Prediction of a common neutralizing epitope of H5N1 avian influenza virus by *in silico* molecular docking

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The H5N1 avian influenza virus (AIV) has widely spread in Asia, Europe and Africa, making a large amount of economic loss. Recently, our research group has screened a common neutralizing monoclonal antibody named 8H5, which can neutralize almost all H5 subtype AIV ever isolated so far. Obviously, this monoclonal antibody would benefit for research and development of the universal AIV vaccine and design of the drug against H5N1 AIV in high mutation rate. In this study, the homology modeling was applied to generate the 3D structure of 8H5 Fab fragment, and “canonical structure” method was used to define the specified loop conformation of CDR regions. The model was subjected to energy minimization in cvff force field with Discovery module in Insight II program. The resulting model has correct stereochemistry as gauged from the Ramachandran plot calculation and good 3D-structure compatibility as assessed by interaction energy analysis, solvent accessible surface (SAS) analysis, and Profiles-3D approach. Furthermore, the 8H5 Fab model was subjected to docking with three H5 subtype hemagglutinin (HA) structures deposited in PDB (ID No: 1j5m, 2ibx and 2fk0) respectively. The result indicates that the three docked complexes share a common binding interface, but differ in binding angle related with HA structure similarity between viral subtypes. In the light of the three HA interfaces with structural homology analysis, the common neutralizing epitope on HA recognized by 8H5 consists of 9 inconsecutive amino acid residues: Asp⁶⁸, Asn⁷², Glu¹¹², Lys¹¹³, Ile¹¹⁴, Pro¹¹⁸, Ser¹²⁰, Tyr¹³⁷, Tyr²⁵² (numbered as for 1j5m sequence). The primary purpose of the present work is to provide some insight into structure and binding details of a common neutralizing epitope of H5N1 AIV, thereby aiding in the structure-based design of universal AIV vaccines and anti-virus therapeutic drugs.

H5N1 avian influenza virus, molecular docking, hemagglutinin, neutralizing epitope

Since late 2003, H5N1 avian influenza virus (AIV) has widely spread all over the world, not only resulting in mass poultry death, but also leading to high mortality in human patients. Hemagglutinin (HA), the major surface protein of AIV, not only mediates viral attachment and entry into the host cell by binding to sialic acid receptors, but also is responsible for protective humoral immunity by neutralizing antibody. However, the high mutation ratio of HA makes it very difficult to control AIV. Recently we have constructed an antibody panel that contained 388 antibodies possessing H5 hemagglutinin inhibited (HI) activity. In this panel, one antibody

8H5 can strongly neutralize all of the 46 viruses isolated from different clades, locations and hosts^[1]. The research on the structure of this common neutralizing antibody may contribute to a breakthrough in seeking methods to overcome the high mutation of H5N1.

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Because antibody has an intrinsic nature with conserved amino acid sequence and typical configuration in 3D structure, the structure can be modeled by some structural templates from Brookhaven PDB where a large number of X-ray resolved antibody structures deposited^[2,3]. The homology modeling method has been widely used to search for the epitopes of antigen and to improve the binding affinity. Frequently 5 of the 6 complementarity-determining regions (CDRs) (all except H3) of antibody fall into one of between 2 and 6 structural classes, referred to as canonical classes^[3,4]. Based on the determinants of the canonical structures, the most accurate method for building the model of antibody can be developed^[5]. Here we will describe the structure modeling of 8H5 using the canonical structure method and the docking pattern between 8H5 and three HAs deposited in PDB. The docking results can shed light on the common neutralizing epitope of H5, thus providing invaluable information for the development of the potential vaccine, design of antiviral drug and research on the broad-spectrum therapeutic antibody.

1 Materials and methods

1.1 Antibody sequence preparation and antigen structure refinement

The monoclonal antibody 8H5 was raised in our lab, and the gene of variable regions was cloned from the hybrid plasmoma cell and verified with sequencing by BoYa Co. in Shanghai^[1]. The primary amino acid sequence of the variable regions is listed below: Variable region of light chain, EIVLTQSPAIMSASLGEKVTMSCRASSSVNFVYWYQQRSDASPKLLIYSSNLAPGVPPRFSGSGSGNSYSLTISGLEGEDAATYYCQHFTSSPYTFGGGT-KLEIKRLE; Variable region of heavy chain, QVQLQQSGAELMKPGASVKISCKATGYTFSNYWIEWIKRPPGHGLEWIGEILPGSDRTNYNGKFKGKATFTADTSSNTAHMQLSSLTSEDSAVYYCANRYDGYFFGLDYWGQGTSVTVSS.

