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Improvement of soluble expression of GM-CSF in the cytoplasm of *Escherichia coli* using chemical and molecular chaperones

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ABSTRACT

The most common approaches to improve soluble expression of heterologous proteins are applications of molecular chaperones such as DnaK, DnaJ, GrpE, GroEL and GroES. The aim of present study was to enhance soluble expression of granulocyte-macrophage colony-stimulating factor (GM-CSF) in *Escherichia coli* by different approaches including modification of cultivation and induction conditions, and thermally, genetically and chemically enhancement of expression of cellular chaperones. To genetically enhance amount of molecular chaperones, co-expression of pET28-GM-CSF and pKJE7 plasmids was performed. The soluble expressed protein was affinity purified and subjected to endotoxin removal. Co-expression with molecular chaperones significantly increased soluble expression of GM-CSF. Addition of chemical chaperones and osmolytes like NaCl (0.5 M), sucrose (0.5 M), sorbitol (0.5 M) and $MgCl_2$ (1 mM) to growing media could improve solubility of GM-CSF. Biological activity of purified GM-CSF was confirmed based on its proliferative effect on HL-60 cell lines. The approach developed in the present study can be applied to improve soluble expression of other recombinant protein proteins.

1. Introduction

Cytokines are soluble and small proteins secreted by different type of cells, mainly certain cells of the immune system. Cytokines are involved in cell signaling and can regulate proliferation, differentiation, movement and responsiveness of other cells [1]. Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine which specifically plays an important role in proliferation and maturation of neutrophil and is mainly used for treatment and prevention of neutropenia in patients who undergo chemotherapy [2]. Recombinant human GM-CSF has been produced in different expression systems including *Escherichia coli* (*i.e.,* molgramostim), *Saccharomyces cerevisiae* (*i.e.,* sargramostim) and Chinese hamster ovary cells (*i.e.,* regramostim) [3].

E. coli is the expression system of choice for production of many recombinant proteins including GM-CSF, both on laboratory and industrial scale, due to its unparalleled advantages. *E. coli* grows uniquely fast, easily reaches high cell densities and does not need expensive and unavailable culture media [4,5]. In addition, it readily accepts foreign genetic materials [6]. Its genetics and molecular biology are well-recognized [7,8]. Finally, its bioprocess can be easily scaled-up [9].

However, as a prokaryotic expression system, *E. coli* shows some serious drawbacks. Since bacterial hosts lack advanced post-translational modifications and eukaryotic chaperones, the heterologous protein may be produced in an insoluble state and aggregated as inclusion bodies [10]. Inclusion body aggregates can be solubilized and refolded to soluble, bioactive protein; nevertheless, the refolding process is burdensome and usually results in low-yield recovery [11]. Furthermore, the refolding conditions must be optimized for each specific protein and *in vitro* solubilization may affect the integrity of protein [12]. Thus, employing methods to enhance soluble protein expression is generally preferred to refolding aggregated protein from inclusion bodies [13].

The most common approaches to improve soluble expression of protein are applications of molecular chaperones such as DnaK, DnaJ, GrpE, GroEL and GroES [14]. Chaperones facilitate correct folding of the nascent polypeptide and also refold and solubilize misfolded and aggregated proteins. There are some reports on the improvement of soluble protein production by reduction of rate of protein synthesis and enhancement of chaperone molecule levels by decreasing cultivation temperature and inducer concentration [15], addition of chemical chaperones like osmolytes [16], application of thermal shock [17] and co-expression of the recombinant protein with chaperones [18].

The purpose of present study was to improve soluble expression of recombinant human GM-CSF in *E. coli* BL21 (DE3) by enhancement of cellular concentration of chaperones. We compered effectiveness of different approaches including modification of cultivation and

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induction conditions, and thermally, genetically and chemically induced expression of cellular chaperones for soluble expression of GM-CSF.

2. Materials and methods

2.1. Bacterial strain and plasmids

Escherichia coli BL21 (DE3) (Novagen, USA) was used as a host strain for expression of GM-CSF. The recombinant pET28-GM-CSF plasmid was constructed in our previous study [19] and pKJE7 plasmid was obtained from Takara (Shiga, Japan). pET28-GM-CSF consists of a codon optimized synthetic gene of molgramostim for its expression in *E. coli* and a hexa-histidine tag was linked via a TEV protease cleavage sequence to the N terminus of GM-CSF protein.

