An Efficient Method for Recovering Recombinant Cephalosporin C Deacetylase from the Cytoplasm of *E. coli* Cells

X. Ma,[a] E. Su,[b,*] S. Deng,[a] Y. Xie,[a] and D. Wei[a,*]

[a]State Key Laboratory of Bioreactor Engineering, New World Institute of Biotechnology, East China University of Science and Technology, Shanghai 200237, P. R. China

[b]Enzyme and Fermentation Technology Laboratory, College of Light Industry Science and Engineering, Nanjing Forestry University, Nanjing 210037, P. R. China

doi: 10.15255/CABEQ.2013.1904

Original scientific paper
Received: December 20, 2013
Accepted: July 24, 2014

In this study, several chemical treatment techniques were verified for releasing cytoplasmic cephalosporin C deacetylase (CAH) from recombinant *Escherichia coli* (*E. coli*) cells. Among all the chemical treatment methods, using cationic detergent, organic solvents and chelating agents as permeabilizing reagents exhibited limited capacities for extraction of cytoplasmic CAH from cells. Combined use of 5.0 % (v/v) Triton X-100 and 0.3 mol L^{-1} NaCl achieved better effect on CAH extraction, and the release yield of CAH reached approximately 88 %. The extraction parameters such as temperature, ionic strength, Triton X-100 concentration and time were separately optimized to enhance the process of CAH release. The optimal permeabilization conditions (Triton X-100: 2.0 %, v/v; extraction temperature and time: 30 °C and 14 h) resulted in 103 % of relative CAH yield when compared with an optimized sonication process. Release yield of more than 100 % indicated that sonication could inactivate a few CAH molecules in the extraction process. This chemical treatment method could avoid the inactivation of CAH caused by sonication, and thus improve the CAH extraction process.

Key words:

chemical treatment method, Triton X-l00, extraction, cytoplasmic protein, Cephalosporin C deacetylase (CAH)

Introduction

As a host for recombinant protein expression, *Escherichia coli* (*E. coli*) is greatly valued because of its rapid growth rate, easy manipulation, capacity for continuous fermentation, low media costs and capacity to hold over 50 % of foreign protein in total protein expression.[1,2] However, only a few recombinant proteins in *E. coli* can be secreted into culture medium by guiding with signal peptide.[2] The recombinant proteins are usually intracellularly expressed in cytoplasm or periplasmic space. Three kinds of methods are developed for recovering intracellular proteins from recombinant *E. coli* cells, such as disruption by high-pressure homogenization or sonication, digestion with lysozyme and permeation with chemical reagents.

Sonication is a fast and simple method for releasing target protein from cells. However, the local heating produced in treating process can denature the target protein, which is disadvantageous to scale up to a certain size in industry.[3,4] High-pressure homogenization using small glass beads is relatively mild, but in addition to higher energy consumption and longer processing time, increasing number of passes causes a reduction in the size of the cell debris.[5] Enzymatic digestion has been widely used in laboratory for a long time. However, in gram-negative bacteria such as *E. coli*, in addition to the high cost of lysozyme, lysozyme's passage to the peptidoglycan layer can be largely prevented by the lipopolysaccharides in the external membrane layer.[5,6] Chemical extraction methods are achieved by changing the permeability of cell membrane with surfactants or organic solvents, such as ethylenediaminetetraacetate (EDTA),[7] urea, Triton X-100,[8] cetyltrimethylammoniumbromide (CTAB)[9] and butyl acetate.[10] They can be adapted on a large scale. Therefore, particular chemical treatment method with mild and selective effect for protein recovering can be an efficient substitute for mechanical or enzymatic method.

Triton X-l00 is a nonionic detergent used for membrane permeabilization, cell lysis and RNA extraction.[11] Previous studies had shown that Triton X-100 had a specific dissolving effect on the cyto-

*Corresponding authors: Erzheng Su, e-mail: ezhsu@njfu.edu.cn, tel.: +86 25 85428906, fax: +86 25 85428906; Dongzhi Wei, e-mail: dzhwei@ecust.edu.cn, tel. +86 21 64252078, fax: +86 21 64250068.

plasmic membrane of *E. coli* cell.[12] Fox and Kennedy[13] solubilized the M protein from envelope fragments of *E. coli* with Triton X-100. When envelope preparations were extracted with Triton X-100 in the presence of small amounts of Mg^{2+}, proteins of the cytoplasmic membrane were selectively solubilized.[14] Additionally, Triton X-l00 was also employed to extract cell wall-associated proteins from the nitrogen-fixing actinomycete *Frankia*.[15] Very recently, Reza Jalalirad studied the effect of various chemicals at low concentration on selective release of three periplasmic proteins from *E. coli*, and the results indicated that approximately 37 %, 20 % and 40 % of β-lactamase, amylase and Fab D1.3 were released from periplasmic space with 0.05 % – 1.0 % (v/v) of Triton X-100.[8]