There were 3 X-ray resolved HA antigen structures depositing in PDB. The PDB IDs were as follows: 1jsm (Virus strain: A/DUCK/SINGAPORE/3/97, Host: Bird), 2fk0 (Virus strain: A/VIETNAM/1203/2004, Host: Human) and 2bix (Virus strain: VN1194, Host: Human)^[6–8]. The structure of 1jsm was refined in Insight II package (Accelrys Company) before docking with the antibody. Because the other 2 structures were deposited as the trimers, the monomers were unmerged and then sub-

jected to refinement before docking.

1.2 Chothia numbering of antibody sequence and canonical structure analysis

According to Chothia numbering scheme, the amino acid insertion and deletion site in antibody were located in residues L31, L95, L106, H31, H52, H82 and H100^[2]. For the variable domain in light chain (VL), the conserved residue cysteine was always located in residues 23 and 88 which were 1 residue ahead of the start point of CDR-L1 and CDR-L3 respectively. The residue after CDR-L1, which ranged from residue 24 to 33, was always Trp and the residues after CDR-L3, which ranged from residue 89 to 97, were always Phe-Gly. CDR-L2, which ranged from residue 50 to 56, always started sixteen residues after the end of L1. The residues before CDR-L2 generally were Ile-Tyr. For the variable domain in heavy chain (VH), the conserved residue cysteine was always located in residues 22 and 92, which were 4 and 3 residues ahead of the start point of CDR-H1 and CDR-H3, respectively. The residue after CDR-H1, which ranged from residue 26 to 32, was always Trp and the residues after CDR-H3, which ranged from residue 95 to 102, were always Trp-Gly. CDR-H2, which ranged from residue 52 to 56, always started 15th residues after the end of H1. The residues before CDR-H2 typically were Leu-Glu-Trp-Ile-Gly and the residues after CDR-H2 were Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala. In spite of the high sequence variability in CDRs, five of them (all except CDR-H3) can assume just a small repertoire of main-chain conformation, called canonical structures, and each of the 5 CDRs fell into 1 of between 2 and 6 canonical structure classes. The conformations for canonical structures were determined by the length of the loops and by the presence of key residues at specific positions in the antibody sequence (either within the loops or in the framework regions)^[5].

1.3 Molecular modeling of L and H chains

In the Homology module of Insight II software package, L and H sequences were aligned with the sequences from PDB using FASTA method^[9,10]. The protein structures with >50% homology to the targets were selected as templates. The conserved residue coordinates in framework were first assigned. For the canonical structures, the coordinates from PDB with the same canonical classes as targets were adopted and assigned to the L or H sequences. For the CDR-H3, the sequence was also sent to PDB to seek the best loop coordinate, and the

one with least RMSD was selected as the template. The terminals and the splice region of the structures were repaired by the Refine program in Homology module in Insight II package. The structures were released with the RMSD less than 0.5 Å before proceeding to the next step. And then the models were subjected to optimization in the consistent-valence force field using the conjugate gradient method, the steepest descent method and the molecule dynamics method in the Discover module.

1.4 Structural pairing of L and H chains

The residues at the L-H interface were highly conserved among almost all antibodies, which were: L33–L39, L43–L47, L84–L90, L98–L104, H34–H40, H44–H48, H88–H94, H103–H109^[3,11]. In order to obtain the fab fragment, the L and H domains were packed together by a least-squares fit of the main-chain atoms of residues in the interface. Only the outcome with RMSD less than 0.5 Å was adopted. The structures of L and H were merged to an assembly named 8H5Fab. In program BUMP, the residues in serious collision were subjected to torsion revision. After that, the backbone atoms of 8H5Fab were fixed, and 1000 steps of optimization in the consistent-valence force field were carried out by the conjugate gradient method and the steepest descent method. The energy minimized 8H5Fab was tested by residue compatibility with Profile-3D program, solvent accessible surface and interaction energy analysis.

1.5 Molecular docking of 8H5Fab and HAs

The 8H5Fab and the optimized antigens were subjected to Zdock in Zdockpro module in Insight II package^[12]. The 8H5Fab was taken as receptor and the antigens were ligand. In order to save the computing time, the regions of HA2 and the constant regions of 8H5Fab were blocked. From the Zdock output file, the first 30 poses were selected for further Rdock analysis^[13]. From the Zdock scores, Rdock scores and the CDR binding regions, the complexes were screened for further study.