2.2. General expression of GM-CSF

E. coli BL21 (DE3) chemically competent cells were transformed with pET28-GM-CSF using heat shock method and were grown on Luria Bertani (LB) agar plates containing 25 μg/ml kanamaycin. A single positive colony was inoculated into LB broth containing antibiotic and incubated overnight. This culture was added to the fresh LB medium at a ratio of 1:10 and incubated at 37 °C and 180 rpm until the optical density at 600 nm (OD_{600}) reached 0.6. Isopropyl β-D-1-thiogalactopyranoside (IPTG) was then added at a final concentration of 0.5 mM to induce expression of GM-CSF. The culture was incubated under the same conditions for 4 h. Samples were collected before and 1, 2, and 4 h after induction store at −70 °C for further analysis.

2.3. Effect of cultivation temperature and IPTG concentration on soluble expression of GM-CSF

E. coli BL21 (DE3) harboring pET28-GM-CSF cells were cultivated overnight. The overnight culture was used to inoculate into LB broth containing kanamycin and incubated at 37 °C and 180 rpm until reaching exponential phase. The cultures were adapted to the different induction temperature (37, 30, 25, 20 and 15 °C) by incubation at that temperature for 30 min; then the protein expression was started by addition of different IPTG concentrations (0.1, 0.25, 0.5, 1 and 2 mM) and the cultures were further incubated for 3, 4.5, 18, 18 and 18 h at 37, 30, 25, 20 and 15 °C, respectively.

2.4. Effect of medium additives on soluble expression of GM-CSF

To evaluate the effect of chemical chaperones on soluble expression of GM-CSF, some chemical additives were added to LB medium (Table 1). The overnight culture of recombinant cells were inoculated into to modified LB media and incubated at 37 °C at 180 rpm until reaching logarithmic growth phase. However, benzyl alcohol, glycerol and betaine were added to the growth medium 20 min before induction.

Table 1
Chemical additives which added to LB broth to improve soluble expression of GM-CSF.

Additive	Concentration
Glucose	0.5% W/V
NaCl	0.5 M
Sorbitol	0.5 M
Sucrose	0.5 M
Glycerol	0.1% V/V
Ethanol	1% V/V
PBS	0.5 M
$MgCl_2$	1 mM
Betaine	1 mM
Benzyl alcohol	0.1% V/V

Then, the cultures were incubated under following conditions: induction with 0.5 mM IPTG at 37 °C at 180 rpm for 3 h or induction with 1 mM IPTG at 20 °C at 180 rpm for overnight.

2.5. Effect of thermal-shock on soluble expression of GM-CSF

The overnight culture of recombinant cells were inoculated into to fresh LB broth and grown at 37 °C at 180 rpm until reaching exponential phase. For cold-shock treatment the culture was incubated on ice (4 °C) and for heat-shock treatment the culture was transferred into a 45 °C water bath. After 20 min, IPTG was added to both samples at a final concentration of 0.5 mM and the cultures were incubated at 37 °C, while shaking at 180 rpm, for 3 h.

2.6. Effect of co-expression with molecular chaperones on soluble expression of GM-CSF

E. coli BL21 (DE3) electrocompetent cells were co-transformed with pET28-GM-CSF and pKJE7 plasmids using electroporation method (voltage = 2500 V, capacitor = 25 μF, resistance = 200Ω) and were grown on LB agar plates containing 25 μg/ml kanamaycin and 34 μg/ml chloramphenicol. A positive colony was grown in LB broth containing antibiotics and incubated overnight. This culture was used for expression of protein under two different conditions. First, the overnight culture was inoculated into LB medium containing 0.5 mg/ml arabinose and incubated at 37 °C and 180 rpm until reaching exponential phase. The expression of protein was initiated by adding of 0.5 mM IPTG and incubation at 37 °C for 3 h. Second, the overnight culture was added into fresh LB medium and incubated at 37 °C and 180 rpm until reaching to OD_{600} of 0.6. Then, expression of GM-CSF and molecular chaperones were simultaneously induced by addition of 0.5 mM IPTG and 0.5 mg/ml arabinose and the culture was shaked at 180 rpm and 37 °C for 3 h.

2.7. Analytical methods

At the final time of protein expression, the cultures were centrifuged at 7,000 × *g* for 15 min and cellular pellets were collected. The pellets were subjected to cell disruption using a Micro Smash MS-100 (Tomy, Japan) as described previously [20]. Soluble and insoluble fractions were separated by centrifugation at 12,000 *g* for 30 min. The samples were analyzed by 15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Amount of soluble protein was estimated by densitometry analysis of the gels using TL120 software (Nonlinear Inc, Durham NC, USA). The soluble protein was subjected to purification using Ni-NTA affinity column, as described previously [21]. The endotoxin removal was performed using Triton X-114 extraction [22]. The concentration of GM-CSF after purification and endotoxin removal was estimated using Bradford method [22]. The secondary structure of the obtained GM-CSF was assessed by circular dichroism (CD) spectroscopy using a J-810 spectropolarimeter (JASCO, USA). Spectra were recorded at room temperature under following conditions: cell length, 1 cm; scanning speed, 200 nm/min; data pitch, 0.2 nm; sensitivity, standard; bandwidth, 1 nm. The CD profile of expressed GM-CSF in the far UV rang (190–250 nm) was compared with CD pattern of standard GM-CSF.

