRLLIYDTSNVAPGVPFRFSGKGKGTSYSLTINRMEA
EDAATYYCQEWSGYPYTFGGGXKXEXKR
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GGGGSGGGGSGGGGS

Modeling of V_H and V_L of Sc3F3 V_H and V_L were modeled separately. Then a linker sequence of 15 residues between them was put to make a complete sequence of Sc3F3. The linker peptide consisted of 12 glycines and 3 inserting serines, thereof increased the flexibility and hydrophilicity of Sc3F3. Since the linker peptide did not keep a stable structure, the contribution of linker peptide in molecular modeling was not considered.

V_H and V_L sequences were sent to the Swiss-Model server (http://www.expasy.ch/swissmod/SWISS-MODEL.html) for analysis (FASTA, BLAST, *etc*). The sorted out proteins with >35 % homology to the V_H and V_L sequences were adopted as modeling templates. The three-dimensional structures were modeled using ProMed software[8-10] , and then optimized respectively under the module Discover[11] in InsightII 98.0 software package from MSI Co on SGI O2 workstation. Firstly, all of the heavy atoms were fixed, 1000 steps of calculation in the consistent-valence forcefield were carried out by the steepest descent method, followed by optimization using the conjugated gradient method. To overcome the regional potential barrier releasing all the atoms, molecular dynamics simulation was performed at 300 K for 20 ps (1 ps=10^{-12} s). The conformation was collected consecutively at 1-ps intervals, then optimized as above-mentioned to the smallest gradient 8.36 kJ· Å^{1}· mol^{-1}, and the lowest-energy conformation was chosen as the ultimate model.

Assembly of the integral structure of Sc3F3 The amino acid sequences of V_H and V_L were aligned with reference to a structurely identified antibody 1 plg[12] to determine the structure conservative regions (SCR). Then the backbone atoms were superimposed respectively on 1-plg for their relative steric positions, thus

the preliminary structure of Sc3F3 was obtained. The final structure was acquired by optimization, and tested for residue compatibility with Profile-3D program[13].

Calculation of solvent accessible surface of Sc3F3 amino acid residues The solvent accessible surfaces were calculated and displayed using programs of Delphi and Insight II (Biosgm, Inc, San Diego, CA). The solvent accessible surface area of each residue was calculated using an implement of Lee and Richards algorithm[15].

Three-dimensional structure optimization of *Torpedo* AChE The crystal structure of *Torpedo* AChE (2ACE, in PDB) has been reported in 1991[16]. However, the data missed 5 amino acid residues (485-489) and some side chain atoms. We sent the sequence of *Torpedo* AChE to Swiss-Model server to model the whole sequence again restricting 2ACE as template. The structure sent back from the server was optimized. The conformation with the lowest energy was reckoned to be the ultimate model.

Docking between Sc3F3 and *Torpedo* AChE The CDR was firstly aimed at the fixed AChE molecule. Then moving Sc3F3 approached to AChE in reasonable contact, and the initial site of docking was determined according to the binding energy calculated. The related amino acid residues of Sc3F3 were released and the whole system was optimized *via* molecular mechanic and dynamic calculations under condition of AChE fixation. Finally, AChE and Sc3F3 were set as the centers respectively, and searched within a radius of 5 Å for the atoms reacting with the modeling structures. The related amino acid residues were further released and optimized to obtain the defined model. The final complex structure had a large solvent accessible buried surfaces between Sc3F3 and *Torpedo* AChE, and the interaction energy was -825.42 kJ/mol.

RESULTS

Detection of CDR of Sc3F3 CDR were identified by multiple sequence comparison[14]. V_H and V_L each contained three CDR and two cysteine residues necessary for the maintenance of the antibody structure (Fig 1). CDR of V_H were estimated at 32-36, 51-66, and 100-108 amino acid residues, while CDR of V_L at 24-34, 46-54, and 88-95 residues.

Structure of the modeled Sc3F3 molecule The modeled structure of Sc3F3 was shown in Fig 1. It had the common features of a classical antibody. Both V_H and V_L were composed of two β-sheets and connecting loops. The two-disulfide bonds located at Cys23-Cys97 in V_H and Cys23-Cys87 in V_L with an average bond-length of 2.0 Åaccording to the standard length of disulfide bond for stabilizing the molecule structure. The total energy of V_H and V_L after optimization came to 5089.15 and 3849.36 kJ, whereas the energy of Sc3F3 (8050.68 kJ) decreased 887.83 kJ (Tab 1) nearly equal to the total interacting energy (-774.14 kJ) between V_H and V_L. It implied that V_H and V_L had a tendency to form a dimer. Nine pairs of hydrogen bonds took part in the formation of the dimer (Tab 2). Those hydrogen bonds played an important role in the stabilization of molecules. All the six loops situated at one side of the dimer to form the CDR. The C-termius of V_H was 35.2 Ådistant from the N-termius of V_L. The linker peptide was 48.8 Åin length residing at the opposite side of CDR and fitting well for linking

