J Biochem Tech (2018) Special Issue (2): 15-20
ISSN: 0974-2328

Optimizing the Culture of Protein Production of Recombinant Human Growth Hormone in Host E. coli

Mehdi Mohammadpour-Aghdam, Ahmad Molaei Rad *, Azadeh Azizi

Received: 22 November 2017 / Received in revised form: 18 May 2018, Accepted: 23 May 2018, Published online: 05 September 2018
© Biochemical Technology Society 2014-2018
© Sevas Educational Society 2008

Abstract

Growth hormone as a drug product that is not limited to its application to the topic of short-term treatment of pediatric patients is made recombinantly. Since the low expression of this heavy protein in the recombinant state influences its effective extraction, various changes have been made on this protein in the culture and expression stage. In this study, optimization based on the concentration of DMSO additive in culture medium, incubation temperature and induction concentration were investigated. The results of PAGE-SDS showed a good percentage for 1% dimethyl sulfoxide and a suitable temperature for incubation of 16 ° C and an appropriate concentration for Isopropylthiogalactoside inducer 0.1 mM. In the next step, the culture specimen was optimized with native gel and dual display (CD) and Visible-UV, and confirmed the expression and production of recombinant protein.

Key words: Growth hormone, additive, dimethyl sulfoxide, isopropyl thiogalactoside, rotational duplex.

Introduction

The single-chain polypeptide human growth hormone protein consists of 191 amino acids, secreted from the previous part of the pituitary gland. The molecular weight of this protein is about 22 kDa (Johnson, 1983). The second structure of the protein consists of alpha helix, which is connected to two basic loops by two disulfide bands (Sindelar and et al, 2013). Growth hormone protein is a non-glycosylated protein and therefore expressible in recombinant forms in both prokaryotic and eukaryotic systems (Blijlevens & Sonis, 2006)). High intake of this protein as a drug has increased the demand for its recombinant production. But the main problem of the recombinant expression of this protein, especially expression in prokaryotic systems, is the insolubility of the protein (Roifman and et al, 1985). The resulting proteins are an abnormal accumulation or ankylosing spondylitis. The formation of these objects is due to the excessive presence of hydrophobic groups at the protein level, which can destroy the biological activity of the protein. On the other hand, the results of the study of the folding of the growth hormone protein also show that the folding of this protein is much faster compared with proteins of similar molecular weight, which is due to the explosive loss of the hydrophobic sequence in the protein structure (Sindelar and et al, 2013). An essential solution for soluble occlusion is reducing the probability of forming hydrophobic bonds between growth hormone proteins before and after protein retrusion (Narhi and et al, 2001). On the other hand, recombination of the recombinant protein of the growth hormone is still a bottleneck in structural biology and the development and development of this protein as a biocidal product. So far, a variety of solutions have been made to solve the problem of correcting and correcting the growth retardation of the growth hormone protein, most notably protein engineering for the administration of surface amino acids to reduce the surface hydrophobicity of the protein (Macmillan and et al, 2001; Zündorf & Dingermann, 2001), which involves chemical and physical changes in the level of the protein without a prescription The gene is about protein expression and also the use of Chaototrop additives. Chaotrope supplements, without altering protein structure, cause protein-level coatings and prevent our hydrophobic interactions between proteins, thus increasing their dissolution. On the other hand, in addition to protein engineering techniques, the use of Chaotrope and organic solvents adds to the growth hormone protein dissolution without altering the protein structure. Also, these additives, as facilitators of the protein retention process, also prevent the formation of protein inactive states. The purpose of this study is to investigate the effect of chaotropic and organic solvents on the amount of solubility of the occlusion of the occlusion due to recombinant expression of the growth hormone protein and the process of retracting the soluble proteins.
In this paper optimization of different concentrations of DMSO and IPTG in the culture step is optimized as additive and then incubation temperature.