The proliferative effect of produced GM-CSF on HL-60 cell line was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay as described previously [21]. Briefly, cells were seeded in 96-well plate (1×10^4 cell/well). Then, standard (R&D systems, USA) and samples at various concentrations were added and the plate was kept at 37 °C in 5% CO_2 for 48 h. To each well, 20 μl of MTT solution (5 mg/ml) was added to each well and incubated for 3 h. Finally, 150 μl DMSO was added to each well to dissolve formazan crystals and the absorbance was measured at 540 nm by a microplate reader (Bio-Tek®,USA). The curve was drawn by plotting cell survivals

(responses) against concentrations (doses). Curves were calculated using a sigmoidal, four parameter logistic fit in the GraphPad software (USA) using following equation:

Y = A + (B-A)/(1 + 10^((LogEC50-X)*C))where A is the minimum signal, B is the maximum signal, C is the slope and EC50 is the concentration of GM-CSF that has 50% of the maximal proliferative effect which defined as one unit of activity. Specific activity was determined as following:

Specific activity (IU/μg) = 1/EC50 (pg/ml) * 10^6 (1 μg = 10^6 pg).

Relative potency (ratio of the potency of the sample to the potency of the standard) was estimated using a parallel line assay software (PLA).

3. Results

3.1. General expression of GM-CSF

The first experiments under general induction conditions (*i.e.,* 0.5 mM IPTG at 37 °C and 180 rpm) showed that GM-CSF could be successfully expressed in *E. coli* BL21 (DE3) under the control of T7 promoter. The apparent molecular weight of GM-CSF (15 kDa) on SDS-PAGE (Fig. 1) confirmed the expression of full-length protein. Four hours after induction, the expressed protein represented 25% of the total protein (Fig. 1).

3.2. Effect of cultivation temperature and IPTG concentration on soluble expression of GM-CSF

To evaluate the effect of temperature on soluble protein expression, induction was carried out at different temperatures 37, 30, 25, 20 and 15 °C; and in each temperature, the effects of five concentrations of inducer on protein expression were also evaluated (*i.e.*, 0.1, 0.25, 0.5, 1 and 2 mM). SDS-PAGE analysis showed soluble expression of GM-CSF improved when induction temperature increased from 20 °C to 30 °C but then reduced at 37 °C (Fig. 2). The results revealed higher soluble GM-CSF achieved at 0.5–2 mM IPTG. Therefore, the following inductions were performed with 1 mM IPTG at lower temperature or 0.5 mM IPTG at higher temperature.

3.3. Effect of medium additives on soluble expression of GM-CSF

Different chemical additives were added to culture medium and induction of protein was carried out at two temperature levels. SDS-PAGE analysis (Fig. 3a) revealed some compounds like sucrose (0.5 M), $MgCl_2$ (1 mM) and sorbitol (0.5 M) significantly improved soluble expression of GM-CSF at 20 °C. As shown in Fig. 3, addition of osmolytes like sorbitol (0.5 M) and NaCl (0.5 M) can increase soluble expression of GM-CSF at 37 °C (Fig. 3b).

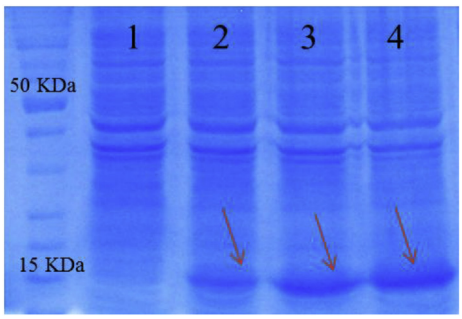

Fig. 1. SDS-PAGE analysis of GM-CSF expression. Total protein from *E. coli* BL21 (DE3) containing pET28-GM-CSF plasmid before induction (*lane 1*) and after induction with 1 mM IPTG for 1 h (*lane 2*), 2 h (*lane 3*) and 4 h (*lane 4*) at 37 °C in LB broth. GM-CSF (15 kDa) is denoted by arrows.

3.4. Effect of thermal-shock on soluble expression of GM-CSF

Effect of thermal-shock on expression of soluble GM-CSF was evaluated and the samples were analyzed by SDS-PAGE. Both heat shock and cold shock did not significantly improve soluble expression of GM-CSF (Fig. 4a).

