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Computer Aided Study of Mechanism of Immunoadsorbent for Myasthenia Gravis

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Abstract: Myasthenia Gravis (MG) is an organ specific autoimmune disease mediated by autoantibodies (AChR Ab) against the acetylcholine receptor (AChR). Literature reported that the AChR Ab can be removed by absorbent linked with tryptophan. It was studied in detail in our lab. With the aid of computer, we docked some ligands into AChR Ab, and the results from scores of docking under different generations showed that there was no specific binding between tryptophan and scFv fragment just as the binding between antigen-antibody. The interaction between Trp and immunoglobulin was a broad-spectrum binding.

Keywords: Myasthenia Gravis, docking, SYBYL6.6.

Myasthenia Gravis (MG) is an autoimmune disease characterized by fatigue and weakness of skeletal muscles. The interaction of autoantibodies with acetylcholine receptors (AChR) leads to the destruction of AChRs at the neuromuscular junction. Anti-AChR antibodies are detected in approximately 90% of MG patients, but are essentially absent in healthy humans. Literature reported that the AChR Ab could be removed by absorbent linked with tryptophan. Tryptophan-Polyvinyl alcohol was used to treat 20 MG patients by Shibuya *et al* in 1992[1] and 16 MG patients were treated by this way by Grob *et al* in 1995[2]. Asahi Medical Center in Tokyo also used the same absorbent in the clinic. Adsorption capacities of various ligands were studied in our Lab. It was also proved that immunoadsorbent prepared by coupling L-tryptophan to epichlorohydrin-activated cellulose bead showed the best result.

Software genetic algorithm-based Flexible Docking (FlexiDock[3]) provides the means of docking ligands into protein active sites. It is a plug-in in software STBYL6.6. Genetic algorithms (GA), as global optimizers, can be grouped with simplex and stochastic methods. Methodology and terminology from biological (or Darwinian) evolution were used in an iterative process in which the most-fit members of a population will have the best chance of propagating themselves into future generations. This evolutionary pressure directs the process, which gives GAs a performance advantage over other global optimization methods[3~4]. FlexiDock is a better software than the others. It can be considered the structure changes both of proteins and ligands. In order to study the selectivity of immunoadsorbent, the structure of the complex

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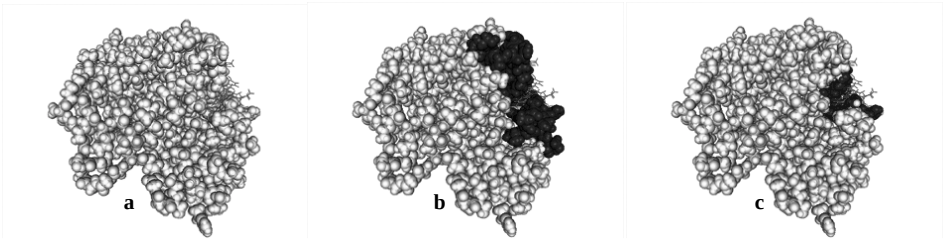
between scMab198 Fv fragment and an analogue of the main immunogenic region of the AChR was found from Protein Data Base, and 3D models of some amino acids were designed as ligands in SYBYL. Then we defined the binding pocket inside the active site of the protein and docked ligands into it. Comparison of scores when docking under the different generations, the difference of capability between ligands was observed.

Materials and Methods

The three-dimensional structure of the complex between an Fv antibody (scMab198) fragment and the analogue of the main immunogenic region (MIR) of the acetylcholine receptor: a combined two-dimensional NMR, homology, and molecular modeling was approached (**Figure 1 a**)[5]. The three-dimensional structures of several amino acids such as Trp, Phe, Glu, Lys, Cys and Tyr *etc* were designed in SYBYL.

Figure 1 Complex between scMab198 Fv fragment and an analogue of the main immunogenic region of the AChR



CDR was marked Atoms of binding pocket were marked

Figure 2 Amino acid sequences of the cloned scMab198 heavy and light variable regions, Using three-letter amino acid code. (CDR was marked by underline, the amino acid of binding pocket was marked by frame)

VH

GLN VAL GLN LEU LEU GLU SER GLY PRO GLY LEU VAL ARG PRO SER GLU THR LEU SER LEU THR CYS THR VAL SER GLY
PHE SER LEU THR SER PHE SER VAL SER TRP VAL ARG HIS PRO SER GLY LYS GLY PRO GLU TRP MET GLY ARG MET TRP
CDR-H1 CDR-H2
TYR ASP GLY TYR THR ALA TYR ASN SER ALA LEU LYS SER ARG LEU SER ILE SER ARG ASP THR SER LYS ASN GLN VAL
CDR-H2
PHE LEU LYS MET ASN SER LEU GLN THR ASP ASP THR GLY THR TYR TYR CYS THR ARG ASP LEU TYR GLY GLY TYR PRO
CDR-H3
LEU GLY PHE TRP TYR PHE ASP PHE TRP GLY PRO GLY THR MET VAL THR VAL SER SER GLY GLY GLY GLY SER GLY GLY
CDR-H3
GLY GLY SER GLY GLY GLY GLY SER