1.6 Solvent accessible surface analysis of complexes and epitope candidates prediction

Based on the complexes and the individual HAs, 8H5Fab, the values of solvent accessible surface (SAS) area were calculated for each residue using a probe radius of 1.4 Å. The residues of HAs with more than 7 Å² decrease in SAS during binding were regarded as the epitope candidates^[14–16]. Moreover, an HA residue was

considered to belong to the epitope if it was significantly buried in the complex and at least one atom of the residue made an atomic contact with an atom of the antibody fragment, at a distance compatible with either a hydrogen bond, a salt bridge or a Van der Waals interaction.

2 Results

2.1 Molecular modeling of 8H5Fab

(i) The templates for 8H5Fab modeling and its “canonical structure” classes. In the light of the homologous similarity, the templates for the V_L or V_H were screened from the PDB. From the multiple sequence alignment between the target sequences and the templates, the conserved residues, the conserved frameworks and CDRs can be identified (Figure 1).

The sequences were labeled with the Chothia numbering method (Table 1). For CDR-L1, there was one amino acid residue deletion located in residue 31. One and three residues insertion occurred in CDR-H2 (P52a) and CDR-H3 (F100a, G100b and L100c), respectively. Based on the length of the loops and the presence of key residues at specific positions in the antibody sequence, the canonical structure classes were defined (Table 1). Three of them (CDR-L1, CDR-L3 and CDR-H1) belonged to class 1 and the other two were to class 2^[5].

(ii) Structure of the 8H5Fab. The modeled structure of the 8H5Fab is shown in Figure 2, its chains L and chain H having the common features of a typical antibody. Chain L contained 211 aa totally with 109 residues located in variable domain and the rest 102 in constant domain. Chain H contained 219 aa with 120 residues were located in variable domain and the rest 99 in constant domain. Each domain was packed with two face-to-face β sheets, linked together by a conserved disulfide bridge and inter-chain loops. The disulfide bonds were located at Cys²³-Cys⁸⁸ in VL and at Cys²²-ys⁹² in VH. Also there were two pairs of disulfide bonds in the constant domains. Like other antibodies, the average bond-length of disulfide bonds was about 2.0 Å which was the standard length to stabilize the molecule structure.

(iii) The verification of the 8H5Fab structure.

(1) Energy analysis. As shown in Table 2, the total energy of model H and model L was 3637.96 and 4551.28 kal, respectively. However the energy of 8H5Fab was 7875.71 kcal, which was 313.53 kal less

Figure 1 Sequence alignment between the V_L, V_H and the templates (clustal X).

Table 1 The sequence numbering and canonical structure classification

	Amino acid	Original numbering	Chothia numbering	Canonical structure
CDR-L1	RASSVNIFY	24–33	24 25 26 27 28 29 30 32 33 34	1
CDR-L2	YSSNLAP	49–55	50 51 52 53 54 55 56	2
CDR-L3	QHFTSSPYT	88–96	89 90 91 92 93 94 95 96 97	1
CDR-H1	GYTFSNY	26–32	26 27 28 29 30 31 32	1
CDR-H2	LPGSDR	52–57	52 52a 53 54 55 56	2
CDR-H3	RYDGYFGLDY	99–109	95–100 100a 100b 100c 101 102	
Others	LSSLT	83–87	82 82a 82b 82c 83	

than the total energy of the L and H models. The loss of energy indicated that the 8H5Fab became more stable after L-H pairing. Moreover, as shown in Table 3, the loss of energy which was almost the same as interacting energy between model H and model L implied that the two models had a tendency to form a dimer, and the pairing was driven by the free energy. This obeys the second law of thermodynamics.

(2) SAS analysis. With 1.4 Å solvent radius, the SAS for model L and H was 11580.41 and 11852.29 Å², respectively. However the SAS for 8H5Fab was 1973709 Å², about 3600 Å² less than the total SAS of

model L and H. For the typical antibody structure, *Cyrus Chothia* reported that the loss SAS between V_H and V_L domains was about 1800 Å² after pairing^[11]. 8H5Fab consisted of V_H-V_L and C_H-C_L, and the buried surface between model L and H was twice that of the typical 1800 Å² between two partner domains, which further verified the model 8H5Fab.