3.5. Effect of co-expression with molecular chaperones on soluble expression of GM-CSF

GM-CSF was co-expressed with pKJE7 which encoded dnaK, dnaJ and grpE (Fig. 4b). Our finding showed molecular chaperones could improve expression of soluble GM-CSF. Interestingly, the amount of soluble protein significantly (up to 3 fold) increased when expression of chaperones induced before addition of IPTG in comparison with the condition that both inducers (*i.e.,* arabinose and IPTG) were added at the same time.

3.6. Purification, endotoxin removal, conformational integrity and bioassay

GM-CSF was successfully purified using Ni-NTA affinity column and subjected to Triton X-114 based endotoxin extraction. SDS-PAGE and western analysis revealed endotoxin removal led to significant protein loss (40%) (Fig. 5). After purification and endotoxin removal, the obtained GM-CSF showed near 95% purity and a final yield of approximately 9 mg/L was achieved (Table 2).

The secondary structure of soluble GM-CSF expressed under the optimum condition was evaluated by CD. The CD pattern of expressed protein was very similar to that of the standard GM-CSF (Fig. 6). This result suggests that secondary structures (*e.g,* alpha helix and beta sheet) were correctly formed, and conformational integrity and primary refolding of GM-CSF were preserved.

The biological activity of purified and endotoxin removed GM-CSF on HL-60 cell line was evaluated by MTT assay and the specific activity of produced GM-CSF was 1.2×10^4 IU/μg with the relative potency of 0.9 as calibrated with standard GM-CSF (Fig. 7).

4. Discussion

GM-CSF is a hematopoietic growth factor with different applications including mobilization of hematopoietic stem cells after bone marrow transplantation, neutropenia induced by chemotherapy, oral mucositis, wound healing and immunotherapy of cancer [23–25]. Due to its wide range of clinical applications, high yield and cost-effective production of this protein have attracted considerable attention. Overexpression of GM-CSF in eukaryotic systems usually led to formation of insoluble and inactive protein [26].

Previous studies evaluated different strategies to improve soluble expression of GM-CSF. One of the most successful approaches was application of fusion proteins including thioredoxin and intein [27,28]. However, it is necessary to remove the fusion partner by enzymatic or chemical treatments and to perform further purification processes to avoid contamination of target protein with the fusion protein. Additionally, most of cleavage methods left some extra amino acids which could affect efficacy and safety of therapeutic proteins [29]. So, fusion-tag approach especially at large scale in industrial bioprocessing is not commonly used.

Alternatively, soluble expression of recombinant protein can significantly improve by co-expression of target protein with molecular chaperones. Chaperones facilitate correct folding process of newly synthesized polypeptide and also unfolding and refolding of misfolded proteins [30]. Therefore, aggregation of overexpressed heterologous protein may be due limited capacity of chaperones of prokaryotic host cells [31]. There are some reports on improvement of soluble production of proteins by co-expression with molecular chaperones using different plasmid sets including pG-KJE8, pGro7, pKJE7, pG-Tf2 and

