ISSN 0233-7657
Biopolymers and Cell. 2021. Vol. 37. N 6. P 428–437
doi: http://dx.doi.org/10.7124/bc.000A6C

UDC 577.112.083+663.18

Development of the method of isolation and purification of the recombinant human interleukin-7

T. M. Lutsenko[1,2], V. V. Motronenko[1]

1 National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"
37, Peremohy Ave, Kyiv, Ukraine, 03056

[2] "PRO-PHARMA"
120/4, bld "Zh" Kozatskaya Str, Kyiv, Ukraine, 03022
tanywalytsenko@gmail.com

Aim. To develop a method for isolation and purification of recombinant human interleukin-7 from bacterial inclusion bodies. **Methods.** Protein synthesis via fermentation, densitometry, refolding method of gel filtration, two-stage chromatographic purification of the protein. **Results.** The inclusion bodies obtained via fermentation in *E.coli* cells contained recombinant human interleukin-7, which was solubilized at 22ºC for 1 hour in the following buffer solution: 7M of guanidine hydrochloride, 100mM of Tris-HCl, 0.1 % Tween-20 and 50mM of dithiothreitol. Renaturation was conducted by replacing the buffer on column XK26 / 40 (GE Healthcare) with Sephadex G-25 fine equilibrated Clark-Labs solution containing L-arginine hydrochloride and tween-20. Subsequent purification of rIL-7 on the gel filtration column was performed in two stages: at the first stage, purification of rIL-7 was performed on a column packed with 20 ml of Q sepharose, at the second stage, the rIL-7 fraction purified on Q sepharose was purified on SP sepharose. Elution of rIL-7 was performed using double-stage gradient NaCL (0.2 and 0.7M) in a buffer 0.05 M of KH_2PO_4/NaOH with pH 6.0; 0.1 M of L-arginine; 0.1 % Tween-80. **Conclusions.** The amount of the target protein after all the proposed stages of purification was 10 % with the purity ratio about 95 %, which is sufficient for further development of the various pharmaceutical forms.

Keywords: interleukin-7, inclusion bodies, refolding, gel filtration, renaturation.

Introduction

Interleukin-7 (IL-7) was first extracted more than 30 years ago. It is a central cytokine of the immune system taking part in modulation of the T and B-cells development and T cell homeostasis [1, 2]. Nowadays numerous laboratories in many countries perform the research of IL-7 as the means of regeneration of the immune system in the conditions of

© 2021 T. M. Lutsenko *et al.*; Published by the Institute of Molecular Biology and Genetics, NAS of Ukraine on behalf of Biopolymers and Cell. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited

immunodeficiency of various origin. IL-7 was proven to manifest the therapeutic activity in case of various diseases, such as solid tumors, immunologic diseases, bacterial and virus infection [3, 4]. Considering the prospects of application of human IL-7 for therapeutic purposes it is of vital importance to develop a technology for extracting biologically active protein IL-7.

Human IL-7 is a single-chain glycoprotein, the aminoacidic sequence of which corresponds to a molecular weight of 17.4 kDa, but due to glycosylation the active form has a molecular weight of 25 kDa. The molecule of IL-7 contains four a-helices (A, B, C, D), which are spatially organized with disulfide junctions, and one mini-helix. Hydrophobic core of IL-7 is constructed with amino acids Leu 16, Met 17, Ile 20, Leu 23, Leu 24 (helix A), Phe 55, Leu 56, Ala 59, Leu 63, Phe 66 (helix B), Leu 79, Val 82, Thr 86, Leu 89, Thr 93 (helix C), Leu 128, Leu 131, Leu 135, Ile 138 and Trp 142 (helix D) [5].

The aminoacidic sequence of IL-7 contains 6 residuals of cysteine able to form intramolecular disulfide bonds. The first bond connects the chain N-end and helix C Cys 1-4 (Cys-2-Cys-92); the second one is formed between the loop AB and the beginning of helix D Cys 3-6 (Cys 47-Cys 141). The third disulfide bond is between the loop AB and the lower part of the helix D Cys 2-5 (Cys 34-Cys 129) The molecule also has 3 sites of N-glycosylation in the positions Asn 95, Asn 116 and Asn 141 [6, 7].

