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Original Article

New Strategy for Solubilization and Refolding of Recombinant Human Interferon α2b Inclusion Bodies from *E. coli* Gene Overexpression System

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ABSTRACT

Objective: New Strategy for solubilization and refolding of recombinant human Interferon α2b inclusion bodies was established to obtain a high solubilization and refolding rate of recombinant human Interferon α2b inclusion bodies from *E. coli* Gene Overexpression System.**Method:** The IFN-α2b inclusion bodies were solubilized with solubilizing buffer of 2mol/L urea. Refolding was performed via two steps, diluting by pulse adding the solubilized IFN α2b sample and dialysing it slowly using ultrafiltration 5K step by step. Finally refolded IFN α2b was purified through Cu^{2+} chelate affinity chromatography. **Results:** The urea concentration of 2mol/L in the solubilizing buffer gave the solubilization rate of 89.73%. Diluting the solubilized IFN α2b sample by pulse adding gave the refolding rate of 78.53%. The multi steps dialysis through ultrafiltration membrane 5K gave the antiviral activity recovery of 98.73%. The purification through one step Cu^{2+} chelate affinity chromatography raised the specific activity of IFN sample to 1.4×10^8U/mg protein and 12% SDS-PAGE showed single band of purified IFN- α2b at expected MW height, whose purity was 99.8%. **Conclusion:** Large-scale production of recombinant human Interferon α2b (IFN-α2b) in *E. coli* with a thermoinducible overexpression system was established by applying an effective solubilizing and refolding processes of interferon α2b inclusion bodies (IBs). Solubilization and refolding rate of IFN-α2b IBs were 89.71% and 79.87% respectively. Refolded IFN-α2b was purified through one-step immobilized metal affinity chromatography to give a pure bioactive IFN-α2b with the specific activity of 1.4×10^8 IU/mg protein and with the recovery rate over 52.89%.

Key words: Recombinant human interferon (rhIFN), Inclusion body (IB), Refolding, Protein purification.

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INTRODUCTION

Recombinant human interferon α2b is a cytokine which has antiviral, antiproliferative and immunomodulator activities. IFN-α2b has been used mainly as a drug for treatment of hepatitis of B and C virus infections. *E. coli* is the most commonly used host for the production of recombinant proteins. High-cell-density culture has been developed as a widely used method for enhancing productivity of desired bioproducts in *E. coli*.[1] However, high expression levels of recombinant proteins in *E. coli* often lead to the formation of inclusion bodies.[2-3] Thus, a protein refolding procedure is usually necessary.[4-5] A good recovery of the recombinant protein production results from a high yield of solubilizing and refolding of inclusion bodies. In general, inclusion bodies are solubilized by the use of a high concentration of denaturants such as urea or guanidine, along with a reducting agent such as β-mercaptoethanol.[6,7,4] Solubilized proteins are then refolded by slow removal of the denaturant in the presence of oxidizing agent.[8,4] Protein solubilization using high concentration of denaturants leads to the loss of secondary structure and bioactivity due to random coil formation of protein structure and exposure of the hydrophobic surface.[9] It results in low yield of refolding. With new information, protein aggregates inside inclusion bodies have native-like secondary structure. It is assumed that maintenance of this native-like secondary structure by use of mild solubilization buffer will help in improved recovery of bioactive protein in comparison to solubilization under a high concentration of chaotropic agent due to more easy restoration of native active structure.[10] Rudolph and Fischer developed a pulse refolding method which decreased the refolding volumes by stepwise addition of solubilized protein into the refolding buffer.[11] By keeping the protein concentration at low level in each aliquot, the high final refolding yields at high final protein concentrations were then obtained. Another simple and efficient means to prevent aggregation is the addition of aggregation inhibitors. The commonly used additives are L-arginine, urea, glycerol, Triton X-100, polyethylene glycol, and some denaturants at low concentration.[12] In this study, we established a simplified productive process for IFN-α2b production. We applied a new strategy for solubilization and refolding of IFN IBs by using a solubilizing buffer containing a low concentration of urea at high pH and a pulse refolding with buffer containing urea, aggregation inhibitors. Finally, a one-step affinity chromatographic purification was implemented to isolate the bioactive IFN-α2b.

MATERIALS AND METHODS

Bacterial stain

The organism used in this study was *E. coli* BL21/pBUG-IFN-α2b made by Institute of Medical Genetics, Pyongyang Medical College of Kim Il Sung University.

Preparation of IFN IBs

The cell pellets were suspended in PBS and disrupted by high-presssure homogenization and the inclusion bodies were collected by centrifugation at 8,000g for 40min. After that, the inclusion bodies were washed by washing buffer containing 4mol/L urea, 1mol/L sodium chloride and 0.5% Triton X-100.