(3) Hydrogen bond analysis. At the interface of the model L and H, there were 13 pairs of hydrogen bonds (Table 4). Seven of them were located in the variable regions and the other 6 in the constant regions. The hydrogen bonds were one of the main forces between the

Figure 2 The 3D structure of the model 8H5Fab.

interacting domains, playing an important role in the stability of the model 8H5Fab.

(4) Ramachandran plot. In Figure 3, the ramachandran plot of the 6 loops showed that 80% residues of the CDRs were in the most favored regions, meaning that the model 8H5Fab was in good 3D-structure compatibility. The 8H5Fab model superimposed with crystallographically-determined antibody structures showed good overall agreement exception to the loops.

(5) Profile-3D analysis. When the 8H5Fab was

subjected to profile-3D test in Insight II package, the overall self-compatibility score for 8H5Fab was 216.91, while the score expected for a valid protein of this size was 196.23 and for a protein of this size, a score of 88.30 or less would indicate a structure that was almost certainly incorrect. Moreover, the test scores of all amino acid residues were above zero (Figure 4). All of this indicated that the model 8H5Fab was in a very good and reasonable conformation.

2.2 The molecular docking between 8H5Fab and the HAs

In Figure 5, the binding sites of 8H5 in HAs were quite near the receptor binding sub-domains^[8], suggesting that the antibody 8H5 might compete for the binding sites with the receptor and block the avian influenza virus infection, i.e. the so-called neutralizing activity.

On keeping the 8H5Fab location still, and superimposing the 3 8H5Fab-HAs in facility for the analysis, it was clear that 8H5Fab bound to 1jasm and 2bix HAs almost in the same way, but for the 2fk0 HA, there was some deviation, and the binding sites were very close to the His/Lys patch. In detail, it showed that the side chains of 8H5Fab for the binding were differently involved (Figure 5(b)) just as the binding angles' difference (Figure 5(a)). Moreover, it was reported that 1jasm was from bird and 2ibx was from human, which indicated that the preference binding of 8H5 was unrelated to the hosts. Comparing 1jasm and 2ibx, we found that the HA structure from 2fk0 was more similar to that from H1, which implied that 2fk0 was the transition

Table 2 The energy analysis of model L, model H and model 8H5Fab

	Model L (kcal)	Model H (kcal)	8H5Fab (kcal)
Van der Waals energy	1903.96936	1830.8492	3506.664
Repulsive energy	9630.30371	9506.7725	19408.84
Dispersive energy	-7726.33447	-7675.923	-15902.18
Coulomb energy	1.61702	789.50665	705.7458
Bond energy	536.83484	457.26556	994.1004
Theta energy	874.28912	1071.5287	1945.818
Phi energy	251.47975	270.37045	521.8502
Out of plane energy	7.66596	15.04769	22.71366
Bond-bond energy	15.56962	33.08727	48.65688
Bond-theta energy	44.38714	81.77821	126.1654
Theta-theta energy	21.44454	31.37887	52.82341
Theta-theta-phi energy	-19.29458	-29.53335	-48.82793
Bond-bond(1-3) energy	0	0	0
Op-op energy	0	0	0
Phi-phi energy	0	0	0
Hydrogen-bond energy	0	0	0
Total energy	3637.96289	4551.2778	7875.707

Table 3 The interaction energy of model L and model H

Summary for objects: L and H	
Van der Waals energy =	-225.68803 kcal
Repulsion energy =	271.72363 kcal
Dispersion energy =	-495.94510 kcal
Coulomb energy =	-85.37601 kcal
Total energy =	-311.06405 kcal

Table 4 Hydrogen bonds formed between models L and H in 8H5Fab

H receptor	H donor	Distance (Å)	Angle (°)
L:38:HE22	H:39:OE1	2.05	155.28
L:1:HE2	H:46:OE1	1.83	156.42
L:94:HG	H:50:OE2	2.18	153.78
H:60:HD22	L:1:OE1	2.41	145.53
H:100B:HN	L:34:OH	1.63	152.72
L:36:HH	H:100C:O	1.93	172.15
L:43:HG	H:104:O	1.93	156.76
L:174:HG	H:164:ND1	2.43	148.73
L:174:HG	H:164:NE2	2.30	151.36
H:167:N	L:162:OG	2.90	NA ^{a)}
L:137:HD22	H:180:OG	2.34	165.49
L:122:HN	H:212:O	1.98	160.82
L:122:HN	H:212:OXT	2.38	130.38

a) NA, No data was detected.

