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The Denaturing and Renaturing are Critical Steps in the Purification of Recombinant Protein in Prokaryotic System

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1. Introduction

Proteins were first described in 1838 by the Dutch chemist Gerardus Johannes Mulder and named by the Swedish chemist Jöns Jacob Berzelius. [1] The term protein comes from the Greek word proteios, meaning "primary". [2] Protein carries primary functions of the organisms, including both structural and physiological functions. One of the appropriate samples of its importance is the enzyme, which catalyzes almost all biochemical process in vivo. 3 Actually, lots of the most vital biological functions of an organism are fulfilled by proteins, so the isolation and expression of proteins become an important issue for both research and application.

Most proteins consist of 20 different L-α-amino acids, which are linked by peptide bonds, and finally form linear chains. There are 4 levels of structures of a protein. The primary structure of protein lies in the sequence of amino acids, the secondary structure of protein means the basic elements like regularly repeating local structures stabilized by hydrogen bonds, the tertiary structure of protein is its 3-demintional structure, and the quaternary structure exists in some proteins, in which a few single polypeptide chains aggregate together and function as a whole complex. [4]

The sequence of amino acids in a protein is defined by the sequence of its genetic code. 5 As a commonly accepted opinion, the primary structure of a protein determines its higher level structure; however, the biological function of a protein is not decided by the amino acids composition, but most by its 3-dimentional structure. That is also why, not like DNA or RNA, to know the exact sequence of amino acids of a protein cannot ensure the successful production of the biological active one. During expression, isolation or purification processes of a protein, the natural structure of the protein must be preserved or recovered for it to exhibit normal biological activities. In this meaning, to develop a general technology or a method that can be used to purify every protein becomes an impossible task.

The production of recombinant proteins in prokaryotic system is a powerful tool that has been developed in the research and production of functional proteins for many years. To

achieve successful production of a recombinant protein, the genetic code which decides the final amino acids sequence in the target protein is firstly sequenced. A vector containing the coding sequence is then cloned or synthesized, and transduced into bacteria, such as Escherichia *coli* (E. *coli*). The protein synthesis system in bacteria then recognizes the specific sequence, and proteins will be produced by the bacteria. Since the post-translational system are different in bacteria than the protein's original organism, the over-expressed recombinant protein are normally not functional, and will aggregate to form inclusion bodies. The inclusion bodies are easily to be isolated, and functional proteins will then be recovered from these inclusion bodies by different denaturing and renaturing techniques. Fortunately, there are some basic principles that can be applied to the expression and purification of protein, and in this chapter, some of them will be discussed. [6]

In general, to achieve successful expression and purification of recombinant protein, the usual steps will be (1) obtain target gene; (2) prepare the expressing vector; (3) clone the target gene into the expressing vector with accuracy ensured by sequencing; (4) transduce host bacteria; (5) induce the expression of target protein; (6) analyze the expressed proteins; and (7) cultivate the engineered strain in large scale and (8) purify the expressed protein.

2. First step: The preparation of engineered bacteria

2.1 The purpose of target protein determines its form

In order to purify a protein, one must first ask themselves a question: what is the purpose of the target protein? If some wants to investigate the biological functions of a protein, it's appropriate to express and purify the protein in its full length or matured form, with natural bioactivity. Or, if the target protein is for antibody production, segment of the protein will serve the purpose well. Since proteins are synthesized as linear polypeptides and undergo post-translational modification and maturation, the denaturing and renaturing must also be considered in the expression and purification of recombinant proteins.

In order to investigate the biological functions of a protein in vitro or in vivo, full length, matured proteins need to be expressed and purified. One good example that can be easily produced and purified in E. *coli* is recombinant human interleukin-1 receptor antagonist (rhIL-1Ra) [7]. rhIL-1Ra can be produced by E. *coli* at large scale in soluble form. The recombinant protein in supernatant of the lyzed bacteria can be purified by 2-steps of ion-exchange chromatography to yield high-output biological functional proteins. However, some other proteins, such as recombinant human Midkine (rhMK), [8] recombinant mouse monokine induced by IFN-γ (rmMig) [9], recombinant human Reg 4 (rhReg-4) [10] or recombinant human Chemerin (rhChemerin), [11] easily form inclusion bodies in E. *coli*, hence, necessary denaturing and renaturing steps must be applied. In some other cases, like recombinant murine CXCL14 (rmCXCL14), [12] full length protein is hard to be purified, and an additional tag will help to get final products with high yield and high purity.