Solubilization of IF Nα2b inclusion bodies

The IFN-α2b inclusion bodies were resuspended in 9mL of solubilizing buffer (2 mol/L Tris, 2 mol/L urea, 2 mmol/L β-mercaptoethanol, pH 12.5) per gram of inclusion bodies and solubilized for 2 h. The supernatant was obtained by centrifugation at 10,000 g for 40 mins. The extent of solubilization was estimated by measure of absorbance at 450 nm before centrifugation of solubilizing solution and at 280 nm after centrifugation and by accounting the rate of the wavelength 280 nm to 450 nm.

Refolding of IFN-α2b inclusion bodies

Refolding of IFN-α2b inclusion bodies was performed via two steps. First, solubilized IFNα2b solution was diluted in refolding buffer (50mmol/L Tris-HCl, pH8.4 containing 2mol/L urea, 0.1mmol/L EDTA and 0.1mmol/L β-mercaptoethanal) by pulse adding with an interval of one hour. Second, the above solution was dialysed using ultrafiltration 5K against dialysing buffer (0.1mol/L phosphate, pH 7.4 containing 0.1mol/L NaCl, 0.025% mannitol, 0.01% Tween80 with 1.5~0.01 mol/L urea and finally without urea) slowly step by step decreasing the concentration of urea, EDTA, and β-mercaptoethanal and finally removing them. Refolding rate was accounted by the following equation: [IFN (U) after refolding/IFN (mg) before refolding $\times 2\times 10^8$(U)] × 100%.

Immobilized metal chelate affinity chromatography

Refolded IFN α2b was purified through immobilized metal Cu^{2+} chelate affinity chromatography. 150mmol/L phosphate buffer, pH 7.2 was used as equilibrating buffer and 0.2mol/L glycine-HCl, pH 2.3 containing 0.3mol/L NaCl as eluting buffer.

Bioactivity assay of IFN α2b

Bioactivity assay of IFN α2b was carried out by WISH/VSV system.[13]

RESULTS

Solubilization of IFN α2b IBs

Influence of urea concentration of solubilization buffer on the solubilization effect of IFN α2b IBs was investigated. Urea concentration of solubilization buffer was set to different values ranging from 1 to 4mol/L and tested for the influence on the solubilization effect of IFN α2b IBs (Figure 1). As shown in Figure 1, at the urea concentration of 2mol/L, A_{280} of solubilized protein was 4.22, solubilization effect (A_{280}/A_{450}) was 23.44 and solubilization rate was 89.73%. No significant difference was detected at higher concentrations.

Influence of concentration of IFN α2b IBs on the solubilization effect was also investigated. Concentration of IFNα2b IBs to be solubilized was set to different values ranging from 2.5 to 15 mg/L and tested for the influence on the solubilization effect of IFN α2b IBs (Figure 2). When the IFN α2b IBs concentration was below 12.5mg/mL, solubilization effect (A_{280}/A_{450}) of IFN α2b IBs was 25.6, solubilization rate was 89.5% and these values decreased at higher IFN α2b IBs concentration. This result shows that the maximum IFN α2b IBs concentration that allows complete solubilization in the buffer containing 2mol/L urea at pH 12.5 is 12.5mg/mL.

Refolding of IFN α2b IBs

We investigated the influence of urea concentration in refolding buffer on the refolding of IFN α2b. Refolding rate of IFN α2b in the solution of concentration of IBs 10mg/ml was tested at different concentrations of urea ranging from 1 to 4mol/L in refolding buffer (50mmol/L Tris-HCl, pH 8.4). The refolding rate at refolding buffer containing 2mol/L urea

