



Regular article

Affinity chromatography of human IgG with octapeptide ligands identified from eleven peptide–ligand candidates



Aiying Xue^a, Wei-Wei Zhao^a, Xiaoguang (Margaret) Liu^b, Yan Sun^{a,*}

^a Department of Biochemical Engineering and Key Laboratory of Systems Bioengineering of the Ministry of Education, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China

^b Department of Chemical and Biological Engineering, The University of Alabama, Tuscaloosa, AL, USA

ARTICLE INFO

Article history:

Received 17 June 2015

Received in revised form

19 November 2015

Accepted 30 November 2015

Available online 2 December 2015

Keywords:

Protein

Affinity

Adsorption

Chromatography

Purification

Human immunoglobulin G

ABSTRACT

We have reported earlier on the characterization of four peptides from a library of 15 peptide ligand candidates for human IgG (hIgG) obtained by a biomimetic design strategy and identified three high-affinity octapeptides. In this work, the left 11 peptides were evaluated and we found two more octapeptides (FYCHWQDE and FYCHNQDE) that showed high affinity for hIgG. The binding pH ranges for the two ligands were different, but the optimum pH values were the same for each other (pH 6.0). Both the ligands showed high specificity and bound hIgG mainly by electrostatic interactions. Ligand binding competition experiments revealed that the binding sites on hIgG for the two octapeptides were similar to those for Protein A. Finally, hIgG was purified from human serum with high purities and recovery yields with the two peptide affinity columns. Thus, among the 15 peptide candidates, a total of five octapeptides were identified as high-affinity ligands of hIgG. The five ligands were all derived from the same peptide model FYxHxxxE (where x denotes any amino acid) and contained four common hot spots F132, Y133, H137, and E143 of the affinity motif of Protein A. Analyses and evaluation of the peptide library would help deepen our understanding of the affinity binding of Protein A to IgG, and promote application of the biomimetic strategy in the design of affinity ligands for different proteins.

© 2015 Published by Elsevier B.V.

1. Introduction

The growing role of antibodies in biopharmaceutical researches and applications [1–3] has paved the way to the industrial manufacture in recent years, and great improvements in upstream production have switched the bottleneck to downstream purification in antibody industry [4,5]. Affinity chromatography plays a vital role in antibody purification on account of its high selectivity [6,7]. Staphylococcal protein A (SpA) is the most commonly used biospecific ligand for the isolation of immunoglobulin G (IgG), the major type of antibodies [8,9]. However, the shortcomings such as toxic ligand leakage at harsh elution condition and expensiveness [5,7] have restricted the application of Protein A affinity chromatography. So finding alternative affinity ligands with improved properties is greatly demanded.

Small peptides [10–14] have been popular alternatives of Protein A to separate antibodies, mainly owing to their advantages of high specificity, stability and low cost [10,15,16]. One major

conventional method to obtain affinity peptide ligands is through high-throughput screening of combinatorial libraries [17–19], but the method has always been time-consuming and costly [20]. With the rapid development of molecular simulations in recent years, rational design has been extensively used in identifying novel high-affinity peptide ligands for target proteins, which is usually on the basis of protein structures and ligand–protein interactions [21–23]. Molecular docking has been a widely accepted technique for the rational design of affinity peptides. Rapid screening of a library of candidate compounds by molecular docking could shrink the scale of candidate peptides and even increase the hit rate [24]. However, although some effective ligands [25–28] have been screened from established promising libraries, the accuracy of docking as a screening technique is still rather limited mainly due to the simplified treatment of target proteins [23]. For the sake of screening a library with high efficiency, molecular dynamics (MD) simulation has been utilized simultaneously to provide insight into the dynamic molecular interactions and to evaluate candidate ligands [24]. For example, Li et al. [16] first screened a constructed peptide library by molecular docking, obtaining 10 potential high-affinity peptides for further confirmation by MD simulations. Eventually an octapeptide DWDLRLLY was validated by affinity

* Corresponding author. Fax: +86 22 27403389.
E-mail address: ysun@tju.edu.cn (Y. Sun).

chromatography to have high affinity for the target protein. Tetrapeptide QDES [29] has also been found by rational design that combined docking and MD simulations, and high-affinity and specificity of the affinity ligand for its target protein were confirmed. Examples stated above have demonstrated that molecular simulations are feasible to acquire affinity peptide ligands.

Recently, we have developed a biomimetic design strategy for affinity peptide ligands of human IgG (hIgG) based on the affinity motif of SpA. In brief, according to the distribution of the six hot spots of the SpA affinity motif, the number of residues inserting between the hot spots was determined. Cysteine was introduced as one of the middle residues of the peptide for ease of coupling reaction onto a solid matrix. Then, amino acid location was performed to identify other amino acid residues for insertion, leading to the construction of a peptide library. Finally, the library was screened by using different molecular simulation protocols, obtaining 15 potential candidates (Table S1 in the Supplementary material) [20]. Till now only four of the 15 peptides have been studied in detail. That is, the three octapeptides, FYWHCLDE, FYCHTIDE, and FYCHWALE, ranking No. 1, No. 8, and No. 9, respectively, in Table S1, have been identified as high-affinity ligands of hIgG, and the peptide FYTHCAKE (No. 14 in Table S1) was confirmed negative due to its weak affinity via MD simulations and experimental verifications. The three high-affinity peptides could selectively bind to the Fc fragment of hIgG mainly by electrostatic interactions [30]. Although the main interactions were different from those of Protein A [31], the three octapeptide ligands could purify IgG from serum feedstocks in a single step with high purities and recovery yields. Then, what about the left 11 potential candidates obtained from the MD simulation analyses? Although the affinity binding mechanism of the SpA-Fc complex was taken into account, there still existed some blindness of the rational design strategy in the process of peptide library construction. Conceivably, the most straightforward and reliable approach to screening of a ligand library is through chromatographic evaluation.