2.2 The preparation of engineered bacteria

2.2.1 Information collection

Once the target protein is decided, the initial step of the expression and purification of a protein is to capture target gene of a protein. This can be simply achieved by acquiring

corresponding information of the genetic code of the gene that determines the amino acids sequence of the target protein from some database. The fragment of target genes can be easily synthesized or cloned, and cloned into different commercial available expressing vectors. The packed vector, containing the target gene, the selective markers and the elements needed for expressing in bacteria is then cloned into expressing strains, such as E. *coli* BL21 (DE3). Once the engineered strain is successfully constructed, it will then use its own resources to produce the alien protein.

2.2.2 The vectors and strains are the keys ensuring successful expression and purification

E. *coli* was discovered by Theodor Escherich in 1885. [13] It is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of endotherms. [14] E. *coli* is one of the most adopted bacteria in the expression and purification of recombinant protein, since it can be easily cultivated in laboratory in a relative cheap way, and has been intensively investigated as a widely studied prokaryotic model organism.

The pET system is developed by Novagen Company as a powerful system for the cloning and expression of recombinant proteins in E. *coli*. In this system, target genes are cloned in pET plasmids under control of T7 transcription. The expression is induced by T7 RNA polymerase in the host cell. When fully induced, target protein may consist more than 50% of the total cell protein in a few hours after induction. On the other hand, in the un-induced state, the expression level of target gene is low. pET system and its host bacteria E. *coli* BL21 (DE3) can be used as an optimized system in the expression and purification of recombinant protein. In order to choose a suitable pET system, a good source of knowledge comes from the manual of pET system offered by Novagen. [15]

3. The cultivation of engineered bacteria and the extraction of recombinant proteins

3.1 The cultivation conditions of E. *coli* should be tested and verified

Although the expression of recombinant protein in E. *coli* is normally high and consists large part of total bacteria protein, the expression could also be low, and the cultivation conditions of bacteria must be tested, including different temperature, vortex speed and induction conditions.

Soluble, highly expressed target proteins are priorities in some cases, because the yield of target protein is high, and the biological activity is normally preserved; however, the down-stream purification may be difficult, because the target protein is mixed with all soluble bacteria protein. Another side effect is that target protein may be digested by protease. On the other hand, if the target protein exists in inclusion bodies, it is non-biological active, but can be easily isolated with high purity, and the recombinant protein will not harmed by the presence of protease. After the optimized denaturing and renaturing processes, the downstream process may be much easier than soluble expressed target protein. In each case, the expressing condition must serve the downstream purification steps.

Normally, the conditions that lead to high growth speed and high expression result in aggregation of target proteins, for there are short time for the protein to fold correctly and so

the inclusion bodies will be formed. Strong induction condition easily leads to high-expressing of target protein, and also leads to the formation of inclusion bodies. [16] On the contrary, lowering the cultivation temperature, adding some additives in cultivation medium and change medium can reduce the formation of inclusion bodies, and the target protein will present in soluble form. [17]

3.2 The extraction of crude protein from cell lysates

Different methods can be applied to isolate the target proteins from the cell lysates maximally. Normally the first step after the induction of target protein is to lyze the bacteria. This could be achieved by different methods including frozen-thaw, ultrasonication, lysozyme treatment, or the combination of them. Depending on the forms of expressed protein, the target protein can be found in supernatant or precipitate of the cell lysate. This could be identified easily by Western Blotting.

In this step, the target protein should be verified by Western Blotting using certain antibodies, and the proportion of the target protein in total cell lysates should be calculated. If the expression level of the recombinant protein is too low, the downstream process will become very difficult. Optimization needs to be considered to enhance the production of the target protein.

The goal of the extraction of recombinant protein is to facilitate the downstream process. The more concentrated proteins in this step, the easier the later purification will be.

4. The denaturing and renaturing of recombinant protein

4.1 The purification of soluble recombinant protein

If the protein exists in soluble form, it will probably possess natural bioactivity. The protein then needs no denaturing and renaturung treatments. For instance, when packed into pET11a expressing vector, rhIL-1ra is induced in a soluble form in E. *coli*. A good example of how to express and purify soluble rhIL-1Ra is indicated below. [18]

The interleukin-1 receptor antagonist (IL-1Ra) is a protein encoded by the IL1RN gene. IL-1Ra was initially called the IL-1 inhibitor and was discovered in 1984. [24] IL-1Ra binds to the cell surface interleukin-1 receptor (IL-1R) hence blocks IL-1 signal pathway.

The full length of secreted rhIL-1Ra gene (gi: 186385) is 534bp with a CDS of 534bp, whereas the first 75 bps encode a 25-amino acid signal peptide. A DNA fragment corresponding to bp 76 to 534 in the gene IL1RN which encodes 152 amino acid residues in the matured rhIL-1Ra is cloned and packed into pET11a for the protein expression in E. *coli*. After induction, rhIL-1Ra mainly exists in the supernatant of the cell lysates.

