Improved Performance of Recombinant Protein A Immobilized on Agarose Beads by Site-Specific Conjugation

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ABSTRACT: Protein A affinity adsorbent with high antibody-binding capacity plays a prominent part in the purification of biopharmaceuticals to decrease the manufacturing costs. We describe a site-specific covalent conjugation strategy for protein A to immobilize on agarose beads. Recombinant protein A, which has one cysteine introduced at the C terminus through genetic engineering technology, was immobilized site-specifically on maleimide-functionalized agarose beads by the thiol–maleimide reaction. As a comparison, the recombinant protein A was randomly immobilized on the aldehyde-functionalized agarose beads via free amino groups on the protein surface. The site-specific conjugation of recombinant protein A on the agarose beads was validated through the assay of free SH groups on the adsorbents using the Ellman’s reagent. Adsorbents containing various amounts of protein A were used to adsorb antibody from human plasma. Analysis of immunoturbidimetry showed that the adsorbed fractions contained the 90.1% IgG, 4.2% IgA, and 5.7% IgM. The maximal antibodies-binding capacities with static adsorption and dynamic adsorption were approximately 64 and 50 mg, respectively, per swollen gram for site-specifically conjugated adsorbent and 31 and 26 mg for randomly conjugated adsorbent. Remarkably, the high antibody-binding capacity for site-specifically conjugated adsorbent outperformed the existing commercial protein A Sepharose (approximately 30 mg/g). The orientation of a protein is crucial for its activity after immobilization, and these results demonstrate that the site-specifically conjugated protein molecule is in a functionally active form to interact with the antibody with weak steric hindrance. The proposed approach may be an attractive strategy to synthesize affinity adsorbents with high-binding capacity.

INTRODUCTION

Affinity chromatography has been described as the most selective method for protein purification because it eliminates purification steps and increases the yields.1,2 The conjugation chemistry between the protein and the solid support plays an essential role in the performance of the chromatographic system,3 and the optimal conjugation chemistry must assure the binding activity of the protein. However, in most covalent conjugation approaches, the protein remains immobilized on the solid support through the reaction of the amine of lysine residues of the protein with electrophiles on the support. Such random amide bond formation between the protein and the solid support can result in the loss of protein activity as a result of improper orientation of the protein on the solid surface.3,4 By contrast, site-specific conjugation strategies provide the defined immobilization of proteins with uniform orientation where the bioactive site (binding epitope) is freely accessible for application.5,6 As a result, recent efforts have focused on site-specific covalent conjugation for protein immobilization.7–9 Several bioorthogonal chemistries are available for protein site-specific conjugation to effect “traceless” protein activities,10 such as expressed protein ligation,11 protein trans-splicing,12 Cu(catalyzed alkyne/azide cycloaddition (CuAAC, click chemistry),13 the Staudinger ligation,14 and the Diels–Alder reaction.15

Protein site-specific conjugation can be conveniently achieved if the protein possesses a single accessible, reactive cysteine (Cys), which is the only naturally occurring amino acid containing a thiol group in its side chain.16–18 The Cys residue is highly suitable for conjugation as its thiol group readily undergoes the nucleophilic substitution reaction with electrophilic reagents or Michael addition to αβ-unsaturated carbon-yls (e.g., maleimides) to form stable thioether bonds.19,20 The thiol addition to maleimide is widely used for the preparation of peptide and oligonucleotide conjugates and arrays, biosensors, fluorescent labeling of proteins and other biomolecules, and so forth.5

*Staphylococcal* protein A (SpA) is a cell-wall-bound pathogenicity factor from the bacterium *Staphylococcus aureus*. It exhibits tight binding to many IgG, IgA, and IgM molecules, and the IgG-binding region includes two highly homologous domains (E-D-A-B-C).21 Affinity chromatography based on
SpA is the most widespread and accepted methodology for antibody purification. The Z-domain, a mutant domain (N23T, F30A) derived from the B-domain of SpA, has been shown to have IgG-capturing efficiency similar to that of B-domain of SpA. The Z-domain contains three α-helices in the polypeptide segments Lys7 to His18 (α1), Glu25 to Asp36 (α2), and Ser41 to Ala54 (α3). The interaction between Z-domain and IgG is such that only α1 and α2 helices bind to the hinge region of the second and third constant domains (CH2 and CH3) of the Fc fragment, without involving the C-terminal α3 helix. Owing to the lack of intrinsic Cys residues for the Z-domain, when Cys residue is introduced into the C terminus of the Z-domain, oriented immobilization of the Z-domain on the solid surface can be conveniently achieved by exposing the IgG-binding epitope to the media.