Cephalosporin C deacetylase (EC 3.1.1.41, CAH) is an important biocatalyst used in pharmaceutical industry for conversion of 7-aminocephalosporanic acid (7-ACA) to deacetyl-7-aminocephalosporanic acid (D-7-ACA) which is the starting material for many expanded-spectrum semi-synthetic cephalosporins.[16] CAHs have been widely isolated from different fungal and bacterial sources.[17] However, only the ones from *Bacillus subtilis* were well characterized with favorable features for industrial production of D-7-ACA because of their high k_{cat} values to various cephalosporins and negligible product inhibition.[16,17]

In our laboratory, a constitutive plasmid of pB1-SIL3-K was constructed for high-level expression of CAH from *Bacillus subtilis* SIL3. This plasmid contained a replication origin derived from pUC19, a B1 insertion sequence between the SIL3 CAH gene and trp promoter and a kanamycin selected marker, which was similar to a previously reported CAH expression plasmid.[16] By optimizing synthetic medium and glycerol feeding strategy, the activity of CAH reached approximately 1340 U mL^{-1} (unpublished results). In consideration of the further industrial application, the cytoplasmic CAH needs be efficiently extracted from recombinant *E. coli* cells with a complete activity. In this study, a simple method by using Triton X-l00 was developed to extract the cytoplasmic CAH from recombinant *E. coli* cells.

Material and methods

Bacterial strain, culture medium, conditions and chemicals

E. coli DH5α strain (Tiangen, Beijing, China) harboring a constitutive plasmid (pB1-SIL3-K) was used for CAH expression. The fed-batch cultivation was carried out at 37 °C for 24 h in a 7.0 L fermenter (Shanghai BaoXing Co., Ltd, China) containing 4.0 L working medium (20 g L^{-1} dextrin, 20 g L^{-1} fish peptone, 20 g L^{-1} angel yeast powder, M9 medium minimal salt and 50 μg mL^{-1} kanamycin). 50 mL of seed culture (LB medium with 50 μg mL^{-1} kanamycin) was cultivated at 37 °C and 200 rpm until the OD_{600} reached 1.8, and then inoculated in the fermenter. The feeding process began at 5 h and stopped at 15 h using 450 mL concentrated feeding medium (400 g L^{-1} glycerol). Filter-sterilized air was purged into the culture at 2.5 L min^{-1} for aeration, and the dissolved oxygen concentration was maintained above 30 % by cascading agitation rate (300–800 rpm). The pH was kept at 6.8 by adding diluted phosphoric acid or ammonia solution.

7-ACA employed to assay the activity of CAH was supplied by the HuaBei Pharmacy Co., Ltd (China). Triton X-100 was purchased from Sangon Biotech Co., Ltd (China). The fermentation medium compositions were obtained from Chinese local market. All other chemicals were of the analytical grade.

Extraction of CAH by different chemical treatment methods

After fermentation, cells were harvested by centrifugation at 8,000 × *g* and 25 °C for 10 min. The cell pellets (10 g) were resuspended in 100 mL 0.1 mol L^{-1} pH 8.0 phosphate buffer. The CAH was extracted as follows (Unless indicated, 5.0 mL resuspended cell pellets solution were treated):

Sonication: the resuspended cell pellets solution was 5-fold diluted with 0.1 mol L^{-1} pH 8.0 phosphate buffer, then disrupted by the ultrasonic cell disruptor (Ningbo Scientz, JY88-II, China) in ice bath at 400 W for 150 cycles (working 5 s and interval 5 s as one cycle). CAH release was considered to be complete until no further activity increase was observed upon extension of sonication cycles, and the highest CAH release yield obtained by sonication (6510 U/5 mL of resuspended cell pellets solution) was set as 100 %.

Permeabilization with CTAB: 5 g L^{-1} CTAB was added into the resuspended solution in the absence or presence of NaCl (0.3 mol L^{-1}), and shaken at 200 rpm at 4 °C for 24 h (HuaLiDa, Refrigerator shaker HZ-9216K, China). Permeabilization with sodium dodecyl sulfonate (SDS) and NaCl: 5 g L^{-1} SDS was added into the resuspended solution in the presence of NaCl (0.3 mol L^{-1}), and shaken at 200 rpm at 4 °C for 24 h. Permeabilization with butyl acetate: butyl acetate was added to the resuspended solution to a concentration of 5 % (v/v) in the absence or presence of NaCl (0.3 mol L^{-1}), and shaken at 200 rpm at 4 °C for 24 h. Permeabilization with ethyl acetate: ethyl acetate was added to the resuspended solution to a concentration of 5 % (v/v), and shaken at 200 rpm at 4 °C for 24 h. Permeabilization with Triton X-100 and NaCl: Triton X-100 was added to the resuspended solution to a