In order to extract IL-7 at industrial scale the most appropriate approach may be the recombinant producer-associated method of obtaining the cytokine [8]. When obtaining recombinant proteins for therapeutic purposes, it is important to isolate the target product with a high degree of purity and stability.

In the literature the use of various recombinant expression systems, such as *Escherichia coli* [9], yeast *Pichia pastoris* [10], baculovirus system [11] and CHO cells [12], is described. However, in terms of technical and economic efficiency, the most optimal today is the technology of obtaining IL-7 using recombinant *E.coli* cells [5].

The main disadvantage of using *E.coli* is accumulation of the recombinant human IL-7 in the form of aggregates, the so-called inclusion bodies, the protein of which is insoluble and thus requires renaturation *in vitro*; this results in additional time losses in terms of purification process [13–15]. There is also a risk of contaminating the extracted material with the cellular components and *E.coli* genetic material. Therefore, the aim of the work was to develop a method for isolation and purification of recombinant human interleukin-7 from bacterial inclusion bodies.

Materials and Methods

The main research material was recombinant human IL-7 (LLC "UA" "PRO-PHARMA", Kyiv). It was obtained using the technology of recombinant DNA in expression system, based on *E. coli BL21(DE3)* producer and plasmid vector pACYC184 [16].

The protein synthesis was performed via fermentation of producer in the induction environment of the following composition (per 1 l): peptone — 17 g, yeast extract — 10 g, $MgSO_4 \times 7H_2O$ — 5 g, $(NH_4)_2SO_4$ solution 0.5 M — 50 ml, 1M KH_2PO_4 — 50 ml, 1M Na_2HPO_4 — 50 ml, solution 5052 (10 % lactose, 25 % glycerol, 2.5 % glucose) —

20 ml, 10 % solution of D-maltose — 50 ml, $NaHCO_3$ — 5 g and chloramphenicol at 37.5 °C with the orbital rotation speed of 160 rpm. It lasted for 14-18 hours and then the cells were deposited via centrifugation at 4000 rpm for 15 min.

In order to purify rIL-7, the cells were destroyed by biochemical method with the addition of DNase. For the vextraction and purification of inclusion bodies we used consecutive washing in buffer solutions of following composition:

Buffer A: 50 mm TRIS-HCl, pH 8.0, 5 mM EDTA, 100 mM NaCl, 1mg/ml of lysozyme (lysozyme is added to the solution right before the application);

Buffer B: 20 mM TRIS-HCl, pH 7.0, 1 mM EDTA, 0.1 % Triton X-100;

Buffer C: 20 mM TRIS-HCl, pH 8.0, 0.14M NaCl;

Buffer D: 20 mM TRIS-HCl, pH 8.0, 0.3 % sodium deoxycholate.

Solubilization of inclusion bodies was performed in a buffer solution with adding the chaotropic agents of the following composition: 7M of guanidine hydrochloride, 100 mM of Tris-HCl, 0.1 % Tween-20 and 50mM of dithiothreitol, pH=5.0. The dissolution was performed considering the following ratio: for 1.2–1.5 mg of IL-7 we used 2 ml of buffer (the amount of protein in the obtained inclusion bodies batch before solubilzation is defined using densitometry of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE)). This solution was then resuspended and incubated at 22 °C for 1–1.5 hours in an impeller (2 rpm). Upon the incubation, the obtained solution was centrifuged at 13.2 thousand rpm for 15 minutes [17, 18].

Resulting supernatant was used for further renaturation of the target protein. The supernatant, obtained upon the solubilization, was placed on a column XK26/40 (GE, Healthcare), filled with sorbent Sephadex G-25 fine. Prior to this the sorbent was balanced with 5 portions of Clark-Labs solution ((50 ml 0.1 M KH_2PO_4 (3.6 g/l) + 13.9 ml 0.1M NaOH, pH 6.0), containing 0.1 M L-arginine HCL and 0.1 % Tween-80 [19].

2 ml of the supernatant from the previous stage of solubilization were applied on a column, the supernatant containing 1.2–1.5 ml of target protein. The volume of column effluent varied from 4.5 to 5.1 ml. The obtained effluent was centrifuged at 13.2 thousand rpm for 15 minutes, the deposits were removed. After centrifugation the supernatant was collected and transferred for further purification. Purification of recombinant IL-7 was performed in two stages with application of sepharose DEAE (weak anion exchanger) at the first stage, and sepharose SP (strong cation exchanger) at the second one.