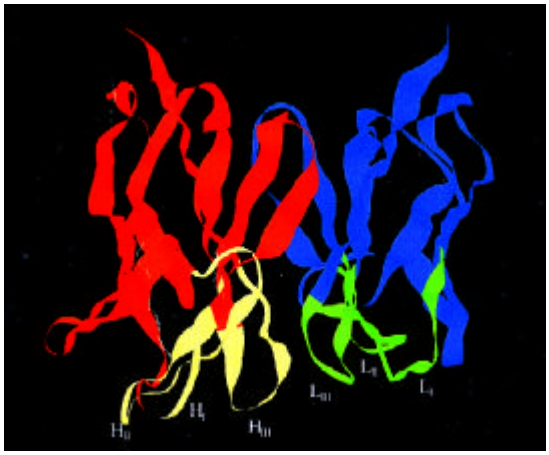

Fig 1. Three-dimensional structure of Sc3F3 constructed by computer homology modeling. The red denoted V_H; the blue denoted V_L; H_I, H_{II}, and H_{III} in yellow color, CDR of V_H; L_I, L_{II}, and L_{III} in green color, CDR of V_L.

Tab 1. The system energy of V_H, V_L, and Sc3F3 (kJ).

	V_L	V_H	Sc3F3
Total energy	3849.17	5089.36	8050.83
Bond energy	1355.40	1475.30	2826.03
Theta energy	1874.57	2362.45	4242.30
Phi energy	470.29	472.07	958.24
Out of plane energy	15.51	23.73	40.46
Nonbond energy	1159.15	1138.38	1680.17
Non-bond repulsion energy	15784.40	17530.47	33805.11
Non-bond dispersion energy	-14625.25	-16392.09	-32124.95
Coulomb energy	-1025.74	-382.57	-1696.59

Tab 2. Hydrogen bonds formed between V_H and V_L in Sc3F3.

Donor[1]	Acceptor	Distance (Å)	Angle (º)
H40:HE21	L86:OH	2.38	133.55
H40:HE22	L37:OE1	2.32	137.55
L86:HH	H44:O	1.70	159.38
H45:HG	L99:O	1.75	165.78
L93:HH	H61:O	1.73	159.40
L95:HH	H103:O	1.81	151.24
L35:HH	H105:OD2	1.81	158.33
H105:HD2	L88:OE1	1.59	170.99
L42:HG	H111:O	2.08	134.31

[1]Amino acids were numbered separately for V_H and V_L

of V_H and V_L without interfering the structures of CDR. Profile-3D compatibility test of Sc3F3 showed a score of 107, higher than the anticipated value 102. All the 3D-1D averaged scores of the residues were above the base line (Fig 2), suggesting that the residues were all compatible in a reasonable fashion.

In the profile-3D test of V_H and V_L monomers, the scores of some amino acid residues were less than zero (Fig 3); it further indicated that the hydrophobic amino acid residues originally exposed outside the molecular surface had been buried inside during formation of the heterodimer. Thus, the modeled Sc3F3 manifested itself a rational integral frame. The solvent-accessible surface areas of amino acid residues in CDR were calculated (Tab 3).

Molecular basis of inhibitory effect of Sc3F3 on acetylcholinesterase activity The optimized model of *Torpedo* AChE was imposed upon the prototype 2ACE, deviations of the space shift (RMS) of all the atoms were 1.70 Å, indicating that the optimized AChE structure was quite similar to that of the crystal

Tab 3. Solvent accessibility of amino acid residues in Sc3F3 complementary determining regions.

	CDR1		CDR2		CDR3	
Heavy chain	GLY_32	42.3671	ARG_51	6.0803	SER_100	0.0000
	TYR_33	39.6309	ILE_52	10.6134	THR_101	30.3471
	TYR_34	24.7437	ASN_53	9.5089	MET_102	138.3842
	ILE_35	0.3859	PRO_54	3.8691	ILE_103	78.8349
	HIS_36	9.7512	TYR_55	144.5701	THR_104	47.3171
			ASN_56	110.1383	ASP_105	10.1426
			GLY_57	44.0734	PHE_106	68.2508
			ALA_58	33.0665	ASP_107	42.4652
			THR_59	57.9069	TYR_108	65.7571
			ARG_60	69.9235		
			TYR_61	35.5650		
			ASN_62	12.9359		
			GLN 63	165.7094		
			ASN_64	98.9747		
			PHE_65	9.2064		
			LYS_66	125.3784		
Light chain	SER_24	38.2647	LEU_46	15.2716	GLN_88	4.9312
	ALA_25	10.3442	ILE_47	0.0000	GLU_89	0.0000
	SER_26	72.1942	TYR_48	90.2434	TRP_90	50.2106
	SER_27	68.2129	ASP_49	36.3973	SER_91	25.1128
	SER_28	92.1655	THR_50	29.5429	GLY_92	35.2516
	ILE_29	1.0396	SER_51	49.8146	TYR_93	80.9178
	ARG_30	184.4778	ASN_52	60.1866	PRO_94	22.7706
	TYR_31	53.3214	VAL_53	60.6449	TYR_95	1.8178
	ILE_32	0.0000	ALA_54	5.8875		
	TYR_33	2.1227				
	TRP_34	0.6546				