concentration of 5 % (v/v) in the presence of NaCl (0.3 mol L^{-1}), and shaken at 200 rpm at 4 °C for 24 h. Permeabilization with guanidine hydrochloride (GH) and EDTA: the procedure was performed according to a previously published method.[18] GH and EDTA were added to the resuspended solution to a concentration of 0.1 mol L^{-1} and 0.01 mol L^{-1}, respectively, and shaken at 200 rpm at 4 °C for 24 h. The disrupted *E. coli* cells or solutions obtained after extraction was centrifuged at 12,000 × *g* and 4 °C for 10 min. The supernatant was collected for determination of CAH activity, protein content and SDS-PAGE analysis.

Determination of CAH activity

The CAH activity was evaluated according to a previously published method with minor modification.[16] The assay was performed as follows: 50 mL of 25 g L^{-1} 7-ACA in 0.1 mol L^{-1} pH 8.0 phosphate buffer was kept at 25 °C in the thermostatic bath. Then 0.3 mL concentrated or diluted enzyme solution was added into the above solution. The mixture was stirred and kept at constant pH 8.0 by titrating with 0.1 mol L^{-1} NaOH solution. The activity of CAH was measured according to the following eq. (1):

$$A\ (\text{U mL}^{-1}) = V_{\text{NaOH}}\, c_{\text{NaOH}} \cdot 10^3 / V_{\text{enzyme}}\, t \qquad (1)$$

where the A was the enzyme activity of CAH (U mL^{-1}), V_{NaOH} (mL) was the volume of NaOH solution consumed, c_{NaOH} (mol L^{-1}) was the concentration of NaOH solution, V_{enzyme} (mL) was the volume of enzyme solution, t (min) was the reaction time. 1 Unit of CAH activity was then defined as the amount of enzyme required to produce 1μmol of acetate per min in 0.1 mol L^{-1} pH 8.0 phosphate buffer at 25 °C. The BCA kit (Shanghai Generay Co., Ltd. China) was used for determination of protein content. 0.12 g mL^{-1} polyacrylamide gels were used for SDS-PAGE analysis, and performed according to the method of Laemmli.[19] Total activity of CAH and protein content obtained by sonication was 6510 U and 31.56 mg, respectively.

The release yield and specific activity of CAH were calculated according to the eqs. (2), and (3), respectively.

$$\text{RY} = \alpha/\beta \cdot 100\ (\%) \qquad (2)$$

where RY was the CAH release yield (%), α was the total enzyme activity (U) of 5 mL solution obtained by different chemical treatment methods as describe in Table 1, β was the total enzyme activity (U) of 5 mL solution obtained by sonication.

$$\text{SA} = \alpha/\beta \qquad (3)$$

where SA was the specific activity (U mg^{-1} protein), γ was the total protein content (mg mL^{-1}).

Table 1 – *Extraction of CAH from recombinant E. coli cells with different chemical treatment methods*

Chemical treatment methods	Protein content (mg)	CAH activity (U)	Specific activity (U mg^{-1})	CAH release yield (%)
5 % (v/v) Triton X-100 + 0.3 mol L^{-1} NaCl	29.06	5700	196.1	87.56
5 g L^{-1} CTAB	11.5	2292	199.7	35.21
5 g L^{-1} CTAB + 0.3 mol L^{-1} NaCl	4.84	1111	229.4	17.07
5 g L^{-1} SDS + 0.3 mol L^{-1} NaCl	7.58	666.7	87.98	10.24
0.1 mol L^{-1} GH + 0.01 mol L^{-1} EDTA	3.44	980	284.9	8.76
5 % (v/v) Butyl acetate + 0.3 mol L^{-1} NaCl	ND	ND	ND	ND
5 % (v/v) Butyl acetate	ND	ND	ND	ND
5 % (v/v) Ethyl acetate	2.89	ND	ND	ND

Data were the means of duplicates. Standard error was less than 5 %. N.D, not detected.

