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BIOTECHNOLOGY FOR OBTAINING THE RECOMBINANT HEAT SHOCK PROTEIN (HSP-60) OF *CHLAMYDIA TRACHOMATIS* AND EVALUATION OF THE PERSPECTIVES OF ITS USE IN SEROLOGICAL DIAGNOSTICS

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Identification of humoral immune responses to 60 kDa heat shock protein of Chlamydia trachomatis (HSP-60) is of great diagnostic value, as evidence of probable autoimmune process and risk of pathology of female reproductive system. To develop a highly ELISA kit for the detection of anti-HSP-60 antibodies should be used recombinant antigen of the pathogen. A methodological development of biotechnology of recombinant HSP-60 (rHSP-60) with its accumulation in the cytoplasm of E. coli in soluble form has been performed: the optimal parameters of cultivation producer (growth medium, temperature, concentration of expression inducer and biosynthesis duration) providing a preferential (79%) accumulation of the target protein in a soluble form. Purification technology was based on the usage of affinity chromatography on glutathione-sepharose and enzymatic hydrolysis of GST-containing proteins using a clotting factor Xa, and gel filtration on Sephadex G-75 for final purification. We carried out comparative studies of the activity of the obtained rHSP-60 as a part of immunosorbent in ELISA for detecting specific IgG antibodies in human serums. The experiment results for the group of positive serums were assessed based on the positivity index (a ratio of the average arithmetic value of optical density in ELISA for positive and negative (cut off) serums). At the same recombinant protein concentration in the immunosorbent for our obtained rHSP-60, we obtained a 30% higher PI compared to the analogue commercial protein. Of interest were the results of a comparison of the immunological activity of our obtained HSP-60 and its conjugate with GST – positivity indices using these two proteins in the immunosorbent composition were comparable – 2.2 and 2.4, respectively. At the same time, no interaction between serum immunoglobulins and GST enzyme was detected. Such data indicate on absolute possibility of the use of the GST-conjugated rHSP-60 protein for immunodiagnostic purposes. It has been proven that the obtained rHSP-60 preparations as well as its GST-conjugate were highly effective when used in the immunosorbent composition in ELISA for the detection of specific IgG-antibodies.

Keywords: heat shock protein, Chlamydia trachomatis, recombinant protein, biosynthesis, chromatographic purification.

Introduction. Urogenital chlamydiosis (UC) is one of the most common sexually transmitted infections. According to the World Health Organization data, approximately 90 million people are infected sexually with the pathogen *Chlamydia trachomatis* every year (Гончарук, 2008). In Ukraine, UC incidence is 80 people per 100 thousand residents. About 16% of pregnant women are infected with *Ch. trachomatis*. The etiological role of chlamydia has been established in 50-60% of tubal infertility cases. A quarter of the cases of ophtalmopathology and respiratory diseases in newborns and young children are related to chlamydia infection (Исаков и др., 2010). One of the most important components of the fight against the UC spread is effective diagnostic of this disease, which is carried out by both direct (detection of antigens, nucleic acids, microscopy, and cultivation of the causative agent) and indirect (detection of specific antibodies) methods. One of the methods, which became widely used in UC diagnostics, is the enzyme-linked immunosorbent assay (ELISA), application of which allows differential diagnostics to determine the stage and nature of the disease course. For this purpose, studies of human blood serum (plasma) and biological secrets for the content of IgM, IgA, and IgG antibodies specific to the causative agent antigens are conducted. With long-term persistence of *Ch. trachomatis*, there is an increase in the expression of a 60 kDa heat shock protein (HSP-60), which has high homology degree with the analogue protein of human cells. Its production results in a molecular mimicry phenomenon that in its turn frequently causes autoimmune processes. An increased level of the immune system stimulation by the heat shock

protein that occurs in a reinfection or persisting infection results in chronic inflammation and tissue cicatrization and can play a role in the pathogenesis of endometrium and uterine appendage lesions. Such immunopathological reactions can be a cause of extrauterine pregnancy and tubal infertility (Исаков и др., 2010; Мавров и др., 2010; Мукантаев и др., 2012). Therefore, an examination of patients infected with *Ch. trachomatis* for the presence of IgG antibodies to HSP-60 is of great importance. It is obvious that for the creation of an appropriate high informative ELISA test-kit, reference antigens of the causative agent are necessary to have. The most modern way to obtain the antigen proteins is genetic engineering and molecular biotechnology methods that ensure not only the standardization of the obtained protein but also give an opportunity to develop progressive technologies of protein extraction and purification.