Figure 3 Ramachandran plot of CDRs.

structure from H5 to H1^[6].

2.3 The prediction of HA epitope

(i) Δ SAS analysis. The solvent accessible surface area of each residue in HA structures was calculated as the monomer and complex and the Δ SAS was developed (Table 5). Some residues, such as Phe⁷⁹ in 2fk0, Ser¹²⁰ in 1jsm and Ser¹²⁴ in 2bix, were deeply buried in the complexes with a fraction larger than 90%. Based on the complexes, the contacting residues within 4 Å were also

Figure 4 Profile-3D test of model 8H5Fab.

identified. Taking consideration of the Δ SAS and the contacting residues, the epitopes were defined as follows (Table 5).

(ii) Sequence alignment analysis. From the experiment we carried out, we found that 8H5 could react with almost all of the H5 influenza viruses, indicating that the binding sites in HA were the common neutralizing epitope. Therefore, in the light of the sequence alignment (Figure 6) and the epitopes in 3 HAs shown in Table 5, the common 8H5 epitope consisted of (aa in 1jsm) Asp⁶⁸, Asn⁷², Glu¹¹², Lys¹¹³, Ile¹¹⁴, Pro¹¹⁸, Ser¹²⁰, Tyr¹³⁷, Tyr²⁵².

(iii) Natural mutation analysis. In order to test the conservation of the common epitope in HAs, natural mutation analysis was performed. By blast on-line, 1000 H5 HA sequences from different virus isolates were screened and subjected to conservation analysis. As Table 6 shows, the amino acids were highly conserved with a ratio as high as 99%, except for the Ser¹²⁰ with 95.9% and Tyr²⁵² with 87.2%. The data also showed that the prediction of the common neutralizing epitope was convincing.

(iv) Hydrogen bond analysis in the epitope. There were many pairs of hydrogen bonds at the interface of HA-8H5Fab complex (Table 7). Most interestingly, the amino acid Lys¹¹³ in 1jsm (Lys¹¹⁷ in 2ibx and Lys¹²⁰ in 2fk0) contributed to the hydrogen bond formation in all the three HAs, indicating the most important role it played in the predicted common epitope. However, the amino acid Lys worked as the proton donor in 2fk0 but as the receptor in 1jsm and 2ibx, which further confirmed the different binding angles mentioned above.

Figure 5 The binding pattern between 8H5Fab and the 3 HAs. (a) White, 8H5Fab; red, ljsm; green, 2ibx; blue, 2fk0. (b)—(d) The coloring region in surface was the binding region in HA. The binding sites in 8H5 were shown in ribbon. (b) Fab8H5-ljsm; (c) Fab8H5-2ibx; (d) Fab8H5-2fk0.

Table 5 SAS analysis of HA epitopes ($\text{\AA}^2$)^{a)}

2fk0			ljsm			2ibx		
Site	aa	Δ SAS	Site	aa	Δ SAS	Site	aa	Δ SAS
A57	Lys	78.361	<u>A68</u>	<u>Asp</u>	27.716	<u>A72</u>	<u>Asp</u>	18.55
A60	Ile	23.262	A71	Leu	37.573	A75	Ile	66.375
A62	Arg	70.997	<u>A72</u>	<u>Asn</u>	86.13	<u>A76</u>	<u>Asn</u>	65.577
A75	Met	8.945	<u>A112</u>	<u>Glu</u>	28.131	<u>A116</u>	<u>Glu</u>	18.239
<u>A77</u>	<u>Asp</u>	40.472	<u>A113</u>	<u>Lys</u>	30.212	<u>A117</u>	<u>Lys</u>	28.056
A78	Glu	26.316	<u>A114</u>	<u>Ile</u>	34.115	<u>A118</u>	<u>Ile</u>	34.576
A79	Phe	143.975	A115	Arg	133.757	A119	Gln	84.019
<u>A81</u>	<u>Asn</u>	126.082	A116	Ile	7.654	<u>A122</u>	<u>Pro</u>	35.802
A82	Val	51.191	<u>A118</u>	<u>Pro</u>	48.655	A123	Lys	52.91
A82	Pro	69.096	A119	Arg	28.444	<u>A124</u>	<u>Ser</u>	104.211
A83	Glu	10.926	<u>A120</u>	<u>Ser</u>	109.74	A125	Ser	41.273
A117	His	18.864	A121	Ser	30.395	A126	Trp	9.254
A118	Phe	7.108	A123	Ser	68.298	A127	Ser	69.711
<u>A119</u>	<u>Glu</u>	56.822	A124	Asn	23.362	A130	Glu	17.771
<u>A120</u>	<u>Lys</u>	49.1	A126	Asp	8.974	A135	Val	11.537
<u>A121</u>	<u>Ile</u>	31.501	A127	Ala	17.753	<u>A141</u>	<u>Tyr</u>	54.689
A122	Gln	73.071	A131	Val	8.408	A142	Gln	28.543
A123	Ile	8.25	<u>A137</u>	<u>Tyr</u>	47.138	A144	Lys	43.149
<u>A125</u>	<u>Pro</u>	12.846	A140	Arg	37.735	A146	Ser	13.358
<u>A125</u>	<u>Ser</u>	12.562	A142	Ser	15.934	A149	Arg	60.859
<u>A141</u>	<u>Tyr</u>	33.14	A145	Arg	66.956	A157	Lys	69.367
A142	Gln	45.85	A153	Lys	50.086	A158	Asn	10.379
<u>A256</u>	<u>Tyr</u>	37.194	A154	Asn	64.563	A255	Glu	11.505
A273	Glu	73.261	A164	Tyr	9.15	<u>A256</u>	<u>Tyr</u>	65.716
A274	Tyr	56.55	A251	Glu	7.993			
A276	Asn	77.474	<u>A252</u>	<u>Tyr</u>	52.789			

