Journal of Chromatography B, 907 (2012) 159–162

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short Communication

Comparison of magnetic carboxymethyl chitosan nanoparticles and cation exchange resin for the efficient purification of lysine-tagged small ubiquitin-like modifier protease

Junhua Li[a,b,1], Yang Zhang[a,b,1], Fei Shen[a,b], Yanjun Yang[a,b,*]

[a] State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China
[b] School of Food Science and Technology, Jiangnan University, Wuxi 214122, China

ARTICLE INFO

Article history:
Received 26 June 2012
Accepted 14 August 2012
Available online 30 August 2012

Keywords:
Poly-lysine tag
Ion exchange
Magnetic particles
Small ubiquitin-like modifier protease
Carboxymethyl chitosan

ABSTRACT

A fusion tag that can be purified by the cheap ion-exchanger based on the ionic binding force may provide a cost-effective scheme over other affinity fusion tags. Small ubiquitin-like modifier (SUMO) protease derived from *Saccharomyces cerevisiae* was fused with a poly lysine tag containing 10 lysine residues at its C-terminus and then expressed in *Escherichia coli*. The ionic binding force provided by the ploy lysine tag allowed the selective recovery of the small ubiquitin-like modifier protease from recombinant *E. coli* cell extracts. A preliminary comparative study of the adsorption and elution of poly lysine tagged SUMO protease on Amberlite Cobalamion and magnetite carboxymethyl chitosan nanoparticles was performed. Amberlite Cobalamion and magnetite nanoparticles had the similar elution profile due to the common functional groups – carboxyl groups. The maximum dynamic adsorption capacity of Amberlite Cobalamion and magnetite nanoparticles reached 36.8 and 211.4 mg/g, respectively. The lysine-tagged protease can be simply purified by magnetite nanoparticles from cell extracts with higher purity than that by Amberlite Cobalamion. The superparamagnetic nanoparticles possess the advantages of highly specific, fast and excellent binding of a larger amount of lysine tagged SUMO modifier protease, and it is also easier to separate from the crude biological process liquors compared with the conventional separation techniques of polycationic amino acids fusion proteins.

1. Introduction

Small ubiquitin-like modifier (SUMO) protease, a highly active cysteinyl protease also known as ubiquitin-like protein specific protease (Ulp), is a recombinant catalytic fragment of ubiquitin-like protein specific protease 1 from *Saccharomyces cerevisiae* [1,2]. SUMO protease, which specifically removes SUMO from fusion proteins and allows the generation of a native N-terminus of the target protein, has been successfully applied to the SUMO fusion system that facilitates the efficient expression of recombinant proteins in *Escherichia coli* [3]. Recombinant proteins expressed as SUMO fusions have demonstrated enhanced stability and solubility, leading to a great increase in yield over the constructs lacking this tag [4,5]. However, the commercially SUMO protease purified by affinity chromatography on a nickel-chelating resin is difficult to meet the demands of large scale preparation of recombinant proteins due to the relatively high price.

If a protein is isolated in an industrial scale, the cost of the purification media may be critical. Cation exchange chromatography (CEC) is a well-established unit operation in the downstream processing of lysine-tagged protein [6,7] without the use of the expensive immobilized metal-chelated affinity resin and toxic chemicals (such as heavy metal ions and imidazole) [8]. Moreover, the poly-lysine tag can be used for the immobilization of enzyme on a solid support without deterioration of its enzymatic characteristics [9–11]. The application of functionalized magnetic adsorbent particles with magnetic separation techniques has received considerable attention in recent years. The magnetically responsive nature of such adsorbent particles makes them possible to magnetically separate selected target species directly from crude biological process liquors (e.g. fermentation broths, cell lysates) using a magnet [12,13].