The development and applications of protein site-specific conjugation have undergone significant advances in recent years, especially for the protein microarray or protein biochip. However, this approach is rarely used for affinity chromatography. In this work, we report the successful site-specific covalent conjugation for recombinant protein A (ZZZ protein, three tandem Z domains) through the reaction of the Cys-modified C terminus of the ZZZ protein with maleimide-functionalized agarose beads. After the affinity adsorbents have been developed, they will be used for IgG-binding from human plasma. The results will then be compared with those obtained using the Schiff-base method, a common amine-based conjugation technique used for recombinant protein A.

## RESULTS AND DISCUSSION

**Characterization of Recombinant Protein A (ZZZ-Cys and ZZZ).** By using Cys-specific modification, a unique Cys was genetically introduced into the C-terminus of ZZZ protein that resulted in engineered ZZZ-Cys. In this study, we engineered a hexahistidine tag at the C-terminus of ZZZ-Cys for protein purification using immobilized-metal affinity chromatography. The cartoon structure of ZZZ-Cys protein is shown in Figure 1. The proteins ZZZ-Cys and ZZZ were analyzed using 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the results are shown in Figure 1. Highly purified proteins were obtained as shown in the SDS-PAGE (Figure 1, lanes 3 and 4) for ZZZ under nonreducing condition and ZZZ-Cys under reducing condition. The protein bands had an apparent molecular mass of approximately 20 kDa, which corresponded to the theoretical value calculated for the ZZZ and ZZZ-Cys. However, some fraction of the purified ZZZ-Cys had spontaneously dimerized when recovered from agarose beads through intermolecular disulfide bond (Figure 1, lane 5). The same phenomenon was also discovered for the Z-Cys and ZZZ-Cys reported by Uhlen et al.

To obtain the monomeric ZZZ-Cys protein bearing the free thiol group, the protein was reduced using 2-mercaptoethanol. However, a trace amount of the dimer was still formed after 1 day, and the amount increased slowly up to approximately 50% in a week (Figure 1, lane 6). The results showed spontaneous dimerization has occurred during the protein storage process. Therefore, the site-specific immobilization of ZZZ-Cys on the agarose beads should be performed using the freshly reduced ZZZ-Cys.

**Oriented and Random Immobilization of the SpA.** The C-terminal Cys residue provided the oriented immobilization of ZZZ-Cys on maleimide-activated agarose beads. Scheme 1 shows the synthetic route of the adsorbent for ZZZ-Cys to site-specifically immobilize on the agarose beads (coded as A-SI). Sepharose 6FF amination was performed using the reactions of agarose beads with epichlorohydrin and then ethylenediamine. The heterobifunctional cross-linking reagent of 3-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) was used to react with amino-modified matrix, resulting in the formation of maleimide-functionalized matrix. The reaction of maleimide with free SH group at the C-terminus of ZZZ-Cys leads to the oriented immobilization of ZZZ-Cys on agarose beads. As a comparison, the random immobilization of ZZZ on agarose beads was carried out using Schiff-base method. Scheme 2 shows the synthetic route of the adsorbent for ZZZ to randomly immobilize on the agarose beads (coded as A-RI). Sepharose 6FF was activated by NaIO4 to obtain the aldehyde-functionalized matrix. The reaction of the aldehyde with one of many amino groups on the ZZZ chain leads to the random immobilization of ZZZ on agarose beads.

The adsorbents with different protein densities were obtained by the addition of increasing amounts of the protein, and the results are shown in Table 1. The coupling efficiency of ZZZ-Cys protein decreased with an increasing concentration of ZZZ-Cys and was higher than 90% for the concentration lower than 4 mg/mL and between 80 and 90% for the concentration between 6 and 10 mg/mL. The coupling efficiencies of ZZZ-Cys were higher than 90% during the concentration of 1 and 10 mg/mL. In our understanding, thiol—maleimide reaction should be more efficient than the aldehyde—amine coupling reaction. The lower coupling efficiencies of ZZZ-Cys are most likely attributed to spontaneous dimerization during the reaction process, especially for the ZZZ-Cys of high concentration. Note that the coupling efficiency of ZZZ-Cys protein was higher than 80% for site-specific immobilization, which demonstrated that the ZZZ-Cys protein was overwhelmingly present as a monomer with the free SH groups for the freshly reduced ZZZ-Cys. Additionally, the adsorbents with different immobilization grades can be obtained simply by varying the amount of added ZZZ-Cys to the activated Sepharose.

