academicJournals

Vol. 13(14), pp. 1588-1596, 2 April, 2014
DOI: 10.5897/AJB12.1495
Article Number: C7F237A47124
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Expression, production and renaturation of a functional single-chain variable antibody fragment (scFv) against human intercellular adhesion molecule-1 (ICAM-1)

Hong Sun[1], Yu Ding[2], Songling Yang[1], Zuoqun Yin[1] and Yuhuan Yue[3]*

[1]Department of Biological Pharmacy, Heilongjiang Vocational College Of Biology Science and Technology, Harbin, 150025, P. R. China.
[2]Laboratory Animal Center, Jilin University, Changchun, 130062, P. R. China.
[3]Institute of Military Veterinary, Academy of Military Medical Sciences of The Chinese People's Liberation Army, Changchun, 130062, P. R. China.

Received 12 April, 2012; Accepted 5 July, 2012

The single-chain variable antibody fragment (scFv) against human intercellular adhesion molecule-1 (ICAM-1) was expressed at a high level in *Escherichia coli* as inclusion bodies. We attempted to refold the scFv by ion-exchange chromatography (IEC), dialysis and dilution. The results show that the column chromatography refolding by Q Sepharose high performance (Q HP) had remarkable advantages over the conventional dilution and dialysis methods. Furthermore, the anti-ICAM-1 scFv yield was higher by this method, which is about 60 mg/l. The purity of the final product was greater than 90%, as shown by denaturing gel electrophoresis. Enzyme-linked immunosorbent assay (ELISA), cell culture and animal experiments were used to assess the immunologic properties and biologic activities of the renatured scFv.

Key words: Intercellular adhesion molecule-1, single-chain variable antibody fragment, expression, purification, renaturation, biological activity.

INTRODUCTION

Intercellular adhesion molecule-1 (ICAM-1) is a member of the immunoglobulin supergene family and is a cell surface ligand for lymphocyte function-associated antigen-1 (LFA-1). ICAM-1 is mainly expressed on the surface of endothelial cells. It is also expressed on activated lymphocytes in inflamed regions; however, the expression in peripheral blood lymphocytes is normally very low. The level of ICAM-1 is upregulated in the presence of various stimuli (for example, inflammatory mediators, oxidative stress and viral infection) (Dustin et

*Corresponding author. E-mail: YuhuanYue0716@163.com. Tel: +86 15104522008.

Abbreviations: scFv, Single-chain variable antibody fragment; **IEC,** ion-exchange chromatography; **Q HP,** Q Sepharose high performance; **ELISA,** enzyme-linked immunosorbent assay; **ICAM-1,** intercellular adhesion molecule-1; **SDS-PAGE,** sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

al., 1986). The interaction of ICAM-1 with the LFA-1 plays an important role in leukocyte adhesion and in the execution of immunological and inflammatory functions mediated by leukocyte adhesion (Dustin et al., 1988). Increased ICAM-1 levels result in the transmigration of neutrophils during the initial phase of inflammation. Inhibition of this process could decrease inflammatory response and tissue damage (Sadowska et al., 2004). Many diseases are associated with the over-expressed ICAM-1, such as acute pancreatitis (Kaufmann et al., 1996), inflammatory bowel disease and colonic neoplasms (Sadowska et al., 2004), organ transplantation (Vainer et al., 2006; Zhao et al., 2007; Aronni et al., 2006), angiocardiopathy (Sadowska et al., 2004; Bowes et al., 1993), ischemia-reperfusion injury (Souza-Moraes et al., 2003) and cancer (Alexiou et al., 2001; Coskun et al., 2006; Thomas and Speight, 2001). These reports suggested that the anti-ICAM-1 strategy has a potential application in the treatment of ICAM-1-mediated immunological and inflammatory diseases.

Today, the single-chain variable antibody fragment (scFv) strategy has become one of the most popular methods in antibody engineering because of its lower level of immunogenicity, and its small molecular size endows scFv with better tissue penetration (Yokota et al., 1992). Thus, scFv has a wide range of applications in diagnosis and therapy. It is possible for anti-ICAM-1 scFv to block the biological activity of ICAM-1, and it may be effective in preventing the progression of the above mentioned diseases. Therefore, it is necessary to prepare large-scale amounts of the anti-ICAM-1 scFv protein for further research and application. In this study, we expressed the anti-ICAM-1 scFv at a high level in the form of the inclusion bodies in *Escherichia coli*. We successfully refolded the denatured scFv by ion exchange chromatography (IEC). The study paves the way for preparing a large amount of anti-ICAM-1 scFv to be used for the application against diseases correlated with inflammation.

MATERIALS AND METHODS

The following reagents were used in this study: isopropyl-β-D-thiogalactoside (IPTG) (Merck), horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG antibody (KeyGen Biotech), reduced glutathione hormone (GSH), glutathione (GSSG), lipopolysaccharide (LPS) and bovine serum albumin (BSA) (Sigma), Ficoll-Paque (Amersham Biosciences), rat ICAM-1 (R and D); Q Sepharose high performance (Pharmacia Biotech), ÄKTA (Prime protein purification system) (Amersham Biosciences), fermenter (10 L, East China University Science and Technology), anti-ICAM-1 mAb (prepared in our laboratory) (Sun et al., 2008a). A cell line (ECV-304) and Kunming mice (weight 18 to 22 g) were purchased from Maisha Biotechnology Limited Company (Shanghai, China) and the Center of Experimental Animal Changchun Institute of Biological Products (Changchun, China), respectively. All the other chemicals used were of analytical grade. Anti-scFv rabbit polyclonal (the antiserum against scFv) was obtained by hypodermic injecting of the rabbit with the purified inclusion bodies protein.

