NI^{2+}-PAA ADSORBENT FOR PURIFYING 6HIS-OMPTS RECOMBINANT PROTEIN

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ABSTRACT

Functional Ni^{2+}-PAA adsorbent has been prepared for metal chelate affinity chromatography. DNA elements coding for adjacent histidines were fused to the *Aeromonas hydrophila* ompTS gene. Subsequent expression in *E. coli* resulted in the production of hybrid protein 6His-OmpTS that could be purified by Ni^{2+}-PAA affinity chromatography.

One of the most versatile purification methods utilizing affinity tags containing six consecutive histidine residues engineered into recombinant proteins, and Ni^{2+} ions immobilized on commercially available nitrilotriacetic acid-agarose were widely used as affinity matrix[1-4]. However, the preparation procedure of Agarose-NTA-Ni^{2+} is a cumbersome one[5] and agarose is notoriously mildew, the cost of purification is quite expensive. Here we describe the application of a simple, stable and cheaper IMAC adsorbent, Ni^{2+} chelate PAA, which displayed low nonspecific binding of irrelevant proteins and was specific for proteins containing 6 His tags.

METHODS

From an aqueous solution of a mixture of the monomers acrylic acid and *N,N*'- methylenebis(acrylamide) a stiff gel is prepared by a block polymerization procedure. The gel is dehydrated by freeze-drying and pulverized. Uniform granular size is obtained by sieving. Sieved powder was allowed to stand in water for 1 hour to ensure thoroughly swell. The swelled powder was then incubated with 0.2 mol/L $NiCl_2$ for 20 min under vigorous stirring at room temperature. Ni^{2+} charged media was rinsed thoroughly with 0.2 mol/L acetic acid and distilled water. 4 mL of the charged media was packed in a 20mL polypropylene spin column. Pre-equilibrate the media in the column with 7 mL Denaturing Binding Buffer twice. Ni^{2+}-PAA affinity matrix made by this method can be stored at room temperature for at least 3 months *prior* to use.

All DNA manipulations were performed using the plasmid pRSET A with the *E. coli* strain XL blue. A target fragment from *Aeromonas hydrophila* ompTS gene, which codes for *A. hydrophila* outer membrane protein Omp TS, was inserted into the linearized plasmid vector pRSET A.

Inoculate 2 mL of SOB + Ampicillin (50 μg/mL) with a single pRSET A-ompTS transformed *E. coli* XL Blue colony. Inoculated SOB was grown overnight at 37℃ with vigorous shaking to an OD_{600}=0.3. The next day, inoculate 500 mL of LB + Ampicillin (50 μg/mL) with 3 mL of the overnight culture. The culture was grown at 37℃ with vigorous shaking to an OD_{600}=0.3. IPTG was added to a final concentration of 1 mmol/L. In the presence of IPTG, the culture was grown for an additional 4 hours.

The Guanidinium Lysis Buffer was equilibrated to 37℃. The *E.coli* was harvested from 500 mL LB culture by centrifugation (6000 rpm for 10 minutes). The bacteria pellet was resuspended in 25 mL of Guanidium Lysis Buffer, pH 7.8. The bacteria was slowly rocked for 5-10 minutes at room temperature to assure thorough cell lysis, sonicated for 5-10 minutes at room temperature to assure thorough cell lysis. The lysate was sonicated on ice with three 5-second pulse at a high intensity setting to shear the DNA and RNA. Insoluble debris was removed by centrifugation at 3,000×g for 15 minutes. The sheared lysate was transferred to a fresh tube. The lysate was stored on ice or at -20℃ until use.

The protein to be purified was batch bound by resuspending the pre-equilibrated Ni^{2+}-PAA column with 5 mL lysate aliquots. The column was gently rocked for 10 minutes to keep the resin to be resuspended and allow the 6 His-OmpTS to fully bind. The resin was settled by gravity and carefully aspirated the supernatant. Repeat with a second 5mL aliquot (Fraction 1 and 2).

