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Affinity Capture of Uropathogenic *Escherichia coli* Using Pigeon Ovalbumin-Bound Fe_3O_4@Al_2O_3 Magnetic Nanoparticles

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***Escherichia coli* and *Staphylococcus saprophyticus* are the most common causes of urinary tract infections, with 80% of these infections caused by uropathogenic *E. coli*. Because the P fimbriae of *E. coli* have specificity toward Gal(α1−4)Gal*β* units, pigeon ovalbumin (POA), whose structure contains terminal Gal(α1−4)Gal*β* moieties, was used as a probe for interaction with P fimbriated *E. coli*. The functional affinity probes for these bacteria by immobilizing POA—a phosphoprotein—onto the surface of magnetic iron oxide nanoparticles (NPs) coated with alumina (Fe_3O_4@Al_2O_3), using the phosphate units of POA as linking groups for the formation of phosphate−alumina complexes. The immobilization process occurred within 30 s when performing the reaction under microwave heating. The magnetic POA−Fe_3O_4@Al_2O_3 NPs generated using this facile approach exhibited specificity toward P fimbriated *E. coli*. The bacteria targeted by the affinity probes were characterized by matrix-assisted laser desorption/ionization mass spectrometry. The detection limit toward uropathogenic bacteria when using this approach was ~9.60×10^4 cfu/mL (0.5 mL).**

Selective enrichment of pathogenic bacteria and their subsequent rapid characterization using suitable analytical methods, such as mass spectrometry (MS), are attracting considerable attention.[1–6] Many approaches have been proposed for the use of functional magnetic nanoparticles (NPs) as affinity probes for bacteria.[6–11] Among the uropathogenic bacterial strains, *Escherichia coli* is the most common cause of urinary tract infection (UTI), with *E. coli* expressing P fimbriae being the cause of acute pyelonephritis. P fimbriae structures associated with pyelonephritis G have binding specificity toward Gal(α1−4)Gal*β* units.[12] Because the oligosaccharides on pigeon ovalbumin (POA) contain high levels of terminal Gal(α1−4)Gal*β* units,[13,14] POA binds to P fimbriated *E. coli*. This feature suggested to us that POA-bound magnetic NPs might be suitable for use as affinity probes for targeting P fimbriated *E. coli* strains from urine samples. Furthermore, because hen ovalbumin is a known phosphoprotein, we suspected that POA might also be a phosphorylated protein and, therefore, might have affinity toward metal oxide NPs.

Magnetic iron oxide NPs coated with metal oxides, such as titanium oxide,[15] zirconium oxide,[16] and aluminum oxide,[17,18] are effective affinity probes for phosphorylated proteins/peptides, which bind to these metal oxides through monodentate, bidentate, and tridentate interactions of the phosphate units to the metal ion centers.[19] Thus, the surfaces of metal oxides are readily modified with phosphorylated molecules through simple mixing; i.e., molecules containing phosphates can self-assemble onto the surface of metal oxides. Among these metal oxides, aluminum oxide is more reliable for use as a platform because titanium oxide and zirconium oxide display photocatalytic activity, which might decrease the stability of the metal oxide-bound phosphates. Furthermore, it has been demonstrated previously that the selectivity of alumina-coated magnetic iron oxide NPs toward phosphorylated molecules is superior to that of those coated with

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titania.[20] Coletti-Previero and Previero[21] were the first to demonstrate that aluminum oxide materials are suitable substrates for immobilization of biomolecules containing phosphate groups through alumina−phosphate chelating. Furthermore, such complexes are stable within a large range of pH. Li et al.[22] also immobilized phosphorylated pepsin onto alumina NPs via the binding of phosphate groups to Al(III) ions. These results inspired us to develop a facile method for generating functional magnetic NPs—ones that are capable of interaction with P fimbriated *E. coli*—through alumina−phosphate complexation. Furthermore, it has been demonstrated that magnetic iron oxide NPs are good microwave absorbers and that microwave heating can accelerate the extraction of target species onto the surfaces of iron oxide magnetic NPs.[23] In this study, this principle was extended to the binding between phosphates with Fe_3O_4@Al_2O_3 magnetic NPs.

EXPERIMENTAL SECTION

Reagents and Materials. Iron(III) chloride hexahydrate, ammonium hydrogen carbonate, trifluoroacetic acid (TFA), phosphoric acid (85%), and urea were purchased from Riedel-de Haën. Iron(II) chloride tetrahydrate and aqueous ammonium hydroxide were purchased from Fluka. Hydrochloric acid and sodium silicate were obtained from J. T. Baker (Phillipsburg, NJ). Aluminum isopropoxide and α-cyano-4-hydroxycinnamic acid (CHCA) were obtained from Aldrich (Germany). Dithiothreitol (DTT), iodoacetic acid (IAA), trypsin, sinapinic acid, and tris(hydroxymethyl)aminomethane (Tris) were obtained from Sigma (St. Louis, MO). POA was purified from pigeon egg white, which was kindly provided by Dr. James Johnson (Minneapolis Veterans Affairs Medical Center and University of Minnesota, Department of Medicine). Luria−Bertani (LB) broth and granulated agar were obtained from Becton Dickinson.