To prove that the ZZZ-Cys protein was site-specifically immobilized on the agarose beads through the SH group at the
C terminus for the adsorbent A-SI, detection of the SH group on the surface of adsorbents was performed, as shown in Scheme 3. As we know, Ellman’s reagent of 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) is able to react with the free SH group but not with the thioether bond. In the case of the reaction of aldehyde with the amino group, the adsorbent is able to react with the reagent DTNB. The reaction product, 2-nitro-5-thiobenzoic acid (TNB), has a high absorbance at 412 nm at pH 8.0. Therefore, the adsorbent A-SI-6 and, as a control group, the adsorbent A-RI-6-Cys reacted with DTNB in the Tris solution, pH 8.0, and subsequently precipitated by centrifugation at 4000 rpm for 10 min. The supernatant was analyzed using UV−vis spectroscopy, and the result is shown in Figure 2. Significant absorbance at 412 nm (A_{412nm}) could only be observed in the case of the adsorbent A-RI-6-Cys. However, upon incubation with the Ellman’s reagent, almost no A_{412nm} signal could be observed, and a modest increase in A_{325nm} of DTNB was detected for the adsorbent A-SI. The results indicated that the ZZZ-Cys protein was site-specifically immobilized on the agarose bead by Cys at the C terminus for the adsorbent A-SI.

**Antibody Binding Capacity.** After confirming the thiol-site specific for immobilization of the adsorbent A-SI, the adsorbents A-SI and A-RI containing increasing ligand densities were subsequently used for antibody-binding from the human plasma using static and dynamic adsorption experiments. Using blank agarose beads without immobilized SpA as a control group, the static antibody-binding capacity for the adsorbents of A-SI and A-RI was determined using batch experiment. For each stationary phase, 1 g of adsorbent and 20 mL of human plasma sample were incubated at room temperature with gentle shaking for 2 h. The bonded materials were eluted by lowering the pH to 2.5 and then were neutralized with Tris to pH 7.0. The eluent content was analyzed using immunoturbidimetry after being concentrated approximately 5-fold by ultrafiltration with millipore filtration.

Table 2 shows the effect of the protein density immobilized on the surface of the agarose beads on the antibody adsorption activity. The agarose only adsorbed 1.6 mg/g antibody, which was far below the performance of adsorbents A-SI and A-RI, indicating that the ZZZ protein played an important role in antibody adsorption. As we know, the SpA exhibits tight binding to many IgG, a small amount of IgA and IgM molecules. Analysis of eluted materials using immunoturbidimetry...
etry showed that the absorbed antibody composed of 90.1% IgG, 4.2% IgA, and 5.7% IgM. The IgG purity of 90.1% was comparable with the performance achieved by conventional protein A affinity resins.

The antibody-binding capacity of adsorbents increased with the increase in ligand density. In the case of adsorbent A-SI, the antibody-binding capacity continued to increase when ZZZ-Cys immobilization content increased from 0.98 to 8.12 mg/g. The maximum antibody-binding capacity was approximately 64 mg/g when the density of ZZZ-Cys on the agarose beads was 6.72 mg/g. In the case of adsorbent A-RI, the antibody-binding capacity similarly continued to increase and the maximum antibody-binding capacity was approximately 31 mg/g when the density of ZZZ on the agarose beads was 7.28 mg/g. The results clearly revealed the significant differences in antibody-binding capacity because of the different conjugating method.

The maximum antibody-binding capacity of 31 mg/g for randomly conjugated adsorbent A-RI-4 was comparable with the commercial protein-A Sepharose.29 Surprisingly, the maximum antibody-binding capacity of 64 mg/g for site-specific adsorbent A-SI-4 was far above the value for commercial protein A Sepharose and the adsorbent A-RI. This is probably due to weak steric effects between the matrix and ZZZ-Cys molecules because of the oriented immobilization resulting in the IgG-binding epitope freely exposed to the surrounding media.