In order to preserve disulfide bonds, oxydation-reduction pair of glutatione (oxidized/reduced) was added to the renaturating mixture until the final concentration 1mM. It was incubated at +4 °C for 30 minutes [20, 21].

The solutions used for chromatographic purification of rIL-7 were filtered through 0.22 μm membrane filter ("Milipore") and decontaminated with vacuum pump [22].

The fraction of recombinant IL-7, which was flown through DEAE sepharose, was further used for purification on SP sepharose.

All fractions obtained as a result of chromatographic purification of rIL-7 were collected and analyzed by gel electrophoresis

under denaturing conditions in 12 % polyacrylamide gel along with using spectrophotometer NanoDrop.

The obtained rIL-7 was monitored for biological activity by a validated technique using human peripheral blood mononuclear cells (PBMCs) [4, 8]. The method of testing human rIL-7 biological activity is based on its ability to cause T-lymphocyte proliferation. Proliferation activity is assessed by the method using 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT test) [23].

After isolation, the mononuclear cells were resuspended in RPMI medium containing 10 % fetal calf serum and 50 μM β-ME. Resuspension was performed at the rate of 2 million cells per 1 ml of medium. To these we added phytohemagglutinin (PHA) to a final concentration of 20 μg/ml and incubated at 37 °C in a CO_2 incubator (5 % CO_2 concentration) for three days. Precipitated at 1000 rpm for 10 min at 4 °C and twice washed the cells with RPMI medium without calf serum and β-ME were resuspended in the previous medium at a rate of 1×10^5 per 50 μl and transferred to 2 96-well tissue culture template (GE Healthcare, UK) 50 μl per well. Then we added to one of the tablets control IL-7 (rhIL-7, "PeproTech", USA) in concentrations of 250 pg/ml, 500 pg/ml, 1 ng/ml, 2 ng/ml, 2 ng/ml, 4 ng/ml, 5 ng/ml, 6 ng/ml, 7 ng/ml, 8 ng/ml, 9 ng/ml, 10 ng/ml. The rhIL-7 obtained by us in concentrations of 2, 5, 10, 15, 20, 25, 30, 35, 40, 50, 100 and 200 ng/ml was added to another plate. The cells were incubated under the previous conditions for 3 days.

After incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazole bromide (MTT) was added to the final concentration of 0.5 μg/ml and incubated for 4 hours under the same conditions. Then 100 μl of dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals formed during the reaction and incubated for 10-15 minutes. After incubation, the absorption measurements were performed on a spectrophotometer at a wavelength of 570 nm [24].

Results and Discussion

As a result of fermentation, we obtained the target protein in a form of intracellular aggregations (inclusion bodies).

Extraction and purification of inclusion bodies from rIL-7 were conducted according to the aforementioned method. At the same time, we selected the samples for polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate to control every stage (Fig. 1). The amount of target protein in the obtained inclusion bodies was 30–35 % of the total amount of protein in *E.coli* cells. The obtained inclusion bodies contained on average 0.7 mg/ml of rIL-7 with reference to *E.coli* stock culture.

Solubilization of inclusion bodies, in which the recombinant protein is in aggregated, inactive form, was conducted in media with high concentration of chaotropic agent guanidine HCl, Tween-80 detergent and reducing agent dithiothreitol, which provides the possibility to leave a substantial amount of impurities in non-dissolved state thus facilitating further purification. In order to remove metal ions, which induce undesirable oxidation of thiol groups of the protein, we added chelant EDTA to the solybilizing buffer. The buffer for dissolving of inclusion bodies contains 7M of guanidine hydrochloride, 100mM of Tris-HCl, 0.1 %

Tween-20 and 50mM of dithiothrietol, pH=5.0. We incubated and rotated it at 22 ºC for 1 hour. After incubation, the obtained solution of inclusion bodies was centrifuged at 13.2 thousand rpm for 15 minutes. Supernatant was collected for further renaturation and purification.