Preparation of Magnetic Iron Oxide NPs. Magnetic nanoparticles were prepared via coprecipitation. That is, $FeCl_3$ (5.4 g) and $FeCl_2$ (2.0 g) were dissolved in aqueous hydrochloric acid (2 M, 25 mL) at room temperature under sonication. After the salts had dissolved completely in the solution, the mixture was degassed using a vacuum pump and then the flask was filled with nitrogen gas. Aqueous ammonia (28%, 40 mL) was slowly injected into the mixture under nitrogen while stirring for 1 h at room temperature, followed by rinsing with deionized water three times and resuspending in deionized water (40 mL).

Preparation of Magnetic Iron Oxide NPs Coated with Alumina (Fe_3O_4@Al_2O_3). Iron oxide NPs (0.2 g) in deionized water (40 mL) were suspended through sonication under nitrogen gas. Aqueous sodium silicate solution (0.6%, pH 9, 40 mL) was added, and then the solution was stirred for 24 h at 37 °C to coat a thin film of SiO_2 onto the surfaces of the magnetic particles. The particles were rinsed with deionized water (3 × 40 mL) and then resuspended in deionized water (40 mL). Aluminum isopropoxide (20 mg) was added, and then the solution was sonicated for 30 min at room temperature. The mixture was placed in a reaction vial and heated at 80 °C in a water bath while stirring vigorously for 1 h; at that point, the cap of the vial was opened to release the generated gas (2-propanol) and was continually heated at 90 °C in a water bath for 30 min. After that, the reaction vial was loosely capped and was heated at 90 °C for another 2.5 h. After the mixture was cooled to room temperature, the Fe_3O_4@Al_2O_3 NPs were isolated by magnetic collection, rinsed with water (3 × 40 mL), and then resuspended in water (40 mL). The suspension was stored in a refrigerator prior to use. Storage of 1 month did not seem to affect the performance of the NPs.

Using Fe_3O_4@Al_2O_3 NPs To Selectively Enrich Phosphopeptides from the Tryptic Digest of POA. POA purified from pigeon egg white was denatured prior to performing the tryptic digestion. A solution of POA (10^{-4} M) in aqueous ammonium hydrogen carbonate (50 mM, 1 mL) containing urea (8 M) was incubated at 37 °C for 30 min, and then DTT (0.4 mg) was added. The mixture was incubated at 45 °C for 1 h. After cooling the solution to room temperature, the Eppendorf tube containing the mixture was wrapped with aluminum foil and placed in an ice bath. IAA (1 mg) was added, and then the contents of the Eppendorf tube were vortex-mixed for 2.5 h at room temperature. Trypsin (1 μg/μL, 1 μL) was added, and then the denatured POA (10^{-5} M, 100 μL) was digested at 37 °C for 18 h; the mixture was then diluted 10-fold using 0.15% TFA. Selective enrichment of the phosphopeptides in the digest was performed by binding them to the Fe_3O_4@Al_2O_3 NPs. An aliquot of the digest (5 μL) was mixed with Fe_3O_4@Al_2O_3 NPs (40 μg/μL, 2 μL), and then the suspension was mixed by pipetting in and out of the Eppendorf tube for 30 s. The probe target species were aggregated to the side of the Eppendorf tube by applying an external magnet; the supernatant was removed, and the remaining NP−target species were rinsed with a solution of 0.15% TFA (2 × 60 μL) in deionized water and acetonitrile (1:1, v/v). After removing the supernatant, the remaining probe−target species were mixed with CHCA (2.5 mg/mL, 2 μL) containing 1% phosphoric acid. The supernatant was deposited onto a MALDI target. After evaporation of the volatile solvent, the sample was ready for MALDI MS analysis. Reflectron mode was operated for obtaining the mass spectra with mass resolution >7500. Bradykinin and melittin were used as the standards for mass calibration.

Preparation of Bacterial Samples. *Staphylococcus saprophyticus* was collected from patients at the General Tzu-Chi Hospital, Hualien, Taiwan. *E. coli* J96, *E. coli* BOS117, *E. coli* Low31, and *E. coli* V21 were kind gifts from Dr. James Johnson (Minneapolis Veterans Affairs Medical Center and University of Minnesota, Department of Medicine). All of these *E. coli* strains express P fimbriae, except for *E. coli* V21. When preparing bacterial samples, it should be handled in a Biosafety Level 2 (BSL-2) laboratory. These bacteria were cultured in LB agar plates, which were prepared by mixing LB broth (10 g) and agar (6.8 g) in deionized water (400 mL). After incubation overnight at 37 °C, the bacterial cells were transferred to Tris buffer (12.5 mM, pH 7.4, 1 mL). The bacterial concentration was adjusted to a desired level by measuring the optical density (OD) at 600 nm and checked by plating serial dilutions of the bacterial samples on LB agar plate and counting the colony forming units (cfu) after incubation overnight at 37 °C. Live bacteria were directly used in all the experiments described in this study.