The natural polysaccharide, carboxymethyl chitosan (CM-CTS), can act as a cation exchanger. We recently reported one-step separation of lysozyme from egg white using superparamagnetic carboxymethyl chitosan nanoparticles [14]. In our laboratory, lysine tagged SUMO protease was also successfully purified by

* Corresponding author at: State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China. Tel.: +86 510 85329080; fax: +86 510 85329080.
E-mail address: jiamaolifang1@163.com (Y. Yang).
[1] Joint first authors: Junhua Li & Yang Zhang.

1570-0232/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jchromb.2012.08.029

classical cation exchange chromatography. However, the studies are still scarce about the comparison of the purification performance between classical ion exchange chromatography and functionalized magnetic adsorbent particles. In this paper, an optimized cation exchanger and superparamagnetic carboxymethyl chitosan nanoparticles were chosen as adsorbents for the purification of the poly lysine tagged SUMO protease.

2. Materials and methods

2.1. Materials

All chemicals were reagent grade, purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, P.R. China), unless otherwise noted. Cation exchange resin (AMBERLITE™ COBALAMION) was purchased from Rohm and Haas (Philadelphia, PA, USA). The magnetic carboxymethyl chitosan nanoparticles, which was designated as Fe_3O_4 (PEG + CM-CTS), were prepared in our laboratory according to the description [14].

2.2. Bacterial strain and plasmids

E. coli Rosetta™ (DE3) (Novagan, Madison, WI, USA) was used for producing the recombinant protein (Novagan, Madison, WI, USA). Auto-induction medium ZYM-5052 [15] with 50 μg/mL kanamycin sulfate was used for *E. coli* growth and protein expression. The oligonucleotides were synthesized by Sangon Biotech Co., Ltd (Shanghai, P.R. China). A catalytic fragment of ubiquitin-like protein specific protease 1 encoding residues 403–621 was amplified from *S. cerevisiae* genomic DNA based on the sequence of *S. cerevisiae* chromosome XVI (512,309–514,174, complementary strand) using the following primers (restriction sites underlined): 5′-CATGCCATGGCACTTGTTCCTGAATTAAATGAA-3′ and 5′-CGCGGATCCGATTTTAAAGCGTCGGTTA-3′. The amplified gene was inserted into pET-28a (Novagen, Madison, WI) via the *Nco*I and *BamH*I sites to construct pET-Ulp1. The poly lysine tag coding 10 lysine residues was annealed by the following oligonucleotides: 5′-GATCCGAAAAAGAAAAAGAAAAAAAAGAAAAAGAAATAAC-3′ and 5′-TCGAGTTATTTCTTTTTCTTTTTTTTCTTTTTCTTTTTCG-3′. The annealed product was inserted into pET-Ulp1 via *BamH*I and *Xho*I to construct pET-Ulp1-K10.

2.3. Recombinant protein expression and purification

The protein expression plasmids pET-Ulp1-K10 were transformed into the *E. coli* strain Rosetta™ (DE3). Cells were grown in 50 mL of auto induction medium supplemented with 50 μg/mL kanamycin sulfate in 250 mL flasks with a rotary shaking rate of 200 rpm at 37 °C for 3–4 h. After another 12 h of cultivation at the decreased temperature of 20 °C, cells (typically 0.8–1 g wet weight) were harvested by centrifugation and suspended in 5 mL of 20 mM sodium phosphate buffer (pH 8.0). The cell pellets were disrupted by ultrasonication. After centrifugation, the cell lysate supernatant was respectively incubated with cation exchange resin and magnetic carboxymethyl chitosan nanoparticles at room temperature on an orbital shaker. After 30 min of incubation, cation exchange resin was collected by discarding the supernatant while carboxylated magnetic particles were separated using a magnet, then both adsorbents were washed three times with 20 mM phosphate buffer (pH 8.0) and further eluted with a step gradient using 20 mM phosphate buffer (pH 8.0) containing 200 mM, 400 mM, 600 mM and 1 M NaCl. The eluate was collected in every elution step and analyzed by SDS-PAGE. The image was analyzed by Quality One software (Bio-Rad Laboratories, USA). The protein concentration was measured by the Bradford method [16].