The supernatant, obtained upon the inclusion bodies solubilization, was placed on a column XK26/40 (GE, Healthcare), filled with Sephadex G-25 fine (100 ml). Before, the sorbent was balanced with 5 portions of Clark-Labs solution. 10 ml of the supernatant from the previous stage were applied on a column, the supernatant containing 7.5–8 ml of the target protein. The application and all procedures of chromatographic fractionation and further centrifugation were conducted at 20–22 °C. The speed of the protein solution appication and supplying the eluating buffer was 15 ml/m. The volume of column effluent varied from 20 to 25 ml. The obtained effluent was filtered through MF Membrane Filters (pore size 0.45 µm), and then through filter with pore size 0.22 µm. After filtration we added 1 mM of oxidized and reduced glutatione into preparations in order to increase the efficiency of renaturation, and then proceeded with purification.

Further purification of rIL-7 on a column for gel filtration was double-staged and was conducted with application of Q sepharose for the first stage, and SP sepharose for the second

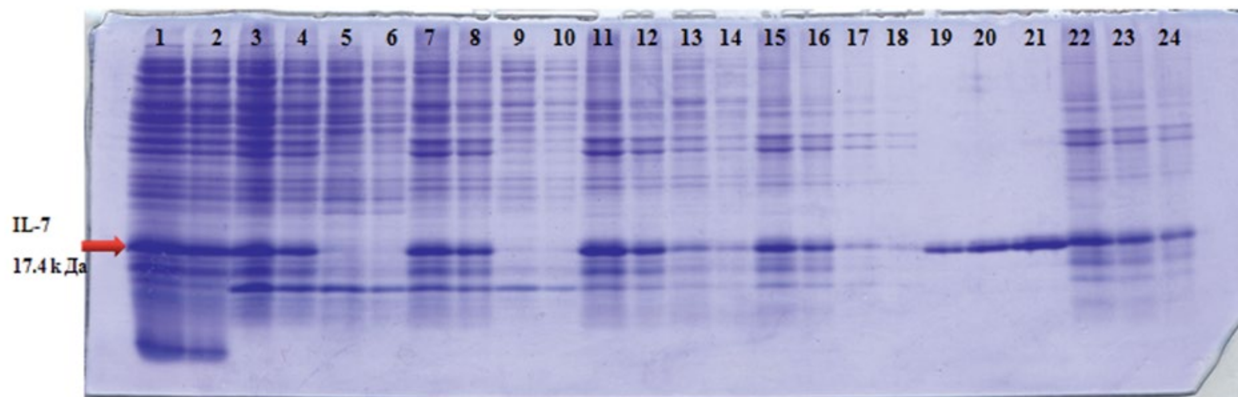

Fig. 1. Electropherogram of protein fractions obtained during isolation and purification of inclusion bodies:
1, 2 — 5 and 2.5 µl of *E.coli* stock culture.
3 and 4 — 5 and 2.5 µl of cells resuspended in Buffers A and B;
5 and 6 — 5 and 2.5 µl of supernatant after IB depositing;
7 and 8 — 5 and 2.5 µl of inclusion bodies resuspended in Buffer C;
9 and 10 — 5 and 2.5 µl of supernatant after depositing;
11 and 12 — 5 and 2.5 µl of inclusion bodies resuspended in Buffer D;
13 and 14 — 5 and 2.5 µl of supernatant after depositing;
15 and 16 — 5 and 2.5 µl of inclusion bodies resuspended in deionised water;
17 and 18 — 5 and 2.5 mcl of supernatant after depositing;
19, 20 and 21 — 0.5, 1, and 2 µlg of marker protein FGF-1 (MW 17,0 kDa);
22, 23 and 24 — 5, 2.5, and 1 µll (in reference to stock culture) of purified inclusion bodies.

one. During the first stage of rIL-7purification 20 ml of Q sepharose were put into a column [18]. The fraction of recombinant IL-7, which was flown through Q sepharose, was further purified on SP sepharose. Elution of rIL-7 was performed using double-stage gradient NaCl (0.2 and 0.7 M) in a buffer 0.05 M of KH_2PO_4/NaOH with pH 6.0; 0.1 M of L-arginine; 0.1 % Tween-80, Fig. 2).

All fractions obtained as a result of chromatographic purification of rIL-7 were collected and analyzed in 12 % polyacrylamide gel (SDS-PAGE) with spectrophotometer NanoDrop. The output of target protein during all stages of purification was 10 %. The purity of preparation was approximately 95 % (fig. 3).