The detailed processes of the expression and purification of rhIL-1a are listed below:

- Inoculate a single colony of transformed bacteria into LB medium containing 100 μg/mL ampicillin at 37 °C overnight with vigorous shaking. Inoculate large-scale of LB medium containing 100 μg/mL ampicillin with small-scale culture mentioned above at 37°C for 2 h with vigorous shaking. 1 mM IPTG was used to induce the strain to

produce rhIL-1Ra afterwards. The incubation continued for additional 4 h at 37 °C and the cell pellets were spun down.

- The supernatant after ultrasonication was collected. 0.284 g ammonium sulfate was used for each milliliter of supernatant to precipitate rhIL-1Ra. Precipitated protein was solubilized 10-fold into the loading buffer 1 (1 mM EDTA, 20 mM Tris-HCl, pH 9.0). After centrifugation the supernatant was collected and named as rhIL-1RaST (Fig. 1)

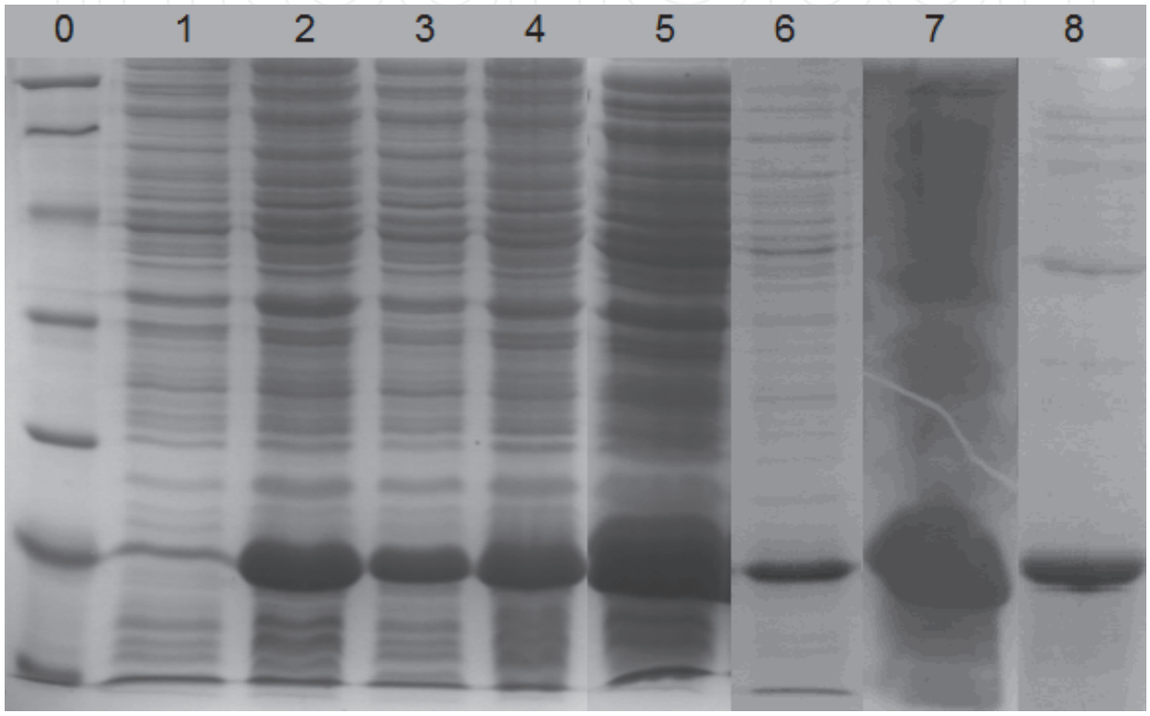

Fig. 1. Picture of Commassie-Blue staining after SDS-Page of induced rhIL-1Ra. (Lane 0: Makers; 1: Engineered E. coli without IPTG inducement; 2: IPTG induced E. *coli*; 3: Inclusion bodies, 4: Supernatant after unltrasonication; 5: Solublized inclusion bodies in 8M Urea; 6: rhIL-1RaIB; 7: Supernatant precipitated by ammonium sulfate; 8: rhIL-1RaST)

- rhIL-1Ra can then be purified by running the solution over DEAE-Sepharose and S-Sepharose, successively. First rhIL-1Ra solution was run over DEAE-Sepharose and flow through solution is collected. rhIL-1Ra purity was determined by Commassie-Blue staining after SDS-Page. The solution was then adjusted to pH 4.5. After centrifugation the supernatant was applied to a cation-exchange S-Sepharose. A 0 - 1 M NaCl gradient (1%/min) in loading buffer 2 (32.26 mM HAc, 17.74 mM NaAc, 1 mM EDTA, pH 4.5) was applied to elute bounded rhIL-1Ra from the column. According to UV absorption peak fractions were collected. The protein purity was determined by Commassie-Blue staining after SDS-Page (Fig. 2, 3, 4)