Effect of different extraction conditions on cytoplasmic CAH release

Extraction of enzymes from recombinant *E. coli* cells is largely influenced by extraction process conditions, thus the effects of different cationic salts, extraction temperature, ionic strength, Triton X-100 concentration and time were separately investigated. Six cationic salts (NaCl, NH_4Cl, $(NH_4)_2SO_4$, KCl, $CaCl_2$, $MgCl_2$) were added to the resuspended cell pellets at a final concentration of 0.3 mol L^{-1} in the presence of 5.0 % (v/v) Triton X-100, and shaken at 200 rpm at 4 °C for 24 h. 5.0 % (v/v) Triton X-100 was added to the resuspended cell pellets in the presence of 0.3 mol L^{-1} NaCl, then stirred at 15 °C, 20 °C, 25 °C, 30 °C and 35 °C, respectively, and 200 rpm for 24 h. NaCl was added to the resuspended cell pellets at a final concentration of 0–0.5 mol L^{-1} in the presence of 5.0 % (v/v) Triton X-100, and shaken at 200 rpm at 4 °C for 24 h. Triton X-100 was added to the resuspended cell pellets at a final concentration of 0.5–6.0 % (v/v), and shaken at 200 rpm at 4 °C for 24 h. Additionally, 2 % (v/v) Triton X-100 in resuspended cell pellets was stirred at 200 rpm and 25 °C for 2.0–24 h.

Results and discussion

Effect of different chemical treatment methods on cytoplasmic CAH release

Although many chemicals, such as detergents (nonionic, anionic and cationic), chelating agents and organic solvents, have been widely used to selectively extract the periplasmic proteins from recombinant *E. coli* cells, there have been no reports on extracting the cytoplasmic CAH from recombinant *E. coli* cells

by using chemical treatment method. In order to investigate the feasibility of chemical extraction of cytoplasmic CAH from recombinant *E. coli* cells, the effects of various chemical extraction methods on CAH release were firstly investigated. Previous studies on extracting periplasmic penicillin G acylase (PGA) from recombinant *E. coli* cells indicated that a certain amount of cation salt is useful for CTAB (cationic detergent) to act on the lipopolysaccharide layer of the bacterial outer membrane, thereby forming a channel in the membrane, and increasing cell permeability.[20,21] Therefore, CTAB (cationic detergent), SDS (anionic detergent), and Triton X-100 (nonionic detergent) were tested for extracting CAH from recombinant *E. coli* cells in the presence or absence of 0.3 mol L^{-1} NaCl in resuspended cell pellets solution. It could be observed in Table 1 that various chemical treatment methods resulted in CAH release from the cytoplasm of *E. coli* to different extents. Combined use of 5 % (v/v) Triton X-100 and 0.3 mol L^{-1} NaCl was the most efficient chemical treatment method for CAH extraction. The release yield of CAH reached 87.56 %. However, the specific activity (196.1 U mg^{-1}) of CAH was not improved in comparison with that (206.3 U mg^{-1}) obtained by sonication. Besides, cationic detergent (CTAB) and anionic detergent exhibited (SDS) a limited capacity for extraction of cytoplasmic CAH from cells, the release yield of CAH only reached 35.21 %, 17.07 % and 10.24 % by using CTAB, CTAB + NaCl and SDS + NaCl, respectively (Table 1). As a less membrane protein or cytoplasmic protein were released from cells, a higher specific activity of CAH (229.4 U mg^{-1}) was achieved by combined use of CTAB + NaCl.

It was reported that GH + EDTA treatment could selectively recover protein from the periplasmic space.[18,21] Cell wall was damaged by interference of guanidine chaotropic effect with hydrophilic and hydrophobic interactions,[18] and EDTA was believed to remove divalent cations resulting in 30 %–50 % shedding of the lipopolysaccharide component of the outer membrane of *E. coli*, which increased the permeability across the outer membrane.[22,23] Recently, 95 % of beta-lactamase, 100 % of alpha-amylase, and 91 % of Fab D1.3 were released by an osmotic shock method using 10 mmol L^{-1} EDTA as permeabilizing reagent when compared to high-pressure homogenization. In this work, combined using of GH + EDTA achieved the highest specific activity (284.9 U mg^{-1}) among all chemical extraction methods, but there was only 8.76 % of CAH extracted from recombinant cells (Table 1). Perhaps, GH or EDTA cannot extract the CAH that is mainly present in the cytoplasm of recombinant *E. coli* cells. Organic solvents such as butyl acetate and ethyl acetate can dissolve lipid substances in the cell envelope, which will increase the permeability of cell membrane.[10]