To clarify the interaction between the IgG molecule and the ZZZ protein coupled to the agarose beads by site-specific and random immobilization, the binding ratio (mol/mol) was calculated assuming that the antibody is composed of IgG because of the IgG purity of more than 90%.

Table 2. Effects of the Protein Density Immobilized on the Surface of Agarose Beads on the Adsorption of Antibody

| adsorbent     | IgG-binding (mg/g) | IgA-binding (mg/g) | IgM-binding (mg/g) | antibody-binding capacity (mg/g) | antibody-binding ratio (mol/mol) |
|---------------|--------------------|--------------------|--------------------|----------------------------------|----------------------------------|
| agarose beads | 1.6                | "                  | "                  | 1.6                              | "                                |
| A-SI-1        | 10.2               | "                  | "                  | 10.2                             | 1.46                             |
| A-SI-2        | 19.2               | 0.9                | "                  | 20.1                             | 1.44                             |
| A-SI-4        | 34.6               | 1.6                | 2.1                | 38.3                             | 1.47                             |
| A-SI-6        | 49.9               | 2.3                | 3.2                | 55.4                             | 1.48                             |
| A-SI-8        | 57.6               | 2.7                | 3.7                | 64.3                             | 1.33                             |
| A-SI-10       | 58                 | "                  | 3.6                | 64.3                             | 1.11                             |
| A-RI-1        | 6.9                | "                  | "                  | 6.9                              | 1.01                             |
| A-RI-2        | 12.1               | "                  | "                  | 12.1                             | 0.88                             |
| A-RI-4        | 19.0               | 0.9                | "                  | 19.9                             | 0.73                             |
| A-RI-6        | 25.3               | 1.2                | 1.6                | 28.1                             | 0.71                             |
| A-RI-8        | 27.8               | 1.3                | 1.7                | 30.8                             | 0.59                             |
| A-RI-10       | 28.1               | 1.3                | 1.6                | 31.0                             | 0.48                             |

*The data are not available because the concentration is too low to exceed the lowest limit of immunoturbidimetry measurement. The antibody-binding ratio is calculated assuming that the antibody is composed of IgG because of the IgG purity of more than 90%.*

Figure 2. UV−vis spectra of the solutions from the reaction between the adsorbents with ZZZ-Cys site-specific immobilization (dash) and random immobilization (solid) and the Ellman’s reagents.

Scheme 3. Detection of SH Group on the Surface of Adsorbent A-SI by Reaction with Ellman’s Reagent
epitopes of the ZZZ protein were too close to the agarose bead, resulting in the difficulty to interact with IgG because of the steric hindrance.

Dynamic adsorption capacity is a very important property to assess the performance of the SpA adsorbent. The adsorbents A-SI-4 and A-RI-4 were used to study the dynamic adsorption of the antibody from human plasma, and the absorbance spectra of both flow through and elution fractions for antibody purification are shown in Figure 3. A glass column with 2 g of adsorbents was used in the experiment and 40 mL of human plasma sample flow through the column with the rate of 2 mL/min. The area of elution fraction for A-SI-4 was clearly higher than that for A-RI-4, which indicated that the dynamic adsorption capacity of the antibody for A-SI-4 was far above that for A-RI-4. The elution fractions composed of approximately 90.1% IgG, 4.2% IgA, and 5.7% IgM by the analysis of immunoturbidimetry. Also, the dynamic adsorption capacity of the antibody was 50 mg/g for A-SI-4 and 26 mg/g for A-RI-4. The adsorption of the antibody in dynamic conditions was lower than that in static conditions, which was likely due to the insufficient contact resulting in the unsaturated adsorption of the antibody.

Figure 4 shows the SDS-PAGE analysis of eluted materials from adsorbent A-SI-4 after dynamic adsorption (the result of A-RI-4 was similar and not shown). The IgG molecule consists of two identical heavy chains and two identical light chains. The molecular weight of the heavy chain is approximately 55 kDa, whereas the light chain is approximately 25 kDa. The result (Figure 4, lane 3) indicated that the eluted fraction contained mainly human IgG, which was comparable with the results achieved by immunoturbidimetry measurement.