Mehdi Mohammadpour-Aghdam, Ahmad Molaei Rad *, Azadeh Azizi
Biotechnology Research Center, Malek Ashtar University of Technology, Tehran

***Email:** molaeirad@gmail.com

Materials and Methods

cultivating bacteria

For this step, prepare 2 liters of LB broth medium separately in 5 identical dishes and add each 0.4 ml ampicillin to each stoic and add the contents of a 5-mL overnight culture-grown falcon and injected into the culture medium in the same manner for 3-4 hours under incubation conditions of 37 ° C and at about 200 rpm to reach a maximum of 0.66 wavelengths at 600 nm. Once assured, the induction is performed by IPTG with the different concentrations listed in Table 1 and the recurrence of 3-4 h incubation is carried out at 37 ° C and at about 200 rpm. After this step, and ensuring the culture of the bacteria containing the recombinant protein, the bacterial collection stage is carried out by centrifugation at 9000 rpm for 15 minutes and at 4 ° C. After centrifugation, the precipitate is discarded and stored in a freezer at -70 ° C. Next, SDS-PAGE electrophoresis is used to ensure protein expression.

Table 1- Different concentrations of IPTG used in the culture stage

A	B	C	D
control	0.5 mM	0.1 mM	0.005 mM

In order to optimize incubation temperature, as in Table 2, different temperatures at 0.1 mM IPTG concentrations were performed as optimized IPTG concentrations and then SDS-PAGE electrophoresis was used to obtain the appropriate temperature.

Table 2- Different temperatures used for incubation

Sample	A	B	C	D	E	F
Temperature (°c)	37	30	Without IPTG	25	20	16

After obtaining a suitable concentration for IPTG at 0.1 mM IPTG concentration and appropriate temperature of 16 ° C for incubation temperature, in the next step, different concentrations of DMSO in the medium were investigated, which was used in Table 3 for different concentrations of DMSO Is expressed.

Table 3- Different concentrations of DMSO used in the culture stage

A	B	C	D	E
1%	1.5%	2%	Control	0.5%

At this stage, we act as before, and after collecting the sediment, SDS-PAGE is used to ensure protein expression. After ensuring the results obtained by SDS-PAGE, re-cultivation was carried out in optimal conditions, and using native gel, circular dichroism (CD) and UV-Visible techniques were used to ensure expression and production.

Cell failure

Recovered bacterial recombinant bacteria from centrifuges at 9000 rpm are dissolved for 15 minutes in a buffer and completely buffered by a mechanical stirrer (it should be noted that PMSF has a finite solubility, so firstly Solve 50 µl of absolute alcohol and then add). After preparing a single-handed bacterial solution, the bacteria break down by using a sonication (ultrasonic, 400 W 20KHz ultrasonic technology development) at 4 ° C and in 10 stages of 40 seconds, which is between 80 seconds each time.

Isolation

Separation is done in three stages of washing, which in the first stage of the sediment, dissolve the cell failure in buffer (50 mM Tris-HCl and 1% Deoxycholic acid), and to separate cells that are not broken out from other cells, first, centrifuge stage runs at 4000 rpm and lasts for 4 minutes. The supernatant is then removed and the precipitate is discarded, then the supernatant is carried out for 20 minutes at 12,000 rpm. In the second step, the precipitates obtained in the buffer (Tris-HCl, 50 mM and 1% Deoxycholic acid) are dissolved, and centrifuges are carried out for 20 minutes at 12,000 rpm. At this stage, after removal of the supernatant, the sediments in the buffer (Tris-HCl mM 50) are dissolved and after centrifugation of the solution with the magnetic stirrer is uniformly adjusted for 20 minutes at 12,000 rpm. After this stage, the sediment is collected and stored at -20 ° C for the soluble stage.