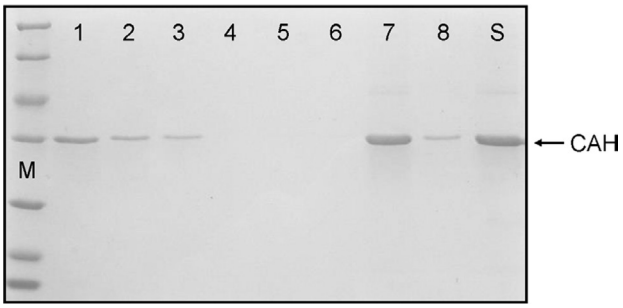

Fig. 1 – *SDS-PAGE analysis of CAH release by various chemical treatment methods. Lane 1–8, 30 μL supernatant of extracting solution treated with 5 g L^{-1}CTAB, 5 g L^{-1} CTAB + 0.3 mol L^{-1} NaCl, 5 g L^{-1} SDS + 0.3 mol L^{-1} NaCl, 5 % (v/v) Butyl acetate + 0.3 mol L^{-1} NaCl, 5 % (v/v) Butyl acetate, 5 % (v/v) Ethyl acetate, 5 % Triton X-100 + 0.3 mol L^{-1} NaCl and 0.1 mol L^{-1} GH + 0.01 mol L^{-1} EDTA, respectively. Lane S, 30 μL supernatant of extracting solution obtained by sonication. M: molecular mass markers, from top to bottom, 116.0, 66.2, 44.0, 35.0, 25.0, 18.4 and 14.4 kDa, respectively.*

However, organic solvents proved to be very inefficient in extracting CAH from the recombinant *E. coli* cells in the present study, as no CAH activity was detected in the permeabilizing solution (Table 1). SDS-PAGE analysis of CAH release by various chemical treatment methods showed the amount of CAH extracted by Triton X-100 + NaCl (Fig. 1, lane 7) was much more than those obtained by other methods (Fig. 1, lane 1–8), which was accorded with the results of CAH activity assay (Table 1). Therefore, the process of extracting CAH by Triton X-100 treatment method was investigated in detail.

Effect of different cationic salts on CAH extraction

High concentration of cation can disrupt the outer membrane, and release significant amount of enzyme in the periplasmic space or cytoplasm.[23] Six cationic salts were employed to investigate their effects on CAH release, Fig. 2 showed the release

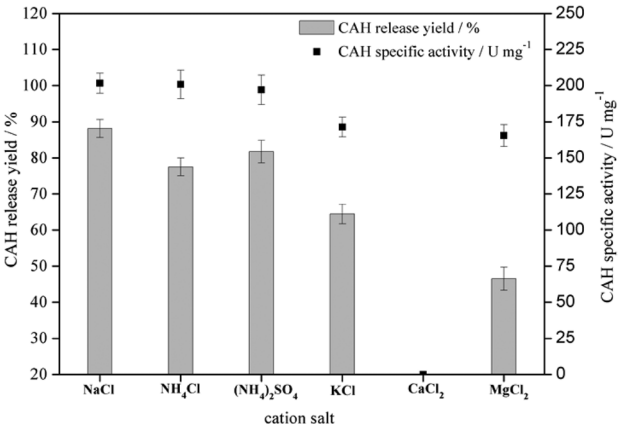

Fig. 2 – *Effect of various cation salts on CAH release yield. 5.0 % (v/v) of Triton X-100 was added into 100 g L^{-1} resuspended cell pellets solution in the presence of 0.3 mol L^{-1} various inorganic salts (NaCl, NH_4Cl, $(NH_4)_2SO_4$, KCl, $CaCl_2$, $MgCl_2$), and extracted at 25 °C, 200 rpm for 24 h.*

yields of CAH achieved by using NH_4Cl, $(NH_4)_2SO_4$, KCl, $MgCl_2$ were obvious lower than that obtained by NaCl. The highest CAH release yield reached 88.13 % by using 0.3 mol L^{-1} NaCl as cationic salt. When 0.3 mol L^{-1} $CaCl_2$ was added to the extracting solution, there was no enzyme activity detected because of insoluble precipitate formed.

Effect of different temperatures on CAH extraction

The degree of membrane lipid insolubility depends on the stability of lipid-lipid interactions relative to lipid-detergent interactions, which is affected by lipid composition, detergent and temperature.[24,25] In the process of CAH extraction, the Triton X-100 will contact with the lipid in outer or inner membrane directly. Therefore, the extraction temperature will influence the release of CAH from cells. The results showed that the release yield of CAH increased significantly when the extraction temperature was over 15 °C (Fig. 3). It was found that high temperature (over 30 °C) was beneficial for the release of CAH. The plausible explanation is that the phospholipids located in membrane of recombinant *E. coli* cells are more soluble in Triton X-100 at high temperature, which leads to the increase of membrane permeability. The highest release yield of CAH attained to 105.9 % at 30 °C (Fig. 3). Yield of more than 100 % indicated that drastic cell disruption conditions (sonication) would inevitably inactivate a few CAH molecules in the extraction process. Therefore, 30 °C was chosen as the optimal extraction temperature.

