Biochemical Engineering Journal 139 (2018) 15–24

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular article

A comparative investigation of random and oriented immobilization of protein A ligands on the binding of immunoglobulin G

Xue-Hui Yang[a], Li-Ming Huan[a], Xin-Shuang Chu[a], Yan Sun[a,b], Qing-Hong Shi[a,b,*]

[a] Department of Biochemical Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin, 300350, China
[b] Key Laboratory of Systems Bioengineering (Ministry of Education), Tianjin University, Tianjin, 300072, China

HIGHLIGHTS

- Maximum hIgG capacity was 120 mg/g by far among Sepharose-based SpA gels.
- Immobilization at C-terminus of SpA is more preferred than at its N-terminus.
- Bound hIgG to oriented SpA ligand had a flat-on orientation *over* surface.
- Each tetrameric Z domain could bind 2.1 hIgG due to steric exclusion effect.
- A critical density was obtained at 20 mg/g gel for of oriented ligand.

ARTICLE INFO

Keywords:
Protein A chromatography
Oriented immobilization
Adsorption capacity for IgG
Multimeric ligand
Binding stoichiometry

ABSTRACT

In this work, a range of investigation were conducted to illustrate the mechanism of hIgG –protein A from *Staphylococcal* aureus (SpA) binding for the development of high-capacity SpA gels. Results of surface plasmon resonance demonstrated that oriented immobilization at carboxyl terminus of Z domain was more preferred than that at amino terminus and bound hIgG with the oriented domain had a flat-on orientation over chip surface. Furthermore, much negative enthalpy changes as well as lower binding stoichiometry were observed in the binding of tetrameric Z domains (denoted as Z4cys) to hIgG, indicating that each of both ligands could bind 2.1 hIgG molecules. Adsorption equilibria of hIgG adsorption showed that by far the maximum adsorption capacity for hIgG in Sepharose-based protein A gels was determined to be about 120.0 mg/g gel. Its excellent performance was further manifested by the result of dynamic binding capacity. In protein A gel, moreover, there was a critical density around 20 mg/g gel, above which the availability of the ligand decreased rapidly due to serious steric exclusion effect. The research provided insight into the IgG – SpA binding and strategic guidance for the development of high-capacity protein A gels.

1. Introduction

Binding between IgG and protein A from *Staphylococcal* aureus (SpA) is of importance for the development of chromatographic materials for antibody purification, biosensor and even the design of biodefense systems for specific binding of IgG to antigens [1–5]. SpA is a cell wall associated protein including five highly homologous IgG-binding domains (designated E, D, A, B, and C, in order from the amino terminus) [6–8], and binds to Fcγ region at the CH2 and CH3 interface of most IgG subclasses from human and animals [9,10]. Since the early 1990, protein A chromatography has shown themselves to be gold standard for antibody production after some early misgivings about its economical efficiency, capacity, and life time [3,11,12]. Despite it has proved to be technical successful, the mechanism and kinetics of IgG – SpA binding is still not well understood, and a couple of important questions remained unsettled.

IgG adsorption onto SpA chromatographic materials is an extremely complicated issue including (1) the binding between IgG and SpA, and (2) the interaction of bound IgG and surface. Since the first observation of SpA by Jenson [13], considerable efforts have been put into structural analysis and biochemical investigation. Around 1980, Deisenhofer and his co-collaborators determined three-dimensional crystal structures of human IgG (hIgG) and the complex between an hIgG Fc fragment and IgG-binding domain B of SpA (B domain) using X-ray crystallography [9,14], and found that B domain formed two contacts with Fc fragment of hIgG molecule at CH2 and CH3 regions, paving the way

* Corresponding author at: Department of Biochemical Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin, 300350, China.
E-mail address: qhshi@tju.edu.cn (Q.-H. Shi).

https://doi.org/10.1016/j.bej.2018.08.002
Received 4 May 2018; Received in revised form 4 August 2018; Accepted 6 August 2018
Available online 07 August 2018
1369-703X/