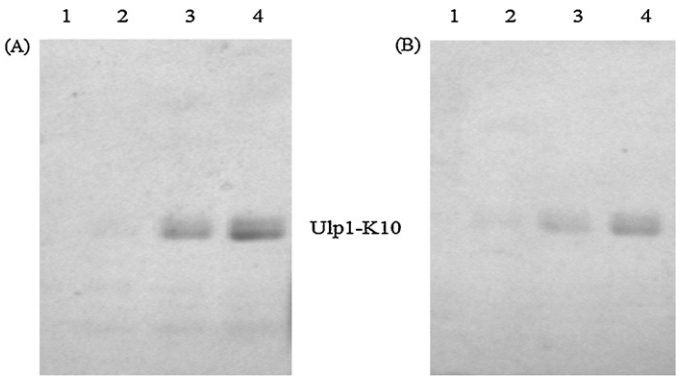

Fig. 1. SDS-PAGE analysis (12%) of Ulp1-K10 expression in *E. coli* BL21 (DE3) and its purification by cation exchange resin (A) and magnetite nanoparticles (B). Lanes 1–4, pooled fractions by stepwise elution at the salt concentration of 200 mM, 400 mM, 600 mM and 1000 mM.

2.4. Ulp1-K10 adsorption studies from aqueous solution

10 mg of magnetic carboxymethyl chitosan nanoparticles or 30 mg of Amberlite Cobalamion (H^+ form) with 1 mL of Ulp1-K10 (0.5–3.5 mg/mL) buffer solution (10 mM phosphate buffer, pH 8.0, 0.2 M NaCl) was respectively added into 50 mL of centrifugal pipe, the mixed suspension was shaken in a thermostated shaker (150 rpm) for 45 min at room temperature during which the adsorption had reached equilibrium. The supernatants obtained from the Ulp1-K10 adsorption studies were used to determine the content of unbound Ulp1-K10. The concentration of Ulp-K10 was measured by the Bradford method. The amount of Ulp1-K10 adsorbed onto the magnetite nanoparticles or Amberlite Cobalamion (H^+ form) was calculated using the following equation:

$$q = \frac{V(C_0 - C_e)}{m} \quad (1)$$

where q is the amount of Ulp1-K10 adsorbed onto the magnetite nanoparticles (mg/g) or Amberlite Cobalamion (H^+ form); C_0 and C_e are the concentrations of the Ulp1-K10 in the initial solution and the supernatant solution after adsorption, respectively (mg/mL); V is the volume of the Ulp1-K10 solution (mL); m is the mass of the magnetite nanoparticles (g) or Amberlite Cobalamion (H^+ form) (g).

3. Results and discussion

3.1. Expression and purification of poly-lysine-tagged SUMO protease

In this study, *E. coli* Rosetta™ (DE3) cells harboring the expression plasmids of pET-Ulp1-K10 were induced and purified under the conditions described in Section 2. This purification procedure resulted in a yield of 3–4 mg of the recombinant enzyme from 50 mL of bacterial culture. Fig. 1 shows that Amberlite Cobalamion and magnetite nanoparticles have almost the same elution profile due to the common carboxyl groups. The lysine-tagged SUMO protease was eluted from the two adsorbents by phosphate buffer (pH 8.0) containing 600 mM NaCl. Magnetite nanoparticles have improved specificity over the ion exchange resin in the stepwise gradient mode, since the purity of lysine-tagged SUMO protease obtained by magnetite nanoparticles (97%, analyzed by Quality One software) was higher than that obtained by Amberlite Cobalamion (93%, analyzed by Quality One software). This result shows that the size of purification matrix may affect the possibility of the contaminants adsorbed on cation exchange adsorbents. The choice of buffer pH and ionic strength is critical for the binding and elution of proteins (both target substances and contaminants) in ion

