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Recognition property and preparation of *Staphylococcus aureus* protein A-imprinted polyacrylamide polymers by inverse-phase suspension and bulk polymerization

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ABSTRACT

Staphylococcus aureus protein A-imprinted polyacrylamide gel beads (SpA-IPGB) and imprinted polyacrylamide particles (SpA-IPGP) were synthesized by inverse-phase suspension polymerization and bulk polymerization, using SpA as template, acrylamide (AM) as functional monomers. Recognition capacity studies of the prepared SpA-IPGB and SpA-IPGP on SpA and *S. aureus* were conducted to determine recognition specificity. Computer imitation docking studies were conducted between template proteins and acrylamide monomer to reveal the recognition mechanism of SpA molecularly imprinted polymer. The results showed that the imprinted gel beads had high adsorption capacity and specificity to the SpA and *S. aureus*, and the adsorption quantity could reach 6.85×10^{-3} μmol/g and 10^3–10^4 CFU/g. The adsorption capacities of SpA-IPGB on SpA and *S. aureus* were higher than that of SpA-IPGP and Non-imprinted polyacrylamide gel beads (Non-IPGB). The recognition specificity of SpA-IPGB and SpA-IPGP on *S. aureus* was much higher than on *Escherichia coli* and *Streptococcus thermophilus*. The formation of complementary shape, and hydrogen bonding, electrostatic, and hydrophobic interactions between the imprinting cavities and the template proteins is the driving force for effective and specific recognition of protein imprinted polymer.

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1. Introduction

Molecularly imprinted polymers (MIPs) are prepared by the molecular imprinted technique that is a method for copolymerization suitable functional monomers around template molecules in a solution containing a ratio of cross-linker [1–3]. The stability, specific recognition, and ease of preparation of molecularly imprinted polymers have led to numerous studies that focused on the specific recognition of small organic molecules, amino acids, peptides, and nucleic acids [4–8]. In addition, protein molecularly imprinted polymers have been prepared and used in diverse applications such as biosensors, bioseparation, and artificial antibodies in immunoassays [9–12]. Different uses and potential applications of the MIP demand different methods to produce molecularly imprinted polymers. Bulk polymerization method is by far the most widely used by groups working on imprinting because of its simplicity and universality [13,14]. But the polymeric block needs to be crushed and ground to obtain particles of irregular shape and size. This process is not only wasteful and time-consuming but also may produce areas of heterogeneity in the polymeric matrix resulting from the lack of control of the process during polymerization, which are low affinity for the adsorption of target molecule. Suspension polymerization is another method that can provide spherical beads in the interface of organic continuous and water medium depending on the stirring speed and the amount of surfactant. After removal of the template, these molecularly imprinted complementary binding sites exhibit high selectivity and affinity for the target molecule [15,16]. Spherical molecular imprinting polymers have large surface, and there exist more effective imprinting sites in the surface or the shallow parts of the gel, it is fast and more effective in aqueous conditions to rebinding template molecules. Thus, MIP can mimic some of the functions of enzymes and antibodies, through the creation of three-dimensional cavities of specific size and shape for the recognition of protein molecules [16]. Some protein molecularly imprinted polymers that are prepared by inverse-phase suspension polymerization and using acrylamide (AM) as functional monomers were recently reported [7,15,17]. It allows large proteins to pass in and out and was shown to have a high adsorption capacity and binding specificity for target proteins [15].

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Recently, People have made some attempts to prepare molecularly imprinted polymers of whole cells such as bacteria, cells and viruses. *Escherichia coli*-imprinted gel granules synthesized by bulk imprinting were able to distinguish between different types and strains of bacteria [18]. The molecularly imprinted polymers of cells and viruses (e.g. yeast cell, erythrocytes and tobacco mosaic virus) have been prepared by Dickert and Bolisaya [19,20]. But the limitations for the template imprinting of whole cells are due to the large molecular size, the fragility and the complexity of the molecules. Rachkov and Minoura have used a short peptide sequence that represents a small and exposed fragment of the whole protein to prepare molecularly imprinted polymers [21]. The resulting polymer was able to recognize not only the small peptide template but also the entire protein. Keegan et al. have shown the specific interaction of the prepared polymers with receptors on the cell surface [22]. These results demonstrate that it may be possible to prepare molecularly imprinted polymers for better selectivity and specific recognition of whole cells using the surface components as template molecules. Certain surface components of different whole cells, such as small organic molecules, peptides, and protein, enable the preparation of imprinted polymers for detection of bacteria and viruses as they can be used as template molecules.

Therefore, we have selected *Staphylococcus aureus* protein A that is a specific surface protein in *S. aureus* [23] as template protein to prepare the SpA-imprinted polymers by inverse suspension polymerization and bulk polymerization. Adsorption experiments of different SpA-imprinted polymers on the target molecules and bacteria were conducted to determine their recognition capacity. In addition, we used chemical computational software docking SpA and AM monomer to study the recognition mechanism of imprinted polymer.

2. Materials and methods

2.1. Materials

SpA, *N*,*N*′-methylene bisacrylamide (BisAM), and ovalbumin were purchased from Sigma–Aldrich Chemical Co. Acrylamide (AM), toluene (Tol) were obtained from Beijing Solarbio Science Co., Ltd. Acetic acid, ethyl cellulose (EC), sodium dodecyl sulfate (SDS), sodium potassium persulfate (KPS), sodium hydrogen sulphite and other chemicals and solvents of analytical grade were provided by Shanghai Chemical Reagents Co., Ltd. All reagents were of analytical grade without further purification. Deionized water was used throughout.