a) The predicted sites shown in bold underlined style were the 9 common epitope amino acid residues from three docking results.


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2ibx .DQICIGYHA NNSTEQVDTI MEKNVTVTHA QDILEKTHNG KLCDLDGVKP LILRDCSVAG
2fk0 GDQICIGYHA NNSTEQVDTI MEKNVTVTHA QDILEKTHNG KLCDLDGVKP LILRDCSVAG
1jxm .DQICIGYHA NNSTEQVDTI MEKNVTVTHA QDILEKTHNG KLCDLNGVKP LILRDCSVAG

2ibx WLLGNPMCDE FLNVPEWSYI VEKANPVNDL CYPGDFNDYE ELKHLLSRIN HFEKIQIIPK
2fk0 WLLGNPMCDE FLNVPEWSYI VEKANPVNDL CYPGDFNDYE ELKHLLSRIN HFEKIQIIPK
1jxm WLLGNPMCDE FLNVPEWSYI VEKDNFVNGL CYPENFNDYE ELKHLLSSTN HFEKIRIIPR

2ibx SSWSSHEASL GVSSACPYQG KSSFFRNWVW LIKKNSTYPT IKRSYNNINQ EDLLVLWGIH
2fk0 SSWSSHEASL GVSSACPYQG KSSFFRNWVW LIKKNSTYPT IKRSYNNINQ EDLLVLWGIH
1jxm SSWSNHDASS GVSSACPYNG KSSFFRNWVW LIKKNMAYPT IKRSYNNINQ EDLLILWGIH

2ibx HPNDAAEQTK LYQNPTTYIS VGTSTLNQRL VPRIATRSKV NGQSGRMEFF WTILKPNDAI
2fk0 HPNDAAEQTK LYQNPTTYIS VGTSTLNQRL VPRIATRSKV NGQSGRMEFF WTILKPNDAI
1jxm HPNDAAEQTK LYQNPTTYVS VGTSTLNQRS VPEIATRPKV NGQSGRMEFF WTILKPNDAI

2ibx NFESNGNFIA PEYAYKIVKK GDSTIMKSEL EYGNCNTKCQ TPMGAINSSM PFHNIHPLTI
2fk0 NFESNGNFIA PEYAYKIVKK GDSTIMKSEL EYGNCNTKCQ TPMGAINSSM PFHNIHPLTI
1jxm NFESNGNFIA PEYAYKIVKK GGSAINKSGL EYGNCNTKCQ TPMGAINSSM PFHNIHPLTI

2ibx GECPKYVKS LVLATGLRN SP
2fk0 GECPKYVKS LVLATGLRN SP
1jxm GECPKYVKS LVLATGLRN VP

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Figure 6 Sequence alignment to analyze the common epitope. Box, The reported receptor binding sites. Gray style means the 8H5 epitope amino acid residues predicted by SAS analysis as shown in Table 5.