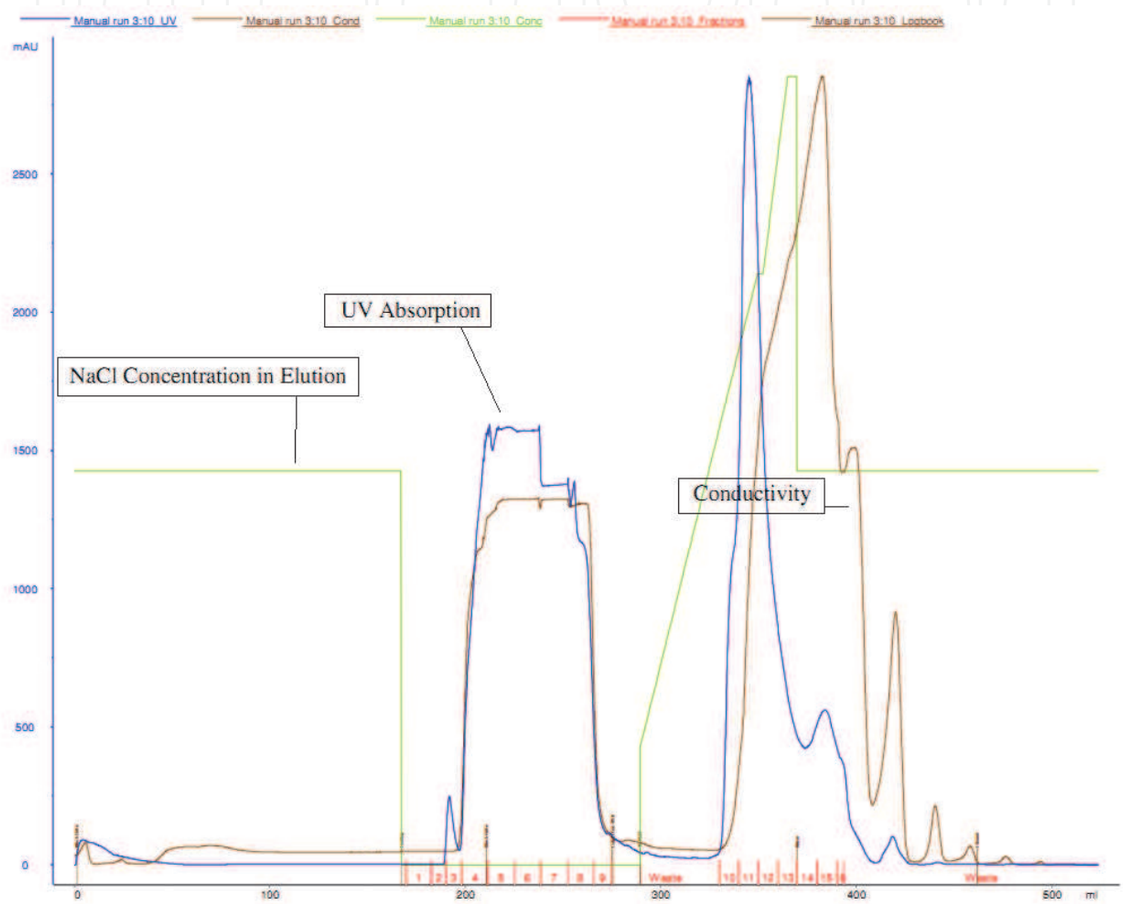

Fig. 2. Purification of rhIL-1RaST by DEAE-Sepharose (Flow through solution marked with 4/5/6/7/8/9 were collected)

