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# A strategy of designing the ligand of antibody affinity chromatography based on molecular dynamics simulation

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ABSTRACT

Designing affinity ligands has always been the development focus of affinity chromatography. Previous antibody affinity ligand designs were mostly based on the crystal structure of protein A (UniProt code number: P38507), and the antibody-binding domains were modified according to the properties of amino acid residues. Currently, more effective bioinformatic prediction and experimental validation has been used to improve the design of antibody affinity ligands. In the present study, the complex crystal structure (the domain D of protein A and the Fab segment of IgM, PDB code: 1DEE) was used as the model. The vital site that inhibits the binding between domain D and IgM was estimated by means of molecular dynamics (MD) simulation, then MM-GBSA calculations were used to design a mutant of domain D (K46E) for improving affinity on the above vital site. The binding analysis using Biacore showed the association and dissociation parameters of K46E mutant that were optimized with IgM. The affinity increase of K46E mutant preferred for IgM, the affinity order is K46E tetramer ($K_D = 6.02 \times 10^{-9}$ M) > K46E mutant ($K_D = 6.66 \times 10^{-8}$ M) > domain D ($K_D = 2.17 \times 10^{-7}$ M). Similar results were obtained when the optimized ligands were immobilized to the chromatography medium. A complete designing strategy was validated in this study, which will provide a novel insight into designing new ligands of antibody affinity chromatography media.

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## 1. Introduction

Due to the high specificity, effectiveness and safety of therapeutic monoclonal antibodies, these drugs become an increasingly large and important part of the global pharmaceutical market. Currently, the antibodies are mainly purified via affinity chromatography. The most widely used affinity ligand is the Staphylococcal protein A (SpA or protein A, UniProt code number: P38507), which has a molecular weight of about 42 kDa. SpA consists of three regions: a signal region that regulates secretion [1], five highy homologous extracellular antibody-binding regions [2], and an X region at the C terminal, which conjugates protein A to the cell wall peptidoglycan via covalent bonds [3]. From the N terminal to the C terminal, SpA comprises the S, E, D, A, B, C, and X domains.

As structural biology develops rapidly, many complex crystals formed by SpA and antibodies have been resolved, and the binding domains or important amino acid residues have also been identified [4–6]. Gülich et al. [7] expressed a recombinant domain Z by introducing site mutants within domain B, and thereby reduced the binding strength between SpA and immunoglobulin G (IgG) with the elution pH from 3.3 to 4.5. The study made the elution condition milder and better prevented protein denaturation. Besides, by joining domain Z head-to-tail, a double domain Z could be constructed. This domain could bind two IgG molecules without steric hindrance [8]. The research on multidomain tandem has become a hotspot in studying affinity ligands because the structure significantly increased the loading capacity of affinity media. As for alkali resistance, asparagine (N) and glutamine (Q) are the major reasons for decreased SpA activity in alkaline solutions. Particularly, in some patents, N is mutated to other amino acid residues in certain domains of the SpA (WO 03/080655 and WO 2008/039141).

Salvalaglio et al. studied the interaction between SpA domain B and IgG-Fc and established MD models both in solution and on

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agarose. The study had identified the amino acid residues that are key to binding [9]. This provided prediction of the binding process and overcame the strategy that screening the mutants by a large amount recombinant expression based on crystal structure only. Though the authors did not report modification of domain B, the study still brought new insights into the research field. In ion-exchange chromatography, the protein retention factors were determined under different pH values and ionic strength levels by establishing a MD model and using two different free energy calculation methods [10]. Wang et al. established MD simulation of DAGG, which is a synthetic ligand that can substitute SpA, and studied the interaction between DAGG and the Fc fragment of IgG [11]. Branco et al. established MD simulation of 22/8, which is a known synthetic ligand affinitive to Fc fragment, and compared the binding of 22/8 with Fc and Fab. They evaluated the influence of pH on the interaction of SpA or 22/8 with antibodies, and proposed the possibility of binding between 22/8 and the Fab fragment [12].

In recent years, the research on Fc-binding SpA domains is a hotspot of the development of antibody affinity ligands, and there also have been some important research on Fab-binding. Sasso et al. concluded that SpA binded Fab in human IgM molecules which contained $V_H3$ H chains [13]. Domain D of SpA has binding interactions with Fab of $V_H3$ family antibodies [14]. In fact, all individual domains of SpA can bind Fab [15]. Starovasnik et al. suggested that Fab and Fc antibody fragments bind to different sites of E domain. The helix-2/helix-3 face of domain E contains negatively charged residues and a small hydrophobic patch which complements the basic surface of the region of $V_H3$ from the structure of hu4D5 contains a positively charged surface [16]. In fact, the Fc and Fab binding surfaces on SpA domains appear to be distinct and nonoverlapping [17]. Until now, there have been so many studies on Fab binding ligands. However, there have been few studies on the MD simulation of Fab fragment and SpA domains.