The goal of this study was to develop a technology for obtaining and purification of *Ch. trachomatis* recombinant HSP-60 as well as to evaluate its use in immune diagnostics.

Materials and methods. *Plasmid*. The recombinant pET42a/ChtHSP-60 plasmid used in our work was constructed based on the pET42a(+) expression vector (Novagen, USA) and contains a full-size insert of the *Ch. trachomatis* HSP-60 gene under control of the lac-operon promoter. The recombinant pET42a/ChtHSP-60 plasmid also contains the kanamycin resistance (Kan) gene, a sequence encoding the glutathione-S-transferase (GST) enzyme and T7 transcription terminator.

Growth media. Three variants of growth media were used in the study: LB medium (0.5% yeast extract, 1.5% tryptone, 0.5% NaCl); TB medium (10% yeast extract, 10% peptone, 0.94% K_2HPO_4, 0.22% KH_2PO_4, 0.8% glycerin), medium no. 3 (2.4% yeast extract, 1.2% tryptone, 0.5% glucose, 0.0195% $MgSO_4$). The antibiotic kanamycin (50 μg/ml) was added to each growth medium.

Recombinant Ch. trachomatis HSP-60 expression in the E. coli bacterial system. E. coli BL21 (DE3) strain cells transformed with the *pET42a/ChtHSP-60* plasmid were cultivated in different growth media and at different temperatures in the presence of kanamycin (50 μg/ml) and with intensive shaking (250 rpm) until obtaining optical density OD_{600} = 0.4. To induce the recombinant protein expression, isopropyl-β-thiogalactoside (IPTG) solution was added. After incubation, the cells were collected by centrifugation at 4000 g for 20 min, the cell pellet was washed with chilled buffered saline, frozen and stored at –70 °C. Cultivation parameters such as temperature, time, IPTG concentration and growth medium composition were the study objects.

Affinity purification of the recombinant Ch. trachomatis HSP-60. The purification was performed at 4°C. The cell pellet (2.0-2.5 g) was re-suspended and lysed in 15 ml of lysing buffer (10 mM tris-HCl, pH 7.5, 150 mM NaCl, 50 mM NaF, 5 mM EDTA, 1% tryptone X-100, 1 mM phenilmethylsulfonyl fluoride) in the presence of the protein kinase inhibitor set (Roche, France). The cells were homogenized by mechanical grinding with sand; the obtained lysate was centrifuged at 17000 g for 20 min. A 50% glutathione-sepharose suspension (GE Healthcare Bio-Sciences AB, Sweden) in lysing buffer was added to the supernatant and incubated for 2 hours while stirring. Glutathione-sepharose was intensively washed with 50 mM tris-HCl, pH 7.5, 150 mM NaCl with 0.05 % Twin-20 three times for 5 min and twice with 50 mM tris-HCl, pH 8.0. The proteins were eluted from the affinity sorbent with 50 mM reconstituted glutathione in 50 mM tris-HCl, pH 8.0, 150 mM NaCl while stirring for 5 min. The glutathione-sepharose was precipitated by centrifugation at 3000 rpm for 1 min. This operation was repeated three times. The obtained eluates were pooled and dialyzed against a buffer containing 20 mM tris-HCl, pH 7.5, 150 mM NaCl, 1 mM dithiothreitol for glutathione extraction. Concentration of GST-conjugated protein was measured by Bradford method. Proteins were stored at –20 °C in 50% glycerin.