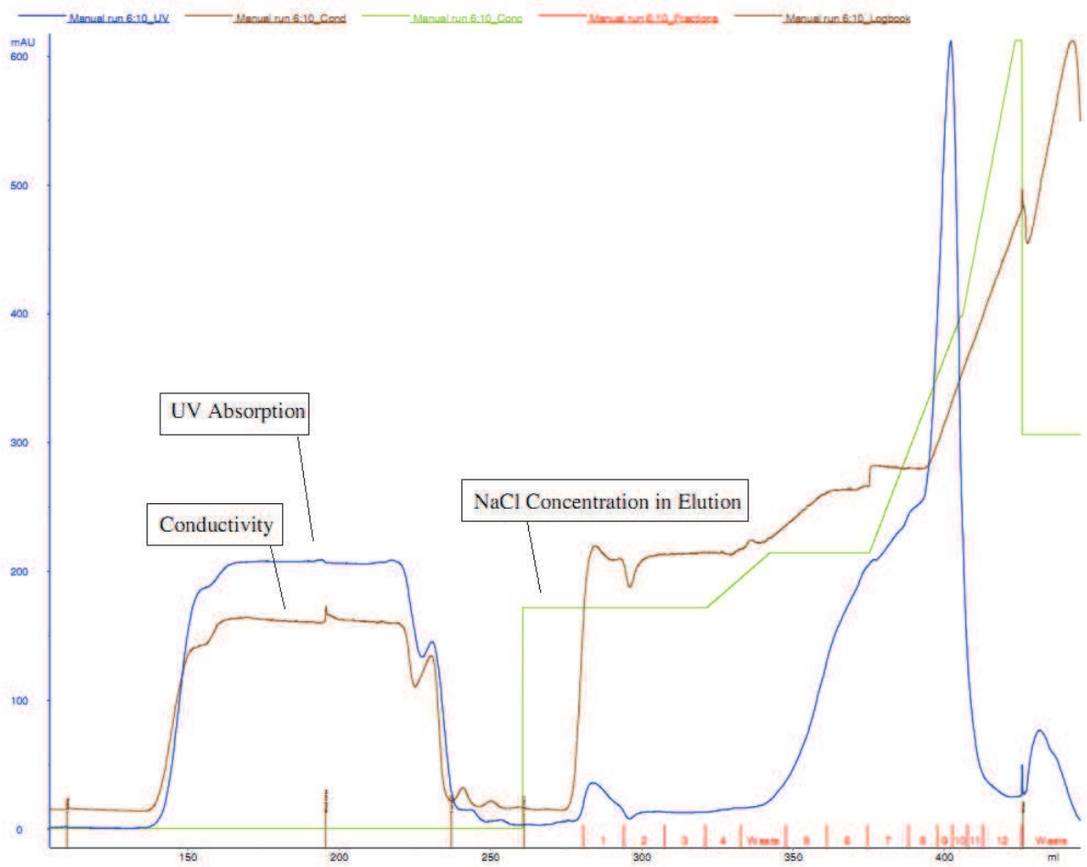

Fig. 3. Purification of rhIL-1RaST by S-Sepharose (According to UV absorption peak, fractions 6, 7, 8, 9 and 10 were collected and determined for its rhIL-1Ra concentration and purity)

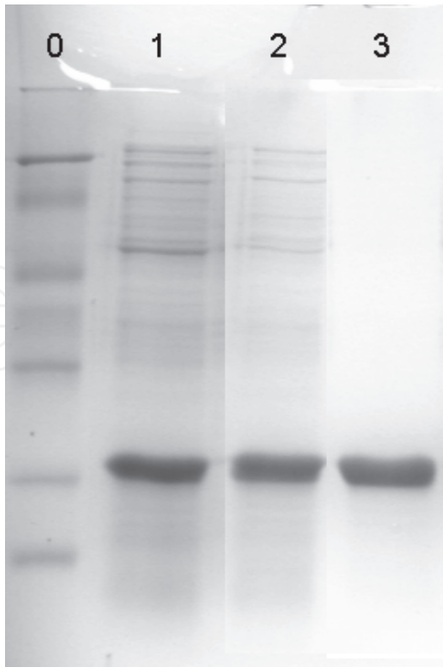

Fig. 4. Picture of Commassie-Blue staining after SDS-Page of purified rhIL-1Ra (Lane 0: Markers; 1: Flow through solution of rhIL-1RaST after DEAE-Sepharose purification; 2: rhIL-1Ra before S-Sepharose purification; 3: rhIL-1Ra after S-Sepharose purification)

4.2 The denaturing and renaturing of recombinant protein in inclusion bodies

If the protein exists in inclusion bodies form, denaturing and renaturung treatments are mandatory for the purification of this protein.

An ideal denaturing and renaturing system must serve the following purpose: high-output, low-loss and normal bio-activity final products. In industrial level, the ideal renaturing technology must also be easily amplified to produce large-scale products. Must commonly used denaturing solution is 6 M guanidine chloride or 8 M urea. There are some renaturing methods including dialysis, dilution, ultra-filtration. Some additives can be applied into the renaturing system to enhance the refolding efficacy such as denaturing agents, PEG, detergents, redox system, L-Arg, chaperone, etc. However, there are no general guidelines that can be applied to all refolding systems and one must test the efficiency of each method to ensure the optimized output. Some commercial available kits can be used to test the optimized renaturing system.