Plasmids and strains

E. coli BL-21(DE3) was obtained from Novagen. The expression plasmid of pET22b-(ICAM-1) scFv was constructed by our laboratory (Sun et al., 2008b).

Expression of anti- ICAM-1 scFv

Bacterial cells with the expression plasmid pET22b-(ICAM-1) scFv were grown overnight in 5 ml of LB with 50 mg/l ampicillin (amp) at 37°C. The overnight culture was used at 1:100 dilutions. Expression was induced by adding IPTG to a final concentration of 0.8 mM when the culture was grown to OD_{600} of 0.6 to 0.8. The mixture was further incubated for another 4 h. Cells were harvested by centrifugation at 5000 rpm for 20 min at 4°C.

Fermentation procedure

Large-scale scFv were produced in the bioreactor as follows; the prepared strain was added to the fermentation medium (pH 7.0 to 7.3). The rate of rotation was 400 rpm and aeration rate was 20. Meanwhile, the dissolved oxygen was controlled at the level of 20 to 30%. Then scFv production was induced by the addition of 0.8 mM IPTG, and the culture was allowed to incubate at 37°C for 4 h.

Preparation of the samples

The scFv was expressed as described above. After extraction from *E. coli* cells by the combination of lysozyme and sonication, the inclusion bodies were washed three times with 100 ml of 0.5% TritonX-100 (v/v) and 2 M urea for 30 min each time. Two grams of the pellet was suspended in 10 ml of denaturing buffer (50 mM Tris-HCl, 50 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 8 M urea, and 10 mM dithiothreitol (DTT), pH 8.0) and kept at room temperature for 2 to 4 h to dissolve the inclusion bodies. Residual insoluble matter was removed by centrifuging at 12000 rpm for 30 min. The supernatant was filtered on 0.22 μm filter (Durapore Membrane Filter, Millipore, USA) before chromatography.

Refolding of scFv

Refolding by dilution

The above solubilized inclusion bodies (7 ml) with a concentration of 7 mg/ml were slowly dropped into the refolding buffer A (30 mM Tris-HCl, 1 mM EDTA, 1 mM GSH, 0.2 mM GSSG, pH 8.0) and adjusted to a protein concentration of 100 μg/ml. The solution was stirred for 2 h at room temperature, followed by incubation at 4°C for more than 48 h (Takai et al., 2005).

Purification by ion-exchange chromatography (IEC): The 500 ml diluted supernatant was applied to the 10 ml IEC (Q HP) which was pre-equilibrated with buffer B (30 mM Tris-Cl, 1 mM EDTA, pH 8.0). The ÄKTA Prime protein purification system was used. The column was eluted with a 50 ml linear gradient of buffer B to buffer B containing 1 M NaCl. Finally, protein concentration was determined with the Bradford assay.

Refolding by urea gradient dialysis

The 100 ml solubilized denatured scFv (0.5 mg/ml) was loaded into a dialysis bag with a membrane molecular weight cutoff of 10000

Daltons, which was dialyzed against 50 columns of buffer A at 4°C for 24 h. Denaturant was slowly removed by a series of equilibrations with buffers of decreasing urea. The urea concentration was reduced as follows: 6 M → 4 M → 2 M → 1 M → 0.5 M → 0 M (Tan et al., 1998). After centrifugation, the supernatant was applied to Q HP for further purification, as described above.

Refolding by IEC

An IEC system was used with the XK16/20 column containing 10 ml of Q HP of the ÄKTA Prime protein purification system. The column was equilibrated with denaturing buffer A (30 mM Tris-Cl, pH 8.0, with 6 M urea, 1 mM EDTA, 1 mM GSH and 0.2 mM GSSG). Following equilibration, urea concentration of the solubilized inclusion bodies (7 ml; 7 mg/ml) was adjusted to 6 M, and the samples were loaded. After sample was loaded, the refolding procedure was performed with a linear gradient of 25 column volumes by decreasing urea concentration from 6 to 0 M, maintaining the flow rate at 0.5 ml/min. Protein was gradually being refolded within the column. Following refolding, another linear gradient of buffer (30 mM Tris-Cl, pH 8.0, 1 mM EDTA) without GSH-GSSG from 0 to 1 M NaCl was performed with the gradient length of six column volumes. The eluate fractions were collected and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The procedure was done at 4°C.

Protein determination

The relative protein concentration of the denatured and purified scFv was determined by Bradford assay using BSA as a standard protein (Bradford, 1976). The refolding yield was calculated as a percentage of the soluble protein after refolding against the total protein of inclusion bodies before refolding.