Table 6 Natural mutation ratio of the common epitope amino acids

	Epitope amino acids in 8H5								
	Asp ⁶⁸	Asn ⁷²	Glu ¹¹²	Lys ¹¹³	Ile ¹¹⁴	Pro ¹¹⁸	Ser ¹²⁰	Tyr ¹³⁷	Tyr ²⁵²
Cys	—	—	—	—	—	—	0.1%	—	—
Asp	99.9%	—	0.1%	—	—	—	0.6%	—	—
Glu	—	—	99.6%	—	—	—	—	—	—
Phe	—	—	—	—	—	—	—	0.1%	—
Gly	0.1%	—	0.2%	—	—	—	0.2%	—	—
His	—	0.1%	—	—	—	—	—	0.7%	0.2%
Ile	—	—	—	—	99.3%	—	—	—	—
Lys	—	0.3%	0.1%	99.9%	—	—	—	—	—
Leu	—	—	—	—	0.5%	—	—	—	—
Met	—	—	—	—	—	—	—	—	—
Asn	—	99.5%	—	—	—	—	3.2%	—	12.6%
Pro	—	—	—	—	—	99.8%	—	—	—
Gln	—	—	—	—	—	—	—	—	—
Arg	—	—	—	0.1%	—	—	—	—	—
Ser	—	—	—	—	—	0.2%	95.9%	0.1%	—
Thr	—	0.1%	—	—	—	—	—	—	—
Val	—	—	—	—	0.3%	—	—	—	—
Tyr	—	—	—	—	—	—	—	99.1%	87.2%

3 Discussion

The HA glycoprotein is present in the viral membrane as a single polypeptide (HA0), which must be cleaved by the hosts trypsin-like proteases to produce two peptides (HA1 and HA2) to make the virus infectious. HA may not only mediate the virus attaching to and entering into the host cell by binding to the sialic acid receptors on the cell surface, but also works as the main antigen that induces the humoral response against the virus^[17]. There-

fore, the HA is an important target for both drug and vaccine development. The epitopes of HA can be used to accurately monitor immune responses and to tease out which influenza responses are specific for a given virus isolate or subtype. They could also be used in detecting and monitoring infections serving to project potential cross-reactive immunity and efficacy against new isolates by existing vaccines and diagnostics. One of the shortcomings of the currently available influenza vaccines is the induction of a strain-specific immunity,

Table 7 The hydrogen bond in the HA-8H5Fab complex

H receptor	H donor	Distance	Angle
8H5Fab:H25:HG1	<u>ljsm:A68:OD2^{b)}</u>	2.36	139.11
<u>ljsm:A72:HD21</u>	8H5Fab:H76:OD1	2.28	156.55
<u>ljsm:A72:HD22</u>	8H5Fab:H27:O	1.78	129.68
8H5Fab:H31:HD22	ljsm:A112:OE2	1.93	139.49
8H5Fab:H28:HG1	<u>ljsm:A113:Q^{c)}</u>	1.78	130.03
ljsm:A115:HN	8H5Fab:H32:OH	1.81	124.02
ljsm:A115:HN	8H5Fab:H97:OD1	2.06	152.29
ljsm:A115:HH22	8H5Fab:H94:OD1	1.70	153.24
8H5Fab:L55:HN	ljsm:A125:O	1.87	138.59
8H5Fab:L55:HN	2ibx:A129:O	2.33	120.20
2ibx:A157:HZ1	8H5Fab:L55:O	1.81	126.66
2ibx:A157:HZ1	8H5Fab:L56:N	2.45	126.52
<u>2ibx:A76:HD22</u>	8H5Fab:H28:N	2.41	135.04
8H5Fab:H28:HG1	<u>2ibx:A117:Q</u>	1.81	123.53
8H5Fab:H31:HD22	<u>2ibx:A116:OE2</u>	1.91	142.38
8H5Fab:H97:HN	2ibx:A119:OE1	2.11	150.73
2ibx:A119:HN	8H5Fab:H97:OD1	1.89	136.25
2fk0:A117:NE2	8H5Fab:L53:OD1	2.57	NA ^{a)}
<u>2fk0:A120:HN</u>	8H5Fab:L54:O	2.37	136.82
2fk0:A256:HH	8H5Fab:L57:O	1.94	147.24
2fk0:A62:HH21	8H5Fab:H26:O	1.68	127.18
8H5Fab:H28:HG1	2fk0:A273:OE1	2.25	143.37
8H5Fab:H28:HG1	2fk0:A273:OE2	1.89	124.43
2fk0:A57:HZ2	8H5Fab:H98:O	1.76	125.63
2fk0:A276:HD21	8H5Fab:H94:OH	2.03	138.56
2fk0:A79:HN	8H5Fab:H102:OH	2.06	151.32

a) NA, No data was detected. b) Underlining style, the atoms of predicted 8H5 amino acid residues are contributing to from hydrogen bold in HA-8H5Fab complex. c) Underlining bold style, the atoms of predicted common 8H5 amino acid residues are contributing to from hydrogen bold in HA-8H5Fab complex.