2.2. Preparation of SpA-imprinted polyacrylamide polymers via inverse-phase suspension and bulk polymerization

SpA-imprinted polyacrylamide gel beads (SpA-IPGB) were prepared as reported in reference by inverse-phase suspension polymerization [15]. The best preparation conditions were selected in our previous experiments. A procedure and selected experimental conditions were given as follows. EC (0.2 g) was dissolved in 100 ml toluene at 60–70 °C for 2 h to prepare the continuous medium. After cooling down 50 °C, the EC solution was then carried out in a 250 ml three-necked flask equipped with a stirrer. AM (9.0 g) and BisAM (1.0 g) (crosslinking degree 10%) were mixed in 30 ml deionized water. SpA (4.2 mg) was then dissolved in the solution. The mixture was added to continuous medium in flask, sonicated for 5 min, and purged with nitrogen for 10 min, and then aqueous solution of KPS (0.1 g) and sodium hydrogen sulphite (0.05 g) were dropped into the reactor with stirring in a water bath at 50 °C. After the polymerization proceeded for 3 h, the gel beads were obtained.

For comparison, Non-imprinted polyacrylamide gel beads (Non-IPGB) were also prepared following exactly the same procedure but excluding the template from the formulation. SpA-imprinted polyacrylamide gel particles (SpA-IPGP) were synthesized by bulk-imprinted polymerization. AM and BisAM were mixed in 30 ml deionized water, SpA was then dissolved in the solution. Aqueous solution of KPS and sodium hydrogen sulphite was dropped into the reactor in a water bath at 50 °C. Once the polymerization is completed, the polyacrylamide gel was baked under vacuum, and then crushed and ground in a mortar to obtain polyacrylamide gel particles before extraction of the template.

The gel beads were immersed in a freshly prepared aqueous solution of acetic acid (10%, v/v) and SDS (1%, w/v) for 24 h. The gel beads were then filtered and washed repeatedly with distilled water until the filtrate was neutral. The same elution process was applied to both the SpA-IPGP and Non-IPGB.

2.3. SEM observations of the wet imprinted polymers

The surface morphologies of the wet gel beads and gel particles with different structures were studied by FEI Quanta 200 SEM. Wet samples mounted on metal stubs were observed under vacuum (200–250 Pa) and at relatively low temperature.

2.4. Adsorption experiments of SpA-imprinted polymers

In adsorption dynamic experiments, the adsorption dynamic curves of proteins onto SpA-IPGB, SpA-IPGP and Non-IPGB were conducted. For this, 1.0 g wet gel beads were placed in glass flasks. Then 10 ml of a known different initial concentration of SpA solution was added and the glass flasks were sealed and shaken at 25 °C. During the incubation period, at 20 min interval, samples were withdrawn from the supernatant, the protein concentration was determined at 280 nm by UV spectrophotometer (UV-1600).

In adsorption isotherm experiments, 1 g of SpA-IPGB, SpA-IPGP and Non-IPGB was introduced into different glass flasks, respectively. 10 ml of SpA solution with initial concentration (C_0) of 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 μmol/L were then added into each glass flask. After 100 min, samples were withdrawn from the supernatant, the protein concentration was determined at 280 nm by UV spectrophotometer.

For determination of static equilibrium adsorption capacity of SpA-IPGB, SpA-IPGP and Non-IPGB for the template molecule (SpA), static adsorption experiments were carried out under equilibrium binding conditions. The glass flasks were shaken for 12 h and the supernatant was analyzed for protein content. The experimental data were presented as the adsorption capacity per unit mass (g) of wet gel beads and the adsorption capacity (denoted as Q) was calculated as follows:

$$Q = (C_0 - C_s) \times V/m$$

where C_0 (μmol/L) is the initial concentration of protein solution, C_s (μmol/L) the protein concentration of the supernatant, V (ml) the volume of the initial solution and m (g) is the mass of the wet gel beads.

The imprinting efficiency (IE) of SpA in polymers could be calculated according to the formula:

$$\mathrm{IE} = Q(\text{imprinting})/Q(\text{non-imprinting})$$

The selectivity adsorption experiments of SpA-IPGB and SpA-IPGP were carried out under equilibrium binding conditions by using ovalbumin as control substrate. The protein concentration of SpA and ovalbumin was determined at 280 nm and 300 nm by UV spectrophotometer, respectively. Recognition selectivity is evaluated by

static distribution coefficient (K_D) and separation factor (α), K_D and α were defined as follows:

$$K_D = C_p/C_s$$

where C_p (μmol/g) is the concentration of substrate on gel beads and C_s (μmol/L) is the substrate concentration in the solution. Here, C_p is equal to Q (μmol/g).

$$\alpha = K_{D1}/K_{D2}$$

Of which K_{D1} and K_{D2} are the static distribution coefficient of templates and control molecules, respectively.

2.5. S. aureus recognition test and bacterial aerobic plate count

Microbial strains of *S. aureus*, *E. coli* and *Streptococcus thermophilus* were kind gifts from the laboratory of Dr. Yu (Anhui Entry-Exit Inspection and Quarantine Bureau, China). They were cultured in 100 ml of Luria Bertani (LB) broth overnight at 37 °C, respectively. The bacterial cells were suspended in PBS (pH 7.4) under gentle vortex mixing, and then were inoculated onto nutrient agar plates and incubated aerobically at 37 °C for 24 h to count colonies. The optical density of different bacterial concentrations was measured at 600 nm by UV spectrophotometer, and a linear relationship was established between bacterial concentration and optical density.