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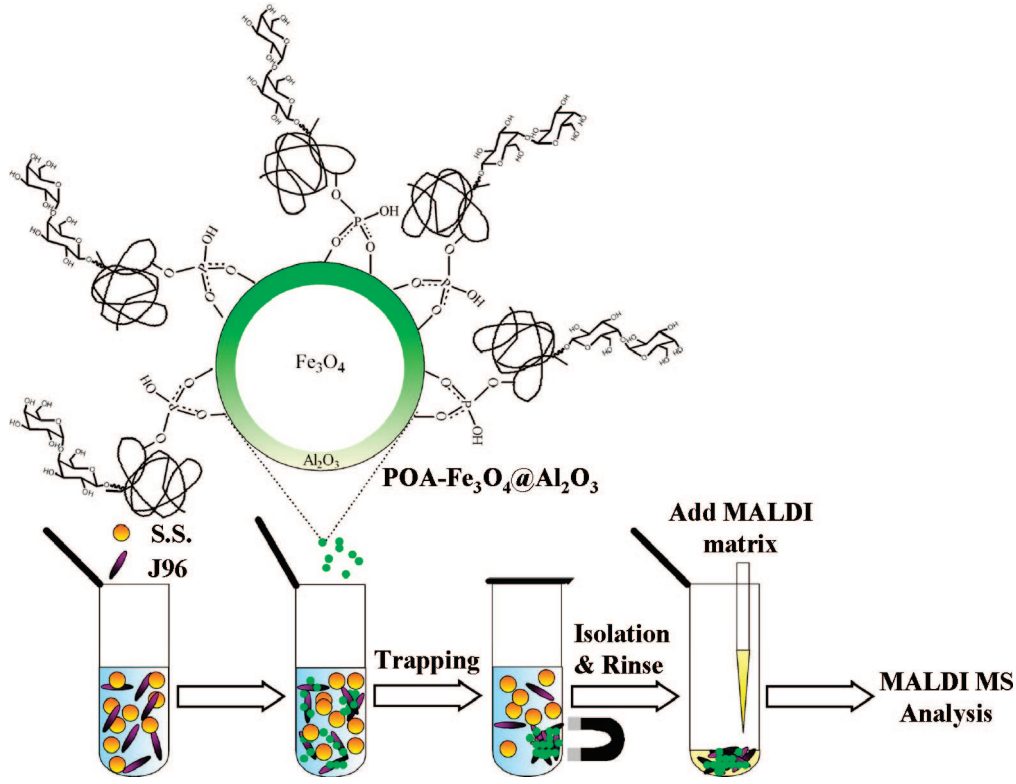
Scheme 1. Cartoon Representation of the Process Employed When Using POA-Fe_3O_4@Al_2O_3 NPs To Selectively Trap Target Bacteria



Preparation of POA-Bound Fe_3O_4@Al_2O_3 NPs. POA (10^{-4} M) was first denatured by reacting with DTT and IAA as described above. The denatured POA stored in a refrigerator was good for two weeks. After the denaturation, POA was acidified by 0.15% TFA aqueous solution (1/4, v/v), and Fe_3O_4@Al_2O_3 NPs (0.4 mg) were incubated with the acidified POA (2×10^{-5} M, 200 μL) in a Eppendorf tube under microwave heating (power, 900 W) for 30 s prior to trapping experiment. The cap of the Eppendorf tube was always kept open during heating. It was found that the binding capacity of the Fe_3O_4@Al_2O_3 NPs for POA under microwave heating (power, 900 W) for 60 s was similar to that obtained under microwave heating for 30 s. The binding capacity was estimated ~2.8 nmol/mg. Furthermore, if the time of the microwave heating was extended to 90 s, the solution in the Eppendorf tube was overheated, which leaded the tube melted. Thus, the heating time for POA binding on the Fe_3O_4@Al_2O_3 NPs was set on 30 s. After magnetic isolation, the POA−Fe_3O_4@Al_2O_3 NPs were rinsed with deionized water (200 μL × 2) and then the NPs were resuspended in deionized water (100 μL) prior to use.

Using Affinity Nanoparticles To Selectively Enrich Uropathogenic Bacteria. Scheme 1 displays the steps followed to trap bacteria from sample solutions using the affinity probes. The POA−Fe_3O_4@Al_2O_3 NPs (unmodified Fe_3O_4@Al_2O_3 NPs, 40 μg) were vortex-mixed with a bacterial sample (1 or 0.5 mL) for 1 h. The NP−bacterium conjugates were isolated through magnetic separation and then rinsed with Tris buffer (12.5 mM, pH 7.4, 3 × 1 mL). The isolated conjugates were mixed with sinapinic acid (15 mg/mL, 2 μL) containing 5% TFA. After standing for 3 min, the supernatant (1 μL) was deposited on a MALDI sample plate for MALDI MS analysis. Linear mode was operated for obtaining the mass spectra. Melittin and ubiquitin were used as internal standards (i.s.) for mass calibration.

Instrumentation. All mass spectra were obtained using an Autoflex III MALDI TOF MS/MS spectrometer (Bruker Daltonics) equipped with a 355-nm Nd:YAG laser. TEM images were obtained using a JEOL transmission electron microscope (2000FX). The absorption spectra were recorded using a Varian Cary 50 spectrophotometer (Melbourne, Australia).