We tested the biological activity of the obtained rIL-7 on human PBMC in comparison with the solution of recombinant human interleukin-7 (Peprotech, Cat.N. 200-07).

The growth curves were constructed for each individual experiment. To construct the cell culture growth curves along the abscissa axis, the decimal logarithm of IL-7 concentration was plotted, and along the ordinate axis, the absorption ratio of the control samples to the absorption of the experimental samples was plotted. Three such experiments were performed. The growth curves, which are constructed from the arithmetic means of these experiments, are represented in fig. 4. On each curve a rectilinear region characterized by

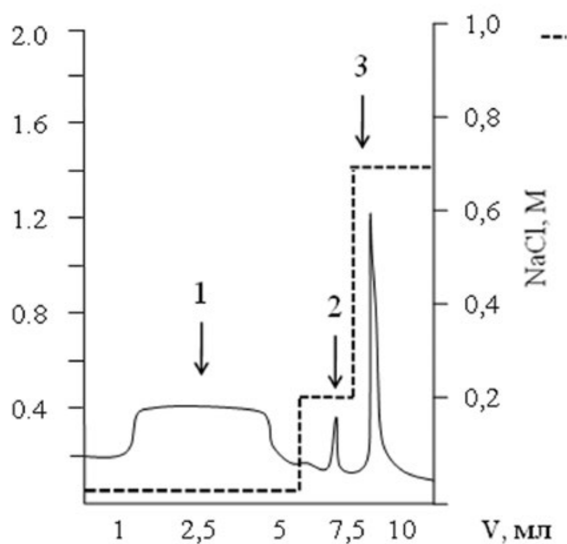

Fig. 2. Profile of rIL-7 elution during chromatographic purification on Q and SP sepharose:
1 — unbound fraction, flown through Q and SP sepharose; 2 — fraction, eluated with 0.2M NaCl; 3 — fraction eluated with 0.7M NaCl.
Full line stands for optical density; dot line — for polarity inversion of NaCl

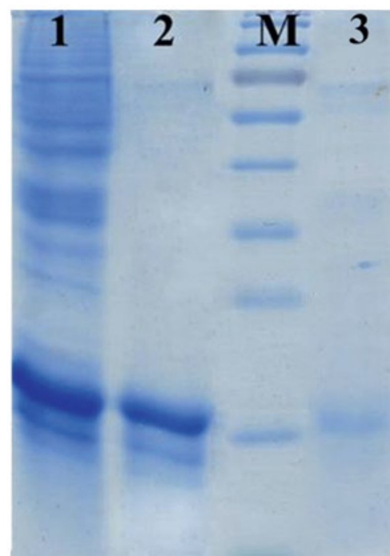

Fig. 3. Electrophoregram of fractions during purification of rIL-7 on Q and SP sepharose:
1 — rIL-7 fraction after renaturation;
2 — rIL-7 fraction after purification on Q and SP sepharose;
3 — unbound fraction;
M — MW markers (250, 130, 100, 70, 55, 25, 15 kDa)

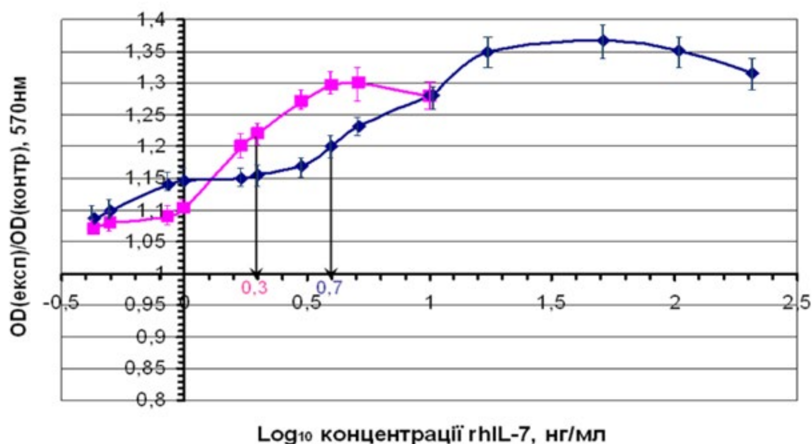

Fig. 4. Verification of specificity of testing the biological activity of rIL-7 — proliferation of human peripheral blood cells at increasing rIL-7 concentration. X axis — common logarithm of rIL-7 concentration, Y axis — ratio between optical density of cells stimulated with rIL-7 and non-stimulated cells (OD(exp)/OD(ref). ($M \pm m$; $n = 3$; $p < 0.05$). Plot –■– conforms to the reference sample, and plot–♦– conforms to the test sample.

exponential growth of cultured cells in response to the action of IL-7 was determined. The midpoint, from which the projection on the abscissa axis determines half the effective dose (Effective dose, ED50), was measured at this site.