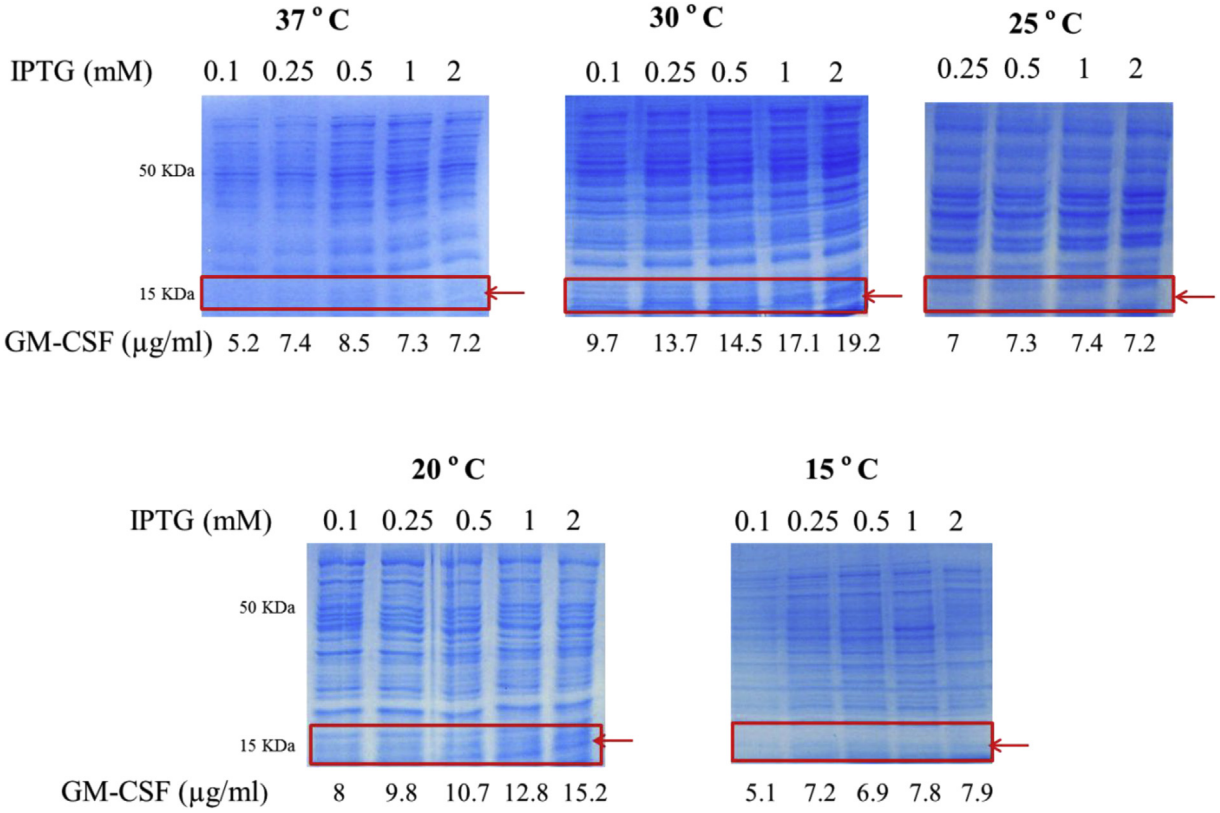

Fig. 2. SDS-PAGE analysis of soluble expression of GM-CSF at different temperatures with various concentrations of IPTG in LB broth. GM-CSF (15 kDa) is denoted by arrows. The intensity of targeted band in the soluble fraction was analyzed by densitometry of the gels to estimate the level of soluble expression for GM-CSF.

pTf16 [32]. In the present study, pKJE7 plasmid was used to express molecular chaperones dnaK/dnaJ/grpE under the control of the *araB* promoter. dnaK, dnaJ and grpE are members of a heat-shock (stress) proteins family (HSP70) which involves in different cellular functions including disaggregation and unfolding of aggregates, stabilization and folding of nascent polypeptide and translocation across cellular compartments [33]. Our results showed co-expression with dnaK, dnaJ and grpE led to a significant increase in soluble expression of GM-CSF especially when expression of molecular chaperones started before expression of the protein. Similar to our findings, Thomson et al., suggested accumulation of chaperons before starting protein expression depending on the type of chaperon promoter [34]. They induced expression of chaperones (*e.g.*, pKJE7) under control of the *araB* promoter from inoculation and reported a significant improvement of soluble expression of polyhydroxyalkanoate synthase [34].