RESULTS AND DISCUSSION

Previously, Fe_3O_4@Al_2O_3 NPs have been demonstrated as effective affinity probes for trapping phosphopeptides from the tryptic digests of phosphoproteins.[17,18,20] To determine whether POA is a phosphorylated protein, Fe_3O_4@Al_2O_3 NPs were employed as affinity probes for the selective enrichment of phosphopeptides from the tryptic digest of POA. Figure 1a displays the MALDI mass spectrum of the sample obtained after using the Fe_3O_4@Al_2O_3 NPs as the affinity probes to capture phosphorylated peptides from a tryptic digest of POA; peaks appear at m/z 2471.56, 2552.97, 2627.16, and 2709.10 in addition to those at m/z 1060.57 and 2845.76 (marked as "i.s.") that represent the standards used for mass calibration. We suspected that the peaks at m/z 2471.56 and 2627.16 corresponded to the fragments obtained from the peaks at m/z 2552.97 and 2709.10, respectively, upon the loss of $H_xPO_3^-$ units. The results were further confirmed using MALDI TOF MS/MS. Figure 1b and c displays the MALDI TOF MS/MS spectra obtained after selecting the peaks at m/z 2709.10 and 2552.97, respectively, as precursor ions. The peaks adjacent to the precursor ions—

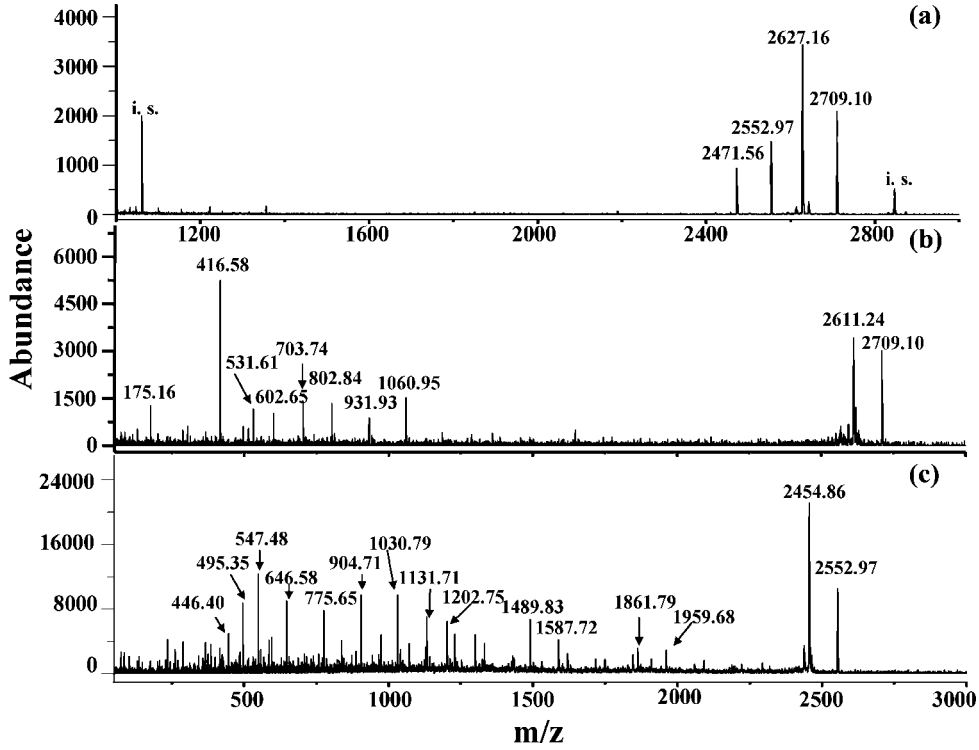


Figure 1. (a) MALDI mass spectrum obtained after using Fe_3O_4@Al_2O_3 NPs as affinity probes to selectively enrich target species from the tryptic digest of POA. (b, c) MALDI TOF MS/MS spectra obtained after selecting the precursor ions at *m/z* (b) 2552.96 and (c) 2709.10. The peaks marked "i.s." represent bradykinin and melittin, which were used as standards for mass calibration.

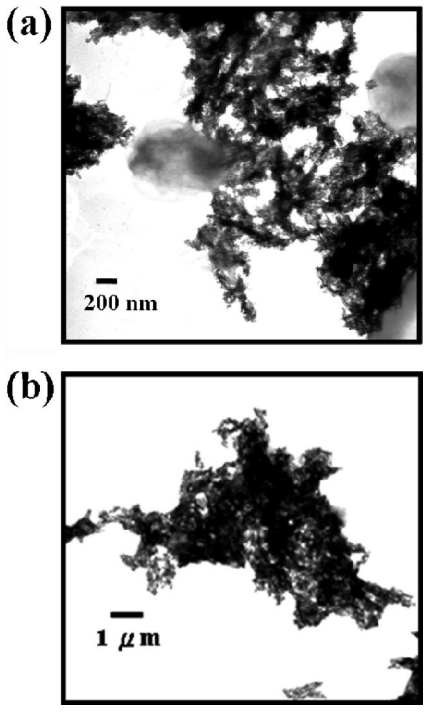


Figure 2. TEM images obtained after incubating (and then magnetically separating) the POA−Fe_3O_4@Al_2O_3 NPs with (a) *E. coil* J96 and (b) *E. coli* V21.