As a result, we acquired the following ED50 values for commercial rIL-7 and obtained by us — 2 ng/ml and 5 ng/ml, respectively. This gives us a reason to believe that the obtained rIL-7 has an appropriate biological activity.

Conclusions

IL-7 has a potential therapeutic field of application for regeneration of lymphocytes during various immunodepressive states (chemotherapy, irradiation, AIDS, transplantation of bone marrow). Nowadays various labs all over the world conduct clinical studies using cylcolized rIL-7 as an immunorestorative factor for treating cancer, lymphocytopenia, and chronic virus infections. These potential ways of rIL-7 usage require big amounts of highly purified protein.

Synthesis of eukaryotic proteins in prokaryotic systems, especially in *E.coli*, is a fast and cost-effective method of large-scale production of recombinant proteins. However, the restrictions of prokaryotic systems for some post-translation modification in connection with the presence of endotoxin in Gram-negative bacteria are the main flaws of this system. Moreover, the formation of inclusion bodies requires an additional stage of refolding, which is a bottleneck in technology and may lead to low rates of restoration.

The suggested fermentative processing of cells with the view of breaking the cell wall provides the possibility to conduct lysis in mild conditions, which is very important during obtaining recombinant proteins. Further addition of DNAase to destroy cellular DNA, and application of consecutive washing in a series of buffers, containing detergents such as EDTA, Triton X-100 and sodium deoxycholate, allow substantial improvement of further dissolution of inclusion bodies and purification.

Dissolution of inclusion bodies is conducted at high concentration of guanidine HCL, tween-80, and dithiothreitol, thus the majority of impurities stay undissolved. In order to remove metal ions, which induce undesirable oxidation of thiol groups of protein we added chelant EDTA to the solubilizing buffer.

Renaturation was performed via gel filtration, which does not result in substantial dilution of protein, this being an important aspect of obtaining recombinant protein. During gel filtration we used a buffer solution, containing arginine amino acids, tween-20 detergent, and phosphate anions. These reagents do not influence the refolding speed, but substantially reduce the probability of development of compounds, taking part in destabilizing intermolecular and hydrophobic interactions. During the next stage we added the oxidation-reduction pair of low-molecular tiols to the obtained protein for efficient formation of disulphide bonds i.e. for renaturation.

The method of purification of a target product, presented in the article, is based on the application of double-stage purification process with usage of Q sepharose as the first stage, and SP sepharose as the second one. This allows the extraction of recombinant human IL-7 with the purity ratio about 95 %, which is acceptable for biological substances. For the development of pharmaceutical forms, an additional purification step will be completed to obtain the protein purity of 98 % and above.