For isolation of the recombinant *Ch. trachomatis* HSP-60 (rHSP-60) from the GST-conjugate (rHSP-60-GST) Xa factor (Sigma, USA) was used. The GST-conjugated protein bound to the glutathione-sepharose and equilibrated with a buffer containing 50 mM tris-HCl, pH 8.0, 100 mM NaCl, 2 mM $CaCl_2$ was subjected to proteolysis. The factor Xa was added to the sorbent suspension at a quantity of 2 μg per 100 μg of protein. The reaction was performed at room temperature for 5 hours while stirring. The sorbent was precipitated by centrifugation at 3000 rpm for 1 hour. The supernatant was collected and transferred to the ultracentrifugation column Ultracel YM-50 (Millipore, USA) and centrifuged for 20 min at 10000 rpm. The filtrate was collected, the protein concentration was measured by Bradford method, and protein electrophotometric analysis was carried out.

Gel-filtration. This type of chromatography was used to transfer the protein into another buffer and as a method for purification of the target product from minor proteins. The procedure was carried out on a 1.5 × 20 cm column with sephadex G-75 (Sigma, USA). The elution of the preparation was performed at a rate of 1 ml/min. Protein peak retention time was registered spectrophotometrically at 280 nm, buffer retention time was measured conductometrically.

Electrophoresis and densitometry. Electrophoretic analysis of cell biomasses and proteins was carried out in a 15% polyacrylamide gel (PAAG) in the presence of 1% sodium dodecyl sulfate (SDS) in tris-tricine buffer system (Рахматулина и др., 2012). Following molecular weight (MW) markers (Sigma, USA) were used: trypsin inhibitor, soybean (20.0 kDa), trypsinogen, bovine pancreas (24.0 kDa), carbonic anhydrase, bovine erythrocytes (29.0 kDa), glyceraldehyde-3-phosphate dehydrogenase, rabbit muscle (36.0 kDa), ovalbumin, chicken egg (45.0 kDa), glutamic dehydrogenase, bovine liver (55.0 kDa), albumin, bovine serum (66.0 kDa), phosphorylase B, rabbit muscle (97.0 kDa), β-galactosidase, *E. coli* (116.0 kDa), myosin, porcine heart (200.0 kDa). Scanning of stained polyacrylamide gels and calculation of the target protein content were carried out by TotalLab 1.10 software.

Indirect ELISA procedure. Antigen sorption was done in 0.05 M carbonate-bicarbonate buffer (pH 9.6) overnight at 4 °C at a concentration of 4 μg/ml. Free areas of the well plate were occupied by incubation with bovine serum albumin solution. One hundred μl of the studied human sera were added into wells, incubated for 1 hour at 37°C, and washed three times with phosphate buffered saline with addition of 0.05% twin-20 (PBST), pH 7.2-7.4. For detection of the conjugated antibodies, the monoclonal anti-human IgG antibodies conjugated with horseradish peroxidase were added to the plate wells and incubated for 30 min at a 37°C. The plate was washed three times with PBST and once with water. As a substrate we used a 0.003% hydrogen peroxide solution in 0.15 M citrate buffer, pH 5.0, and 3,3',5,5'-tetramethylbenzidine as a chromogen. The reaction was stopped with 2 M sulfuric acid. The absorbance at a wavelength of 450/620 nm was measured on a spectrophotometer.

Results and discussion. *E. coli* BL21 (DE3) strain cells were transformed with the recombinant pET42a/ChtHSP-60 expression plasmid as well as with a vector without the *Ch. trachomatis HSP-60* fragment. Kanamycin-resistant bacterial transformants were examined for their ability to synthetize the recombinant protein in the presence of 0.1 mM IPTG by electrophoretic analysis. The analysis of the obtained results showed that the protein with a MW of 85 kDa (corresponds to the MW of the GST-conjugate of *Ch. trachomatis* HSP-60) was presented in the soluble and insoluble protein fractions in the detergent solution (Fig. 1). Thus, the target product was accumulated both in inclusion bodies and in a soluble form. In this regard, we conducted studies aimed to optimize the cultivation conditions for the recombinant protein producer and to increase its expression in *E. coli* cells in the soluble form. Among optimization parameters, we determined the followings: temperature and cultivation time, inducer concentration (IPTG) as well as the growth medium composition.