■ CONCLUSIONS

In this work, we have developed a novel affinity adsorbent by oriented immobilization of a cysteine-containing recombinant protein A molecule to maleimide-functionalized Sepharose. Meanwhile, protein A was randomly immobilized on the surface of Sepharose using Schiffs-base method as a comparison. The assay of free SH group on the adsorbent showed that the ZZZ-Cys protein was validated to react with maleimide-functionalized Sepharose by the thiol group at the C terminus of ZZZ-Cys. As demonstrated in this work, the affinity adsorbent, with the site-specific immobilization at the C terminus of ZZZ-Cys, afforded the higher antibody-binding from human plasma and outperformed the existing commercial protein-A Sepharose. The high antibody-binding capacity was attributed to the weak steric hindrance of oriented ZZZ-Cys with the antibody-binding epitope exposed. This method opens up the opportunity to fabricate the adsorbents with high-binding performance by oriented and covalent immobilization.

■ EXPERIMENTAL SECTION

Materials. Agarose bead (Sepharose 6FF, 45−165 μm), Ni Sepharose 6FF (45−165 μm), and Sephadex G-25 (50−150 μm) were purchased from GE Healthcare (Sweden). MBS was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). Fresh-frozen human plasma was obtained from a local plasma donation center. Other reagents and solvents were obtained from Xinya Reagent (Shandong, China). All reagents used were of analytical grade unless otherwise stated.

Expression and Purification of the ZZZ-Cys and ZZZ. The two recombinant gene sequences, ZZZ-Cys and ZZZ, were synthesized using polymerase chain reaction technique and then inserted into NdeI/XhoI sites of the pET22b plasmid with the 6 × His tag at the C terminus. The plasmids pET22b-ZZZ-Cys and pET22b-ZZZ were constructed and transformed into...
Escherichia coli BL21 (DE3) cells, and then, the cells were cultivated in Luria–Bertani medium containing 100 μg mL⁻¹ ampicillin at 37 °C. Then, β-d-1-thiogalactopyranoside (IPTG) was added when A₆₀₀ is at the range of 0.6–0.8 to induce expression of target protein. Four hours later the cells were harvested by centrifugation, resuspended in 50 mM Tris-HCl and 150 mM NaCl at pH 7.5, and then lysed by ultrasonication. The lysate was centrifuged, and the supernatant was purified using Ni Sepharose 6FF according to the manufacturer’s instructions. The pooled fraction was desalted using Sephadex G-25 and then analyzed using 15% SDS-PAGE.

To obtain the monomeric ZZZ-Cys protein with the free thiol groups, the ZZZ-Cys protein was reduced using 0.5 M 2-mercaptoethanol in 50 mM Tris/HCl, pH 8.5, for 1 h at 50 °C and then purified using Sephadex G-25.

Oriented Immobilization of ZZZ-Cys on Agarose Beads. Agarose beads used in this work were Sepharose 6FF (GE Healthcare, Sweden). Oriented covalent immobilization of ZZZ-Cys on Sepharose 6FF was carried out essentially following a protocol as shown in Scheme 1.

Briefly, an agarose-epoxide matrix was synthesized: 10 g of Sepharose 6FF was resuspended in 15 mL of 2.5 M NaOH solution containing 0.02% (w/v) NaBH₄ and 10 mL of epichlorohydrin. Then, the suspension was then gently stirred for 4 h at 35 °C, and then, it was washed with massive distilled water. The agarose-epoxide matrix was then modified with ethylenediamine (2 mL, 30 mmol) in 20 mL of boric acid buffer, pH 3–5, and then, it was washed with massive distilled water to obtain the amino-modified matrix. Finally, the amino-modified matrix was treated with the reagent of MBS, following the next procedure: 10 g of amino-modified matrix was resuspended in 30 mL of 20 mM phosphate buffer (pH 7.2), and 2 mL of 10 mM MBS in dimethyl sulfoxide (DMSO) was added. The suspension was gently stirred for 4 h at 20 °C. Afterward, the maleimide-functionalized matrix was obtained by washing with an excess of 20 mM phosphate buffer (pH 7.2) containing 10% DMSO and water. The freshly reduced ZZZ-Cys in 20 mM phosphate buffer solution, pH 7.4, was added to the maleimide-functionalized matrix. The reaction was at 20 °C for 24 h with shaking. The amount of protein coupled was monitored by analyzing of the protein content at 280 nm before and after immobilization, and protein concentration was calculated using a theoretical extinction coefficient of 0.18 (mg/mL)⁻¹ cm⁻¹ for ZZZ-Cys. The remaining free reactive groups were blocked with 10 mM cysteine overnight and then washed thoroughly before being stored in 20 mM phosphate buffer, pH 7.4 with 0.2% NaN₃ at 4 °C until needed. The obtained adsorbent using site-specific immobilization technique was coded as A-SI.