Adsorption dynamic curves and adsorption isotherm of *S. aureus* were determined according to the same procedure of experiment 2.4.

The wet gel beads (5.0 g) were placed in a 10 ml column and mixed with 10 ml bacteria suspension. The mixture was incubated on the column for 80–100 min, and then rapidly washed with PBS. The optical density of initial suspension and elution solution of the three bacteria was measured and the bacterial count bound to the SpA-IPGB, SpA-IPGP and Non-IPGB was calculated from the linear plot, respectively.

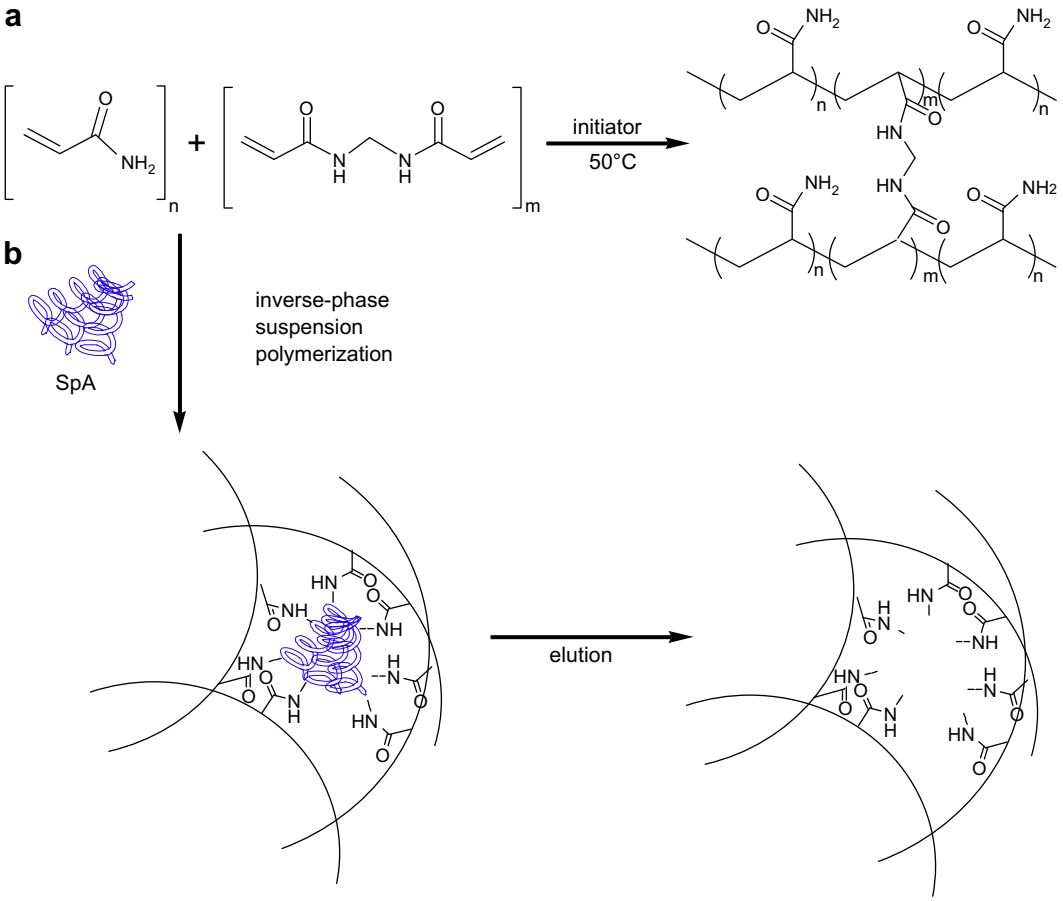
2.6. Computer imitation docking between template proteins and PAM monomer

The three-dimensional structure of SpA was obtained from RCSB protein data bank. Flexible docking was performed by using chemical computational dock software for studying and predicting the interaction between PAM monomer and template proteins.

In computational docking, ligand is flexible, the minimum RMS is 0.100, and the heating/cooling rat is 1.00 kcal/atom/p. Protein binding site is flexible (side chains and protein backbone). The orientation may be energy minimized using a rigid-body simplex minimization. A Score is based on terms taken from the HP Score piece of X Score. The energy could be calculated according to the formula:

$$\Delta G_{bind} = \Delta G_{vdw} + \Delta G_{hydrophobic} + \Delta G_{H\text{-}bond} + \Delta G_{H\text{-}bond(chg)} + \Delta G_{deformation} + \Delta G_0$$

The general parameters used by the docking were that grid resolution is 0.3 Å, binding site radius is 15 Å, population size is 50, max iterations is 2000, scaling factor is 0.50, and crossover rate is 0.90.



Scheme 1. Preparation scheme of SpA-imprinted polyacrylamide gel beads. (a, the crosslinking reaction of polyacrylamide; b, the formation of SpA-imprinted polyacrylamide gel beads).

3. Results and discussion

3.1. Morphology and preparation of SpA molecularly imprinted polymers

The crosslinking reaction between AM and BisAM can be proceeded with initiating of initiator and stirring in a water bath at 50 °C. The binding reaction between SpA template and amine groups of PAM chain takes place when the SpA was added. Then the template was removed with the aqueous solution of acetic acid and SDS. As the polymer crosslinks around the imprint molecule, specifically sized and shaped cavities were formed. The SpA template was being removed by washing the hydrogel with a base and these cavities were emptied, and the SpA-imprinted gel beads had many imprinting sites, which allowed the passage and close rebinding of molecules similar or identical to the SpA template. The preparation scheme of SpA-imprinted polyacrylamide gel beads is shown in Scheme 1.