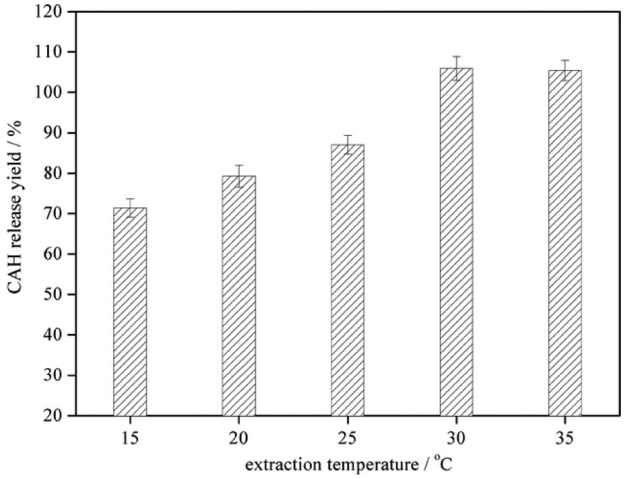

Fig. 3 – *Effect of extraction temperature on CAH release yield. 5.0 % (v/v) of Triton X-100 was added into 100 g L^{-1} resuspended cell pellets solution in the presence of 0.3 mol L^{-1} NaCl, and extracted at 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 200 rpm for 24 h.*

Effect of NaCl and Triton X-100 concentrations on CAH extraction

The effect of ionic strength (NaCl concentration) on the CAH release was also investigated. Fig. 4 showed that the release yield of CAH was not significantly affected by the change of ionic strength in permeabilizing solution, which implied that the permeability of cells treated with Triton X-100 could not be reinforced by cation salt. NaCl was removed from the permeabilizing solution in following experiments. Moreover, the effects of different Triton X-100 concentrations on CAH release yield were investigated. The results were shown in Fig. 5. When the concentration of Triton X-100 reached 2.0 % (v/v), the CAH release yield did not change significantly. The highest CAH release yield

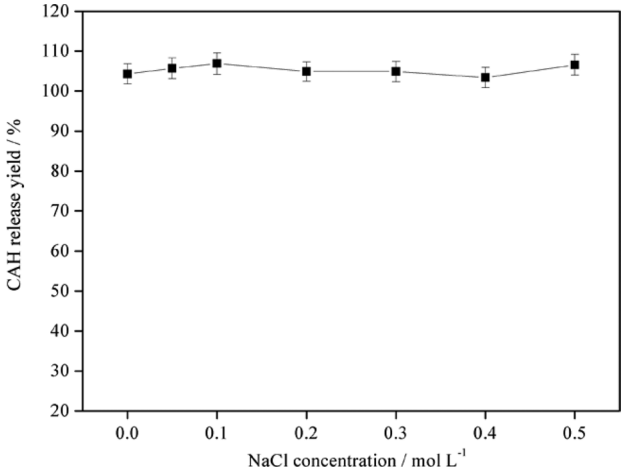

Fig. 4 – *Effect of NaCl concentration on CAH release yield. 0–0.5 mol L^{-1} of NaCl was added into 100 g L^{-1} resuspended cell pellets solution in the presence of 5.0 % (v/v) Triton X-100, and extracted at 30 °C, 200 rpm for 24 h.*

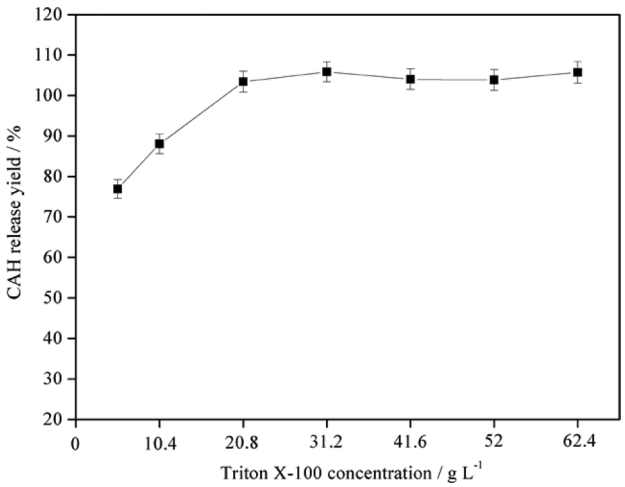

Fig. 5 – *Effect of Triton X-100 concentration on CAH release yield. 5.2–62.4 g L^{-1} (i.e. 0.5–6.0 %, v/v) of Triton X-100 was added into 100 g L^{-1} resuspended cell pellets solution, and extracted at 30 °C, 200 rpm for 24 h.*

attained 105.8 % by using 3.0 % (v/v) of Triton X-100. Considering the utilization efficiency of extracting reagent, 2.0 % (v/v) of Triton X-100 was selected for extraction.