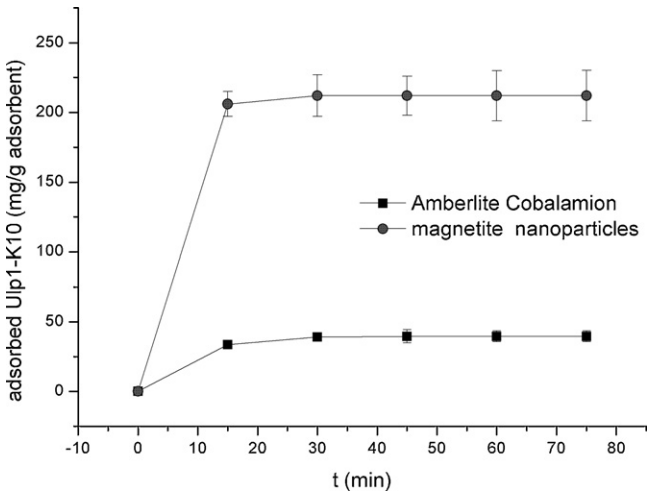

Fig. 2. Kinetic curves of the adsorption of Ulp1-K10 by cation exchange resin and magnetite nanoparticles (initial concentration of Ulp1-K10: 0.25 mg/mL; pH: 8.0; temperature: 25 °C; ionic strength: 0.2 M NaCl).

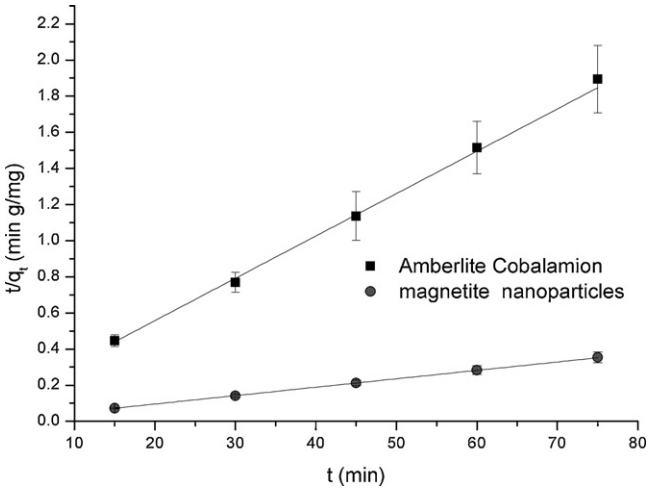

Fig. 3. Pseudo-second-order kinetic plots for the adsorption of Ulp1-K10 on cation exchange resin and magnetite nanoparticles (initial concentration of Ulp1-K10: 0.25 mg/mL; pH: 8.0; temperature: 25 °C; ionic strength: 0.2 M NaCl).

exchange chromatography. In our study, we selected the high pH binding buffer (pH 8.0) to lower the nonspecific adsorption. Therefore, the eluate had less contaminants in each elution step. Using magnetite nanoparticles was also obviously easier to obtain high purity recombinant protein due to its lower nonspecific adsorption compared with Amberlite Cobalamion.

3.2. Adsorption of Ulp1-K10 from aqueous solution

3.2.1. Kinetic properties

To ascertain the time required to reach adsorption equilibrium of the lysine-tagged SUMO enzyme, binding experiments were performed using 10 mg of magnetite nanoparticles or 30 mg Amberlite Cobalamion and 15 mL of Ulp1-K10 solution (10 mM phosphate buffer, pH 8.0, 0.2 M NaCl) with initial concentration of 0.25 mg/mL at room temperature. Fig. 2 shows that more than 95% and 80% of the Ulp1-K10 had been respectively adsorbed onto the magnetic particles and Amberlite Cobalamion in the initial 15-min incubation. The time needed to achieve the adsorption equilibrium was around 30 min for both magnetic particles and Amberlite Cobalamion. This phenomenon may be attributed to the small diameter (excellent dispersibility) and large specific surface area of superparamagnetic carboxymethyl chitosan nanoparticles [14]. According to the literatures [14,17,18], the pseudo-second-order model usually describes the adsorption kinetics well, so in this study, we use it to evaluate the adsorption data of Ulp1-K10 on adsorbents. The pseudo-second-order model is expressed as follows:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \left(\frac{1}{q_e}\right) t \quad (2)$$