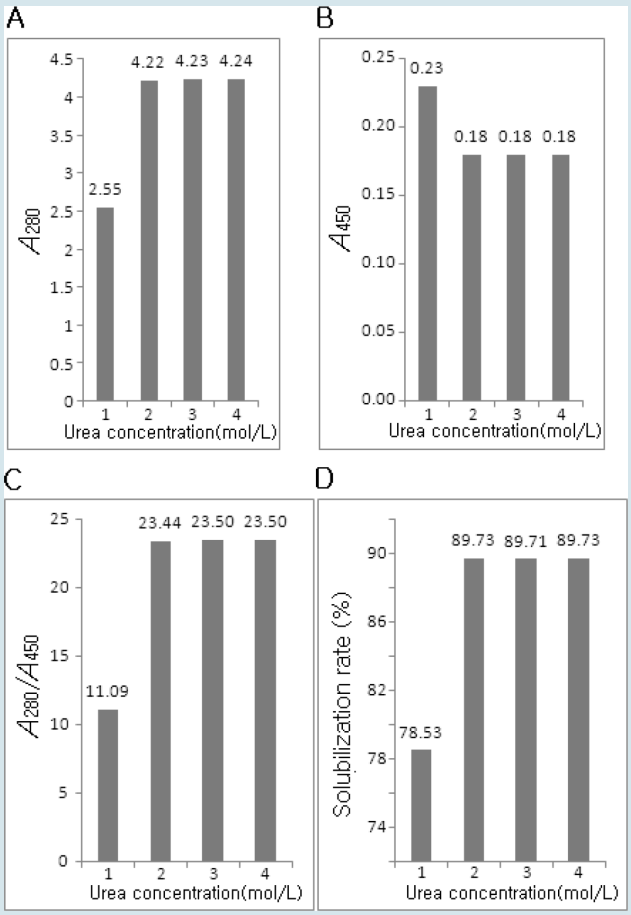


Figure 1: Solubilization rate of IFN IBs at different urea concentrations (A) Optical absorption rates of IB-solubilized IFN α2b solutions containing urea of different concentrations at wavelength of 280nm. (B) Optical absorption rates of IB-solubilized IFN α2b solutions containing urea of different concentrations at wavelength of 450nm. (C) Ratio of A_{280} to A_{450} of IB-solubilized IFN α2b solutions containing urea of different concentrations. (D) Solubilization rate of IFN IBs at different urea concentrations. At the urea concentration of 2mol/L, solubilization rate was 89.73% and no significant difference was detected at higher concentrations.

was 78.81%, the maximal value and it decreased at higher urea concentrations due to the prolonged dialysis (Figure 3).

Refolding effect according to dilution method was investigated. Solubilized IFN α2b sample was diluted in two ways, at one step dilution at the speed of 1mL/min and pulse dilution with fixed intervals. As shown in Figure 4, pulse dilution gave the refolding rate of 78.53%, which was significantly higher than that at one step dilution.

Influence of the quantity of IFN α2b in each aliquot added in refolding buffer on the refolding rate of IFN α2b in pulse dilution was also investigated. The quantity of solubilized IFN that had to be added to refolding buffer in each aliquot was set to different values ranging from 0.1 to 1.0mg/mL to determine the optimal quantity of solubilized IFN α2b. When the quantity of solubilized IFN α2b added in refolding buffer in each aliquot was set to 0.25mg/mL, refolding rate was 79.87%, which was significantly higher than those at bigger quantities (Figure 5).

Influence of final IFN α2b concentration in refolding buffer on the refolding

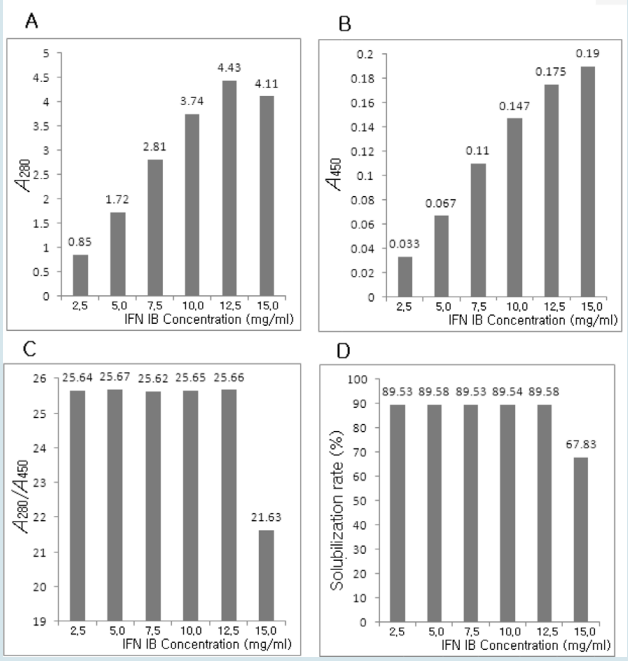


Figure 2: Solubilization rate of IFN IBs at different IBs concentrations (A) Optical absorption rates of IB-solubilized IFN α2b solutions of different IBs concentrations at wavelength of 280nm. (B) Optical absorption rates of IB-solubilized IFN α2b solutions of different IBs concentrations at wavelength of 450nm. (C) Ratio of A_{280} to A_{450} of IB-solubilized IFN α2b solutions of different IBs concentrations. (D) Solubilization rate of IFN IBs at different IBs concentrations. Maximum IFN α2b IBs concentration that allows complete solubilization of IFN IBs in the buffer containing 2mol/L urea at pH 12.5 is 12.5mg/ml.