at *m/z* 2611.24 and 2454.86, respectively, dominate these two MS/MS mass spectra. There is a mass difference of ~98 Da, corresponding to the mass of an H_3PO_4 unit, between the fragments and their precursor ions. Thus, these spectra confirmed that POA contains phosphate units. The remaining peaks in Figure 1b and c correspond to peptide residues derived from the precursor ions, but elucidating their detailed peptide sequences exceeds the scope of our current investigation.

After demonstrating that POA is a phosphoprotein, POA was immobilized onto the surfaces of the Fe_3O_4@Al_2O_3 NPs through its phosphate units. Initially, this immobilization process was carried out through vortex mixing for 1 h. Unfortunately, the Fe_3O_4@Al_2O_3 NPs readily adhered to the wall of the Eppendorf tube during vortex mixing, making it difficult to rinse and collect the NPs. Furthermore, the protein solution generated bubbles during vortex mixing, making it impossible to estimate the amount of POA bound to the NPs. Previously, it has been demonstrated that functional magnetic iron oxide NPs are good absorbers of microwaves.[23] Taking the advantage of this phenomenon, microwave heating was employed to accelerate (~30 s) the extraction of oligonucleotides from sample solutions onto these affinity probes. Those results tempted us to employ a similar concept to immobilize POA onto the surfaces of magnetic NPs. Again, it was found that functionalization of the magnetic NPs took only 30 s under microwave irradiation, with no adhering of the NPs onto the wall of the vial and no generation of bubbles.

Next, the ability of the POA−Fe_3O_4@Al_2O_3 NPs to trap P fimbriated *E. coli* was examined, using P fimbriated *E. coli* J96 and non-P fimbriated *E. coli* V21[24] as representative samples.

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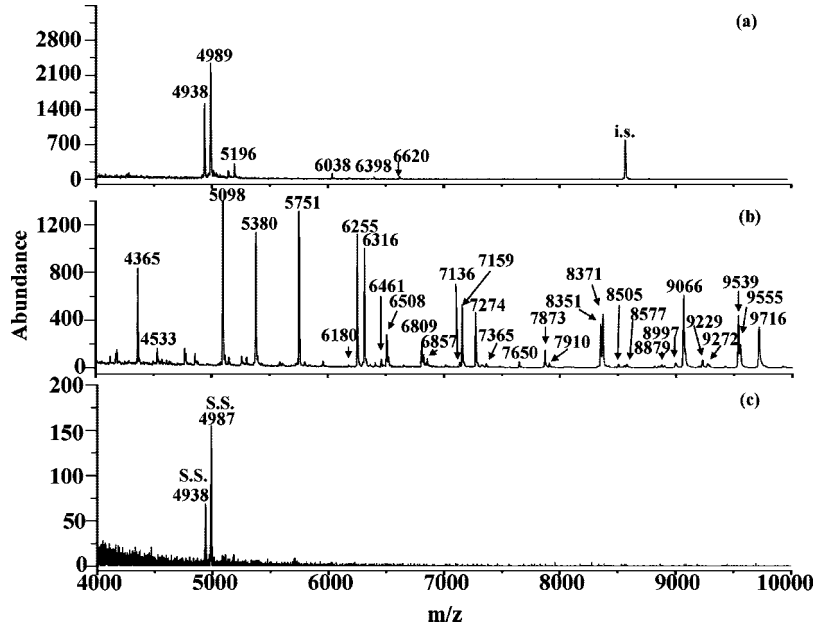

Figure 3. Direct MALDI mass spectra of (a) *S. saprophyticus* (9.60×10^8 cfu/mL, 1 μL), (b) *E. coli* J96 (6.90×10^8 cfu/mL, 1 μL), and (c) direct MALDI mass spectrum of a mixture (1 μL) of *S. saprophyticus* (3.23×10^8 cfu/mL) and *E. coli* J96 (1.13×10^8 cfu/mL). Sinapinic acid (15 mg/mL) containing 5% TFA was used as the MALDI matrix. Melittin and ubiquitin were used as standards for mass calibration. The peak marked i.s. represents ubiquitin. The peaks marked S.S. were derived from *S. saprophyticus*.