Indirect cellular ELISA for antigen-binding activity of anti-ICAM-1 scFv

The antigen-binding activity of the refolded scFv was detected and identified routinely by noncompetitive ELISA. Cultured ECV-304 cells were seeded overnight in the 96-well culture plate at 10^5 cells/well. Cells were fixed in 10% formalin-phosphate buffered saline (PBS) (pH 7.4) for 15 min at room temperature, washed three times with 1% BSA-PBS and blocked by 3% BSA-PBS for 2 h at 37°C. After washing, the refolded scFv concentration was diluted serially and added to the plate. The control well was prepared without scFv, and the plate was incubated for 1 h at 37°C. The second antibody (anti-rabbit IgG antibody) and HRP-conjugated goat anti-rabbit IgG were added in turn and incubated for 1 h at 37°C. Finally, tetramethylbenzidine (TMB) was used as the color developer, and absorbance was measured at 450 nm (Chen et al., 2006). The binding of purified scFv to rat ICAM-1 was also determined by indirect ELISA (the 96-well culture plate was coated with 1 μg/ml rat ICAM-1), as described above, which paved the way for further active research.

Cell adhesion assay for peripheral mononuclear cells (MCs) to ECV-304

ECV-304 cells grown in monolayers in a 96-well plate were treated with LPS (100 ng/ml) for 24 h at 37°C (Beck-Schimmer et al., 2002), followed by adding the anti-ICAM-1 mAb (5 μg/well), purified scFv (5 μg/well), or left untreated (PBS control). The 96-well plate was cultured for 0.5 h. Human MCs were isolated from healthy human peripheral blood using Ficoll-Paque according to the manufacturer's instructions. MCs were adjusted to a concentration of 10^6/ml with RPMI 1640, and added to the monolayers of MC in a final volume of 100 μl. After a 0.5 h co-culture in a CO_2 incubator, the populations of non-adherent cells was removed from the plate, and were manually counted under the microscope at ×20 magnification (Takai et al., 2005). The inhibition (%) was calculated by: 100 × [1 - (the cell population of non-adhesion) / (total cellular score)].

Inhibitory effect on the swelling of mouse auricle that was induced by dimethyl benzene

The Kunming mice weighing 18 to 22 g (females and males) were randomly divided into five groups, namely: experiment group 1 (intraperitoneal injection scFv, 2 mg/kg), experiment group 2 (intravenous injection scFv, 2 mg/kg), experiment group 3 (intravenous injection mAb, 2 mg/kg), hexadecadrol group (intraperitoneal injection hexadecadrol, 2 mg/kg), and the control group (0.9% saline). Thereafter, dimethyl benzene was dropped onto the left ear conch uniformly 1 h after injection. After another 2 h, the mice were sacrificed, and both ears were cut by scissors. The ear pieces were punched with a puncher at the same place and weighed. The inhibition (%) of the engorgement was calculated by: 100 × [(the average tumescent degree of control group - the average tumescent degree of experimental group) / the average tumescent degree of control group]. The animal experiment was conducted under applicable laws and guidelines and after approval by the Animal Care and Use Committee of Jilin University.

RESULTS

The expression of scFv

The constructed expression vector was transferred into BL21 (DE3) and induced with IPTG at 37°C. As shown in Figure 1, a protein of 30 kDa was strongly expressed after 4 h incubation with 0.8 mM IPTG. It was found that the scFv was expressed as insoluble inclusion bodies. The wet weight cell was about 5 g in 1 l of *E. coli* flask culture. After large scale production of anti-ICAM-1 scFv in the fermentor, the overall yield of the harvested cells (wet weight) was about 10 g/l.

Preparation of the samples

Isolation is the first step for the recovery of active protein from the *E. coli* which involves breaking the cells to release the cell contents, including the inclusion bodies. Lysis is more efficient if the cells are pretreated with lysozyme, which weakens the cell walls. Therefore, in our experiments, the cells were treated by lysozyme before sonication. Washing can remove non-target protein and may also remove proteases that could degrade the expressed product. In this study, we washed the inclusion bodies three times with buffer containing 0.5% TritonX-100 buffer and 2 M urea. Finally, 350 mg of inclusion body protein per liter of culture were obtained by fermentor, and the purity of inclusion bodies was up to 70%, as determined by SDS-PAGE (Figure 2A).

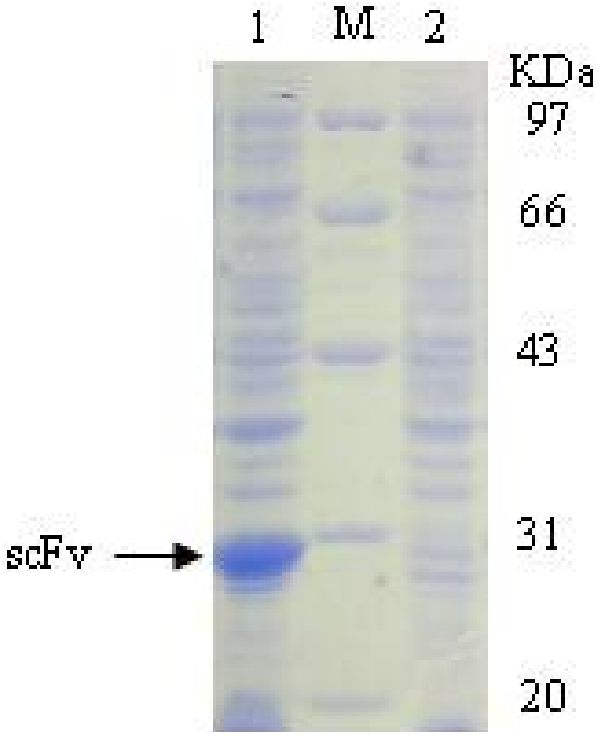

Figure 1. Analysis of scFv expressed in *E. coli.* The amount of inclusion bodies was about 30% of the total cell proteins of *E. coli*. Lane 1, induced total cellular protein; lane 2, non-induced pET22b-anti ICAM-1 scFv/BL21 (λDE3); M, protein molecular weight markers.