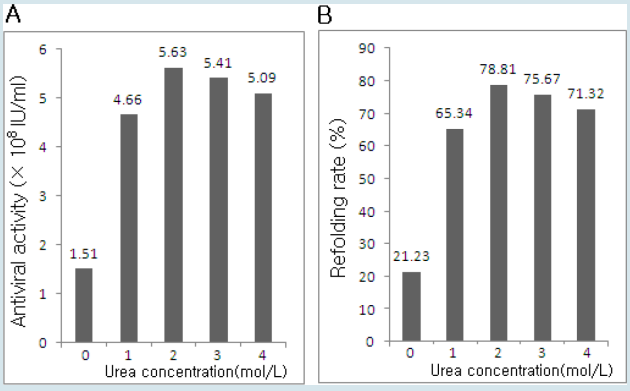


Figure 3: Refolding rate of IFN at different urea concentrations (A) Antiviral activities of refolded IFN solutions with different concentrations of urea. (B) Refolding rates of solubilized IFN IBs at different concentrations of urea. The refolding rate at refolding buffer containing 2mol/L urea was 78.81%, which was the maximal value and it decreased at higher urea concentrations due to the prolonged dialysis.

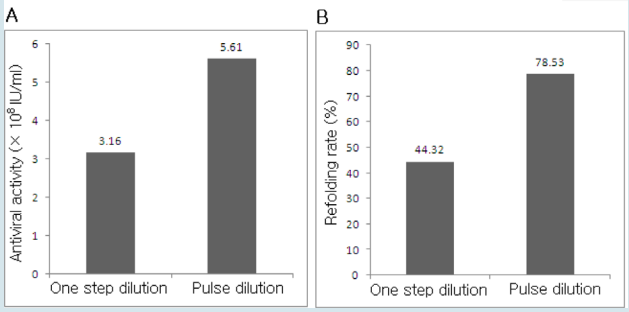


Figure 4: Refolding rate of IFN according to the dilution method (A) Antiviral activities of refolded IFN solutions according to the dilution method. (B) Refolding rates of solubilized IFN IBs according to the dilution method. Pulse dilution gave the refolding rate of 78.53%, which was significantly higher than that at one step dilution.

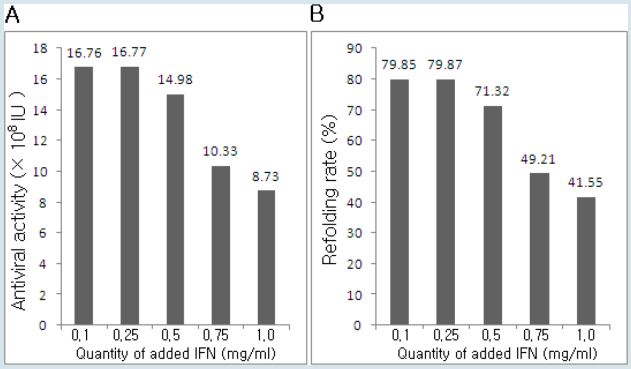


Figure 5: Refolding rate of IFN according to the quantity of added IFN (A) Final antiviral activities of refolded IFN according to the quantity of added IFN. (B) Refolding rates of solubilized IFN IBs according to the quantity of added IFN. When the quantity of solubilized IFN α2b added in refolding buffer in each aliquot was set to 0.25mg/mL, refolding rate was 79.87%, which was significantly higher than those at bigger quantities.

rate of IFN α2b was investigated. IFN α2b concentration in refolding buffer after the final pulse dilution was set to different values ranging from 0.5–1.5mg/mL and tested for the relationship with refolding rate. The IFN α2b concentration below 1.25mg/mL in refolding buffer gave the refolding rate of 79.45%, which was significantly higher than those at higher concentrations (Figure 6).