Random Immobilization of ZZZ on Agarose Beads. Random covalent immobilization of ZZZ on Sepharose 6FF was carried out using Schiff base method, as shown in Scheme 2. Briefly, 10 g of Sepharose 6FF were resuspended in 20 mL of 1.0 M NaOH solution. Then, the suspension was gently stirred for 2 h at 40 °C in the dark, and then, it was washed with massive distilled water to obtain the aldehyde-functionalized matrix. The ZZZ protein in 0.2 M borate buffer solution, pH 8.5, was added to the aldehyde-functionalized matrix. The reaction was at 20 °C for 24 h with shaking. The amount of protein coupled was monitored as mentioned above. After coupling, 30 g of phosphate buffered saline (PBS) containing 1.0% NaBH₄ was used to reduce the beads at 20 °C for 4 h.

The obtained adsorbent using random immobilization technique was coded as A-RJ.

Detection of the Free SH Group on the Adsorbent. To evaluate the thiol-site-specific immobilization of ZZZ-Cys protein on Sepharose, a modified version of an Ellman’s reagent-based assay was used to determine whether or not the free SH group exists on the adsorbents of A-SI. As a comparison, the ZZZ-Cys protein was randomly immobilized on the agarose beads using Schiff-base method. This assay was performed as shown in Scheme 3. To 0.5 g of adsorbents, 4.5 mL of 0.1 mM DTNB in 0.1 M Tris solution, pH 8.0, was applied and incubated at room temperature with gentle shaking for 5 min. Afterward, the suspension was centrifuged at 4000 rpm for 10 min to obtain the supernatant. UV–vis absorption spectroscopy was carried out using a UV–vis spectrophotometer (UV-1780, Shimadzu) from 200 to 600 nm for the supernatant.

Static Adsorption of Antibodies from Human Plasma. Static adsorption of antibodies from human plasma on the adsorbents was studied batchwise. The human blood plasma was thawed, diluted with the same volume of 20 mM PBS solution, pH 7.4, and then centrifuged at 4000 rpm for 10 min before using. To 1 g of adsorbent, 20 mL of plasma samples was applied and incubated at room temperature with gentle shaking for 2 h. Afterward, the adsorbents were packed into a glass column (50 × 10 mm). The column was then washed with PBS solution to remove any unbound materials. Bound materials were then eluted using 0.1 M citrate buffer, pH 2.5. The eluted acidic fractions were immediately neutralized with 1 M Tris. The eluted fraction was checked using SDS-PAGE under reducing conditions. Meanwhile, the eluted fraction was concentrated approximately fivefold by ultrafiltration with millipore filtration (Mₘ 3000) at 6000 rpm and then analyzed using immunoturbidimetry in Beckman Au680.

Dynamic Adsorption of Antibody from Human Plasma. To evaluate the dynamic adsorption capacity of antibody from human plasma, 2.0 g of adsorbent suspension in PBS solution was packed into a glass column (50 × 10 mm). Forty milliliters of PBS solution was used to equilibrate the column at a rate of 2 mL/min, which was controlled by a peristaltic pump. Subsequently, 40 mL of human plasma was perfused into the column with 2 mL/min flow rate. PBS solution was used to wash the nonspecific adsorption of plasma away. Bound materials were then eluted using 0.1 M citrate buffer, pH 2.5. The measurement of eluted fraction was similar to the one mentioned above.

■ ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00362.

Sequences for B domain of SpA, ZZZ, and ZZZ-Cys (PDF)

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Notes

The authors declare no competing financial interest.
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REFERENCES

(1) Shukla, A. A.; Thomas, J. Recent advances in large-scale production of monoclonal antibodies and related proteins. Trends Biotechnol. 2010, 28, 253–261.