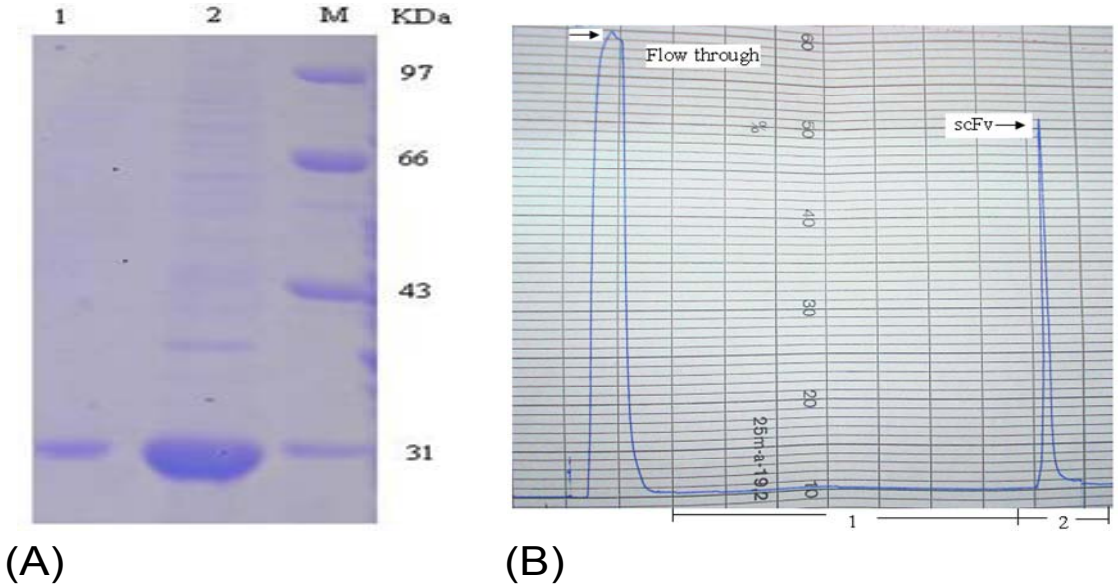

Figure 2. (A) Analysis of purified and refolded scFv on SDS–PAGE. Lane 1, scFv refolding by Q HP; lane 2, solubilized inclusion bodies; M, protein molecular weight markers. (B) Chromatographic elution profile of refolding scFv by Q Sepharose HP column. Protein peaks were observed at 280 nm. Process 1, refolding procedure; process 2, gradient elution. The arrow indicates flow through protein and interest protein.

Table 1. The comparison of three refolding methods.

Refolding method	Dilution	Dialysis	IEC
The protein yield (%)	22	28	17
Protein concentration (mg/ml)	0.03	0.15	0.4-0.5
Purity (%)	>70	>70	>90
Activity (OD)	1.86	2.05	2.53
Time required (h)	>48	>72	<10
Reagent consumption (relative amount)	2	100	1

Activity is the OD value of refolded and purified scFv analyzed by indirect cell-ELISA at the same protein concentration (2.5 μg/mL). (The OD value of negative control was 0.080); reagent consumption is relative amount that is calculated on the basis of 1 g inclusion bodies.

Table 2. Purification summary of scFv.

Purification step	Total protein (mg)	scFv (mg)	Purity (%)	Recovery yield (%)
Total cell lysate[a]	1200	372	31	100
Inclusion bodies after washing	494	346	70	93
Refolded scFv after IEC column	67	60	90	17

[a]Approximately, 10 g of wet-weight cells and a total cell lysate containing 1200 mg protein were obtained from 1000 ml cell culture.

Refolding of scFv

For comparison of the methods, the dilution and dialysis were both carried out. During the two processes, a slight protein aggregation was found. Perhaps due to the prolonged experimental time, the activities of the refolded scFv proteins by dilution and dialysis were lower than that refolded by IEC. Comparing the renatured scFv by three refolding methods (Table 1), and considering purity, activity, time required and the consumption of reagents, the column chromatography method for refolding was the most suitable for large scale production. After refolding by IEC, we obtained a single protein peak during the elution procedure and collected approximately 20 ml of the protein (Figure 2B). The overall purification is summarized in Table 2. The purity of product was about 90%, as shown on the 12% SDS-PAGE (Figure 2A).