Here are a few samples of protein expressed in inclusion bodies:

Monokine induced by IFN-γ (Mig) is a CXC-chemokine (CXCL9). It plays important roles in regulation of immune activities, and knowledge of the protein in areas of allograft transplants, autoimmune diseases, and cancer therapy is evolving quickly. Mature murine MIG is a protein of 106 amino acids with molecular weight of 14.4 kDa. It contains 4 cysteines and 2 disulfide-bonds. The mature mouse MIG cDNA covers the coding sequences of the gene without the signal peptide coding sequences. After induction, rmMig mainly exists in inclusion bodies of the cell lysates. [19]

Chemerin is a novel chemokine that binds to the G protein-coupled receptor (GPCR) ChemR23. It is secreted as a precursor and executes pro-inflammatory functions when the last six amino acids are removed from its C-terminus by serine proteases. Mature human Chemerin is a protein of 137 amino acids with molecular weight of 16 kDa. The mature human Chemerin cDNA covers the coding sequences of the gene without the signal peptide coding sequences and the last 21 bps coding the peptide need to be removed during maturation. After induction, rhChemerin mainly exists in inclusion bodies of the cell lysates. [20]

Regenerating gene (Reg) IV is a newly discovered member of the regenerating gene family belonging to the calcium (C-type) dependent lectin superfamily and was firstly isolated

Recombinant Protein	**rmMig**	**rhChemerin**	**rhReg-4**	**rhMK**	**rmCXCL-14**
Protein Forms	Full length without signal peptide	Full length without signal peptide and last 6 amino acids	Full length without signal peptide	Full length without signal peptide	Full length without signal peptide
Tags	N/A	N/A	N/A	N/A	His-Tag
Engineered Bacteria	E. coli BL21 (DE3)	E. coli BL21 (DE3)	E. coli BL21 (DE3)	E. coli BL21 (DE3)	E. coli BL21 (DE3)
Expressing Vector	pET28a	pET28a	pET30a	pET30a	pET28a
Cultivation Medium and Temerature	LB @ 37 °C, 3 h	LB @ 42 °C, 4 h	LB @ 37 °C, 4 h	LB @ 42 °C, 3 h	LB @ 37 °C, 6 h
Induction	1 mM IPTG	1 mM IPTG	1 mM IPTG	1 mM IPTG	0.1 mM IPTG
Cell Lysate Preparation	Ultraso-nication	Ultraso-nication	Ultraso-nication	Ultraso-nication	Ultrasonication
Forms	Inclusion bodies	Inclusion bodies	Inclusion bodies	Inclusion bodies	Inclusion bodies
Denaturing	8 M urea, 20 mM Na2HPO4, 1 mM EDTA, 50 mM NaCl, pH 9.0.	6 M guanidine chloride, 1 mM EDTA, 50 mMNaCl, 50 mM Tris–HCl; pH 8.0.	6 M guanidine chloride, 1 mM EDTA, 50 mM NaCl, 50 mM Tris–HCl, pH 8.0.	6 M guanidine chloride, 20 mM Na2HPO4, 1 mM EDTA, 50 mM NaCl, 0.1 mM PMSF, pH 8.0.	6 M guanidine hydrochloride, 50 mM imidazole, 0.5 M NaCl, 0.02 M Tris-HCl, 0.1% Tween-20, 1 mM PMSF, pH 7.9.

Renaturing	The protein solution was dropped by pumping into 10-fold volume refolding Buffer (20 mM NaPO4, 1 mM EDTA, pH 6.0) under vigorous agitation. pH of the solution was maintaine d at 6.0. Then an equal volume dilution Buffer (20 mM NaPO4, 1 mM EDTA, pH 8.5) was added into the folding buffer. The refolded protein solution was set at 4°C for about 24 h.	The protein was refolded by slow dilution of the denaturing buffer into 100 volumes of renaturing buffer (1 mM reduced glutathione (GSH), 0.1 mM oxidized glutathione (GSSG), 0.5 M guanidine chloride, 0.4 M sucrose, 0.1 M Tris–HCl; pH 9.5), and concentrated eightfold using the tangential flow concentrator with a 10 kDa cutoff filter (Minimate TFF System; Pall, USA). The protein solution was further diluted into 10 volumes of dilution buffer (1 mM GSH, 0.1 mM GSSG, 0.1 M Tris; pH 9.5) and the pH was adjusted to 7.5 with 1 M HCl.	The denatured protein was added dro-pwise to a total of 500 ml of the optimized refolding buffer and left to stand for 24 h. The buffer at pH 10.5 consists of 50 mM Tris–HCl, 10 mM KCl, 240 mM NaCl, 2 mM MgCl2, 2 mM CaCl2, 0.5 M gua-nidine–HCl, 400 mM sucrose, 500 mM L-argi-nine–HCl, 1 mM reduced glutathione, and 0.1 mM oxidized glutathione. The refolding buffer was concentrated and changed to the phosphate buffer (20 mM, pH 6.5) using the tangential flow concen-trator with a 10 kDa cutoff filter (Mini-mate TFF System, Pall, USA).	The protein refolding was proceeded by drop-wise dilution into defined protein folding buffer The protein solution was dropped by pumping into 10-fold volume refolding buffer (20 mM Na2HPO4 and 1 mM EDTA, pH 7.4) under vigorous (magnetic stirrer) agitation. pH value of the solution was maintained at 7.4. Then an equal volume dilution buffer (20 mM Na2HPO4 and 1 mM EDTA, pH 8.0) was added into the folding buffer, and final pH of the solution was maintained at 8.0. This protein solution was stored at 4 °C for 24 h.	Three ml Ni-Sepharose (Pharmacia, USA) was loaded onto column and recharged by 5 column volumes (CV) of 50 mM NiSO4 and equilibrated by 3 CV of denatured binding buffer. Cell lysate was loaded onto the purifi-cation column at rate of 0.5 ml/min at 4 °C. Then, the column was washed with 10 CV of denatured wash buffer (60 mM imidazole, 0.5 M NaCl, 0.02 M Tris-Cl pH 7.9, 0.1% Tween 20, and 6 M Guanidine hydro-chloride) before elution by denatured elute buffer (1 M imidazole, 0.5 M NaCl, 0.02 M Tris-Cl pH 7.9, 0.1% Tween-20, and 6 M Guanidine hydrochloride). The intermolecular disulfi-de bonds were reduced by adding dithiothreitol (DTT) into protein solu-tion to final concen-tration of 0.2 M. After overnight incubation at 4 °C, the solution was gradually diluted with refolding buffer (20 mM Tris-Cl pH 7.9, 0.1 M NaCl, 1 mM reducing glutathione, 1 mM EDTA, 10% sucrose, 0.1% Triton-X114) to 20-fold of the initial volume over 1 h with gentle stirring at room temperature and then incubated at room temperature for 1 h. Precipitate was removed by centrifugation at 20,000×g for 30 min.