where k_2 is the pseudo-second-order rate constant (min^{-1}) of adsorption; q_e is the maximum adsorption capacity for pseudo-second-order (mg/g); q_t is the amounts of Ulp1-K10 adsorbed at any time (mg/g).

Fig. 3 is obtained by plotting t/q_t versus t according to pseudo-second-order model. The adsorption kinetics were expressed as follows: $y = 0.00469x + 0.00165$ (for magnetic particles), $q_e = 213.2$ mg/g adsorbent, $k_2 = 0.0113$, $R^2 = 0.999$; $y = 0.02427x + 0.05994$ (for Amberlite Cobalamion), $q_e = 41.2$ mg/g adsorbent, $k_2 = 0.00983$, $R^2 = 0.998$. The pseudo-second-order equation fit with the experimental data well and could be used to describe the adsorption kinetics of Ulp1-K10 on carboxyl adsorbents by electrostatic interaction.

3.2.2. Adsorption isotherms

The adsorption model was studied using 10 mg of magnetic particles or 30 mg of Amberlite Cobalamion (H^+ from) and 1 mL of Ulp1-K10 solution with different initial concentration of Ulp1-K10. Since the Langmuir model describes the adsorption behavior of basic protein-lysozyme on superparamagnetic nanoparticles well [14], Ulp1-K10 which is also a basic protein (pI 9.18, computed by DNAStar software) may have the similar adsorption behavior on the same adsorbent. So the absorption behaviors of Ulp1-K10 on magnetite nanoparticles and Amberlite Cobalamion were described by the Langmuir model, and the equations were as follows:

$$q_e = \frac{q_m k C_e}{1 + k C_e} \quad (3)$$

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{q_m k} \quad (4)$$

where C_e (mg/mL) and q_e (mg/g adsorbent) are Ulp1-K10 concentration in the aqueous phase at equilibrium and the amount of Ulp1-K10 adsorbed on the adsorbent at equilibrium, respectively; q_m is the maximum adsorption capacity (mg/g adsorbent) and k is the adsorption constant (mL/mg).

The experimental data was analyzed by the nonlinear and linear regression method according to Eqs. (3) and (4). Fig. 4A was obtained from non-linear fits can be expressed as follows: $q_e = 784.1491\, C_e/(1 + 21.5425\, C_e)$, $q_m = 36.4$ mg/g Amberlite Cobalamion, $k = 21.54$, $R^2 = 0.971$; $q_e = 2496.135\, C_e/(1 + 11.1074\, C_e)$, $q_m = 224.73$ mg/g magnetite nanoparticles, $k = 11.11$, $R^2 = 0.938$. Fig. 4B was obtained from linear fits can be expressed as follows: $C_e/q_e = 0.02721\, C_e + 0.00153$, $q_m = 36.8$ mg/g Amberlite Cobalamion, $k = 17.76$, $R^2 = 0.998$; $C_e/q_e = 0.00473\, C_e + 0.000372$, $q_m = 211.4$ mg/g magnetite nanoparticles, $k = 12.72$, $R^2 = 0.987$. The Langmuir model was fit to describe the adsorption behavior of Ulp-K10 on Amberlite Cobalamion due its high R^2 values in both fitting equations. As can be seen in Fig. 4, it is more suitable to adopt linear fit to describe the adsorption behavior of Ulp-K10 on magnetite nanoparticles. The Langmuir constant k value from Amberlite Cobalamion was little more than that from magnetite nanoparticles. This result shows Amberlite Cobalmion has a higher affinity force for Ulp1-K10 than magnetite particles. This result may be due to the free amino groups exist in N,O-carboxymethyl chitosan which decrease the affinity force of magnetite nanoparticles for Ulp1-K10. The maximum equilibrium adsorption capacities of the two adsorbents were very close to that calculated from the previous