The SEM image of SpA-imprinted polymers involves morphology and amplified surface photograph, as seen shown Fig. 1. Fig. 1a shows that the soft-wet gel beads by inverse-phase suspension polymerization which are spherical morphology, homogeneously distributed and well proportional. The diameter of the soft-wet gel beads were mainly ranged from 50 μm to 100 μm. Fig. 1b shows that the SpA-imprinted polyacrylamide gel particles were synthesized by bulk imprinted polymerizations which are irregular and partly lump. In the amplified SEM photograph of the SpA-IPGB side surface (Fig. 1c), a large quantity of macropores could be observed. There are not macropores in the side surface of the Non-IPGB (Fig. 1d).

PAM gel with low crosslinking degree has soft and macroporous structure, which permits large proteins to pass in and out, is known to be biocompatible. Moreover, there are many amide functional groups in PAM chains and they may be capable of forming hydrogen bonds even in polar solvents [7,15]. The soft-wet PAM gel beads prepared in our experiment had this characteristic. Spherical molecular imprinting polymers have large surface, and there exist more effective imprinting sites in the surface, it is a fast and more effective in aqueous conditions to rebinding template molecules. The fact that the SpA-IPGB showed higher imprinting efficiency (Table 1) than SpA-IPGP evidenced, to some extent, that the SpA-IPGB had more surface area and binding sites to rebind the template protein.

3.2. Adsorption properties and recognition specificity of the SpA molecularly imprinted polymers

3.2.1. Adsorption properties of the SpA-imprinted polymers for SpA

Fig. 2 shows the adsorption dynamic curves of SpA solution onto SpA-IPGB, SpA-IPGP and Non-IPGB, respectively. Fig. 2a shows the adsorption capacity of SpA-IPGB and SpA-IPGP have a rapid increase in 100 min, 100 min later, the adsorption process reached equilibrium, and then it increases slowly with the time extension.

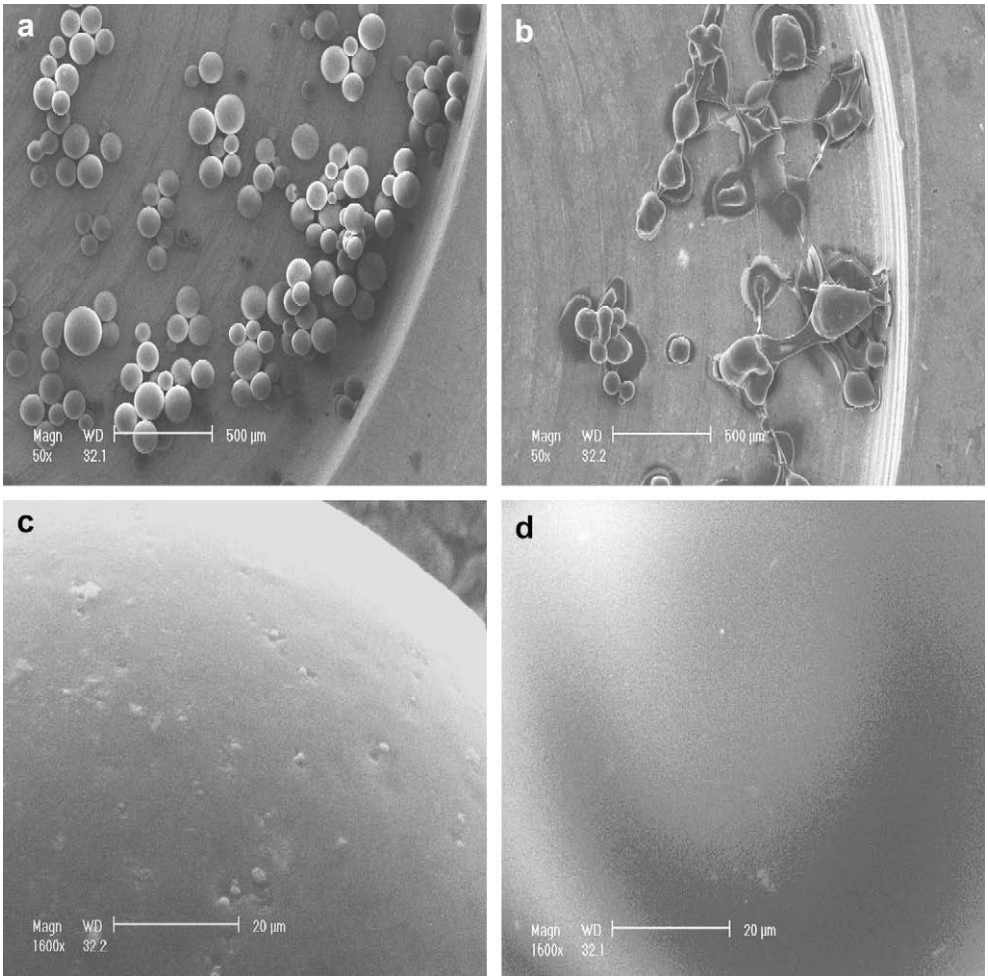


Fig. 1. Morphology and surface magnification SEM photographs of SpA-imprinted polymer in wet condition. (a, SpA-IPGB; b, SpA-IPGP; c, surface magnification of SpA-IPGB; d, surface magnification of Non-IPGB).

Table 1
The imprinting efficiency and adsorption capacities of SpA-IPGB, SpA-IPGP and Non-IPGB for SpA ($\bar{x} \pm s$).