Finally, we investigated the influence of removal method of denaturant and stabilizing agent on the activity recovery of IFN α2b. The simultaneous and sudden removal of denaturant and stabilizing agent may influence on the stability of refolded protein. In order to completely remove 2mol/L urea and others from refolding buffer, one or multi steps dialysis were done through ultrafiltration membrane 5K. At multi steps dialysis, urea and others concentration were decreased step by step. Dialysis buffer was replaced every hour. At one step dialysis, the same dialysis buffer with multi steps dialysis was used. The multi steps dialysis gave the antiviral

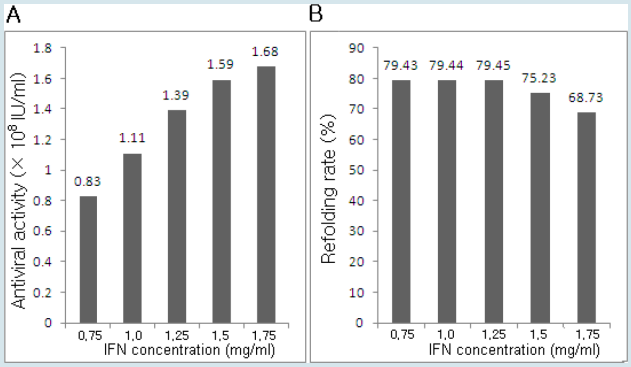


Figure 6: Refolding rate of IFN according to the IFN concentration in the refolding buffer
(A) Final antiviral activities of refolded IFN solutions according to the IFN concentration in the refolding buffer. (B) Refolding rates of solubilized IFN IBs according to the quantity of added IFN concentration in the refolding buffer. The IFN α2b concentration below 1.25mg/mL in refolding buffer gave the refolding rate of 79.45%, which was significantly higher than those at higher concentrations.

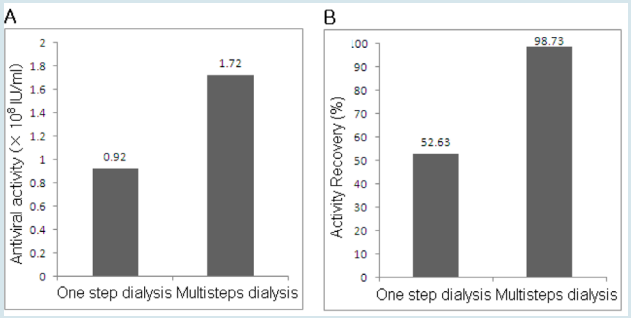


Figure 7: Antiviral activity recovery of IFN according to the dialysis method
(A) Final antiviral activities of refolded IFN solutions according to the dialysis method. (B) Antiviral activity recovery according to the dialysis method. The multi steps dialysis gave the antiviral activity recovery of 98.73%, which was maintained at the initiation level.

activity recovery of 98.73%, which was maintained at the initiation level (Figure 7).

Establishment of IFNα2b purification procedure through immobilized metal Cu^{2+} chelate affinity chromatography.

First, we investigated the influence of elution buffer on the recovery of IFNα2b. In order to test the influence of elution buffer on the IFNα2b recovery through immobilized metal Cu^{2+}chelate affinity chromatography, different kinds of low pH elution buffers were used which have high ion concentration or not (Table 1, Figure 8). The elution buffer which has the composition of 0.2mol/L Glycine-HCl, pH 2.3, 0.3mol/L NaCl gave the highest recovery of 83.71%. Like this, we established the IFNα2b purification procedure through one step immobilized metal Cu^{2+} chelate affinity chromatography.

Table 1: IFN recovery for different kinds of elution buffers

Elution buffer	Eluted IFN (mg)	Recovery (%)
50mmol/L Acetate, pH 4.8	84.41±0.13	67.53±0.67
50mmol/L acetate, pH 4.8/0.3mol/L NaCl	89.69±0.17	71.75±1.04
0.2mol/L Glycine-HCl, pH 2.3	98.91±0.19	79.13±1.15
0.2mol/L Glycine-HCl, pH 2.3/0.3mol/L NaCl	104.64±0.25	83.71*±1.21

Loaded IFN: 125mg, * p<0.01(Compared with other conditions), n=5

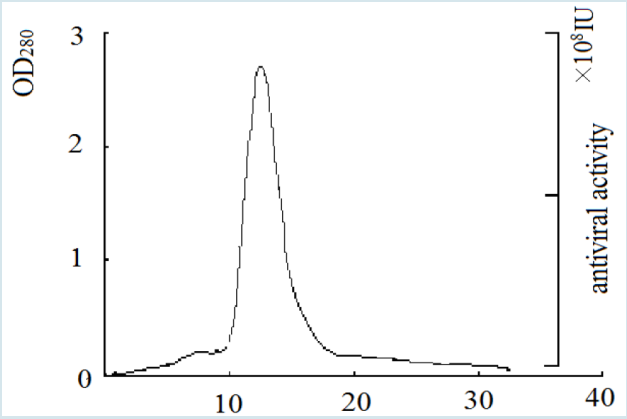


Figure 8: IFNα2b eluate chromatogram on immobilized metal Cu^{2+} chelate affinity column
Antiviral activity of IFNα2b was shown between 10~15mL of eluate volume on immobilized metal Cu^{2+}chelate affinity column.(column size 2×10 cm)

IFN α2b recovery at every purification step was shown in Table 2. The final recovery was 52.89% and specific activity was raised to 1.4×10^8U/mg protein after Cu^{2+} affinity chromatography. As shown in the Figure 9, 12% SDS-PAGE showed single band of purified IFN- α2b at expected MW height, whose purity was 99.8%.