Alternatively, some chemicals like salts, sugars, amino acids and

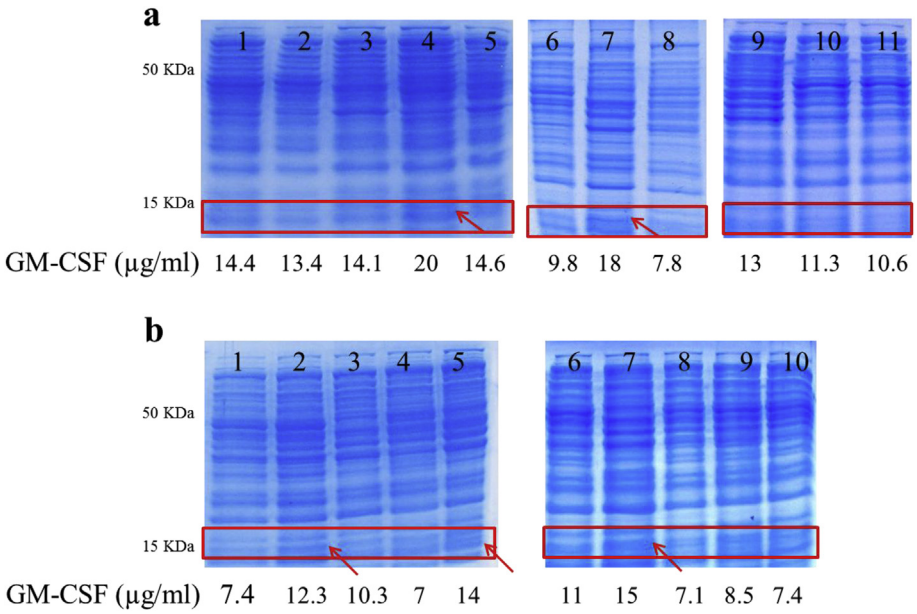

Fig. 3. SDS-PAGE analysis of soluble expression of GM-CSF in LB broth supplemented with different additives. **a)** Protein expression was induced with 0.5 mM IPTG at 37 °C for 3 h in simple LB (*lane 1*), LB supplemented with 1 mM $MgCl_2$ (*lane 2*), LB supplemented with 0.5 M glucose (*lane 3*), LB supplemented with 0.5 M NaCl (*lane 4*), LB supplemented with 0.5 M sucrose (*lane 5*), LB supplemented with 10% glycerol (*lane 6*), LB supplemented with 0.5 M sorbitol (*lane 7*), LB buffered with PBS (*lane 8*), LB supplemented 0.1% benzyl alcohol (*lane 9*), LB supplemented with 1 mM betaine (*lane 10*) or LB supplemented with 1% ethanol (*lane 11*). **b)** Protein expression was induced with 1 mM IPTG at 20 °C overnight in simple LB (*lane 1*), LB supplemented with 1 mM $MgCl_2$ (*lane 2*), LB supplemented with 0.5 M glucose (*lane 3*), LB supplemented with 0.5 M NaCl (*lane 4*), LB supplemented with 0.5 M sucrose (*lane 5*), LB supplemented 0.1% benzyl alcohol (*lane 6*), LB supplemented with 0.5 M sorbitol (*lane 7*), LB supplemented with 1 mM betaine (*lane 8*), LB supplemented with 10% glycerol (*lane 9*) or LB buffered with PBS (*lane 10*). GM-CSF (15 kDa) is denoted by arrows. The intensity of targeted band in the soluble fraction was analyzed by densitometry of the gels to estimate the level of soluble expression for GM-CSF.

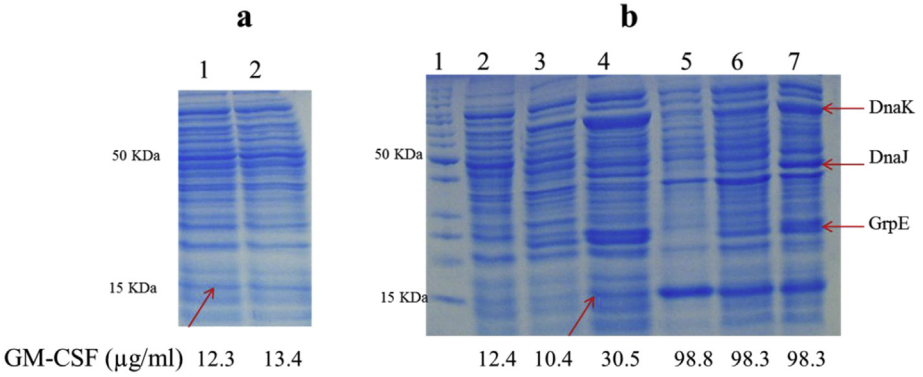

Fig. 4. SDS-PAGE analysis of soluble expression of GM-CSF with 0.5 mM IPTG at 37 °C in LB broth. **a)** before induction the culture was exposed to heat-shock (*lane 1*) or to cold-shock (*lane 2*) **b)** Soluble protein from *E. coli* BL21 (DE3) containing pET28-GM-CSF plasmid after induction with 0.5 mM IPTG at 37 °C (*lane 1*), soluble protein from *E. coli* BL21 (DE3) containing pET28-GM-CSF and pKJE7 plasmids after induction simultaneously with 0.5 mM IPTG and 0.5 mg/ml arabinose at 37 °C (lane 2), soluble protein from *E. coli* BL21 (DE3) containing pET28-GM-CSF and pKJE7 plasmids when 0.5 mg/ml arabinose was added from inoculation and 0.5 mM IPTG was added after reaching logarithmic phase (lane 3), total protein from *E. coli* BL21 (DE3) containing pET28-GM-CSF plasmid after induction with 0.5 mM IPTG at 37 °C (*lane 4*), total protein from *E. coli* BL21 (DE3) containing pET28-GM-CSF and pKJE7 plasmids after induction simultaneously with 0.5 mM IPTG and 0.5 mg/ml arabinose at 37 °C (*lane 5*) and total protein from *E. coli* BL21 (DE3) containing pET28-GM-CSF and pKJE7 plasmids when 0.5 mg/ml arabinose was added from inoculation and 0.5 mM IPTG was added after reaching logarithmic phase (*lane 6*). The intensity of targeted band in crud extract was analyzed by densitometry of the gels to estimate the level of expression for GM-CSF.