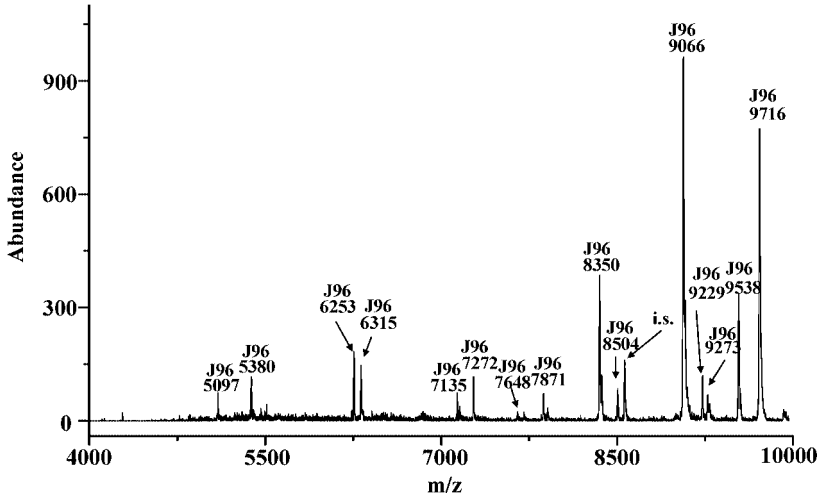

Figure 4. MALDI mass spectrum obtained after using the POA−Fe_3O_4@Al_2O_3 NPs (40 μg) as affinity probes to selectively enrich target bacterial cells from a mixture (1 mL) of *S. saprophyticus* (3.23×10^8 cfu/mL) and *E. coli* J96 (1.13×10^8 cfu/mL). Sinapinic acid (15 mg/mL) containing 5% TFA was used as the MALDI matrix. Melittin and ubiquitin were used as standards for mass calibration. The peak marked i.s. is ubiquitin. The peaks marked J96 were derived from *E. coli* J96.

Panels a and b in Figure 2 display TEM images obtained after incubating the POA−Fe_3O_4@Al_2O_3 NPs (40 μg) with *E. coli* J96 and V21, respectively, followed by magnetic isolation. Several bacterial cell-POA-Fe_3O_4@Al_2O_3 NP conjugates are visible in Figure 2a. Furthermore, the NPs appear to be attached to the fimbriae of the *E. coli*. In contrast, there are no bacterial cells visible in Figure 2b. We confirmed these results by culturing the isolated magnetic NPs used to obtain Figure 2a and b on LB agar plates. After diluting the isolated NP−target species conjugates for 10^5-fold, no bacterial cells were found on the LB agar plate after culturing overnight using the NPs brought into contact with *E. coli* V21, whereas bacterial cells grew on the LB plate when UTI *E. coli* J96 was used as the sample. From a count of the bacterial growth on the LB agar plate, we estimated that the binding capacity of the POA−Fe_3O_4@Al_2O_3 NPs toward *E. coli* J96 was $\sim 2.8 \times 10^8$ cfu/mg. If the dilution of the isolated conjugates was only 10^4-fold, a few *E. coli* V21 cells were visible on the LB agar plate. The binding capacity of POA−Fe_3O_4@Al_2O_3 NPs for *E. coli* V21 was estimated at $\sim 1.6 \times 10^7$ cfu/mg, which is much lower than that of the NPs for *E. coli* J96. These results suggest that POA−Fe_3O_4@Al_2O_3 NPs have high specificity for P fimbriated *E. coli* J96.

MALDI MS was employed to further confirm these findings. Linear mode was operated when carrying out the MALDI MS

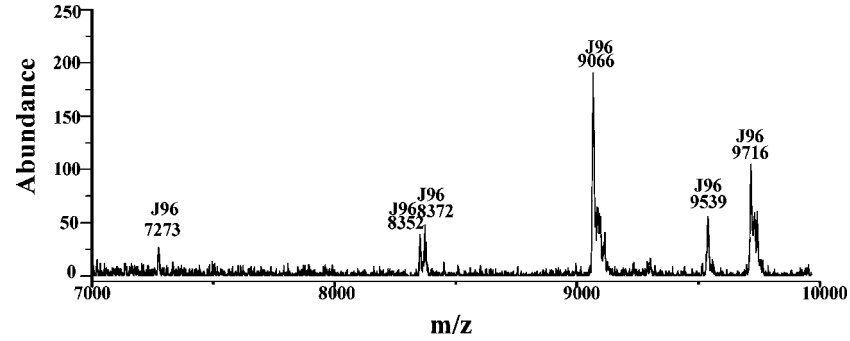

Figure 5. MALDI mass spectrum obtained after using the POA−Fe_3O_4@Al_2O_3 NPs as affinity probes to trap target bacteria from an aqueous sample solution (0.5 mL) (40 μg) containing *E. coli* J96 (9.60×10^4 cfu/mL). Sinapinic acid (15 mg/mL) containing 5% TFA was used as the MALDI matrix. All the peaks were derived from *E. coli* J96.