Effect of pH on the refolding by IEC

The protein yield was affected by the pH value significantly. To study the influence of the pH on the refolding recovery, denatured scFv was loaded and eluted by a buffer with different pH (7.0, 7.5, 8.0 and 8.5) each time. As shown in Figure 3, the pH 8.0 elution buffer led to a significantly increased yield of the functional anti-ICAM-1 scFv (17%). It is suggested that the condition is suitable for the native disulfide bond formation. So the condition of pH 8.0 was used throughout the experiment.

The activity of refolded scFv

The non-competitive ELISA results demonstrated that refolded anti-ICAM-1 scFv was able to bind specifically to human ICAM-1-expressing cells in a dose dependent manner. Specifically, when gradually increasing the concentrations of refolded scFv, the extent of ICAM-1 and scFv binding increased (Figure 4). Meanwhile, the indirect ELISA showed the specific antigen-binding activity of the refolded scFv to rat ICAM-1 (Figure 5).

Cell adhesion assay for peripheral MCs to ECV-304

Statistical analysis was carried out using SPSS 13.0, and the statistical significance was set at $P < 0.05$. It can be seen from the data that the MCs adhesion to LPS-stimulated ECV-304 monolayers was largely inhibited in the presence of a neutralizing ICAM-1 mAb and scFv, both at 30 min, compared with the untreated condition (with PBS). As a result, the ratio of adhesion in the presence of mAb and scFv were 31 and 36% (data not shown), respectively, which was a statistically significant difference compared with the control (42%) ($P < 0.05$). Although both mAb and scFv could inhibit the MCs adhesion to ECV-304, the effectiveness of scFv was lower than that of the mAb ($p > 0.05$).

The analysis of the inflammation depressant effect

Statistical analysis was carried out as described above. We found that injection of anti-ICAM-1 scFv or mAb reduced the severity of swelling of auricle to a different degree, but the depressant effect of scFv and mAb was a little weaker than that of hexadecadrol. As shown in Table 3, there was a statistically significant difference in

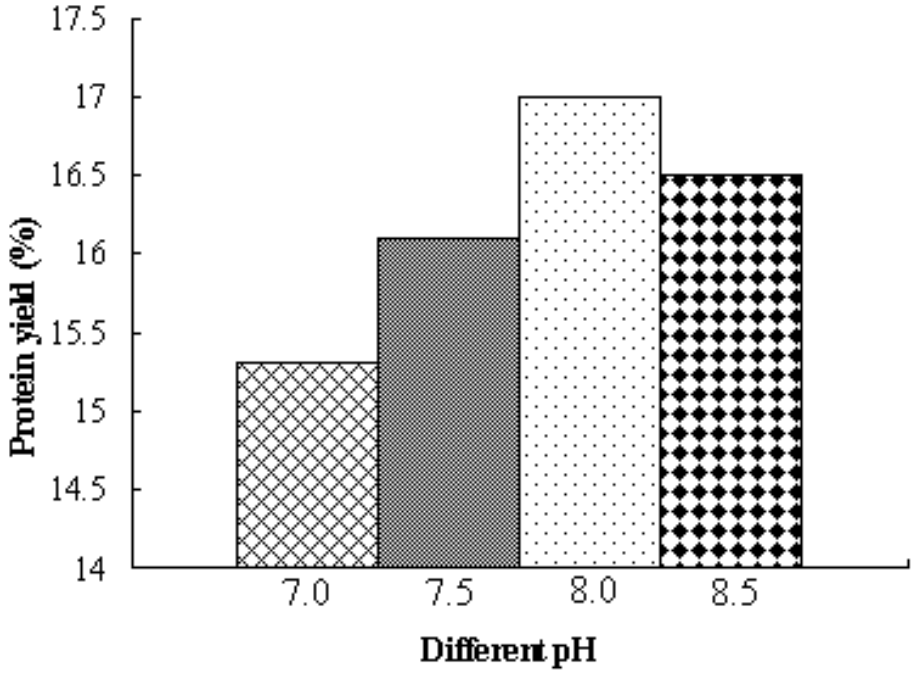

Figure 3. Effect of pH on refolding yield. Refolding was performed at different pH, 7.0, 7.5, 8.0 and 8.5, to measure pH dependence of refolding. The data indicates that the optimal pH for refolding of scFv may be 8.0.