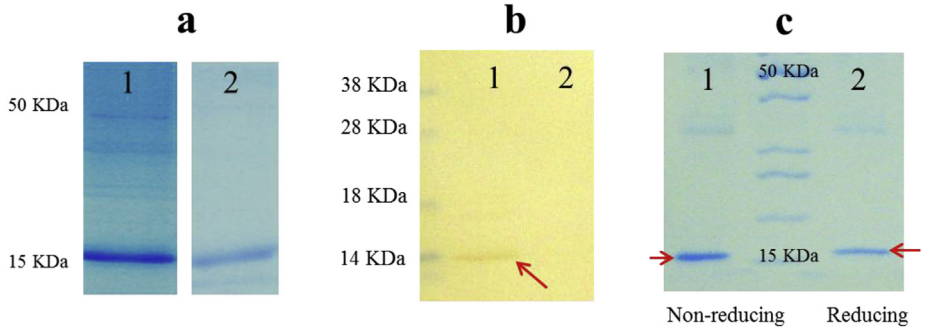

Fig. 5. a) SDS-PAGE analysis of purified soluble GM-CSF (*lane 1*) and endotoxin removed GM-CSF (*lane 2*). **b)** Western blot analysis with anti-his antibody: purified soluble GM-CSF (*lane 1*) and total protein from *E. coli* BL21 (DE3) containing plasmid pET28-GM-CSF before induction (negative control) (*lane 2*).

Table 2
Summary of the yields of GM-CSF protein during procedures of purification and endotoxin removal.

Procedure	GM-CSF (mg)	Purity (%)	Yield (%)
Whole cell lysate[a]	5	25	100
Soluble protein	1.5	3.5	30
Native NIT purification	0.75	92	15
Endotoxin removal	0.45	95	9

[a] From pellet obtained from 50 ml cell culture.

polyoles are used to improve soluble expression of heterologous proteins [35]. These compounds also known as osmolytes when added to growth media at sufficient concentration could lead to increase in osmotic pressure. As a response, bacterial cells synthesize or uptake the osmolytes to adopt with osmotic shock [36]. Osmolytes can stabilize the native conformation of protein so they are also called chemical chaperons or pseudochaperones. In the present study, addition of osmolytes like NaCl, betaine, sucrose and sorbitol to the culture medium led to improvement of solubility of GM-CSF. In agreement with our results, de Marco et al., reported addition of NaCl (0.5 M) and betaine (5 mM) to culture media resulted in up to 5.5 folds enhancement in soluble expression of O36 protein [37]. They also found that increase in soluble expression of dnak (*i.e.*, 2.5 fold) following addition of the osmolyte was less than the target protein. Therefore, they concluded that the effect of osmolytes on soluble expression of proteins is not only limited to enhancement of molecular chaperones production [37]. Prasad et al., also evaluated the effect of sorbitol on solubilization of GFP and reported the concentration of the osmolyte and pH of medium significantly influencing the solubility of the target protein [35]. Our results showed that addition of magnesium (1 mM) also increased soluble expression of GM-CSF at 20 °C. The positive effect supplementing the culture media with some metal ions such as magnesium on solubility of some recombinant proteins was reported in some previous studies. Sina et al., reported that addition of magnesium chloride to media led to increase of soluble expression of *anti*-TNF-α single chain variable fragment (scFv) in *E. coli* Origami (DE3) [38]. They concluded that magnesium might act as a cofactor for correct folding of recombinant protein.

Bacterial cells produce heat-shock proteins, as a defense mechanism in response to thermal stress. Many of these proteins are molecular chaperones that protect native proteins from misfolding and aggregation. Thus, one potential approach for improvement of soluble production of recombinant proteins is applying heat-shock or cold-shock before induction of expression [39]. However, this approach did not significantly improve solubility of GM-CSF. In agreement with our finding other studies reported that heat-shock did not influence soluble expression of the recombinant protein [38].

Modification of cultivation temperature could also result in improvement of soluble protein expression. In the present study, the production of protein at 30 °C and 20 °C significantly increase the soluble expression of GM-CSF. Other studies have previously reported more soluble expression of proteins at temperature around 30 °C as the activity and expression of molecular chaperones enhanced at this temperature [40]. Expression of protein at reduced temperature like 20 °C could also increase soluble expression and decreases formation of misfolded proteins (*i.e.*, inclusion bodies).