REFERENCES

1. *Grigorieva SM, Starosyla DB, Rybalko SL, Motronenko VV, Lutsenko TM, Galkin OYu.* Effect of recombinant human interleukin-7 on Pseudo-monas aeruginosa wound infection. *Ukr Biochem J.* 2019; **91**(5): 7–15.
2. *Galkin OY, Lutsenko TM, Gorshunov YV, Motronenko VV.* Development of the method for microbiological purity testing of recombinant human interleukin-7-based product. *Ukr Biochem J.* 2017; **89**(3): 52–9.
3. *Slyvka AV, Okunev OV.* Molecular mechanisms of versatile biological activity of interleukin-7. *Biopolym Cell.* 2014; **30**(5): 349–57.
4. *Lutsenko TN, Kovalenko MV, Galkin OY.* Validation of biological activity testing procedure of recombinant human interleukin-7. *Ukr Biochem J.* 2017; **89**(1): 82–9.
5. *Lutsenko TM, Galkin OYu.* Substantiation of biotechnological approaches of producing interleukin-7 recombinant human. *Proceedings of the Belarusian State Technological University.* Series “Chemistry, technology of organic substances and biotechnology”. 2015; **4**(177): 188–97.
6. *McElroy CA, Dohm JA, Walsh STR.* Structural and biophysical studies of the human IL-7/IL-7Ra complex. *Structure.* 2009; **17**(1): 54–65.
7. *Galkin OY.* Comparative characteristic of the methods of protein antigens epitope mapping. *Ukr Biochem J.* 2014; **86**(4): 164–77.
8. *Natochii T, Motronenko V.* Comparative characteristics of biotechnological approaches to obtaining recombinant human cytokines in bacterial expressing systems. *Innov Biosyst Bioeng.* 2019; **3**(3): 128–45.
9. *Ouellette T, Destrau S, Ouellette T, Zhu J, Roach JM, Coffman JD, Hecht T, Lynch JE, Giardina SL.* Production and purification of refolded recombinant human IL-7 from inclusion bodies. *Protein Expr Purif.* 2003;**30**(2):156-66
10. *Luo Y, Kong X, Xu A, Jin S, Wu D.* Expression, purification, and functional characterization of recombinant human interleukin-7. *Protein Expr Purif.* 2009;**63**(1):1–4.
11. *Mirzaei M, Jardin B, Elias CB, Prakash S.* Expression and production of human interleukin-7 in insect cells using baculovirus expression vector system (BEVS). *Appl Biochem Biotechnol.* 2008;**151**(1):93–103.
12. *Toghraie FS, Sharifzadeh SM, Ramezani A, Mahmoudi Maymand E, Yazdanpanah-Samani M,*

Ghaderi A. Cloning and Expression of Recombinant Human Interleukin-7 in Chinese Hamster Ovary (CHO) Cells. *Rep Biochem Mol Biol.* 2017;**6**(1):66–73.

13. *Motronenko V, Lutsenko T, Galkin A, Gorshunov Y, Solovjova V.* Optimization of the culture medium composition to increase the biosynthesis of recombinant human interleukin-7 in Escherichia coli. *J Microbiol Biotechnol Food Sci.* 2020; **9**(4): 761–8.
14. *Galkin OY, Besarab OB, Gorshunov YV, Ivanova OM.* New monoclonal antibodies to the Chlamydia trachomatis main outer membrane protein and their immunobiological properties. *Ukr Biochem J.* 2019; **91**(3): 90–8.
15. *Sokol AA, Grekov DA, Yemets GI, Galkin AYu, Shchotkina NV, Dovghaliuk AA, Telehuzova OV, Rudenko NM, Romaniuk OM, Yemets I M.* Comparison of bovine pericardium decellularization protocols for production of biomaterial for cardiac surgery. *Biopolym Cell.* 2020; **36**(5): 392–403.
16. *Patent of Ukraine No.*112442 “Method of obtaining of recombinant human IL-7 via recombinant molecule, containing cDNA copy of a structural part of spliced cDNA of human IL-7 gene and expression DNA vector”
17. *Thapa A, Shahnawaz M, Karki P, Raj Dahal G, Sharoar MG, Yub Shin S, Sup Lee J, Cho B, Park IS.* Purification of inclusion body-forming peptides and proteins in soluble form by fusion to Escherichia coli thermostable proteins. *Biotechniques.* 2008;**44**(6):787–96.
18. *Nikolaenko IV, Goncharenko VS, Shimko NN, Galkin AIu.* [Isolation of surface antigen of hepatites B virus]. *Ukr Biokhim Zh (1999).* 2007;**79**(2):114–22.
19. *Gilchuk PV.* Evaluation of renaturation methods for industrial obtaining of recombinant proteins from Escherichia coli inclusion bodies in biologically active form. *Biopolym Cell.* 2004; **20**(3): 182–91.
20. *Sokol AA, Grekov DA, Yemets I, Galkin O, Shchotkina N, Rudenko N, Yemets I.* Biocompatiblibility analysis of the decellularized bovine pericardium. *Cell Organ Transplantol.* 2020; **8**(2): 193–7.
21. *Galkin A, Komar A, Gorshunov Y, Besarab A, Soloviova V.* New monoclonal antibodies to the prostate-specific antigen: obtaining and studying biological properties. *J Microbiol Biotechnol Food Sci.* 2019; **9**(3); 573–7.
22. *Golembiovska OI, Galkin AYu, Besarab AB.* Development and validation of a dissolution test for ursodeoxycholic acid and taurine from combined formulation. *Scientific Study & Research — Chemistry & Chemical Engineering, Biotechnology, Food Industry.* 2019; **20**(3); 377–94.
23. *van de Loosdrecht AA, Beelen RHJ, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MMAC.* A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. *J Immunol Meth.* 1994; **174**(31): 311–20.