IgM forms polymers where multiple immunoglobulins are covalently linked together with disulfide bonds, mostly as a pentamer. Fc binding ligands can bind antibodies such as IgG. In contrast, IgMs are pentamers, with Fc region inside the molecule. So Fc binding ligands do not apply to IgM purification. The purification of IgM is in need of the binding of Fab region. So designing Fab-binding ligands may be an option for optimizing the purification process. So the Fab binding ligands based on SpA can be used for some $V_H3$ family IgM or IgG purification, which may provide a choice for antibody purification.

As compared to previous studies, a crystal structure formed of SpA domain D and IgM Fab segment was used in the present study as the model [5], and possible factors that would inhibit binding were analyzed using the Amber 11 software. Thus, targeted mutant design could be performed, and specifically the binding process between ligands and antibodies was optimized. According to the MD simulation, the association and dissociation rate constants of mutants were evaluated at the molecular level using surface plasmon resonance (SPR).

## 2. Materials and methods

### 2.1. Baseline data for domain D and IgM-binding simulations

#### 2.1.1. Preparation of molecular systems

The initial crystal structure was taken from the Protein Data Bank (PDB ID: 1DEE). The complex structure of domain D and IgM was generated based on the 1DEE using Discovery Studio 2.5 software [18].

#### 2.1.2. Molecular dynamics simulations

The MD simulations were performed using Amber 11 [19] software package. The force-field is Amber 99SB (ff99SB) force field [20]. The sodium ions ($Na^+$) or chloride ions ($Cl^-$) were added by the t-Leap to be the explicit net neutralizing counterions based on a coulomb potential grid. For those complexes that were further subjected to MD simulations in explicit solvent, each system was solvated with TIP3P waters in a truncated octahedron box [21], with an 8.0 Å distance around the solute. The protein was fixed with a 100 kcal mol$^{-1}$ Å$^{-2}$ positional restraints, and the energy of the water and the ions was minimized for 1000 steps of steepest descent (SD) method and 2000 steps of conjugate gradient (CG) algorithms. Later the minimization was repeated with restraints on the proteins only for 1000 steps SD and 2000 steps CG. The minimization was repeated for 2000 steps SD and 3000 steps CG without any restraints. Thereafter, the heating dynamic was performed with restraint using a 10 kcal mol$^{-1}$ Å$^{-2}$ weight. Thereby, the temperature was increased gently from 50 to 300 K and then equilibrated for 300 ps. Finally, a 50 ns simulation for each system under NPT ensemble condition was performed. All the systems were treated within periodic boundary conditions. Long-range electrostatic interactions were calculated using the Particle-Mesh Ewald (PME) technique [22] with a non-bonded cutoff of 12.0 Å to limit the direct space sum. An integration time step of 2 fs was used, and the SHAKE algorithm was used to constrain bonds involving hydrogen atoms during dynamics. All the data given in the tables and figures were obtained from the final 15 ns of the MD simulations, unless otherwise mentioned. The PyMOL [23], Chimera [24], and VMD [25] softwares were used to visualize the trajectories and to depict structural representations.

#### 2.1.3. MM-GBSA calculations

Binding free energies for each complex were estimated using molecular mechanics generalized Born (MM-GBSA) approaches, using the MM-PBSA protocol in the Amber 11. The final 1000 snapshots taken from the trajectories of each simulation for the energy calculation were extracted. The interaction energy was calculated according to the following equation:

$$\Delta G_{bind} = G_{complex} - G_{domainD} - G_{IgM} \quad (1)$$

Here, $G_{complex}$, $G_{domainD}$, and $G_{IgM}$ are the free energies of complex, domain D, and IgM, respectively. The free energy ($G_{x=complex,domainD,IgM}$) of each species can be estimated using MM-GBSA methods:

$$G_{x=complex,domainD,IgM} = E_{MM} + G_{solv} - TS \quad (2)$$

$$E_{MM} = E_{ele} + E_{vdw} + E_{int} \quad (3)$$

$$G_{solv} = G_{gb} + G_{nonp} \quad (4)$$

Here, the $E_{MM}$ is the gas-phase molecular mechanical energy, $G_{solv}$ is the solvation free energy, and $E_{ele,}$ $E_{vdw,}$ and $E_{int}$ are the electrostatic energy, the van der Waals interaction energy, and the internal energy, respectively. $G_{solv}$ can be separated into an electrostatic solvation free energy ($G_{gb}$) and a nonelectrostatic solvation energy ($G_{nonp}$). $G_{gb}$ can be calculated with Generalized Born (GB) method [26]. $G_{nonp}$ is considered to be proportional to the molecular solvent accessible surface area buried on binding [27].

For obtaining the detailed view of domain D and IgM interactions, the MM-GBSA method was used to calculate the binding free energy of each residue as well. Every single residue can be partitioned into two parts in the calculation, backbone, and side chain. The snapshots used in the binding free-energy decomposition are the same as those used in the binding free-energy calculation.

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