IPGB	Analyte	C_s (μmol/L)	$Q \times 10^3$ (μmol/g)	IE
SpA-IPGB	SpA	0.32 ± 0.053	6.85 ± 0.534	5.34
SpA-IPGP	SpA	0.55 ± 0.028	4.54 ± 0.280	3.55
Non-IPGB	SpA	0.87 ± 0.008	1.28 ± 0.080	

Notes: 1.0 g of wet gels incubated with 10 ml of protein solution ($C_0 = 1.0$ μmol/L) at 25 °C for 12 h. s is standard error.

Fig. 2b shows the experimental equilibrium isotherms for adsorption of 0.4–1.6 μmol/L SpA solution onto SpA-IPGB, SpA-IPGP and Non-IPGB, respectively. It can be seen that the adsorption capacities of the SpA-IPGB and SpA-IPGP on SpA are increased with SpA concentration. When the SpA concentration was 1.4 μmol/L, the adsorption of SpA solution onto SpA-IPGB and SpA-IPGP almost reached saturation. The SpA could scarcely adsorb onto Non-IPGB. The reason probably is that the recognition sites of the SpA-IPGB and SpA-IPGP were more, and more SpA molecules were absorbed into the pores of the beads before equilibrium. The adsorption amount of the proteins or bacteria by the imprinted gel beads was low when the initial protein concentration was low. With the increase in the concentration of the SpA, the recognition sites on the surface of the beads were decreased correspondingly. After saturation, the pores on the surface of the beads were sealed by the SpA molecules, and could not bind other molecules.

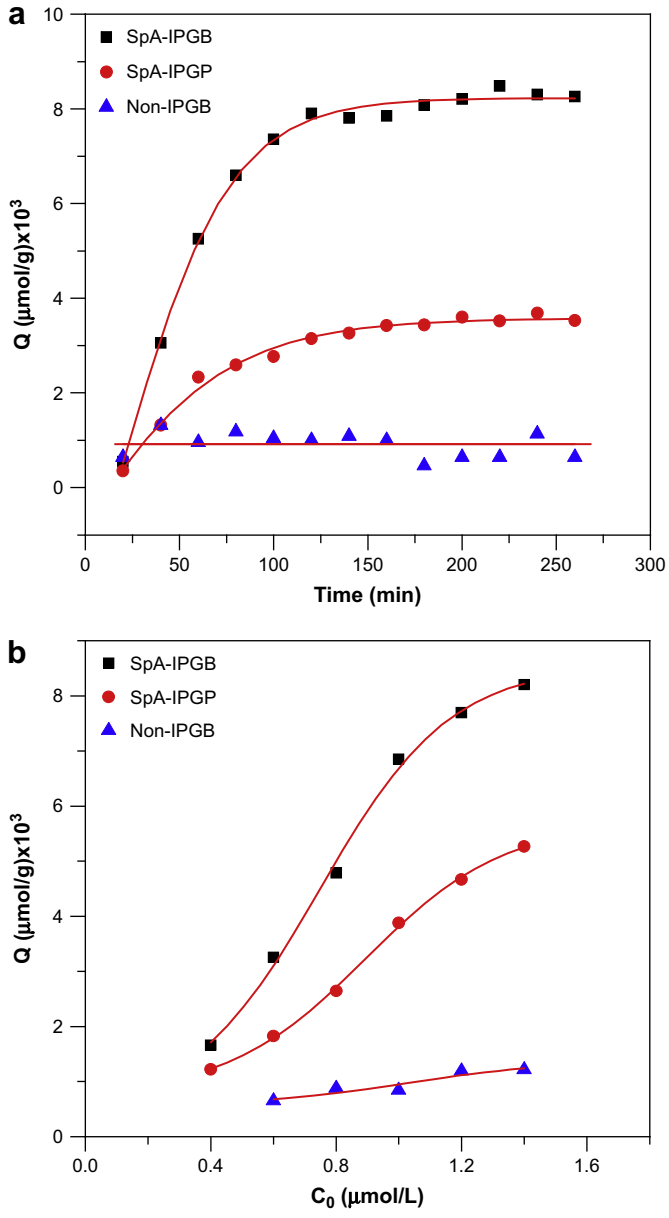


Fig. 2. (a) Adsorption dynamic curves and of SpA on different imprinted polymers. $V = 10$ ml, $m = 1.0$ g, $C_0 = 1.0$ μmol/L, temperature 25 °C. (b) Adsorption isotherms of SpA on different imprinted polymers. $V = 10$ ml, $m = 1.0$ g, $C_0 = 0.4$–1.6 μmol/L, temperature 25 °C.

It is shown in Table 1 that the SpA-IPGB had good imprinting effect, and the adsorption capacities could reach 6.85×10^{-3} μmol/g. The adsorption capacities of SpA-IPGP were 4.54×10^{-3} μmol/g. But the adsorption capacities of Non-IPGB only reached 1.28×10^{-3} μmol/g. It can be seen in experiments that the adsorption capacities of the SpA-IPGB were higher than SpA-IPGP and Non-IPGB. The adsorption capacities of SpA-IPGB and SpA-IPGP to SpA were much higher than that of to ovalbum (Table 2). These results indicated that SpA-IPGB possessed good recognition property to SpA due to its good imprinting efficiency (IE = 5.34). The spherical surface of SpA-IPGB had many complementary bonding sites in shape or structure, which allowed SpA to have close contact and strongly bound to the beads. In contrast, because some imprinted templates were embedded in SpA-IPGP that were synthesized by bulk imprinted polymerization, and not fully eliminated from the polymerization, the imprinting efficiency and the adsorption capacities of SpA-IPGP were lower than the SpA-IPGP. As for the Non-imprinted gel beads, the physical adsorption shows dominant effect due to the lack of imprinting process.