First of all, we conducted studies to determine an optimal cultivation temperature based on the maximum output of the target product in soluble form. From described in literature different temperature schemes for the cultivation of the prokaryotic producers of recombinant proteins, two basic approaches can be highlighted: first of them proposes the cultivation of recombinant bacteria at a stable temperature of 37°C (both for biomass accumulation and for the target product synthesis) (Славченко и др., 2003), another – transition from the temperature of biomass increase (37°C) to the temperature of recombinant protein synthesis (25-28°C) (Чінов, 2005) that ensures more optimal conditions for folding of the polypeptide chain of the recombinant protein and can have effect on its solubility degree and thus on further strategy of its extraction and purification. Another methodological approach is the so-called thermal shock (40-43°C) using a strain with thermoinducible recombinant gene (Gardella et al., 1990), which was not applicable in our case.

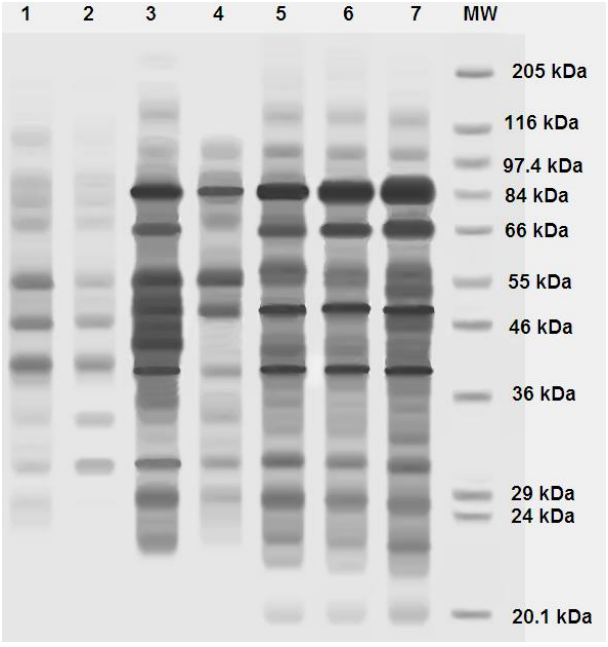

Fig. 1. Electrophoregram of rHSP-60-GST expression results in E. coli BL21 (DE3) strain cells.

Note: 1 – cells transformed with the vector without insert; 2 – culture without expression induction; 3 – fraction of total cell proteins; 4 – fraction of triton-soluble proteins; 5-7 – cells after 2, 3, and 4 hours of expression induction; MW – molecular weight markers.

A study of the effect of cultivation temperature*E. coli* cell concentration in the cultural fluid regime on target protein accumulation in the soluble(OD_{600} decreased by 30%) and gradual increase in the fraction was conducted in parallel with thesoluble recombinant protein content up to the maximum determination of optimal concentrations of thevalue of 0.41 mg/ml. When IPTG concentration was expression inducer: a series of parallel studies wasincreased in a range of 0.3÷0.5 mM, we observed a carried out at different temperature regimes (37°C;decrease in not only the biomass output but also in the 37°C/28°C) and different IPTG concentrations (0÷0.5target product concentration. We relate such a mM). Herein, the inducer was added when OD_{600} = 0.4phenomenon to an increase of the toxic effect of the was reached and cultivation proceeded for 2 morerecombinant product on producer cells: extremely high hours; LB medium was used. The process was assessedaccumulation rate of the target protein (foreign for based on cultural fluid optical density values and target*E. coli*) results in not only a switch of the recombinant protein concentrations in the soluble fraction calculatedprotein accumulation predominantly in the form of by the densitometry method (Fig. 2). The study resultsinclusion bodies but also in a delay of the accumulation showed that the most effective temperature regime atof the biomass itself. Fig. 3 shows results of the almost all IPTG concentrations was bacterial culturecalculation of the total cell-produced recombinant growing at a stable temperature of 37°C. Theprotein (at a stable cultivation temperature of 37°C) and recombinant protein concentrations at differentits soluble fraction ratio. As it is seen from the obtained temperature regimes after adding 0.4 mM IPTG wereresults, the highest portion of the target protein in the statistically equivalent. However, this concentration ofsoluble form (almost 79%) is identified at an IPTG the IPTG was not optimal – the highest output of theconcentration of 0.3 mM. Thus, the results of this stage soluble recombinant protein (0.41 mg/ml) was recordedof the study showed that the optimal cultivation after adding 0.3 mM IPTG. It is important that with anconditions were a temperature of 37°C and IPTG increase of the inducer concentration in the medium in aconcentration of 0.3 mM.
range of 0÷0.3 mM, we observed a gradual decrease in