Solubilization

At this stage, after separation, the precipitate was dissolved in a buffer (50 mM Tris-HCl, 2% deoxycyclic acid and 5 mM EDTA), and then washed twice, and each time washed the centrifuge With 16000 rpm for 15 minutes, then the precipitate obtained from the previous step in a buffer (100 mM Tris-HCl, 2 M urea, 10% glycerol, 2% sucrose, 1% triton x100 and 1 mM EDTA) We soluble it at room temperature for 1 hour and then centrifuge for 20 minutes at a speed of 16000 rpm and then collect the precipitate in the weave (100 mM Tris-HCl, 2 M urea, 10% glycerol) refolding solution and we will be ready after the prototype stage. To ensure the correct solution of the growth hormone protein, 12.5% SDS-PAGE was used.

Refolding

This stage is one of the most important stages in the production of protein. Therefore, at the beginning of retrusion, the retrusion of the protein collected from the previous step is performed, first, the precipitate obtained from the previous step in a reverse buffer (100 mM Tris-HCl, urea 2 Molar, 10% glycerol, 5% sucrose, 0.5 mM EDTA and 0.5 mM L-Argenin). The dissolved sediment ratio in the buffer should be 1 to 10. The protein was dissolved using a pump (PUMP P-1 pharmacia Biotech) at 0.5 ml / min (lowest speed of the device) at 4 ° C. After dissolving the protein inside the buffer, place the supernatant at 4 ° C for 24 hours. In this step, we use native electrophoresis gel to ensure proper protein reagent.

Result

SDS-PAGE

The results of electrophoresis in Figure 1 show the different concentrations of IPTG. According to the bands formed, the concentration of 0.1 mM can best be expressed. Fig. 2 shows the results of different temperatures, which can be deduced from Fig. 2. Temperature Suitable for 16 degrees centigrade. In the next step, considering the optimized environmental conditions in the previous steps, different concentrations of DMSO were considered, which is the most appropriate according to Fig. 3. 1% concentration.

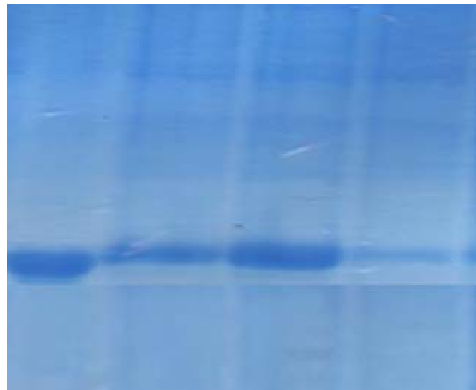

Fig. 1: In SDS-PAGE, the effect of different concentrations of IPTG on the expression of the human growth hormone protein. From left to right: control, 0.5, 0.1, 0.05 (in mM)

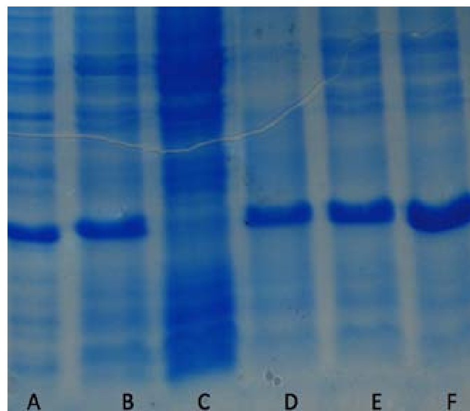

Fig. 2: Temperature effect on growth hormone protein expression A: temperature 37 ° C, B: temperature 30 ° C, C: no induction IPTG, D: temperature 25 ° C, E: temperature 20 ° C, F: temperature 16 ° C

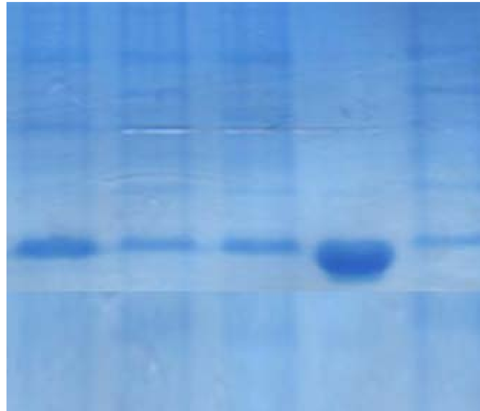

Fig. 3: In SDS-PAGE, the effect of different concentrations of DMSO on expression of human growth hormone protein. Left to right: 1%, 1.5%, 2%, control, 0.5%