(2) Batalla, P.; Bolivar, J. M.; Lopez-Gallego, F.; Guisan, J. M. Oriented covalent immobilization of antibodies onto heterogeneous surfaces: A highly efficient immuno-affinity chromatography platform. J. Chromatogr. A 2012, 1262, 56–63.

(3) Liu, Y.; Yu, J. Oriented immobilization of proteins on solid supports for use in biosensors and biosips: A review. Microchem. Acta 2016, 183, 1–19.

(4) Wang, H.-C.; Yu, C.-C.; Liang, C.-F.; Huang, L.-D.; Hwu, J.-R.; Lin, C.-C. Site-Selective Protein Immobilization through 2-Cyano-benzothiazole-Cysteine Condensation. ChemBioChem 2014, 15, 829–835.

(5) Wong, L. S.; Khan, F.; Micklefield, J. Selective Covalent Protein Immobilization: Strategies and Applications. Chem. Rev. 2009, 109, 4025–4053.

(6) Jonkheijm, P.; Weinrich, D.; Schröder, H.; Niemeyer, C. M.; Waldmann, H. Chemical Strategies for Generating Protein Biochips. Angew. Chem., Int. Ed. 2008, 47, 9618–9647.

(7) Shimizu, D.; Taguchi, E.; Iwano, J.; Yamaguchi, T.; Masuda, K.; Enokizono, J.; Shiraiishi, Y. One-Step Conjugation Method for Site-Specific Antibody–Drug Conjugates through Reactive Cysteine-Engineered Antibodies. Bioconjugate Chem. 2016, 27, 1324–1331.

(8) Duckworth, B. P.; Xu, J.; Tatton, T. A.; Guo, A.; Distefano, M. D. Site-Specific, Covalent Attachment of Proteins to a Solid Surface. Bioconjugate Chem. 2006, 17, 967–974.

(9) Zhou, Y.; Guo, T.; Tang, G.; Wu, H.; Wong, N.-K.; Pan, Z. Site-Selective Protein Immobilization by Covalent Modification of GST Fusion Proteins. Bioconjugate Chem. 2014, 25, 1911–1915.

(10) Slotten, E. M.; Bertozzi, C. R. Bioorthogonal Chemistry: Fishing for Selectivity in a Sea of Functionality. Angew. Chem., Int. Ed. 2009, 48, 6974–6998.

(11) Camarero, J. A.; Kwon, Y.; Coleman, M. A. Chemoselective attachment of biologically active proteins to surfaces by expressed protein ligation and its application for “protein chip” fabrication. J. Am. Chem. Soc. 2004, 126, 14730–14731.

(12) Kwon, Y.; Coleman, M. A.; Camarero, J. A. Selective Immobilization of Proteins onto Solid Supports through Split-Intein-Mediated Protein Trans-Splicing. Angew. Chem., Int. Ed. 2006, 45, 1726–1729.

(13) Lin, P.-C.; Ueng, S.-H.; Tseng, M.-C.; Ko, J.-L.; Huang, K.-T.; Yu, S.-C.; Adak, A. K.; Chen, Y.-J.; Lin, C.-C. Site-Specific Protein Modification through Cu(I)-Catalyzed 1,2,3-Triazole Formation and Its Implementation in Protein Microarray Fabrication. Angew. Chem., Int. Ed. 2006, 45, 4286–4290.

(14) Watzke, A.; Köhn, M.; Gutierrez-Rodriguez, M.; Wacker, R.; Schröder, H.; Breinbauer, R.; Kuhlmann, J.; Alexandrov, K.; Niemeyer, C. M.; Goody, R. S.; Waldmann, H. Site-Selective Protein Immobilization by Staudinger Ligation. Angew. Chem., Int. Ed. 2006, 45, 1408–1412.

(15) de Aratijo, A. D.; Palomo, J. M.; Cramer, J.; Köhn, M.; Schröder, H.; Wacker, R.; Niemeyer, C.; Alexandrov, K.; Waldmann, H. Diels–Alder Ligation and Surface Immobilization of Proteins. Angew. Chem., Int. Ed. 2006, 45, 296–301.

(16) Badescu, G.; Bryant, P.; Swierkoss, J.; Khayrzad, F.; Pawlisz, E.; Farys, M.; Cong, Y.; Muroni, M.; Rumpf, N.; Brocchini, S.; Godwin, A. A New Reagent for Stable Thiol-Specific Conjugation. Bioconjugate Chem. 2014, 25, 460–469.