The column was washed twice with 4 mL of Denaturing Binding Buffer by resuspending the resin, rocking for two minutes and then separating the resin from the supernatant by gravity (Fraction 3 and 4). The column was washed with 4 mL of Denaturing Wash Buffer 6.0 by resuspending the resin, rocking for two minutes and then separating the resin from the supernatant by gravity. This wash procedure was repeated once more for a total of two pH 6.0 washes (Fraction 5 and 6). The column was washed with 4mL of Denaturing Wash Buffer 5.3 by

resuspending the resin, rocking for two minutes and then separating the resin from the supernatant by gravity. This wash procedure was repeated once more for a total of two pH 5.3 washes. The column was clamped in a vertical position and snapped off the cap on the lower end (Fraction 7 and 8)

The protein was eluted by applying 4 mL of Denaturing Elution Buffer. This elution procedure was repeated twice more for a total of three elution (Fraction 9,10 and 11). All of the elution and flow through fractions were collected, monitored by taking OD reading through Pharmacia Ultrospec 2000 UV/visible spectrophotometer. Fractions for analysis on SDS/polyacrylamide gel electrophoresis were dialyzed against 10 nmol/L Tris, pH 8.0, 0.1% Triton X-100 overnight at 4℃ to remove the urea. The dialyzed material was concentrated by any standard method.

RESULTS AND DISCUSSION

Since 6 His-Omp TS was expressed with an affinity tag consisting of six consecutive histidine residues, metal ion affinity chromatography was applied as a key step for its purification. The 6 His-Omp TS containing *E. coli* lysate was subjected to affinity chromatography on Ni^{2+}-PAA column. 6 His-Omp TS is completely retarded by the column and remains bound to it during the washing procedure until eluted with pH4.0 Denaturing Elution Buffer (**Table 1** and **Figure 1**) to a purity about 90%. In this case, between 800-1000 μg of homogeneous 6His-OmpTS could be obtained according to one batch mode procedure described in Experimental after dialyzed.

Ni^{2+} have a coordination number of six. In most of the cases, polyacrylic acid occupy four coordination sites of Ni^{2+}, remain two sites free for interactions with biopolymers. This made Ni^{2+}- PAA an ideal chelate resin for the purification of biopolymers. On the one hand polyacrylic acid strongly complex Ni^{2+}, and on the other hand it permit reversible interactions between Ni^{2+} and proteins.

Here, we have proposed a simplified and economical method for purifying hexa-His tagged recombinant protein with Ni^{2+}-PAA. 6 His-OmpTS fusion protein can be purified very efficiently on the novel Ni^{2+} chelate polymer matrix under denaturing washing and elution condition. This indicates that Ni^{2+}-PAA is selective for proteins and peptides which have a structural element neighbouring histidines. The simplifier preparation procedure and higher mildew-resistance of Ni^{2+}-PAA compared to that of the Agarose-NTA-Ni^{2+} adsorbent make the new resin an attractive addition to the range of sorbents for metal chelate chromatography.

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Table 1 Protein concentration of fractions from Ni^{2+}-PAA column

Fractions	No.	OD_{260}	OD_{280}	OD_{320}	Protein Concentration(mg/mL)
Guanidinium Lysis Buffer	1	3.558	3.427	0.921	1.879
Flow through fractions	2	3.481	3.282	0.460	2.079
Denaturing Binding Buffer	3	3.935	3.805	1.853	1.443
Flow through fractions	4	2.080	1.328	0.148	0.360
Denaturing Wash Buffer	5	3.153	2.165	0.209	0.795
6.0 Flow through fractions	6	1.062	0.662	0.107	0.134
Denaturing Wash Buffer	7	0.483	0.302	0.051	0.061
5.3 Flow through fractions	8	0.268	0.173	0.036	0.036
Denaturing Elution Buffer 4.0 elute fractions	9	1.106	0.681	0.059	0.169
	10	2.437	1.567	0.265	0.368
	11	0.474	0.380	0.164	0.100

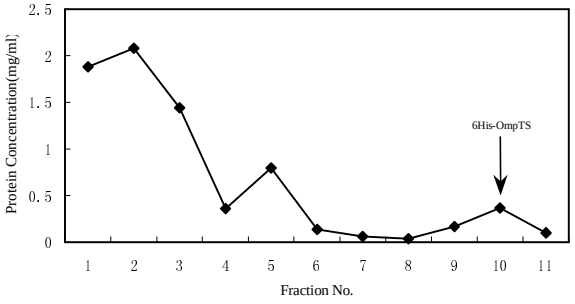

Figure 1 Purification of the recombinant protein
Ni^{2+}-polyacrylic acid chromatography. The Guanidinium lysate fraction was obtained from XL Blue *E.coli.* transformed with pRSET A. The lysate fractions were pooled and mixed with Ni^{2+}-polyacrylic acid. The following column binding, washing and elution were processed under denaturing condition. For more details see Experimental.