Therefore in this work, in order to find more high-affinity ligands of hIgG and totally analyze and evaluate the peptide library, all the left 11 potential candidates (Nos. 2–7, 10–13 and 15 in Table S1) were evaluated on peptide columns packed with the peptide-modified Sepharose gels. The effect of pH in the range of 4.0–8.0 on hIgG binding to the peptide columns was examined for each peptide. After the peptides that could bind hIgG effectively were determined, the binding specificity of the high-affinity ligands was validated with bovine serum albumin (BSA) at the proper pH values. Then, the binding sites of the high-affinity peptides were probed by the competition with Protein A in binding to the Fc fragment of hIgG. Finally, the identified high-affinity peptide ligands were used to capture and purify IgG from human serum.

2. Materials and methods

2.1. Materials

Thiopropyl Sepharose 6B, rProtein A Sepharose Fast Flow (rPA-SFF) and HR 5/5 column were purchased from GE Healthcare (Uppsala, Sweden). Human immunoglobulin G (hIgG) in lyophilized form, human serum, bovine serum albumin (BSA), Micro-BCA assay kit were obtained from Beijing Dingguo Changsheng Biotechnology (Beijing, China). Staphylococcal protein A and Fc fragment of hIgG were obtained from Sigma–Aldrich (St. Louis, MO, USA) and Jackson (West Grove, PA, USA), respectively. Peptides used in this research were synthesized by the solid-phase synthesis method by GL Biochem Ltd. (Shanghai, China). Pre-stained molecular weight markers were the products of TransGen Biotech (Beijing, China).

The Durapore filters with nominal pore sizes of 0.45 μm and 0.2 μm were supplied by Millipore (Billerica, MA, USA).

2.2. Synthesis of peptide gels

In this research, peptides were coupled onto Thiopropyl Sepharose 6B by the method described previously [20]. All the peptide-Sepharose gels were synthesized at a ligand density of 10 $\mu\text{mol/g}$ drained gel. After equilibration with the coupling buffer (0.5 mol/L NaCl and 1 mmol/L EDTA in 0.1 mol/L Tris–HCl buffer, pH 7.5), 1.0 g of the drained gel was transferred into a flask containing 6 mL peptide solution. The mixture was reacted in an air-bath shaker at 25 °C and 170 rpm for 1.5 h. After that, 2.4 mg cysteine was added into the flask, and the reaction continued for 30 min to block the residual sulfhydryl groups. After centrifugation at 3000 rpm for 3 min, the supernatant was collected to determine the content of the residual peptide and then the density of the immobilized peptide ligand. The collected gel was washed with excess distilled water and stored in 20% ethanol aqueous solution for use in chromatographic experiments.

The density of the immobilized peptide was determined by the following method. The peptide concentration decrease in the reaction solution was monitored at 220 nm by reversed-phase high performance liquid chromatography (RP-HPLC) with a Waters C18 reversed-phase column (Waters, Milford, MA, USA) connected to an Agilent 1100 (Agilent Technologies, Santa Clara, CA). Then, the ligand density was calculated by mass balance. Taking FYCHWQDE (No. 2 in Table S1) as an example, the RP-HPLC chromatogram for FYCHWQDE coupling to Thiopropyl Sepharose 6B is shown in the Supporting material (Fig. S1). As seen in Fig. S1, the peptide peak disappeared completely by a 1.5-h reaction, indicating that all the peptide could be coupled on the resin and the coupling reaction was highly effective. As listed in Table S2, the density data were converted into the value of 10.4 $\mu\text{mol/mL}$ -drained gel with the drained gel density of 1.037 g/mL.

2.3. Effect of pH on peptide affinities

The retention behaviors of hIgG and BSA on the peptide gels were investigated in different binding buffers (mobile phases). The concentration of binding buffers was constant (20 mmol/L), but the pH values varied from 4.0 to 8.0. The tested buffers were citrate buffer (pH 4.0 and 5.0), phosphate buffer (PB, pH 6.0 and 7.0), and Tris–HCl buffer (pH 8.0).

An HR5/5 column connected to an ÄKTA Purifier 10 system (GE Healthcare, Uppsala, Sweden) was used in chromatographic experiments as described previously [20]. The column packed with the peptide gel (1 mL) was equilibrated at least 10 column volumes (CVs) of a binding buffer. After the UV baseline reached stable, 100 μL of 1.0 mg/mL protein (hIgG or BSA) solution in the binding buffer was injected into the column, followed by washing off the unbound protein with 6CVs of the binding buffer. Thereafter, the column was eluted with 50 mmol/L citrate buffer (pH 3.0) or 0.5 mol/L NaCl in the binding buffer. All the chromatographic experiments were performed at a constant flow rate of 0.5 mL/min (2.5 cm/min).

The adsorption behaviors of the confirmed high-affinity ligands were also studied in other binding buffers of different pH values. That is, besides the binding buffers stated above, citrate buffers of pH 4.5 and 5.5, phosphate buffers of pH 6.5 and 7.5 were tested in the chromatographic experiments as well to determine the optimal binding condition.

ID	Title	Pages
2771	Affinity chromatography of human IgG with octapeptide ligands identified from eleven peptide-ligand candidates	8

Download Full-Text Now



<http://fulltext.study/article/2771>



Categorized Journals

Thousands of scientific journals broken down into different categories to simplify your search



Full-Text Access

The full-text version of all the articles are available for you to purchase at the lowest price



Free Downloadable Articles

In each journal some of the articles are available to download for free



Free PDF Preview

A preview of the first 2 pages of each article is available for you to download for free

<http://FullText.Study>