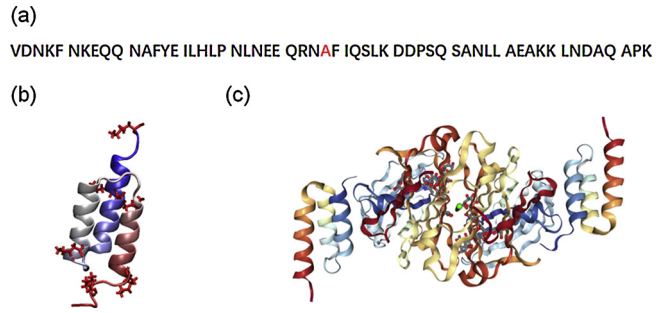

Fig. 1. The amino acid sequence of Z domain from protein A (a), three-dimensional structure of Z domain (b) and its complex with IgG Fc fragment (c). The complex of IgG Fc fragment and Z domain was obtained from PDB website with a PDB ID of 5Y4U.

for the elucidation of the mechanism of IgG- SpA binding. Inspired partly by the crystal structure of the complex between an hIgG Fc fragment and B domain, Nilsson et al develop an engineered variant of B domain (denoted as Z domain) to eliminate the hydroxylamine sensitive site by substituting non-critical Gly residue at Helix II with Ala (G29 A substitution) presented in Fig. 1 [15]. The engineered Z domain retained strong affinity for IgG – Fc fragment. This evolution brings about the development of alkali-stabilized protein A chromatographic materials [3,16]. By analyzing the interaction between B domain and the Fc fragment of IgG with the help of computer-based molecular modeling and molecular dynamic simulation, moreover, peptides and nonpeptidyl mimic for SpA were also designed to develop affinity chromatography for high-capacity IgG capture [17–19].

On the other hand, good structural knowledge of IgG Fc-SpA complex is also essential for optimizing the immobilization of protein A ligands and the bound IgG – surface interactions. In general, most of available immobilization methods for proteins relies on the reaction of naturally occurring amino, carboxyl and thiol groups on proteins with active groups coupled on the matrices [20], and plays a fundamental role in determining the performance of protein A ligand during chromatography process [1,21,22]. However, it often suffers from random and uncontrolled orientation of SpA molecules and results in steric exclusion effect for hIgG binding. Tajima et al observed that oriented SpA molecules by tyrosinase catalysis on a well-defined phospholipid polymer surface controlled antibody orientation much more strictly than random protein A ligand by physi-sorption [23], meaning that SpA molecules immobilized by tyrosinase catalysis had controllable molecular orientation. von Roman and Berensmeier compared the influence of random and oriented immobilization of hexameric B domains on hIgG adsorption and found that *Prof*-B6$_{cys}$ resin with oriented hexameric B domain immobilized at the carboxyl terminus had 40% higher adsorption capacity for hIgG than *Prof*-B6$_{high}$ resin with random immobilized hexameric B domain at the similar ligand densities [24]. In current status, oriented immobilization of protein A ligand has become a well-accepted strategy to improve the performance of protein A chromatography and provide an efficient platform for hIgG immobilization [25–27]. Although plenty of experimental evidence has demonstrated the advantages of oriented immobilization of protein A ligand, the mechanism of hIgG – SpA binding, particularly at the interface, still remains somewhat elusive.

In this work, a range of investigation was conducted with surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC) as well as batch adsorption and chromatography experiments to illustrate the mechanism of hIgG – SpA binding for the development of high-capacity protein A gels. In the experiment, the oriented immobilization of Protein A ligands was achieved with thiol coupling by introducing additional cysteine at the amino or carboxyl terminus of protein A ligands, and four protein A ligands including mono-, di-, tetra- and hexameric Z domains (denoted as Zcys, Z2cys, Z4cys and Z6cys, respectively) were used for the comparison with corresponding random immobilized ligands by amine coupling via Lys residues and an amino group at terminus as shown in Fig. 1(b). The researches provided the guideline for the design of high-capacity protein A gels for IgG purification.