DISCUSSION

High expression levels of recombinant proteins in *E. coli* often lead to the formation of inclusion bodies and a protein refolding procedure is usually necessary. A good recovery of the recombinant protein production results from a high yield of solubilizing and refolding of inclusion bodies. In general, inclusion bodies are solubilized by the use of a high concentration of denaturants such as urea or guanidine, along with a reducting agent such as β-mercaptoethanol. With new information, protein aggregates inside inclusion bodies have native-like secondary structure. It is assumed that maintenance of this native-like secondary structure by use of mild solubilization buffer containing low concentration of denaturant will help in improved recovery of bioactive protein in comparison to solubilization under a high concentration of chaotropic agent due to more easy restoration of native active structure. We estimated the solubilizing effect of low concentration urea, denaturant, on IFN IBs. As shown in Figure 1, at the urea concentration of 2mol/L, A_{280} of solubilized protein was 4.22, solubilization effect (A_{280}/A_{450}) was 23.44 and solubilization rate was 89.73%. No significant difference was detected at higher concentrations. The rational IFN IBs concentration to be solubilized in solubilization buffer was below 12.5mg/mL. At this time, solubilization

Table 2: IFN recovery at every purification step.

Purification step	Total IFN yield(g)	Total IFN activity ($\times 10^{11}$U)	Specific activity ($\times 10^{8}$U·mg^{-1}protein)	Recovery of procedure (%)	Total recovery (%)
Washing	6.91±0.53			88.70	
Solubilization	6.19±0.61	-	-	89.58	79.46
Refolding	4.94±1.93	3.53±0.02	1.28±0.08	79.81	63.41
Cu^{2+}column	4.12±2.23	2.94±0.01	1.40*±0.06	83.4	52.89

Mass of start IFN IBs 7.79g, Bacterial mass 190g, * p<0.01(Compared with others), n=5

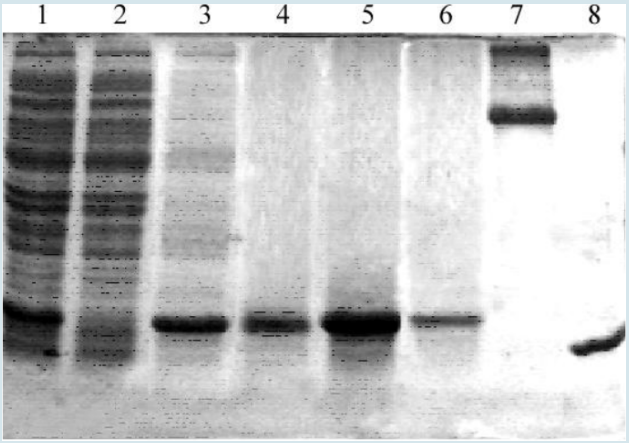
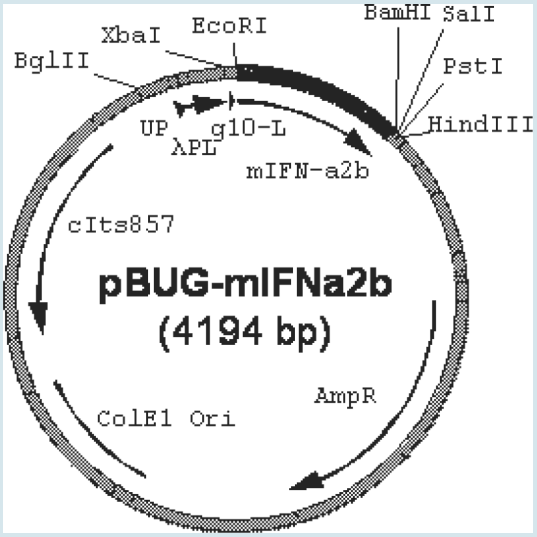


Figure 9: IFN samples at different purification steps run on 12% SDS-PAGE
line1, cell lysate; line2, supernatant of cell lysate; line3, washed IBs; line4, refolded IFN; line5, IFNα2b eluent from Cu^{2+}affinity column; line 6, IFN standard; line7, BSA(control); line8, lysozyme(control).