Native-PAGE

In the refolding stage, using the native gel, the results of the purification of the growth hormone protein can be considered. According to Fig. 4, it can be concluded that according to the native nature of the gel, the location of the band is lower than normal and in the presence of SDS (Wittig and et al, 2006).

Fig. 4. Native gel after soluble inclusion body

Circular Dichroism Spectroscopy (CD)

With the help of Circular Dichroism Spectroscopy (CD) spectrophotometric studies, the pattern of the second structure of the protein is determined. Thus, by comparing the pattern of the second structure of the protein purified by the control protein purchased from LG Life Sciences, by two colorimetric exponential (Aviv model 215; Lakewood, NJ, USA) in the range of 195-260 nm with 1 mm quartz tubes and A concentration of 0.4 mM protein was performed and structural changes in protein were examined.

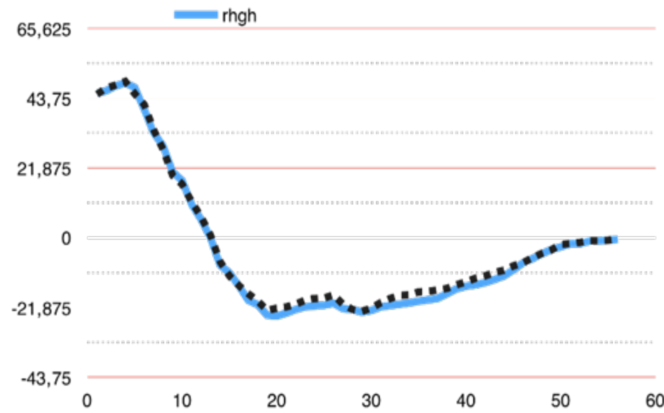

Fig. 5: CD range of protein region. (▬) Protein Control; (▬) Protein Samples Purified by Diffuse Meandering Method. All samples were measured at 0.4 mM protein concentration

Spectrophotometer

The experiment was performed with a model of UV model Shimadzu 3100, Japan. The recombinant growth hormone protein sample was examined at 0.8 mM concentration with recombinant growth hormone purchased and absorbed at 640-260 nm. The absorption change was recorded at 278 nm.

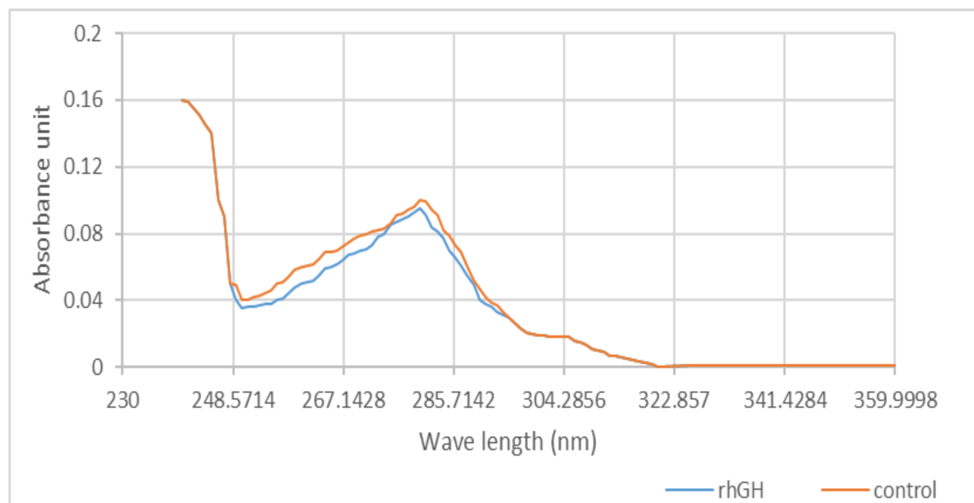

Fig. 6: UV-Visible Spectrum Purified Protein. (▬) standard protein purchased; (▬) Protein samples purified by Visible-UV were analyzed. All specimens were measured at 0.8 mM protein concentration