Table 1.

from a cDNA library of ulcerative colitis (UC) tissues by Hartupee et al. Human Reg IV is synthesized as a 158 amino acid (aa) precursor with a 22 aa signal sequence and a 136 aa mature chain. The molecular weight (MW) of mature Reg IV protein is about 16 kDa. The mature form of human Reg IV is encoded by the cDNA sequences of the gene from 354 to 743 bp without the signal peptide coding sequences. After induction, rhReg IV mainly exists in inclusion bodies of the cell lysates. [21]

Midkine is a heparin-binding growth factor, which plays important roles in the regulation of cell growth and differentiation. The non-tagged recombinant human midkine (rhMK) is therefore required to facilitate its functional studies of this important growth factor. The mature human midkine protein is a protein contains 122 amino acids with a molecular weight of 17 kDa. The mature form of human midkine is encoded by the full-length sequence from 289 to 654 bp without the signal peptide coding sequences. After induction, rhMidkine mainly exists in inclusion bodies of the cell lysates. [22]

Mouse CXCL14/BRAK is a monocyte-selective chemokine which is expressed in almost all normal tissues. The mouse cxcl14 mRNA encodes 99 amino acids, in which N-terminal 22 amino acids serve as a signal peptide which is cleaved as the protein is secreted. The remaining C-terminal 77 amino acids construct the mature chemokine with a calculated molecular weight of 9.4 kDa and pI 9.9. The mature form of mouse CXCL14 is encoded by the full-length sequence from 445 to 678 bp without the signal peptide coding sequences. After induction, rmCXCL14 mainly exists in inclusion bodies of the cell lysates. [23]

The detailed processes of the denaturing and renaturing of those proteins were already published, and can be brifly introduced below Table 1.:

5. The purification of recombinant protein: Chromatography technologies

Like the denaturing and renaturing, lots of technologies had been developed to purify recombinant. Among which chromatography technologies are the most powerful tools, and can give satisfactory purity and yield of final protein products. According to the proteins pI, Ion exchange chromatography can be adopted. According to the hydrophilic and hydrophobic properties and molecular size, HPLC can be used. pET system carries certain fusion tag and can be easily identified by certain types of Affinity Chromatography. The most important guideline is, the purification of recombinant protein may not be achieved by simply using one technology mentioned above; on the contrary, the combination of them can lead to optimized results.

Like the above section, the detailed processes of the purification of the above-mentioned proteins were already published, and can be briefly introduced in table 2.