Виділення та очистка рекомбінантного інтерлейкіну-7 людини

Т. М. Луценко, В.В. Мотроненко

Мета. Розробити метод виділення та очищення рекомбінантного інтерлейкіну-7 людини з бактерійних тілець включення. **Методи.** Синтез білка шляхом ферментації, денситометрирування, ренатурація методом гель-фільтрації, двоетапна хроматографічна очистка білка. **Результати.** Отримані шляхом ферментації в клітинах *E.coli* тільця-включення, що містять рекомбінантний інтерлейкін-7 людини солюбілізували в буфері наступного складу: 7М гуанідин гідрохлорид, 100мМ Тріс-HCl, 0,1 % Твін-20 та 50мМ дітіотріетолу протягом 1 год при температурі 22ºС. Ренатурацію проводили заміною буферу на колонці XK26/40 (GE Healthcare) із Sephadex G-25 fine врівноваженій розчином Кларка-Лабса, який містив L-аргінін гідрохлорид та твін-20. Наступну стадію очищення рІЛ-7 проводили у два етапи: на першому етапі для проведення очистки рІЛ-7 в колонку упаковували 20 мл Q сефарози. Фракцію рІЛ-7, очищену на Q сефарозі очищали на SP сефарозі. Елюцію рІЛ-7 проводили двохступінчастим градієнтом NaCl (0,2 і 0,7 М) в буфері 0,05 М KH_2PO_4/NaOH pH 6,0; 0,1 М L-аргініну, 0,1 % Твін-80. **Висновки.** Вихід цільового білка після всіх запропонованих етапів очистки становив 10 %, чистота близько 95 %, що дозволяє його використання для подальшої розробки різноманітних фармацевтичних форм.

Ключові слова: інтерлейкін-7, тільця-включення, рефолдинг, гель-фільтрація, ренатурація.

Разработка метода выделения и очистки рекомбинантного интерлейкина-7 человека

Т. Н. Луценко, В. В. Мотроненко

Цель. Разработать метод выделения и очистки рекомбинантного интерлейкина-7 человека из бактериальных телец включения. **Методы.** Синтез белка проводили путем ферментации, денситометрирування, рефолдинг методом гель-фильтрации, двухэтапная хроматографическая очистка белка. **Результаты.** Полученные путем ферментации в клетках *E.coli* тельца-включения, содержаще рекомбинантный интерлейкин-7 человека солюбилизировали в буфере следующего состава: 7М гуанидин гидрохлорид, 100 мМ Трис-HCl, 0,1 % Твин-20 и 50 мМ дитиотриетола течение 1 ч при температуре 22ºС. Ренатурацию проводили заменой буфера на колонке XK26 / 40 (GE Healthcare) с Sephadex G-25 fine уравновешенной раствором Кларка-Лабса, содержащий L-аргинин гидрохлорид и твин-20. Следующую стадию очистки рИЛ-7 проводили в два этапа: на первом этапе для проведения очистки рИЛ-7 в колонку упаковывали 20 мл Q сефарозы. Фракцию рИЛ-7, очищенную на Q сефарозе очищали на SP сефарозе. Элюцию рИЛ-7 проводили двухступенчатым градиентом NaCl (0,2 и 0,7 м) в буфере 0,05 М KH_2PO4 / NaOH pH 6,0; 0,1 М L-аргинина, 0,1 % Твин-80. **Выводы.** Выход целевого белка после всех предложенных этапов очистки составил 10 %, чистота около 95 %, что позволяет его использование для дальнейшей разработки различных фармацевтических форм.

Ключевые слова: интерлейкин-7, тельца-включення, рефолдинг, гель-фильтрация, ренатурация.

Received 31.05.2021