Thermoinducible overexpression vector , pBUG-mIFN-α2b

rate was 89.5% and these values decreased at higher IFN IBs concentration. This result shows that the maximum IFN IBs concentration that can be solubilized completely in the solubilization buffer containing 2mol/L urea at pH 12.5 is 12.5mg/mL (Figure 2).

Rudolph and Fischer developed a pulse refolding method which decreased the renaturation volumes by stepwise addition of solubilized protein into the refolding buffer. By keeping the protein concentration at low level in each aliquot, the high final refolding yields at high final protein concentrations were obtained. Another simple and efficient means to prevent aggregation is the addition of aggregation inhibitors. The commonly used additives are L-arginine, urea, glycerol, Triton X-100, polyethylene glycol, and some denaturants at low concentration. We estimated the effect of the pulse refolding method and the low concentration urea as aggregation inhibitor on the refolding of IFN IBs. The concentration of 2mol/L urea at the refolding buffer (50mmol/L Tris-HCl, pH 8.4) gave the maximal refolding rate of 78.81%, which decreased at higher urea concentrations due to the prolonged dialysis (Figure 3). The pulse adding of an aliquot of solubilized IFN IBs gave the refolding rate of 78.53%, which was significantly higher than that at one step dilution (Figure 4). When the quantity of solubilized IFN added in refolding buffer in each pulse adding was 0.25mg/mL, the refolding rate was 79.87%, which was significantly higher than those at bigger quantities (Figure 5). IFN concentration in refolding buffer after the final pulse adding was set to different values ranging from 0.5–1.5mg/mL and tested for the relationship with refolding rate. The final IFN concentration below 1.25mg/mL in refolding buffer gave the refolding rate of 79.45%, which was significantly higher than those at higher concentrations (Figure 6). The simultaneous and sudden removal of denaturant and stabilizing agent may influence on the stability of refolded protein. In order to completely remove 2mol/L urea and others from refolding buffer, one or multi steps dialysis were done through ultrafiltration membrane 5K.

The multi steps dialysis gave the antiviral activity recovery of 98.73%, which was maintained at the initiation level (Figure 7).

We purified the refolded IFN through immobilized metal Cu^{2+}chelate affinity chromatography. In order to test the influence of elution buffer on the IFN recovery through immobilized metal Cu^{2+}chelate affinity chromatography, different kinds of low pH elution buffers were used which have high ion concentration or not. The elution buffer which has the composition of 0.2mol/L Glycine-HCl, pH 2.3, 0.3mol/L NaCl gave the highest recovery of 83.71% (Table 1, Figure 8). The final recovery was 52.89% and specific activity was raised to 1.4×10^{8}U/mg protein after Cu^{2+} affinity chromatography (Table 2). 12% SDS-PAGE showed single band of purified IFN- α2b at expected MW height, whose purity was 99.8% (Figure 9). Large-scale production of Recombinant human Interferon α2b in *E. coli* with a thermoinducible overexpression system was obtained by applying an effective solubilizing and refolding processes of interferon α2b inclusion bodies.

PS:

Sequence of codon-optimized human IFN-α2b gene

PS:

Sequence of codon-optimized human IFN-α2b gene

ATGTGCGATCTGCCGCAGACCCATAGCCTGGGTAGCCGTC-
GCACCTTGATGCTGCTGGCACAGATGCGTCGTATCTCTCT-
GTTCTCCTGCTTGAAAGATCGCCATGATTTTGGCTTTCCG-
CAGGAAGAATTTGGCAACCAGTTCCAAAAAGCCGAAAC-
CATCCCGGTGCTGCATGAGATGATCCAGCAGATTTTTA-
ATCTCTTTAGCACGAAAGACTCGTCTGCCGCGTGGGATGAGA-
CCCTGCTGGATAAATTCTATACGGAACTCTATCAGCAGCT-
GAATGATCTGGAAGCCTGTGTGATTCAGGGTGTGGGCGT-
GACCGAAACGCCGCTGATGAAGGAGGACTCCATTCTG-
GCCGTGCGTAAATATTTCCAACGCATCACTCTGTATCT-
GAAAGAAAAAAAATACAGCCCGTGCGCCTGGGAGGTTGTGC-
GTGCAGAAATCATGCGCTCTTTTTCTTTGAGCACAAACTTG-
CAGGAAAGTTTACGCAGCAAGGAATAA

Compliance with Ethical Standards

Author[2], Pak Yong Ju, declares that he has no conflict of interest.

Author[2,] Yun Song Jin, declares that he has no conflict of interest.

This article does not contain any studies with human participants or animals performed by any of the authors.