3.2.2. Recognition specificity of the SpA-imprinted polymers

The selectivity adsorption tests of SpA-IPGB were carried out under equilibrium binding conditions. The MW of the two kinds of protein molecules is as follows: ovalbumin (MW 43 kDa), and SPA (MW 42.7 kDa). Thus, we select the ovalbumin as contrastive proteins, which had no same shape or structure, but have same MW as the template proteins. Table 2 shows Q and K_D of SpA-IPGB for SpA which were much higher than that for ovalbumin, and Non-IPGB showed low Q and K_D for both SPA and ovalbumin. In addition, separation factor of SpA-IPGB ($\alpha = 5.17$) was much higher than that of SpA-IPGP ($\alpha = 2.65$) and Non-IPGB ($\alpha = 0.91$). These results indicated that both SpA-IPGB and SpA-IPGP possessed distinct adsorption selectivity to SpA. But the selectivity of SpA-IPGB to SpA was much higher than that of SpA-IPGP. Compared to ovalbumin, SpA molecules have complementary shape with imprinting cavities of SpA-IPGB and gain selectivity adsorption. Therefore, the recognition efficiency of imprinted gel beads was not related to molecule weight of proteins, but was closely related to shape and structure of proteins. The proteins similar to template ones in structure were easier to diffuse into the imprinting sites and caused specific adsorption.

Table 2
The selectivity adsorption of SpA-IPGB, SpA-IPGP and Non-IPGB for SpA and ovalbumin ($\bar{x} \pm s$).

IPGB	Analyte	C_s (μmol/L)	$Q \times 10^3$ (μmol/g)	K_D (g/ml)	α
SpA-IPGB	SpA	0.38 ± 0.007	6.19 ± 0.078	16.29	5.17
	Ovalbumin	0.76 ± 0.004	2.39 ± 0.025	3.15	
SpA-IPGP	SpA	0.57 ± 0.006	4.43 ± 0.040	7.95	2.65
	Ovalbumin	0.77 ± 0.006	2.31 ± 0.056	3.00	
Non-IPGB	SpA	0.89 ± 0.005	1.09 ± 0.045	1.22	0.91
	Ovalbumin	0.88 ± 0.007	1.18 ± 0.070	1.34	

Notes: 1.0 g of wet gels incubated with 10 ml of protein solution ($C_0 = 1.0$ μmol/L) at 25 °C for 12 h. s is standard error.

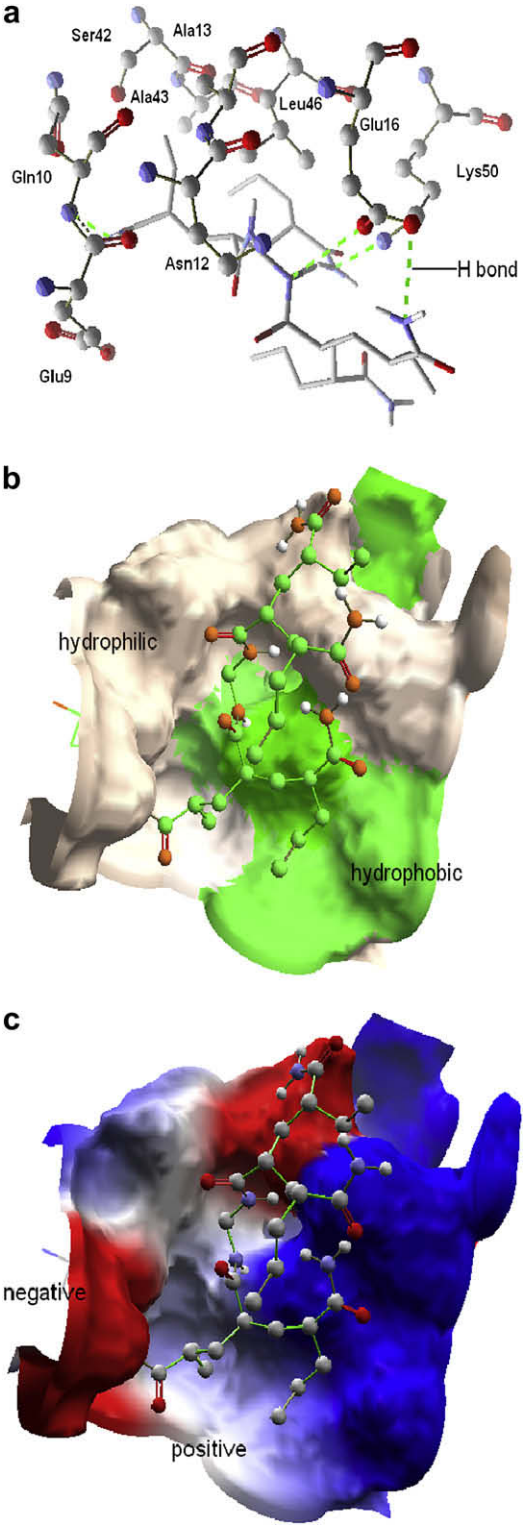


Fig. 3. Imitation docking views of binding sites between template proteins and AM monomer with computer imitation docking. (a, binding sites of hydrogen bonds; b, hydrophobic interaction; c, electrostatic interaction).