Time course of CAH extraction process

The time course of the cytoplasmic CAH extraction process was performed at 30 °C with 2.0 % (v/v) of Triton X-100 in permeabilizing solution. The CAH release yield increased rapidly before 12 h, and then increased gently until 14 h, after that, the release yield of CAH remained almost unchanged. The highest CAH release yield attained about 103 % (Fig. 6). Several extraction conditions such as biomass amount, expression level of target protein, extracting reagents and operational parameters resulted in different extraction time. In the previous studies, the optimal extraction time of PGA achieved by different treatment methods was usually over 15 h. [9,10,18] Recently, approximately 37 %, 20 % and 40 % of β-lactamase, amylase and Fab D1.3 could be released from periplasmic space with 0.05 % –1.0 % (v/v) of Triton X-100 in 1 h, but only 1 mL culture medium was used for recombinant protein extraction.[8] However, due to a high extraction biomass amount (5 mL concentrated culture medium) was used in this work, consequently led to the increase of total CAH extraction time.

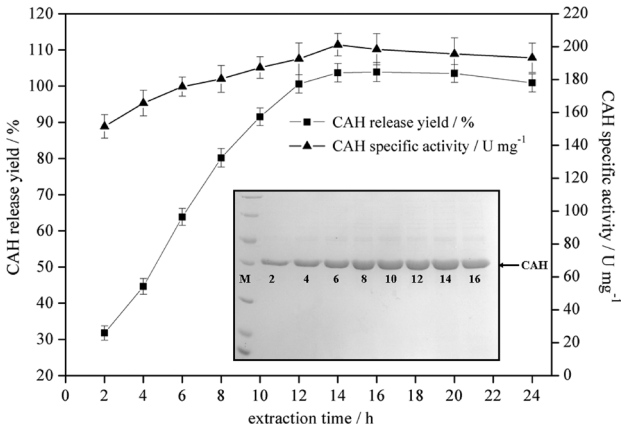

Fig. 6 – *Time course and SDS-PAGE profile of CAH extraction process. 2 % (v/v) of Triton X-100 was added into 100 g L^{-1} resuspended cell pellets solution, and extracted at 30 °C, 200 rpm for 2.0–24 h. Lane 2–16, 40 μL supernatants extracted after 2 h–16 h with 2 % (v/v) of Triton X-100. M: molecular mass markers, from top to bottom, 116.0, 66.2, 44.0, 35.0, 25.0, 18.4 and 14.4 kDa, respectively.*

The specific activity of CAH also increased along with the release yield until 14 h, then remained almost the same (Fig. 6). The highest specific activity of CAH reached to 201.2 U mg^{-1}, which was near to that obtained by sonication method. Besides, the process of CAH extraction was verified by SDS-PAGE analysis, and the band size of CAH was consistent with the CAH release yield during the extraction course (Fig. 6). Triton X-100 was not a selective chemical treatment method for releasing the CAH from cells as other reported methods, but the specific activity of CAH obtained from Triton X-100 treatment (201.2 U mg^{-1}) in this work completely meet the further industrial process of immobilization (Fig. 1, lane S).

Conclusions

In this work, a simple method by using Triton X-l00 to extract the cytoplasmic cephalosporin C deacetylase from recombinant *E. coli* cells was successfully developed. The results indicated Triton X-100 concentration and extraction temperature had significant influences on CAH release. When compared to an optimized sonication process, approximately 103 % of CAH could be extracted from cells by treating with 2.0 % (v/v) of Triton X-100 at 30 °C for 14 h. This chemical treatment method, which avoided the enzyme denaturation by sonication, was a feasible method for the efficient extraction of other cytoplasmic enzymes from the recombinant *E. coli* cells.

ACKNOWLEDGEMENTS

This work was supported by the National Basic Research Program of China (NO.2012CB721103), National High Technology Research and Development Program of China (NO.2012AA022206B), the Fundamental Research Funds for the Central Universities and the Open Funding Project of the State Key Laboratory of Bioreactor Engineering.