Another approach to produce soluble GM-CSF is to recover and refold active protein from inclusion bodies. Thomson et al. reported efficient refolding of GM-CSF by dialysis against a buffer containing arginine, reduced and oxidized glutathione. They could achieve to a final yield of approximately 7 mg/L bioactive protein [41]. In the

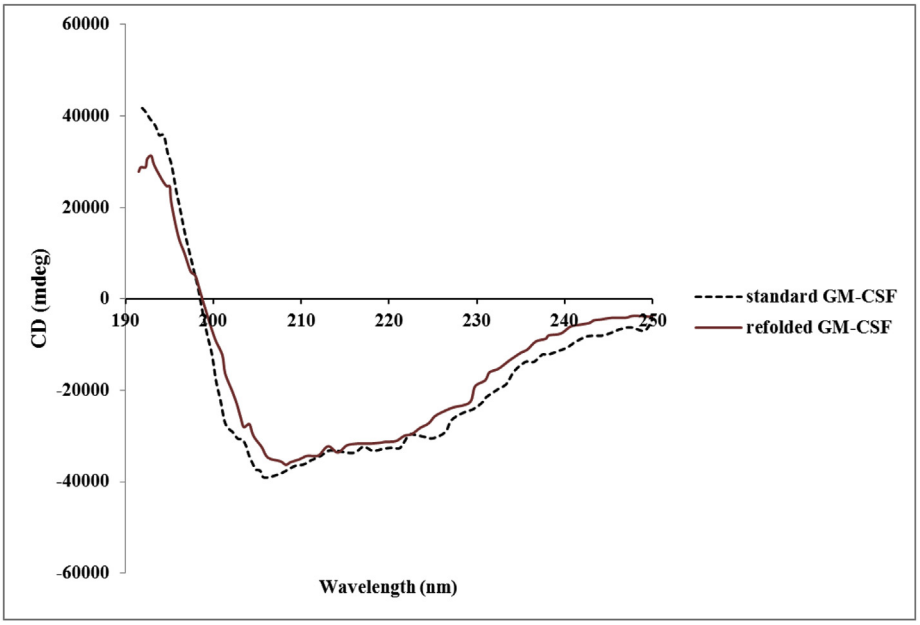

Fig. 6. Far-UV CD spectra of expressed GM-CSF compared with standard GM-CSF.

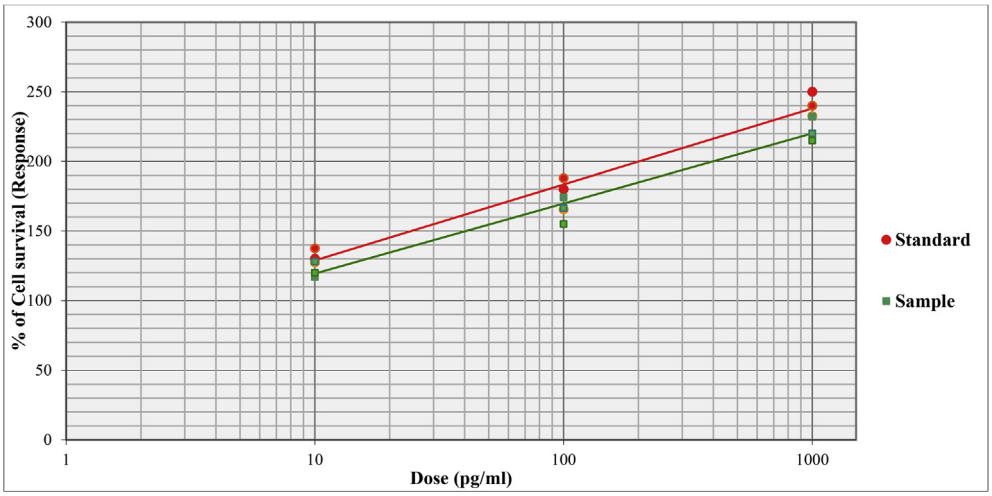

Fig. 7. Parallel line assay of the biological activity of GM-CSF on HL-60 cell line.

current work, instead of refolding of the protein from inclusion bodies which is a difficult and time-consuming task, we applied different strategies to improve soluble expression and prevent formation of inclusion bodies which resulted in the yield 9 mg bioactive GM-CSF from 1 L of culture under the optimum condition.

5. Conclusion

Here, we compared different strategies to enhance soluble expression of GM-CSF. Significant improvement of soluble expression of GM-CSF in *E. coli* BL21 (DE3) could be achieved by genetically enhancement of cellular concentration of chaperones. Furthermore, addition of chemical chaperones like NaCl, sucrose, sorbitol and betaine to growing media could increase solubility of GM-CSF. Additionally, reducing inducer concentration and expression temperature can improve soluble expression of the protein probably due to inhibition of inclusion body formation. The soluble GM-CSF was subjected to purification and endotoxin removal. Biological activity of purified GM-CSF was confirmed based on its proliferative effect on HL-60 cell lines. The approach developed in the present study can be applied to improve soluble expression of other recombinant protein proteins.

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