3.2.3. *Recognition mechanism of the SpA-imprinted polymers for SpA*

For the present study, the imprinting process created some imprinting cavities, which existed on effective imprinting sites and could selectively adsorb the template proteins [15,16,24,25].

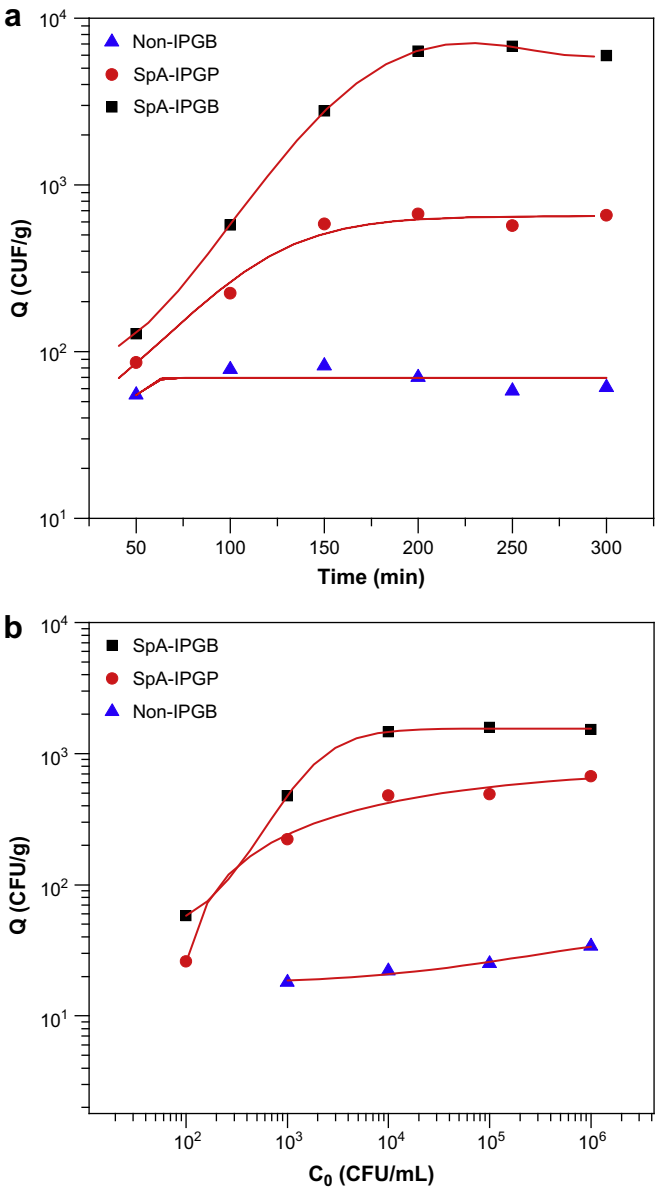


Fig. 4. (a) Adsorption dynamic curves and of *S. aureus* on different imprinted polymers. $V = 10$ ml, $m = 1.0$ g, $C_0 = 2.3 \times 10^5$ CFU/ml, temperature 25 °C. (b) Adsorption isotherms of SpA on different imprinted polymers. $V = 10$ ml, $m = 1.0$ g, $C_0 = 1.8 \times 10^2$–10^6 CFU/ml, temperature 25 °C.

Therefore, complementary shape or structure is an important aspect in protein imprinting and specific recognition. But it is unclear about the interaction between template proteins and PAM monomer in the imprinting cavities. In order to study the interaction mechanism of template proteins and PAM monomer in the imprinting cavities, we do docking between template proteins SpA and PAM monomer with dock software. The docking results are shown in Fig. 3. Fig. 3a shows interactions of the PAM monomer and SpA is partially held in position, and its N-terminal NH_2 group is covalently linked to the O, N-terminal of Lys, Gln, Trp, etc. group in SpA by the hydrogen bonding joining. The interactions of hydrogen bonding between the template and active sites of amide groups in SpA can subsequently drive the specific molecular recognition process. Fig. 3b shows that PAM monomer may bind hydrophobic amino acids such as Ala, Leu, Phe by hydrophobic interaction. Fig. 3c shows that PAM monomer may bind amino acid in negative and

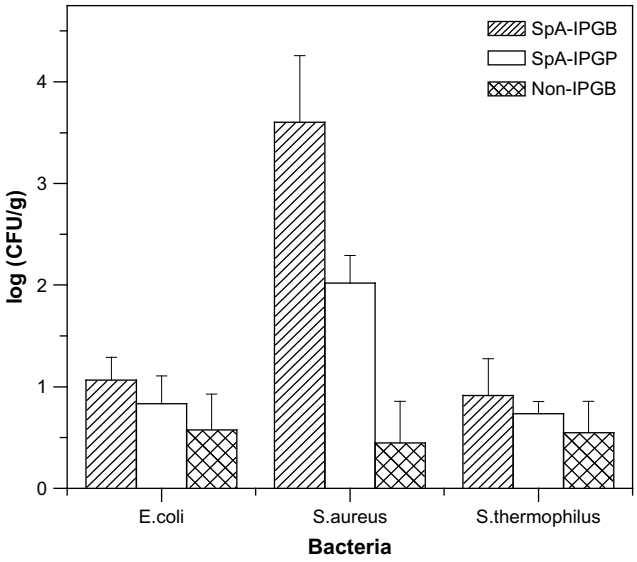


Fig. 5. Recognition specificity of the prepared SpA-IPGB, SpA-IPGP and Non-IPGB on *S. aureus*, *E. coli* and *S. thermophilus*.