Recombinant Protein	rmMig	rhChemerin	rhReg-4	rhMK	rmCXCL-14
Protein Forms	Full length without signal peptide	Full length without signal peptide and last 6 amino acids	Full length without signal peptide	Full length without signal peptide	Full length without signal peptide

Recombinant Protein	rmMig	rhChemerin	rhReg-4	rhMK	rmCXCL-14
Tags	N/A	N/A	N/A	N/A	His-Tag
Purification	The supernatant of refolding was loaded on an S-Sepharose column with a volume of 20 ml. The column was pre-equilibrated with Buffer A (20 mM NaPO4, 1 mM EDTA, pH 7.2). Sample was loaded at speed of 0.5 ml/min and the column was then washed with 2 column volumes of Buffer A. The column bound proteins were eluted using a programmed gradient of Buffer B (20 mM NaPO4, 1 mM EDTA, 1 M NaCl, pH 7.2) at a speed of 3 ml/min.	The supernatant of refolding was loaded onto a Q Sepharose FF column equilibrated with wash buffer 1 (20 mM Tris–HCl, 1 mM EDTA, 25 mM NaCl; pH 7.5) at a speed of 5 ml/min. The flow-through was collected and adjusted with glacial acetic acid to pH 4.5. After centrifugation, the supernatant was loaded onto an S-Sepharose FF column equilibrated with wash buffer 2 (50 mM NaAc–HAc buffer, 1 mM EDTA, 25 mM NaCl; pH 4.5) at a speed of 5 ml/min. The protein was eluted using a 0.3–1.0 M NaCl gradient in wash buffer 2 (1%/min) at a speed of 1 ml/min.	The supernatant of refolding was loaded on an S-Sepharose column in a volume of 20 ml. The column was pre-equilibrated with Buffer A (20 mM phosphate buffer at pH 6.5, 1 mM EDTA). Sample was loaded at speed of 3 ml/min and the column was then washed with 2 column volumes of the Buffer A. The column bound proteins were eluted using a programmed gradient of Buffer B (20 mM phosphate buffer at pH 6.5, 1 mM EDTA, 1 M NaCl) at a speed of 1 ml/min.	The supernatant of refolding was loaded on an S-Sepharose column with a volume of 20 ml. The column was pre-equilibrated with Buffer A (20 mM Na2HPO4 and 1 mM EDTA, pH 8.0). Sample was loaded at a flow rate of 0.5 ml/min and the column was then washed with 2 column volumes of Buffer A. The column-bound proteins were eluted using a programmed gradient of Buffer B (20 mM Na2HPO4, 1 mM EDTA, and 1 mM NaCl, pH 8.0) at a flow rate of 1 ml/min.	The supernatant of refolding was loaded onto an SP Sepharose column with a volume of 20 ml, which had been pre-equilibrated with Buffer A (20 mM Tris-HCl, pH 8.0), with flow rate maintained at 5.0 ml/min. After washing with 5 CV of Buffer A, protein was eluted using programmed gradient Buffer B (20 mM Tris-Cl and 1.0 M NaCl, pH 8.0), which was linearly increased from 20% to 80% with a flow rate of 1.0 ml/ml. The eluted fractions containing the target protein were desalinated using a Sephadex G25 column (height 60 cm and diameter 1.5 cm), which was pre-equilibrated with 2 CV of Buffer A.
Target Protein Purity	99%	99%	97%	98%	97%
Final Yield	5.2%	14%	28%	6.2%	11%

Table 2.

6. Purified recombinant proteins: The quality control and the storage

6.1 The storage of recombinant proteins

Protein samples are easily to lose their bioactivity, so it's important to test appropriate storage conditions and buffers of this protein. After purification, the composition of storage solution and storage condition can both affect the bio-activity of recombinant proteins. For instance, rhIL-1Ra can be safely stored at 4°C for nearly half a year in 10 mM sodium citrate, 140 mM sodium chloride, 0.5 mM EDTA, while rhChemerin, when stored in PBS, pH 7.4, must be frozen in -80°C refrigerator. A good source of the knowledge of the suitable storage of the recombinant protein comes from the manual of commercialized recombinant protein.

6.2 The quality control of recombinant proteins

After protein expression and purification, some quality control items should be tested to serve the later in vivo and in vitro experiment, or other application. These test items include but not limit to:

Concentration (measured by BSA or Bradford methods, etc.)

Purity (measured by silver staining after SDS-Page, HPLC, or SEC-HPLC etc.)

Sequence confirmation (measured by GC-Mass or etc.)

Endotoxin (measured by TAL or LAL test)

Bioactivity (measurements depends. Bioactivity should be expressed as EC50, if applicable).

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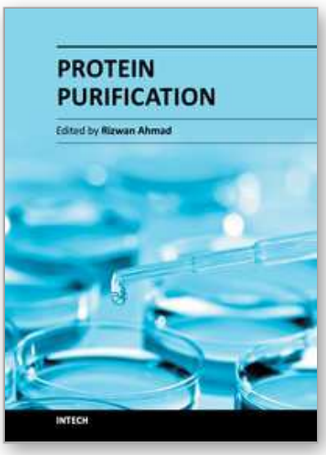

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The current volume entitled Protein Purification is designed to facilitate rapid access to valuable information about various methodologies. It aims as well to provide an overview of state-of-art techniques for the purification, analysis and quantification of proteins in complex samples using different enrichment strategies.

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