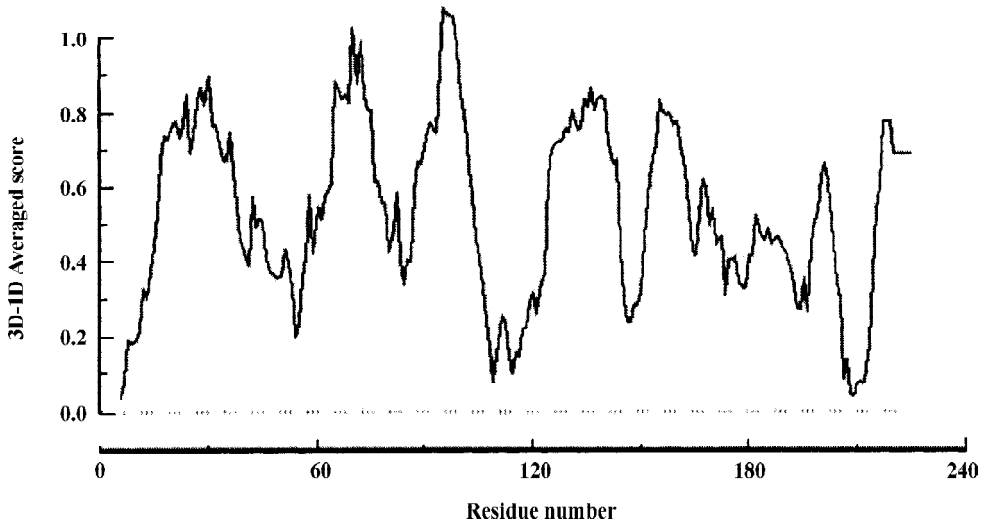

Fig 2. Profile-3D rational evaluation of Sc3F3. Score>0, residues are compatible.