VL

ASP ILE LYS LEU THR GLN SER PRO SER LEU LEU SER ALA SER VAL GLY ASP ARG VAL THR LEU SER CYS LYS GLY SER
CDR-L1
GLN ASN ILE ASN ASN TYR LEU ALA TRP TYR GLN GLN LYS LEU GLY GLU ALA PRO LYS LEU LEU ILE TYR ASN THR ASN
CDR-L1 CDR-L2
SER LEU GLN THR GLY ILE PRO SER ARG PHE SER GLY SER GLY SER GLY THR ASP TYR THR LEU THR ILE SER SER LEU
CDR-L2
GLN PRO GLU ASP VAL ALA THR TYR PHE CYS TYR GLN TYR ASN ASN GLY TYR THR PHE GLY ALA GLY THR LYS LEU GLU
CDR-L3
LEU LYS ALA ALA GLU GLN LYS LEU ILE SER GLU GLU ASP LEU ASN

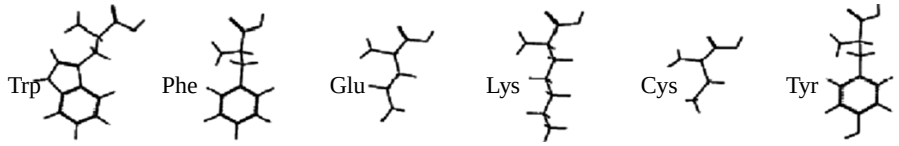
Select analogue atoms in the complex. All atoms of Fv fragment within 4Å radius of the any atoms selected were defined as binding pocket (**Figure 1 b**) (**Figure 1 c**).

Amino acid sequences (**Figure 2**) showed that amino acids of binding pocket laid on the complementarity determining regions (CDR)[6].

The analogue was removed, and the active cavity was discovered. All valences were filled with hydrogen and all atomic charges were computed. The following methods were used in this version: Kollman all-atom for the protein, Gasteiger-Hückel for the ligand.

Trp, Phe, Glu, Lys, Cys and Tyr *etc* were designed in the plug-in of SYBYL6.6. All valences were filled with hydrogens. Then compute all atomic charges.

Figure 3 Structures of amino acids made by SYBYL6.6



A protein and ligand molecule for docking *via* genetic algorithm were prepared as follows.

(1) Rotatable Bonds were selected: All of the rotatable bonds in the amino acids were selected and treated as flexible during the docking process. Analogue and Fv fragment were treated as rigid structures.
(2) Defining H-Bond Sites: All of the atoms in the binding pocket and ligands were selected to identify hydrogen binding centers and their associated, complementary extension (site) points.
(3) Ligand was pre-positioned in cavity: The ligand inside the protein cavity was manually positioned.

Steady-state GA genetic algorithm was used to determine the optimum ligand geometry. The fitness function used a subset of the Tripos force field: the van der Waals, electrostatic, torsional and constraint energy terms were used to calculate the energy of the important atoms in the supermolecule. This force field is described in the SYBYL Force Field manual. The bond stretching, angle bending and out-of-plane bending terms are invariant in torsional-space optimization, and are ignored. The score was the sum of the energy terms for those atoms which interact with the FLEXIDOCK_ATOMS set. Ligands were Trp, Phe, Glu, Lys, Cys, Tyr and analogue. Generation number was 30,000.

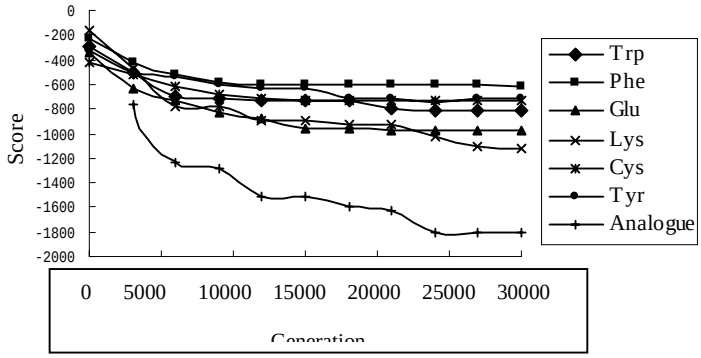
Results and Discussion

Figure 4 showed that the curves of amino acids are very smooth. Their scores fell together into a small range after 30,000 times of calculations. Because of the large structure, the scores of analogue were not ideal in the beginning, but gradually the scores got better and better. It showed that a specific binding between anagloue and scFv fragment finally existed.

The AChR of the neuromuscular junction is an integral membrane of glycoprotein (MW~290,000 Da), consisting of five homologous subunits in the stoichiometry $\alpha_2\beta\gamma\delta$ or $\alpha_2\beta\varepsilon\delta$, which form the cation channel. Anti-AChR antibodies in MG patients, are against an extracellular region of α-subunit of the AChR directly, namely the main immunogenic

region[7]. AChR loss is caused mainly by complement-mediated destruction of the postsynaptic membrane and cross-linking of the membrane-bound AChR by bivalent antibodies, resulting in an increased rate of internalization and degradation (antigenic modulation)[6]. On the other hand, the specific binding of antibody based on complementarity determining regions (CDR) which is in Fv antibody fragment. The binding pocket defined by us is just in this region. There must be a low binding energy between binding pocket and idea ligand. By comparison with the scores we obtained, MIR analogue has a lower binding energy indeed. But Trp has no obvious advantage when we docking it to the cavity. So, conclusion can be made, that there is no specificity between tryptophan and scMab198 Fv fragment. The interaction between Trp and immunoglobulin is a broad-spectrum binding.

Figure 4 Compare of scores when docking under different generation



ScMab198 is not human's antibody, but it has the same binding mechanism with human anti-AChR antibody when they work on human AChR MIR. These data should be valuable in the rational design of adsorbent with much improved affinity for the anti-AChR antibody, which may be benefit for the treatment for MG patients.

Acknowledgments

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