2. Materials and methods

2.1. Materials

In this work, engineered Z domains comprising an additional cysteine at the amino or carboxyl terminus were synthesized by Ziyubio Co., Ltd (Shanghai, China). Z domain comprising an additional cysteine at the amino terminus was denoted as cysZ domain and Z domain comprising an additional cysteine at the carboxyl terminus was denoted as Zcys domain. The BL21 *E. coli* strains transfected with pET-30a plasmids encoding the gene for Z2cys, Z4cys and Z6cys comprising six histidine at the amino terminus and an additional cysteine at the carboxyl terminus (called respectively as pET-30a(+)−Z2cys, pET-30a(+)−Z4cys and pET-30a(+)−Z6cys plasmids) were received from Sangon Biotech Co., Ltd (Shanghai, China). Sensor chip CM5, thiol coupling kit, Sepharose FF, Q Sepharose FF, CM Sepharose FF, rProtein A Sepharose FF (SepFF-rProtein A), Tricorn 5/50 and HK 16/20 column, Sephadex G25 and HisTrap HP (5 mL) were purchased from GE Healthcare (Uppsala, Sweden). hIgG (M_w ~ 150 kDa, > 99%), 1,4-butanediol diglycidyl ether (BDDE), sodium cyanoborohydride ($NaBH_3CN$) and *N*- hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) was purchased from J&K Scientific Ltd (Beijing, China) and pyridine dithioethylamine hydrochloride (PDEA) was received from Dibai Biological Technology (Shanghai, China). Tryptone and yeast were purchased from ThermoFisher Scientific Inc. Tris(hydroxymethyl) aminomethane (Tris), sodium chloride (NaCl), isopropyl-*β*-D-thiogalactoside (IPTG) and kanamycin sulfate were purchased from Sangon Biotech Co., Ltd (Shanghai, China). Other reagents were of analytical grade and received from local sources unless otherwise stated.

2.2. Expression and purification of recombinant multimeric Z domains

The BL21 *E. coli* strains respectively with the pET-30a(+)−Z2cys, pET-30a(+)−Z4cys and pET-30a(+)−Z6cys plasmids were grown in 1 L of Luria-Bertani (LB) medium (tryptone 10 g/L, yeast 5 g/L, NaCl 10 g/L) containing 50 μg/mL kanamycin sulfate at 37 °C and 180 rpm after being inoculated with 20 mL of fresh overnight culture. When OD_{600} reached to 0.6–0.8, IPTG was added to induce protein expression at a final concentration of 0.5 mmol/L. The cells were harvested after 6 h by centrifugation at 5000 rpm and 4 °C for 20 min, and then the collected pellets were resuspended in binding buffer (50 mmol/L Tris-HCl, 500 mmol/L NaCl, 50 mmol/L imidazole, pH 7.4). After the cells was lysed by sonication in an ice-water mixture bath for 5 min (4 s pulse with 6 s intervals), the insoluble debris was removed by centrifugation at 10,000 rpm for 30 min. The supernatant was then applied directly onto a HisTrap HP column precharged with nickel ion. After the sample was loaded, the column was washed with binding buffer and the target was eluted with the elution buffer (50 mmol/L Tris-HCl, 500 mmol/L NaCl, 230 mmol/L imidazole, pH 7.4). Finally, the eluted fraction was pooled and desalted in HK 16/20 column packed with Sephadex G25 gel and the product was freeze-dried for the following experiments.

2.3. SPR measurements

SPR measurements were conducted on a BIAcore 3000 SPR system from GE Healthcare (Uppsala, Sweden) at room temperature to evaluate the affinity and kinetics of hIgG – Z domain binding under conditions of random and oriented immobilization. Prior to the