AFFINITY CAPTURE USING FUNCTIONAL MAGNETIC NANOPARTICLES FOR THE MALDI-MS ANALYSIS OF UROPATHOGENIC ESCHERICHIA COLI

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Introduction

Uropathogenic *Escherichia coli* are the most common pathogenic bacteria that cause urinary tract infections. Urinary tract infections may result in cystitis and pyelonephritis. Most pyelonephritis patients are usually infected by P fimbriated *E. coli*. P fimbriae contains papG adhesion, which can specifically recognizes Galα(1-4)Galβ of glycolipid existing on the epidermal of kidney [1]. It is of great interest to develop a method that can be used to rapidly characterize the bacteria from clinical samples. Because pigeon ovalbumin (POA), whose structure contains terminal Gal(α1–4)Galβ moieties [2], it can be used as a probe for interaction with P fimbriated *E. coli*. Thus, the goal of this work is to develop analytical methods for rapid characterization of the bacteria causing urinary tract infections using nanotechnology combined with matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS).

Methods

The functional affinity probes for P fimbriated *E. coli* 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 were employed to trap target bacteria. 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 approach exhibited specificity toward P fimbriated *E. coli*. The bacteria targeted by the affinity probes were characterized by MALDI MS. Figure 1 shows the steps of using affinity probes to selectively trap target bacteria from sample solutions. That is, POA-Fe_3O_4@Al_2O_3 NPs were vortex-mixed with bacterial samples for 1 h. The NP-bacterium conjugates were then isolated by magnetic separation, followed by rinsing 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). After standing for 3 min, the supernatant (1 μL) was deposited on a MALDI sample plate for MALDI MS analysis.

Results and Discussion

Figure 2a presents the direct MALDI mass spectrum of a urine sample spiked with *S. saprophyticus* and *E. coli* J96. Only the peaks at *m/z* 4938 and 4987 (marked "S.S.") derived from *S. saprophyticus* appear in the mass spectrum. However, after enrichment by the affinity probes, peaks (marked "J96") derived from *E. coli* appear in the mass spectrum (Fig. 2b). The results indicate that the affinity probes can readily trap *E. coli* J96 from the solution with good specificity. Additionally, the peaks derived from *E. coli* J96 still can be observed in the MALDI mass spectra after affinity capture as the cell concentration in aqueous samples is lowered to ca. 10^5 cells/mL.

Conclusions

POA-Fe_3O_4@Al_2O_3 NPs have been demonstrated to have the capability to selectively concentrate P fimbriated *E. coli* from sample solutions. Furthermore, the bacteria trapped by the NPs can be readily characterized using MALDI MS based on their mass spectral fingerprinting. The detection limit of this approach for the uropathogenic bacteria is ca. 10^5

cells/mL (0.5 mL). It is potentially possible to employ this approach for rapidly characterizing P fimbriated *E. coli* from clinical samples.

References

[1] Johnson, *J. Clin. Microbiol. Rev.*, **4** (1991) 80-128.
[2] Suzuki, N.; Khoo, K.-H.; Chen, H.-C.; Johnson, J. R.; Lee, Y. C. *J. Biol. Chem.* **276** (2001) 23221-23229.

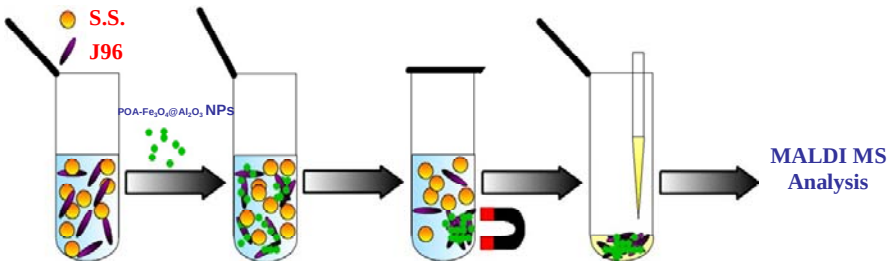

Figure 1. Steps of using POA-Fe_3O_4@Al_2O_3 NPs as affinity probes to selectively trap target bacteria from a sample solution containing *S. saprophyticus* (S. S.) and *E. coli* J96 (J96) followed by characterization of MALDI MS.

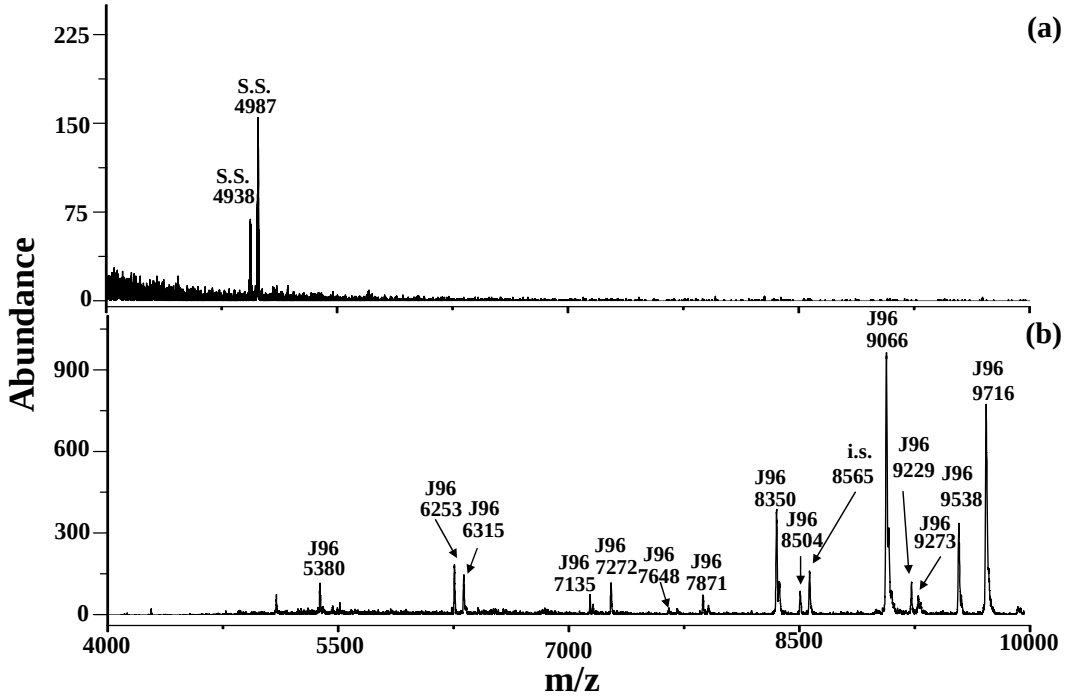

Figure 2. (a) Direct MALDI mass spectrum of the sample containing *S. saprophyticus* (3.23×10^8 cells/mL) and *E. coli* J96 (1.13×10^8 cells/mL) (b) MALDI mass spectrum obtained using POA-Fe_3O_4@Al_2O_3 NPs as affinity probes to trap target species from the sample containing *S. saprophyticus* (3.23×10^8 cells/mL) *E. coli* J96 (1.13×10^8 cells/mL).