Abbreviations

CAH – cephalosporin C deacetylase
E. coli – *Escherichia coli*
EDTA – ethylenediaminetetraacetate
CTAB – cetyltrimethylammoniumbromide
7-ACA – 7-aminocephalosporanic acid
D-7-ACA – deacetyl-7-aminocephalosporanic acid
LB medium – lysogeny broth medium
OD_{600} – absorbance of supernatant
SDS – sodium dodecyl Sulfonate
GH – guanidine hydrochloride
PGA – penicillin G acylase

References

1. *Yin, J. C., Li, G. X., Ren, X. F., Herrler, G.*, J. Biotechnol. **127** (2007) 335. http://dx.doi.org/10.1016/j.jbiotec.2006.07.012
2. *Yoon, S. H., Kim, S. K., Kim, J. F.*, Recent. Pat. Biotechnol. **4** (2010) 23. http://dx.doi.org/10.2174/187220810790069550
3. *Jambrak, A. R., Mason, T. J., Lelas, V., Herceg, Z., Herceg, I. L.*, J. Food. Eng. **86** (2008) 281. http://dx.doi.org/10.1016/j.jfoodeng.2007.10.00
4. *Stathopulos, P. B., Scholz, G. A., Hwang, Y. M., Rumfeldt, J. A., Lepock, J. R., Meiering, E. M.*, Protein Sci. **13** (2004) 3017. http://dx.doi.org/10.1110/ps.04831804
5. *Kamioka, T., Sohya, S., Wu, N., Maki, T., Matsuda, T., Ikegami, T., Nakamura, H., Kuroda, Y.*, Anal. Biochem. **439** (2013) 212. http://dx.doi.org/10.1016/j.ab.2013.04.007
6. *Canfield, R. E.*, J. Biol. Chem. **238** (1963) 2698.
7. *Falconer, R. J., O'Neill, B. K., Middelberg, A. P. J.*, Biotechnol. Bioeng. **53** (1997) 453. http://dx.doi.org/10.1002/(SICI)1097–0290(19970305)53:5<453::AID-BIT2>3.0.CO;2-G
8. *Jalalirad, R.*, J. Ind. Microbiol. Biotechnol. **40** (2013) 1117. http://dx.doi.org/10.1007/s00216–013–7170–0
9. *Cheng, S. W., Wei, D. Z., Song, Q. X.*, Biochem. Eng. J. **32** (2006) 56. http://dx.doi.org/10.1016/j.bej.2006.08.011
10. *Wang, P. X., Gong, X. Y., Su, E. Z., Xie, J. L., Wei, D. Z.*, Biochem. Eng. J. **56** (2011) 17. http://dx.doi.org/10.1016/j.bej.2011.04.011
11. *Ban, E., Chae, D. K., Song, E. J.*, Anal. Bioanal. Chem. **405** (2013) 7535. http://dx.doi.org/10.1007/s00216–013–7170–0
12. *Schnaitman, C. A.*, J. Bacteriol. **108** (1971) 545.
13. *Fox, C. F., Kennedy, E. P.*, Proc. Nat. Acad. Sci. USA. **54** (1965) 891. http://dx.doi.org/10.1073/pnas.54.3.891
14. *DePamphilis, M. L., Adler, J.*, J. Bacteriol. **105** (1971) 396.
15. *Tavares, F., Sellstedt, A.*, J. Microbiol. Methods. **39** (2000) 71. http://dx.doi.org/10.1016/S0167–7012(99)00115–3
16. *Takimoto, A., Takakura, T., Tani, H., Yagi, S., Mitsushima, K.*, Appl. Microbiol. Biotechnol. **65** (2004) 263. http://dx.doi.org/10.1007/s00253–004–1595–5
17. *Barber., M. S., Giesecke. U., Reichert, A., Minas, W.*, Adv. Biochem. Engin/Biotechnol. **88** (2004)179. http://dx.doi.org/10.1007/b99261
18. *Kheirolomoom, A., Ardjmand, M., Fazelinia, H., Zakeri, A.*, Biochem. Eng. J. **8** (2001) 223. http://dx.doi.org/10.1016/S1369–703X(01)00111–5
19. *Laemmli, U. K.*, Nature **227** (1970) 680. http://dx.doi.org/10.1038/227680a0
20. *Felix, H.*, Anal. Biochem. **120** (1982) 211. http://dx.doi.org/10.1016/0003–2697(82)90340–2
21. *Falconer R. J., O'Neill, B. K., Middelberg, A. P. J.*, Biotechnol. Bioeng. **57** (1998) 381. http://dx.doi.org/10.1002/(SICI)1097–0290(19980220)57:4<381::AID-BIT1>3.0.CO;2-I
22. *Nikaido, H., Vaara, M.*, Microbiol. Rev. **49** (1985) 1.
23. *Vaara, M.*, J. Bacteriol. **148** (1981) 426.
24. *London, E., Brown, D. A.*, Biochim. Biophys. Acta. **1508** (2000) 182. http://dx.doi.org/10.1016/S0304–4157(00)00007–1
25. *Ribeiro, A. A., Dennis, E. A.*, Biochim. Biophys. Acta. **332** (1974) 26. http://dx.doi.org/10.1016/0005–2736(74)90118–7