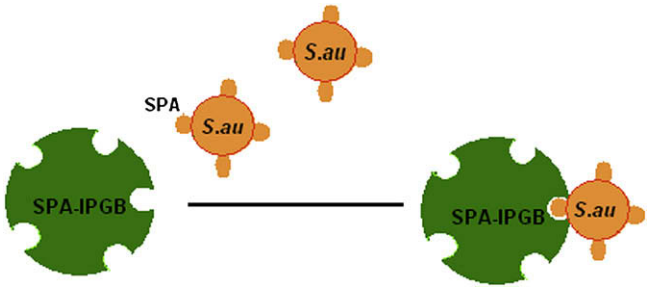
positive electricity areas, electrostatic interaction is not the dominant driving force. From the results in Fig. 3, it is concluded that the surface imprinting cavities of SpA-IPGB had many multiple hydrogen bonding and hydrophobic interaction sites, which are the dominant driving force for the molecular interaction between the SpA and PAM monomer, allowed SpA to have close contact and strongly bound to the beads. Thus, the SpA-imprinted gel beads had higher adsorption capacity.

For the present study, hydrogen bonding is the dominant driving force for the molecular recognition between proteins and the monomers [11,21,26]. For molecular imprinting, we think, the directional nature of a hydrogen bond demanded a high degree of structural or shape complementary and the multiple-point hydrogen bonds could be produced only when the cavities in the gel beads had complementary interaction surface with the proteins. Moreover, the weak interactions such as electrostatic interaction and hydrophobic interaction may also contribute to the interaction between template molecule and monomer. Therefore, complementary shape or structure and hydrogen bonding, electrostatic, and hydrophobic interactions are important aspects in protein imprinting and specific recognition.

3.3. Recognition of the prepared SpA molecularly imprinted polymers on S. aureus

Fig. 4 shows the adsorption dynamic curves of *S. aureus* onto SpA-IPGB, SpA-IPGP and Non-IPGB, respectively. Fig. 4a shows the adsorption quantity of three imprinted polymers on *S. aureus* reached equilibrium after 100–150 min, and then it increases slowly with the time extension. Fig. 4b shows the experimental equilibrium isotherms for adsorption of 10–10^6 CFU/g *S. aureus* solution onto SpA-IPGB, SpA-IPGP and Non-IPGB, respectively. It can be seen that the adsorption capacities of the SpA-IPGB and SpA-IPGP on *S. aureus* are increased with *S. aureus* concentration. When the *S. aureus* concentration was 10^4 CFU/g, the adsorption of *S. aureus* solution onto SpA-IPGB and SpA-IPGP almost reached saturation. The *S. aureus* could scarcely adsorb onto Non-IPGB.

In order to identify whether the SpA-IPGB specific bind on target bacteria, *E. coli* (gram-negative bacteria) and *S. thermophilus* (gram-positive bacteria) were chosen as control bacteria, SpA-IPGB and



Scheme 2. Adsorption scheme of SpA-imprinted polyacrylamide gel beads on *S. aureus*.

two control polymers that were SpA-IPGP and Non-IPGB were examined for their binding capabilities and recognition specificity. Fig. 5 shows the adsorbing capacity of SpA-IPGB, SpA-IPGP and Non-IPGB on *S. aureus*, *E. coli* and *S. thermophilus*. The adsorbing capacity of SpA-IPGB on *S. aureus*, in this study, is 10^3–10^4 CFU/g, and the adsorbing capacity of SpA-IPGP on *S. aureus* only is 10^2–10^3 CFU/g, but their adsorbing capacity on *E. coli* and *S. thermophilus* are under 10 CFU/g. On the other hand, Non-IPGB similarly showed low adsorbed quantity for *S. aureus*, *E. coli* and *S. thermophilus*.

These results indicate that SpA-IPGB, having the same shape cavities as the SpA of *S. aureus*, exhibited high recognition property for *S. aureus* compared to other bacteria. These cavities are correctly sized and shaped and appropriately functionalized to bind or allow the passage of the imprint or its analog and give rise to the variation in binding capacities and specificities (Scheme 2). They have a little the same shape cavities as the SpA in SpA-IPGP, only a little *S. aureus* was adsorped into SpA-IPGP. In contrast, as for the non-imprinted gel beads, they have no imprinted cavities due to the lack of imprinting process, so it was difficult for the non-imprinted gel beads to adsorb *S. aureus*. Both *E. coli* and *S. thermophilus* had no surface proteins of complementary shape or structure with imprinted cavities, which led to they could not be adsorbed onto SpA-imprinted polymers. Therefore, prepared protein imprinted PAM gel beads by using bacterial surface characteristic protein as template molecules may demonstrate specifically and selectively capture ability to target bacteria based on complementary binding in shape (geometrical) and binding cites.

4. Conclusions

SpA-imprinted polyacrylamide polymers were prepared by inverse-phase suspension polymerization and bulk imprinted polymerization. The imprinted gel beads had high adsorption capacity and specificity to the SpA and *S. aureus*, and the adsorption quantity could reach 6.85×10^{-3} μmol/g and 10^3–10^4 CFU/g. The adsorption capacities of SpA-imprinted polyacrylamide gel beads by inverse-phase suspension polymerization were higher than SpA-imprinted polyacrylamide gel particles by bulk imprinted polymerization and Non-imprinted polyacrylamide gel beads. Complementary shape or structure of imprinting cavities and hydrogen bonding, electrostatic, and hydrophobic interactions are important driving force for effective and specific recognition of protein imprinted polymer.

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