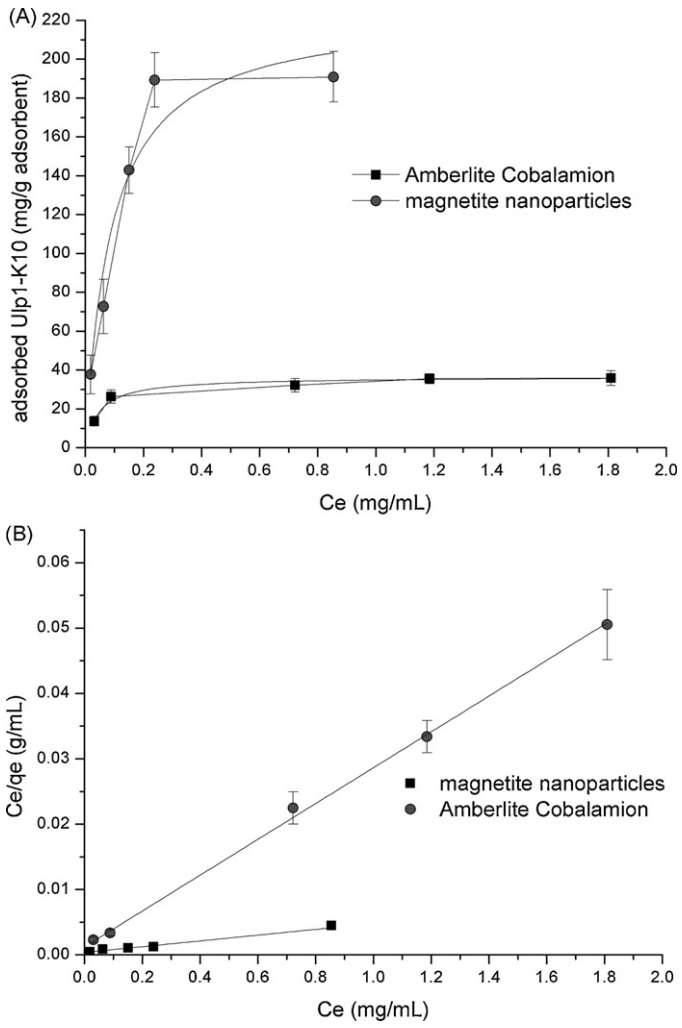

Fig. 4. Adsorption isotherms for Ulp1-K10 on magnetite nanoparticles and cation exchange resin. (A) Non-linear fit and (B) linear fit (pH: 8.0; temperature: 25 °C; ionic strength: 0.2 M NaCl).

pseudo-second-order kinetic models. The maximum adsorption capacity of Ulp1-K10 on magnetite nanoparticles was less than that of lysozyme on magnetite nanoparticles described by Sun et al. [14]. The reason for this result may be the higher pI value of lysozyme (pI 10.6) than that of Ulp1-K10 (pI 9.18). So lysozyme has more positive charge at pH 8, and the electrostatic interactions between lysozyme and magnetite nanoparticles were much stronger which lead to the higher adsorption capacity. Our study has a good agreement with the data obtained by Staby et al. which had the results that the lysozyme (pI 11.35) had the higher static capacity than aprotinin (pI 10.0) on SP Sepharose XL [19].