which requires new vaccines to be produced each year for each different strain. For this reason, if conserved common epitope can be defined, different immunization regimens and vaccine candidates could be evaluated due to their capacity to induce immune responses to those specific conserved determinants^[18].

Damien Flury group reported that the epitopes of hemagglutinin were recognized by two distinct antibodies^[14]. But what they have done was focused on H3 that caused pandemics in 1968. However, the damage caused by avian influenza viruses since 1997 mostly derives from H5, and currently there is no X-ray resolved structure in the seeking for neutralizing epitope of H5. In this study, based on the experiment screening of the common monoclonal antibody 8H5, we constructed the structure using the molecule modeling and analyzed the binding pattern between the antibody and the three HAs so as to shed light on the conserved neutralizing epitope in HA.

The function of the antibody was mainly derived from the variable domains, and a lot of work was focused on

the single-chain Fv antibody^[19,20]. In order to better screen results from the Zdock and Rdock poses which were carried out to find the interacting characteristic of the antibody and the antigen, we modeled the whole Fab structure. By analyzing the primary sequences of the variable domains, the framework and the canonical structure were determined for molecule modeling (Table 1). Based on the high consensus, the structure of framework could be predicted with high accuracy. And the CDRs could be predicted with the most accurate canonical structure method whose class was based on the length of the CDR and the key residue. Each canonical structure class possesses its individual conformation. This is the basic principle for the CDRs conformation determination. The structure modeled by canonical structure method was in the reasonable conformation after being tested by energy analysis, SAS analysis, Ramachandran plot, profile-3D and superimposing with the X-ray resolved structures. As one of the best programs, Zdock was employed in the 8H5Fab and HAs

study. To verify the accuracy, the HA and antibody were separated from the H3 complex mentioned above and then subjected to the Zdock and Rdock analysis. The result was almost the same as that of X-ray resolved complex, indicating that the Zdock and Rdock program could perform very well in the antibody and antigen docking.

From the complexes of 8H5Fab and HAs, it is easy to find out that the binding pattern of 8H5Fab-1jsm and 8H5Fab-2ibx was almost the same although their hosts were different. This result also indicated that the epitope against 8H5 was unrelated to the host, which implied that 8H5 can be extensively used in the diagnostic of HA antigens from different H5 influenza viruses. However, the binding pattern of 8H5Fab-2fk0 was quite different from the other two. The 2fk0 structure was more similar to H1 HAs than H5 HA, and from the experiment, we found that 8H5 cannot react with HA form H1 subtype. This result showed that there was HA subtype binding preference for 8H5, which could provide crucial information for the research of HA subtype transition from H5 to H1. Taking consideration of the Δ SAS at the interface of the complexes and the homologous sequence alignment, the epitope against 8H5 was: (the residue No.

in 1jsm) Asp⁶⁸, Asn⁷², Glu¹¹², Lys¹¹³, Ile¹¹⁴, Pro¹¹⁸, Ser¹²⁰, Tyr¹³⁷, Tyr²⁵². The epitope was located in three small regions near the receptor binding sub-domain, and all of epitope amino acids were in the random coil with exception of about 4 residues in beta sheet. The one ranging from Glu¹¹² to Ser¹²¹ in 1jsm was the largest one that possessed 10 residues. Interestingly, the two epitopes regions from Glu¹¹² to Ser¹²¹ and from Tyr¹³⁷ to Lys¹⁴⁰ in 1jsm had been confirmed as the favorite antigen binding sites reported by *James Stevens* group in 2006^[6]. Besides, the natural mutation ratio also showed that the conservation ratio of the predicted common neutralizing epitope was very high. Because the hydrogen bond played an important role in the protein-protein interaction, Lys¹¹³ might be the key amino acid in the antibody and antigen binding, which might offer crucial information for the anti-virus drug design. However, more gene mutation, epitope analysis^[21] and structure resolved experiments should be performed to verify the structure and binding pattern.

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