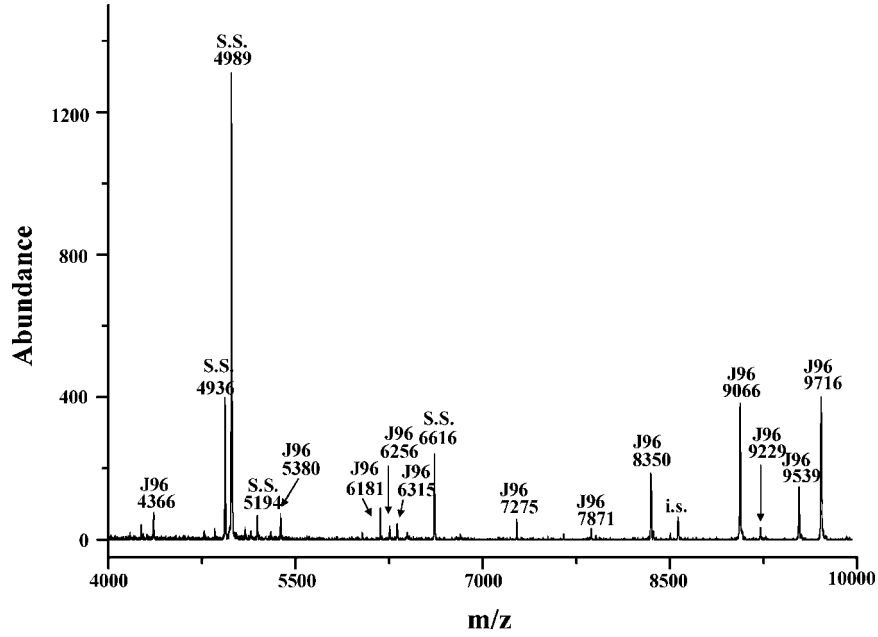

Figure 6. MALDI mass spectrum obtained after using the unmodified Fe_3O_4@Al_2O_3 NPs (40 μg) as affinity probes to enrich target species from a mixture (1 mL) of *S. saprophyticus* (3.23×10^8 cfu/mL) and *E. coli* J96 (1.13×10^8 cfu/mL). Sinapinic acid (15 mg/mL) containing 5% TFA was used as the MALDI matrix. Melittin and ubiquitin were used as standards for mass calibration. The peak marked i.s. represents ubiquitin. The peaks marked J96 and S.S. were derived from *E. coli* J96 and *S. saprophyticus*, respectively.

analysis. Panels a and b in Figure 3 display the direct MALDI mass spectra of *S. saprophyticus* and *E. coli* J96, respectively. There are more peaks found in the mass spectrum of *E. coli* (Figure 3b). The peaks marked i.s. represent melittin and ubiquitin, which we added as internal standards for mass calibration. Figure 3c presents the direct MALDI mass spectrum of the mixture containing *S. saprophyticus* (3.23×10^8 cfu/mL) and *E. coli* J96 (1.13×10^8 cfu/mL). Because the cell concentration of *S. saprophyticus* was higher than that of *E. coli* J96, only the peaks at m/z 4938 and 4987 (marked as "S.S.") derived from *S. saprophyticus* appear in the mass spectrum. In Figure 3c, the baseline rose because a high laser power was required to obtain the mass spectrum. Figure 4 presents the MALDI mass spectrum obtained after using the POA−Fe_3O_4@Al_2O_3 NPs as affinity probes (40 μg) to trap the target bacterial cells from the same sample (1 mL) used to obtain Figure 3c. All the peaks appearing in the mass spectrum matched those derived from *E. coli* J96 in Figure 3b. We detected no ions derived from *S. saprophyticus*. Because of the limited mass resolution in high masses operated in linear mode, the matched masses of these peaks may have ±3 mass difference from those appearing in Figure 3. This result indicates that the POA−Fe_3O_4@Al_2O_3 NPs can concentrate *E. coli* J96 specifically from bacterial mixtures, even when the cell concentration of *S. saprophyticus* is higher. We also employed *E. coli* BOS117 and *E. coli* Low31, which are two other P fimbriated *E. coli* strains, as samples in place of *E. coli* J96 in the bacterial mixture. Again, only peaks derived from *E. coli* appeared in the resulting MALDI mass spectra after using the POA−Fe_3O_4@Al_2O_3 NPs as affinity probes to probe the target species from bacterial mixtures containing *S. saprophyticus* and P fimbriated *E. coli* BOS117 or Low31 (results not shown).

S. saprophyticus is the most common cause of urinary tract infections among Gram-positive bacteria.[25] Previously, it was

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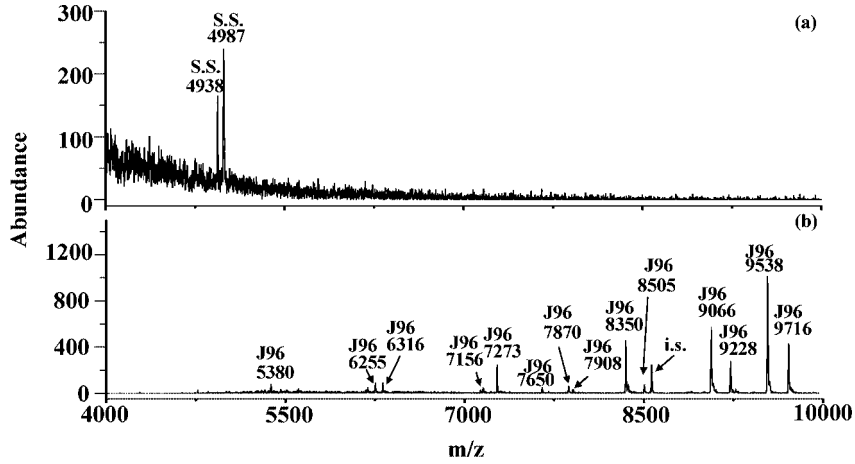