4. Conclusion

The addition of a 10× lysine tag to the SUMO protease results in an increase in pI of the product expressed in the *E. coli* expression system. This polycationic amino acid tag facilitates the SUMO protease recovery in high yield and purity employing inexpensive cation-exchangers. A preliminary comparative study of the elution profile, adsorption kinetic, static adsorption capacity of poly lysine tagged SUMO protease on Amberlite Cobalamion and magnetite nanoparticles was performed. The magnetite nanoparticles show relatively higher static adsorption capacity than traditional cation-exchanger. The carboxymethyl chitosan magnetite nanoparitcles also have the higher adsorption capacity than the traditional immobilized metal-chelating affinity chromatography (such as Ni Sepharose High Performance, 68 mg maltose binding protein-$(His)_6$/mL medium, Ni-NTA Superflow, 13 mg maltose binding protein-$(His)_6$/mL medium, GE Healthcare, Data file 18-1174-40 AE) which was often used to purify the hexa-histidine tagged recombinant proteins. Moreover, the purification process avoiding expensive equipment makes the routine preparation of lysine-tagged SUMO protease feasible. It is expected that the superparamagnetic carboxymethyl chitosan nanoparticles may be a potential alternative to affinity chromatography.

Acknowledgment

This work was supported by grants from the Chinese National High Technology Research and Development Program 863 (2007AA10Z330).

References

[1] S.J. Li, J. Cell Biol. 160 (2003) 1069.
[2] S.J. Li, M. Hochstrasser, Nature 398 (1999) 246.
[3] M.P. Malakhov, M.R. Mattern, O.A. Malakhova, M. Drinker, S.D. Weeks, T.R. Butt, J. Struct. Funct. Genomics (2004) 75.
[4] T.R. Butt, S.C. Edavettal, J.P. Hall, M.R. Mattern, Protein Expr. Purif. 43 (2005) 1.
[5] J.G. Marblestone, Protein Sci. 15 (2006) 182.
[6] C.L. Wu, Y.P. Chen, J.C. Yang, H.F. Lo, L.L. Lin, J. Mol. Catal. B: Enzym. 54 (2008) 83.
[7] G. Levin, F. Mendive, H.M. Targovnik, O. Cascone, M.V. Miranda, J. Biotechnol. 118 (2005) 363.
[8] E. Hochuli, W. Bannwarth, H. Dobeli, R. Gentz, D. Stuber, Nat. Biotechnol. 6 (1988) 1321.
[9] D.H. Kweon, S.G. Kim, N.S. Han, J.H. Lee, K.M. Chung, J.H. Seo, Enzyme Microb. Technol. 36 (2005) 571.
[10] J. Jeong, T.H. Ha, B.H. Chung, Anal. Chim. Acta 569 (2006) 203.
[11] C.L. Huang, W.C. Cheng, J.C. Yang, M.C. Chi, J.H. Chen, H.P. Lin, L.-L. Lin, J. Ind. Microbiol. Biotechnol. 37 (2010) 717.
[12] M. Franzreb, M. Siemann-Herzberg, T.J. Hobley, O.R.T. Thomas, Appl. Microbiol. Biotechnol. 70 (2006) 505.
[13] S. Berensmeier, Appl. Microbiol. Biotechnol. 73 (2006) 495.
[14] J. Sun, Y. Su, S. Rao, Y. Yang, J. Chromatogr. B 879 (2011) 2194.
[15] F.W. Studier, Protein Expr. Purif. 41 (2005) 207.
[16] M.M. Bradford, Anal. Biochem. 72 (1976) 248.
[17] M. Namdeo, S.K. Bajpai, Colloids Surf., A 320 (2008) 161.
[18] M.Y. Arıca, M. Yılmaz, E. Yalçın, G. Bayramoğlu, J. Chromatogr. B 805 (2004) 315.
[19] A. Staby, M.B. Sand, R.G. Hansen, J.H. Jacobsen, L.A. Andersen, M. Gerstenberg, U.K. Bruus, I.H. Jensen, J. Chromatogr. A 1034 (2004) 85.