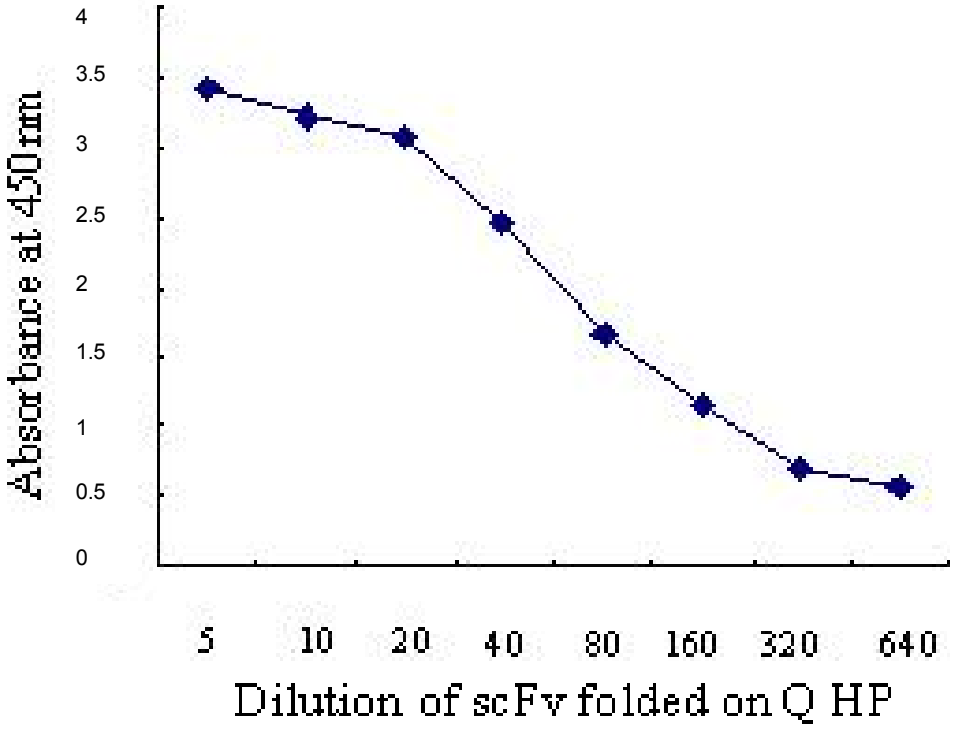

Figure 4. Antigen-binding activity of refolded anti-ICAM-1 scFv. Specific antigen-binding activity was determined by indirect cellular ELISA. The 96-well culture plate was coated by EC-304 cells and 2-fold serially diluted, purified scFv was added to the wells. As can be seen, when gradually increasing the concentrations of refolded scFv, absorbance values increased.

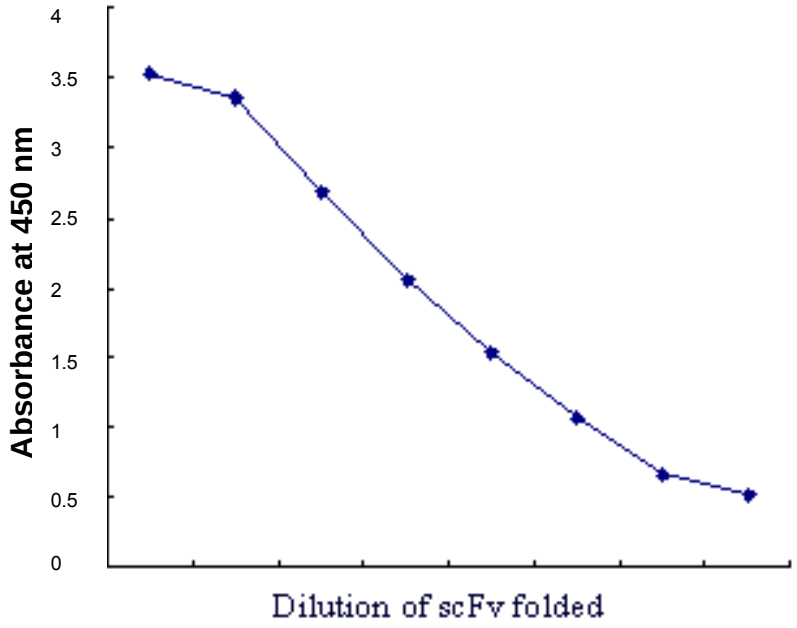

Figure 5. Indirect ELISA for antigen-binding to rat ICAM-1. The 96-well culture plate was coated with 1 μg/ml rat ICAM-1, the refolded scFv concentration was diluted serially and added to the plate.

Table 3. The effect of anti-ICAM-1 scFv and mAb on dimethyl benzene-induced swelling of auricle ($\overline{X} \pm S$).

Group	n	Dosage (mg/kg)	Tumescent degree (mg)	Inhibition (%)
Control group	10	-	12.6±1.05	-
Hexadecadrol group	10	2	5.15±1.23*	59
Experiment group 1	10	2	6.95±1.52*	45
Experiment group 2	10	2	7.61±2.02*	39
Experiment group 3	10	2	5.94±1.69*	53

Statistically significant reduction by *t*-test compared with control group, *P < 0.01.

the inhibition (%) between the experimental group and the control group.

DISCUSSION

In previous studies, it has been shown that increasing ICAM-1 *in vivo* contributed to the pathogenesis of the inflammation-related diseases. Many reports had suggested the protective effects of anti-ICAM-1 mAb, which could block the inflammation response *in vitro* or *in vivo*. The results in our laboratory also indicate that ICAM-1 and its receptors exhibit a high expression level in highly pathogenic avian influenza (H5N1) and viral pneumonia (HPAIV), and may play an important role in the pathogenesis (Coskun et al., 2006). Moreover, we gained excellent results in curing mice of avian influenza with the anti-ICAM-1 antibody we applied for a patent in 2007, in which we named the anti-ICAM-1 antibody as a treatment for avian influenza. However, mAb molecules are large and have more immunogenicity, so it may be advisable to use the anti-ICAM-1 scFv in diagnostic and therapeutic applications. In our present work, the active scFv against human ICAM-1, successfully prepared from the inclusion bodies by chromatography renaturation, had a significant effect on the aseptic inflammation.