(17) Escorihuela, J.; Bañuls, M.-J.; Grijalvo, S.; Eritja, R.; Puchades, R.; Maquieira, A. Direct Covalent Attachment of DNA Microarrays by Rapid Thiol—“Click” Chemistry. Bioconjugate Chem. 2014, 25, 618–627.

(18) Hu, H.; You, J.; Gan, W.; Zhou, J.; Zhang, L. Synthesis of allyl cellulose in NaOH/urea aqueous solutions and its thiol–ene click reactions. Polym. Chem. 2016, 7, 1467–1477.

(19) Yu, F.; Cao, X.; Li, Y.; Chen, X. Diels–Alder Click-Based Hydrogels for Direct Spatiotemporal Postpatterning via Photoclick Chemistry. ACS Macro Lett. 2015, 4, 289–292.

(20) Fontaine, S. D.; Reid, R.; Robinson, L.; Ashley, G. W.; Santi, D. V. Long-term stabilization of maleimide–thiol conjugates. Bioconjugate Chem. 2015, 26, 145–152.

(21) Deis, L. N.; Pemble, C. W.; Qi, Y.; Hagarman, A.; Richardson, D. C.; Richardson, J. S.; Oas, T. G. Multiscale Conformational Heterogeneity in Staphylococcal Protein A: Possible Determinant of Functional Plasticity. Structure 2014, 22, 1476–1485.

(22) McCue, J. T.; Kemp, G.; Low, D.; Quiñones-García, I. Evaluation of protein-A chromatography media. J. Chromatogr. A 2003, 989, 139–153.

(23) Nilsson, B.; Moks, T.; Jansson, B.; Abrahmsén, L.; Elmblad, A.; Holmgren, E.; Henrichson, C.; Jones, T. A.; Uhlen, M. A synthetic IgG-binding domain based on staphylococcal protein A. Protein Eng. 1987, 1, 107–113.

(24) Jendeberg, L.; Tashiro, M.; Tejerio, R.; Lyons, B. A.; Uhlen, M.; Montelione, G. T.; Nilsson, B. The mechanism of binding staphylococcal protein A to immunoglobulin G does not involve helix unwinding. Biochemistry 1996, 35, 22–34.

(25) Yang, H.-M.; Bao, R.-M.; Cheng, Y.-Z.; Tang, J.-B. Site-specific covalent attachment of an engineered Z-domain onto a solid matrix: An efficient platform for 3D IgG immobilization. Anal. Chem. Acta 2015, 872, 1–6.

(26) Wong, L. S.; Thirlway, J.; Micklefield, J. Direct site-selective covalent protein immobilization catalyzed by a phosphopantetheinyl transferase. J. Am. Chem. Soc. 2008, 130, 12456–12464.

(27) Viswanathan, R.; Labadie, G. R.; Poullier, C. D. Regioselective covalent immobilization of catalytically active glutathione S-transferase to a synthetic peptoid support. J. Am. Chem. Soc. 2007, 129, 13317–13318.

(28) Viswanathan, R.; Labadie, G. R.; Poullier, C. D. Regioselective covalent immobilization of catalytically active glutathione S-transferase to a synthetic peptoid support. J. Am. Chem. Soc. 2007, 129, 13317–13318.

(29) Viswanathan, R.; Labadie, G. R.; Poullier, C. D. Regioselective covalent immobilization of catalytically active glutathione S-transferase to a synthetic peptoid support. J. Am. Chem. Soc. 2007, 129, 13317–13318.

(30) Viswanathan, R.; Labadie, G. R.; Poullier, C. D. Regioselective covalent immobilization of catalytically active glutathione S-transferase to a synthetic peptoid support. J. Am. Chem. Soc. 2007, 129, 13317–13318.

(31) Viswanathan, R.; Labadie, G. R.; Poullier, C. D. Regioselective covalent immobilization of catalytically active glutathione S-transferase to a synthetic peptoid support. J. Am. Chem. Soc. 2007, 129, 13317–13318.

(32) Viswanathan, R.; Labadie, G. R.; Poullier, C. D. Regioselective covalent immobilization of catalytically active glutathione S-transferase to a synthetic peptoid support. J. Am. Chem. Soc. 2007, 129, 13317–13318.