Discussion

In countless studies, the effects of different additives such as solvent types, water vapor diffusion agents, different types of anionic and cationic micronuclei and polymer are measured on the medium and the retenuation process of this protein is measured. Various strategies in these cells are used to increase the amount of production used, such as adjusting the temperature of the culture, adding chemicals and engineering bioreactors, including chemical compounds used to stop the cell cycle. In this study, dimethylsulfoxide (DMSO) and incubation temperature as well as IPTG concentration change were investigated. According to the results of the native gel and further with the use of ultraviolet and UV-Visible techniques, the protein expression There was no significant difference with the purchased sample. Because the presence of two CD signals of region 208 and 225 nm indicates the presence of a growth hormone protein, and also the presence of a control sample also proves it. It has also been shown in this study that DMSO and glycerol compounds cause protein to stabilize in its natural configuration and also act as a chemical chalcopyron with the effect of protein retrusion. On the other hand, it should be noted that in connection with the protein expression stage, DMSO also inhibits cell growth, prevents apoptosis, and increases the phenotypic differentiation of M-dandruff, and its polar nature makes it possible to enter the membrane without causing significant damage. Cell. DMSO causes the cell cycle to stop in the G1 phase and, by loosening non-polar reactions between histone and chromatin,

increases RNA synthesis by RNAPol. Glycerol is widely used in the pharmaceutical industry and biotechnology as a protein stabilizer. Glycerol as osmolyte can form hydrogen bonds and forms a blue layer around protein molecules, which increases the surface tensile and dissolved viscosity, thus preventing the formation of protein aggregates during retinalization. Many salts, as well as other amino acids, such as glycine and arginine, facilitate the growth reuptake of the growth hormone protein.

References

Blijlevens, N., & Sonis, S. (2006). Palifermin (recombinant keratinocyte growth factor-1): a pleiotropic growth factor with multiple biological activities in preventing chemotherapy-and radiotherapy-induced mucositis. *Annals of Oncology*, *18*(5), 817-826.

Johnson, I. S. (1983). Human insulin from recombinant DNA technology. *Science*, *219*(4585), 632-637.

Macmillan, D., Bill, R. M., Sage, K. A., Fern, D., & Flitsch, S. L. (2001). Selective in vitro glycosylation of recombinant proteins: semi-synthesis of novel homogeneous glycoforms of human erythropoietin. *Chemistry & biology*, *8*(2), 133-145.

Narhi, L. O., Arakawa, T., Aoki, K., Wen, J., Elliott, S., Boone, T., & Cheetham, J. (2001). Asn to Lys mutations at three sites which are N-glycosylated in the mammalian protein decrease the aggregation of Escherichia coli-derived erythropoietin. *Protein engineering*, *14*(2), 135-140.

Roifman, C. M., Mills, G. B., Chu, M., & Gelfand, E. W. (1985). Functional comparison of recombinant interleukin 2 (IL-2) with IL-2-containing preparations derived from cultured cells. *Cellular immunology*, *95*(1), 146-156.

Sindelar, R. D., Crommelin, D. J., & Meibohm, B. (2013). *Pharmaceutical biotechnology*. Springer.

Wittig, I., Braun, H. P., & Schägger, H. (2006). Blue native PAGE. *Nature protocols*, *1*(1), 418.

Zündorf, I., & Dingermann, T. (2001). From cattle, swine, and horse insulin to human insulin: the biotechnology and genetic technology of insulin production. *Pharmacy in our time* , *30* (1), 27.