To obtain the active protein, refolding the expressed products from the inclusion bodies is the most important and fundamental procedure. If we can develop a refolding strategy *in vitro* with lower cost, higher yield and activity, then less expensive and easier prokaryotic expression, namely, bacterial fermentation systems, may become

feasible for the inclusion body protein production. In the past, dilution and dialysis were convenient and traditional refolding strategies. Now, there are many reports about the two methods, which had been used for refolding many proteins. Most of them were prepared at the laboratory scale but not industrial scale production. These refolding techniques had some disadvantages, such as that the dialysis procedure needed large amounts of reagents, long treatment times (Tan et al., 1998), and also can cause the adhesion of protein on the membrane (Verma et al., 1998). Sometimes, it is easy to form protein aggregates (West et al., 1998). The dis-advantages of the dilution method are the large processing volumes involved, and the increasing costs and the "step-change" in denaturant concentration to native conditions may result in aggregation (Chaudhuri, 1994). Moreover, the concentration of denatured protein during the two refolding process has to be controlled at a low level to prevent aggregate formation, which restricted their application on a large scale production.

In recent years, chromatographic methods have been developed for the refolding of the inclusion body proteins from *E. coli*. In many cases, they appeared to be more effective than traditional refolding strategies (Lanckriet and Meddelberg, 2004; Fahey et al., 2000; Gu et al., 2002). IEC has the characteristics of simple operation, good biological compatibility and high capacity. Furthermore, the medium could be reused, which decreases the cost of materials. In addition, the IEC method is the concomitant purification of the target protein during the refolding process (Müller and Rinas, 1999), so considered from all perspectives, the IEC process is the more valuable method for refolding of the engineered proteins. In the case of proteins containing cysteine, the isolated inclusion bodies usually contain a certain amount of interchain disulfide bonds (Schoemaker et al., 1985). There are two disulfide bonds in anti-ICAM-1 scFv. For disulfide-containing proteins, the refolding yields are strongly dependent upon the redox environment, which helps to form the proper disulfide bond and to associate different domains (Wetlaufer et al., 1987).

In our study, 1 mM GSH and 0.2 mM GSSG were included in the first gradient buffers during the refolding procedure. In the second gradient, we investigated two conditions and found that introducing redox conditions to the refolding buffer did not lead to a significantly increased yield of functional anti-ICAM-1 scFv (data not shown). So GSH-GSSG was not added in the second gradient. It may be that the first gradient helps to form the disulfide bonds. The stage is a critical refolding period for native disulfide bond formation, not the post refolding stage. Due to the lack of Fc domain in the structure of scFv, the refolded scFv did not bind with common HRP-conjugated second antibody. In the noncompetitive ELISA experiment, we prepared second antibody (rabbit anti-mouse IgG antibody) which can bind to scFv.

Conclusion

A high-level production of scFv in the *E. coli* expression system has been successfully established, and an inexpensive convenient refolding strategy for scFv recovery has also been developed. We could obtain 60 mg of active scFvs from 1 liter cultivation of *E. coli* cells by fermentation. The established on-column refolding procedure for the efficient recovery of anti-ICAM-1 scFv from the inclusion bodies had a practical significance for further research on other scFv or recombinant protein. The advantages of this method include the biophysical and biochemical characteristics, and the fact that the purified scFv can markedly suppress the MCs adhesion to LPS-stimulated ECV-304 monolayers. Moreover, we have established the mouse model of aseptic inflammation and showed that the scFv significantly inhibited the inflammatory swelling of auricle in mice induced by dimethyl benzene. The renaturation process was significant; it is possible that the IEC could be very useful in refolding inclusion body protein on a large scale but refolding efficiency still needs to improve so as to magnify its commercial application on other recombinant proteins.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

We express our appreciation to Cairong Jiang, Ph.D, who revised the manuscript. This work was supported by a grant from the Academy of Military Medical Sciences of the People's Liberation Army (20060929).

REFERENCES

Alexiou D, Karayiannakis AJ, Syrigos KN, Zbar A, Kremmyda A, Bramis I, Tsigris C (2001). Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients:correlations with clinicopathological features, patient survival and tumour surgery. Eur. J. Cancer 37(18):2392-2397.

Aronni S, Cortes M, Sacchetti M, Lambiase A, Micera A, Sgrulletta R, Bonini S (2006). Upregulation of ICAM-1 expression in the conjunctiva of patients with chronic graft-versus-host disease. Eur. J. Ophthalmol. 16(1):17-23.

Beck-Schimmer B, Madjdpour C, Kneller S, Ziegler U, Pasch T, Wüthrich RP, Ward PA, Schimmer RC (2002). Role of alveolar epithelial ICAM-1 in lipopolysaccharide-induced lung inflammation. Eur. Respir. J. 19:1142-1150.

Bowes MP, Zivin JA, Rothlein R (1993). Monoclonal antibody to the ICAM-1 adhesion site reduces neurological damage in a rabbit cerebral embolism stroke model. Exp. Neurol. 119(2):215-219.