Figure 7. (a) Direct MALDI mass spectrum of a urine sample (1 μL) spiked with *S. saprophyticus* (3.23×10^8 cfu/mL) and *E. coli* J96 (1.13×10^8 cfu/mL). Sinapinic acid (15 mg/mL) containing 5% TFA and citric acid (50 mM) was used as the MALDI matrix. (b) MALDI mass spectrum obtained after using the POA−Fe_3O_4@Al_2O_3 NPs (40 μg) to selectively trap target species from the same urine sample (1 mL). Sinapinic acid (15 mg/mL) containing 5% TFA was used as the MALDI matrix. Melittin and ubiquitin were used as standards for mass calibration. The peak marked i.s. represents ubiquitin. The peaks marked J96 and S.S. were derived from *E. coli* J96 and *S. saprophyticus*, respectively.

demonstrated that IgG-bound magnetic NPs (IgG@Fe_3O_4)[26] can interact with *S. saprophyticus*. Thus, combining the complementary affinity probes POA−Fe_3O_4@Al_2O_3 and IgG@Fe_3O_4 with MALDI MS analysis would allow the rapid identification of pathogenic bacteria from patients having urinary tract infections. Furthermore, the detection limit of this approach for P fimbriated *E. coli* was $\sim 9.60 \times 10^4$ cfu/mL (Figure 5), which is similar to the populations of these bacteria ($\sim 10^5$ cfu/mL) in the urine of patients experiencing urinary tract infections.[25] That is, the detection limit of this approach may be sufficient to detect P fimbriated *E. coli* in authentic clinical samples. However, further studies are required to validate the feasibility of this approach for the analysis of clinical samples.

As a control experiment, Figure 6 presents the MALDI mass spectrum obtained after using the unmodified Fe_3O_4@Al_2O_3 NPs as affinity probes to trap target bacterial cells from the same sample as that used to obtain Figure 4. The peaks marked S.S. and J96 were derived from *S. saprophyticus* and *E. coli* J96, respectively. Thus, the presence of ions generated from both these strains of bacteria after enrichment with unmodified Fe_3O_4@Al_2O_3 NPs reveals that the unmodified Fe_3O_4@Al_2O_3 NPs exhibit no specificity toward either *S. saprophyticus* or *E. coli* J96. It is no surprise to know that unmodified Fe_3O_4@Al_2O_3 NPs can interact with both bacterial strains. The basic compositions of the cell wall of Gram-positive bacteria such as *S. saprophyticus* include teichoic acid and lipoteichoic acid, which contain negatively charged functionalities.[27] Lipopolysaccharide and phospholipids with negatively charged functionalities are major components of the outer membrane of Gram-negative bacteria such as *E. coli*.[27,28] The isoelectric point of alumina is ~9.1,[29] so the net charge of the surface of the alumina-coated magnetic nanoparticles is positive at pH 7.4. Thus, it is understandable why electrostatic interactions between both *E. coli* and *S. saprophyticus* and the unmodified Fe_3O_4@Al_2O_3 NPs take place when mixing the NPs with these bacteria in a solution at pH 7.4.

To examine the utility of this approach under clinical conditions, Figure 7a presents the direct MALDI mass spectrum of a urine sample containing *E. coli* J96 and *S. saprophyticus*. Only two peaks at m/z 4938 and 4987 derived from *S. saprophyticus* appear in the mass spectrum. Urine contained high amounts of salts, which affected the crystallization of MALDI matrix samples and led to poorer mass resolution, a desalting agent, i.e., citric acid, was added in the sample to slightly improve the mass resolution. Figure 7b displays the MALDI mass spectrum obtained after using the POA−Fe_3O_4@Al_2O_3 NPs as affinity probes to trap target species from the same urine sample. In this mass spectrum, all of the peaks were derived from *E. coli* J96. These results indicate that the POA−Fe_3O_4@Al_2O_3 NPs have the capability to selectively trap P fimbriated *E. coli* from a complex urine sample.

CONCLUSIONS

After determining that POA is a phosphoprotein, its phosphate units were used as linking groups for coordination to Fe_3O_4@Al_2O_3 NPs. The immobilization process can be performed under microwave heating for only 30 s. This microwave-assisted linking method for fabrication of functional magnetic NPs avoids the problems of the NPs adhering onto the walls of the vials and the generation of the bubbles, which generally arise when vortex mixing is used to fabricate functional magnetic NPs. This present approach toward generating functional magnetic NPs is faster than any other reported previously. Furthermore, this is the first report of combining magnetic iron oxide NPs with accelerated microwave-assisted immobilization to generate phosphoprotein-functionalized magnetic NPs. In addition, the resulting POA−Fe_3O_4@Al_2O_3 NPs had the capability to selectively concentrate P fimbriated *E. coli* from sample solutions. This approach has potential ap-

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plicability for determining the presence of P fimbriated *E. coli* in urine samples of patients suffering from urinary tract infections.

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