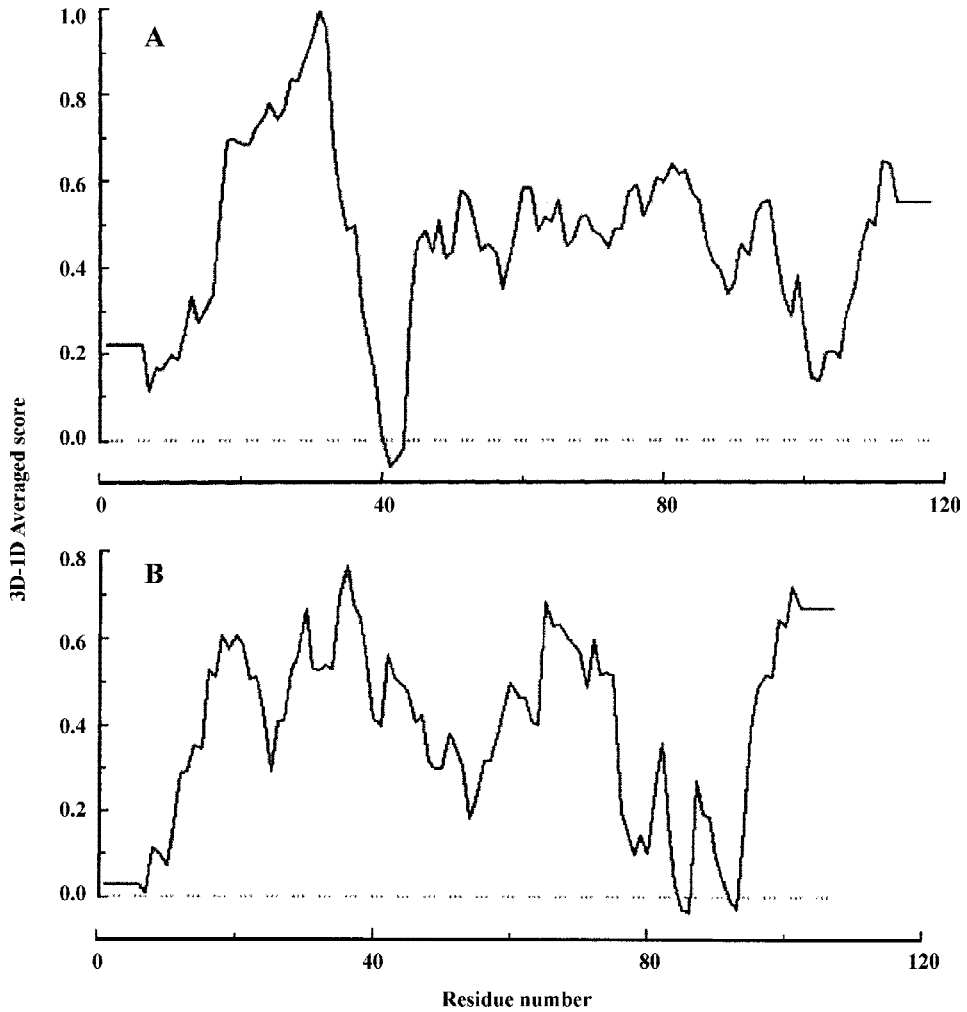

Fig 3. Profile-3D rational evaluation of V_H and V_L. Score<0, residues are incompatible. A: Heavy chain; B: Light chain.

structure. It declared that the modeling AChE was reliable. Besides, the optimized AChE consisted of all the atoms in the molecule, much more complete than 2ACE. This model was selected as the antigen for next

step in molecular docking.

Setting the regions that did not interact with the ligand fixed, and allowing regions interacted with the ligands flexsible would be easy and timesaving in procedure of docking [17]. The initial site was chosen in reference to the mode of action between 3F3 and AChE[4] and the crystal structure of the complex of fasciculin II and AChE[18]. The docking profile was obtained (Fig 4) *via* optimization and dynamic simulation. The total energy of the Sc3F3-AChE complex decreased 836 kJ, demonstrating the formation of a stable structure. The van der Waals force played an important role (Tab 4) suggesting that the complex formed mainly via hydrophobic interactions between Sc3F3 and AChE molecules. Eight pairs of hydrogen bonds were also formed between Sc3F3 and AChE (Tab 5). It could be seen that Sc3F3 overlaid the entrance of the active center gorge of AChE blocking the access of substrate

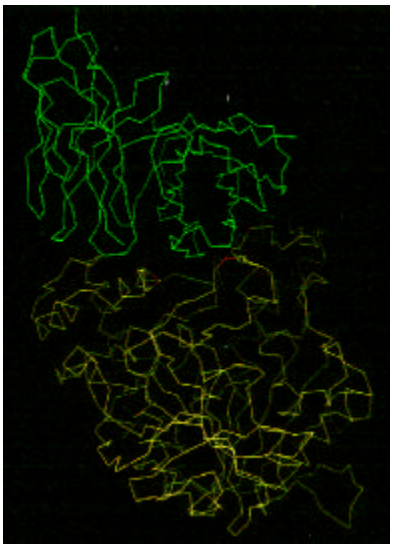

Fig 4. Docking of Sc3F3 and AChE. AChE and Sc3F3 were shown in yellow and green color, respectively. Trp 279 and Tyr 70 were shown in red color.

Tab 4. Energy of the docking complex of Sc3F3 and AChE.

Van der Waals energy =	-646.52 k J
Repulsion energy =	384.66 kJ
Dispersion energy =	-1029.18 kJ
Coulomb energy =	-178.90 kJ
Total energy =	-825.42 kJ

(Fig 4). This constructs the molecular basis of inhibitory effect of Sc3F3 on AChE activity.

Tab 5. Hydrogen bonds formation between Sc3F3 and AChE (B means heavy chain).

Donor[1]	Acceptor	Distance (Å)	Angle (°)
Anti-59B:HN	AChE-70:OH	1.89	167.92
AChE-73:HE2	Anti-66B:NZ	2.14	160.27
AChE-269:HZ1	Anti-91:O	2.48	150.03
Anti-60B:HH22	AChE-273:OE1	2.13	146.69
AChE-273:HE2	Anti-90:NE1	2.16	159.77
Anti-34B:HH	AChE-280:OD1	1.68	169.68
Anti-55B:HH	AChE-285:O	1.71	166.23
AChE-285:HD2	Anti-56B:OD1	1.72	174.67

DISCUSSION

In a previous research, Sc3F3 expressed in *E coli* HB2151 had been purified. The immuno-reactivity test indicated that the purified Sc3F3 could well reacted with AChE inhibiting the AChE activity, though it was much weaker than that of the prototype 3F3 as expected[6]. The computer-aided molecular docking predicted that Sc3F3 would not only keep the binding ability with AChE, but also conserve the inhibitory action on the enzymatic activity *via* steric blockade of the catalytic gorge entrance hampering the access of the substrate. The genetic engineering product Sc3F3 intensively verified that it was the case, the recombinant single-chain antibody Sc3F3 remained both the immunoreactivity and the inhibitory ability towards AChE, thereof well confirmed the modeling anticipation.

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