Bradford MM (1976). A rapid method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 7:248-254.
Chaudhuri JB (1994). Refolding recombinant proteins: process strategies and novel approaches. Ann. N.Y. Acad. Sci. 721:374-385.
Chen LH, Huang Q, Wan L (2006). Expression, purification, and *in vitro* refolding of a humanized single-chain Fv antibody against human CTLA4 (CD152). Protein Expr. Purif. 46:495-502.
Coskun U, Sancak B, Sen I, Bukan N, Tufan MA, Gulbahar O, Sozen S (2006). Serum P-selection soluble vascular cell adhesion molecule-1(sVCAM-1) and soluble intercellular adhesion molecule-1 (sICAM-1) levels in bladder carcinoma patients with different stages. Immunopharmacology 6(4):672-677.
Dustin ML, Rothlein R, Bhan AK, Dinarello CA, Springer TA (1986). Induction by IL-1 and interferon-gamma: tissue distribution, biochemistry, and function of a natural adherence molecule (ICAM-1). J. Immunol. 137:245-254.
Dustin ML, Staunton DE, Springer TA (1988). Supergene families meet in the immune system. Immunology 9(7-8):213-215.
Fahey EM, Chaudhuri JB, Binding P (2000). Refolding and purification of a urokinase plasminogen activator fragment by chromatography. J. Chromatogr. B. 737(1-2):225-235.
Gu ZY, Weidenhaupt M, Ivanova N, Pavlov M, Xu B, Su ZG, Janson JC (2002). Chromatographic methods for the isolation of, and refolding of proteins from. *Escherichia coli* inclusion bodies. Protein Expr. Purif. 25:174-179.
Kaufmann P, Tilz GP, Smolle KH, Demel U, Krejs GJ (1996). Increased plasma concentrations of circulating intercellular adhesion molecule-1(ICAM-1) in patients with necrotizing pancreatitis. Immunobiology 95(2):209-219.
Lanckriet H, Meddelberg AP (2004). Continuous chromatographic protein refolding. J. Chromatogr. A 1022(1-2):103-113.
Müller C, Rinas U (1999). Renaturation of heterodimeric platelet-derived growth factor from inclusion bodies of recombinant *Escherichia coli* using size-exclusion chromatography. J. Chromatogr. A 855(1):203-213.
Sadowska AM, van Overveld FJ, Luyten C, Germonpré P, De Backer WA (2004). Use of ICAM-1 antibodies and antisense oligonucleotides to inhibit transmigration of neutrophils. Inflamm. Res. 53(4):143-149.
Schoemaker JM, Brasnett AH, Marstion FAO (1985). Examination of calf prochymotrypsin accumulation in *Escherichia coli*: disulfide linkages are a structural component of prochymotrypsin-containing inclusion bodies. EMBOJ 4:775-780.
Souza-Moraes MR, David-Filho R, Baptista-Silva JC, Ullian M, Franco MF, Gabriel Jr A, Smith B, Burihan E (2003). Effect of antibodies to intercellular adhesion molecule type 1 on the protection of distant organs during reperfusion syndrome in rats. J. Med. Biol. Res. 36(5):605-612.
Sun H, Wan ZH, Zhang GL, Wu GM, Zhu P, Yue YH (2008). Construction and expression of anti-human ICAM-1 scFv in *E.coli*. Chin. J. Biologicals 21(5):82-86.
Sun H, Wan ZH, Zhang GL, Wu GM, Zhu P, Yue YH (2008). Preparation of monoclonal antibody against human ICAM-1 and identification of its activity. Chin. J. Biologicals 21(4):308-311.
Takai T, Takaoka M, Yasueda H, Okumura K, Ogawa H (2005). Dilution method to refold bacterially expressed recombinant Der f 2 and Der p 2 to exhibit the secondary structure and histamine-releasing activity of natural allergens. Int. Arch. Allergy Immunol. 137(1):1-8.
Tan PH, Sandmaier BM, Stayton PS (1998). Contributions of ahighly conserved VH/VL hydrogen bonding interaction to scFv folding stability and refolding efficiency. Biophys. J. 75:1473-14821.
Thomas GJ, Speight PM (2001). Cell adhesionmolecules and oral cancer [J]. Crit. Rev. Oral. Biol. Med. 12:479-498.
Vainer B, Horn T, Nielsen OH (2006). Colonic epithelial cell expression of ICAM-1 relates to loss of surface continuity: a comparative study of inflammatory bowel disease and colonic neoplasms. Scand. J. Gastroenterol. 41(3):318-325.
Verma R, Boleti E, George AJ (1998). Antibody engineering: comparison of bacterial yeast insect and mammalian expression systems. J. Immunol. Methods 216(1-2):165-181.
West SM, Chaudhuri JB, Howell JA (1998). Improved protein refolding using hollow-fibre membrane dialysis. Biotechnol. Bioeng. 57(5):590-599.
Wetlaufer DB, Branca PA, Chen G (1987). The oxidative folding of proteins by disulfide plus thiol does not correlate with redox potential. Protein Eng. 1:141-146.
Yokota T, Milenic DE, Whitlow M, Schlom J (1992). Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. Cancer Res. 52:3402-3408.
Zhao X, Song GM, Xu JL, Wang XJ, Song HM, Qu X (2007). Expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule in cardiac allografts and significance thereof: experiment with rats. Zhonghua Yi Xue Za Zhi 87(37):2657-2659.