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CONCLUSION

Large-scale production of recombinant human Interferon α2b (IFN-α2b) in *E. coli* with a thermoinducible overexpression system was established by applying an effective solubilizing and refolding processes of interferon α2b inclusion bodies (IBs). Solubilization and refolding rate of IFN-α2b IBs were 89.71% and 79.87% respectively. Refolded IFN-α2b was purified through one-step immobilized metal affinity chromatography to give a pure bioactive IFN-α2b with the specific activity of 1.4×108IU/mg protein and with the recovery rate over.

ACKNOWLEDGEMENT

We acknowledge the support of Ministry of Public Health of Democratic People's Republic of Korea and the cooperation of the Institute of Medical Genetics, Pyongyang Medical College of Kim Il Sung University.

CONFLICT OF INTEREST

The authors declare no conflcit of interest.

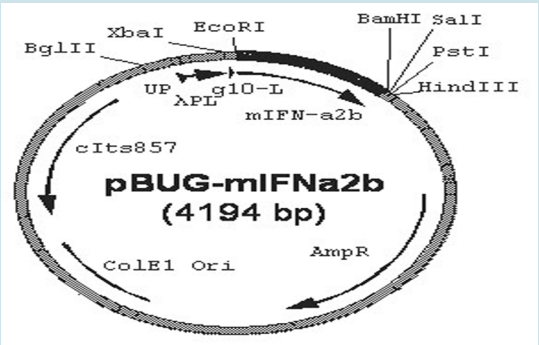
ABBREVIATIONS

IFN: Interferon; **IBs:** Inclusion Bodies.

REFERENCES

1. Lee SY. High cell-density culture of *E. coli*. Trends Biotechnol. 1996;14(3):98-105.
2. Fahnert B, Lile H, Neubauer P. Inclusion bodies: Formation and utilization. Adv Biochem. Eng / Biotechnol. 2004;89:93-142.
3. Kane JF, Hartley DL. Formation of recombinant protein inclusion bodies in *E. coli*. Trends Biotechnol. 1988;6(5):95-101.
4. Rudolph R, Bohm G, Lilie H, Jaenicke R 1997 Folding proteins. Protein function, a practical approach. IRL-Press Oxford University Press Oxford. 1996;10(1):49-56.
5. Vallejo LF, Rinas U. Strategy for recovery of active protein through refolding of bacterial inclusion body proteins. Microb Cell Fact. 2004;3:2-12.
6. Clark ED. Refolding of recombinant proteins. Curr Opin Biotechnol. 1998;9(2):157-63.
7. Lilie H, Schwarz E, Rudolph R. Advances in refolding of proteins produced in *E. coli*. Curr Opin Biotechnol. 1998;9(5):497-501.
8. Fischer B, Sumner I, Goodenough P. Isolation, renaturation and formation of disulfide bonds of eukaryotic proteins expressed in *E. coli* as inclusion bodies. Biotechnol. Bioeng. 1993;41(1):3-13.
9. Dill KA, Shortle D. Denatured states of proteins. Annu Rev Biochem. 1991;60(1):795-825.
10. Surinder MS, Amulya KP. Solubilization and Refolding of Bacterial Inclusion Body Proteins. J of Bioscience and Bioengineering. 2005;99(4):303-10.
11. Rudolph R, Fischer S. Process for Obtaining Renatured Proteins. US Patent. 1990;4:933.
12. Ya LS, Xiao XX, Yue Z, Jian WL, Dong ZW, Sheng LY. Refolding and purification of Apo2L/TRAIL produced as inclusion bodies in high-cell-density cultures of recombinant E coli. Biotechnology Letters. 2003;25(24):2097-101.
13. Hirai N, Hill NO, Motoo Y, Osther K. 1985 Antiviral and Antiproliferative activities of human leukocyte interferon potentited by cimetidine *in vitro* J-Interferon-Res. 1985;5(3):375-82.

PICTORIAL ABSTRACT



SUMMARY

- We applied an effective solubilizing and refolding processes of interferon α2b inclusion bodies (IBs) to establish the large-scale production of recombinant human Interferon α2b (IFN-α2b) in E. coli with a thermoinducible overexpression system. The IFN-α2b inclusion bodies were solubilized with solubilizing buffer of 2mol/L urea. Refolding was performed via two steps, diluting by pulse adding the solubilized IFN α2b sample and dialysing it slowly using ultrafiltration 5K step by step. Finally refolded IFN α2b was purified through Cu2+ chelate affinity chromatography. The final yield of a pure bioactive IFN-α2b was 52.89%, its specific activity 1.4×108IU/mg protein.

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