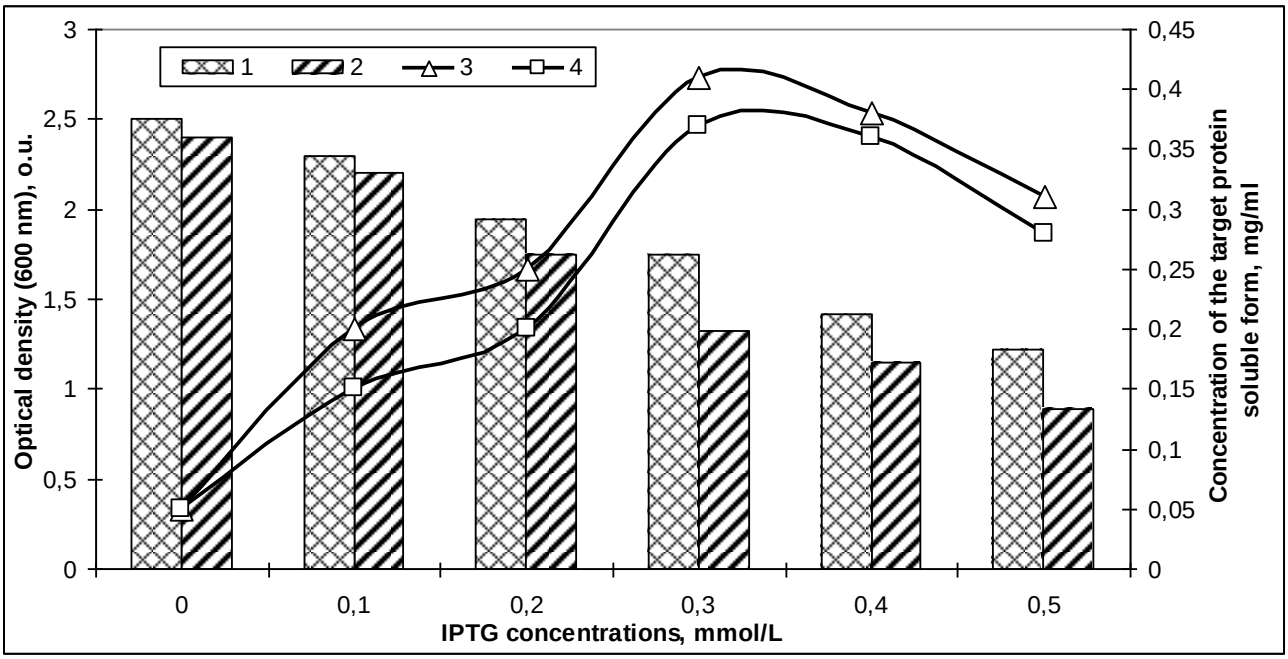

Fig. 2. Determination of the optimal IPTG concentrations and temperature parameters for the cultivation of the recombinant protein producer strain

Note: 1 and 2 – OD_{600} for temperature regimes of 37°C and 37°C/28°C, respectively; 3 and 4 – dynamics of the accumulation of a soluble form of the target protein for temperature regimes of 37°C and 37°C/28°C, respectively.

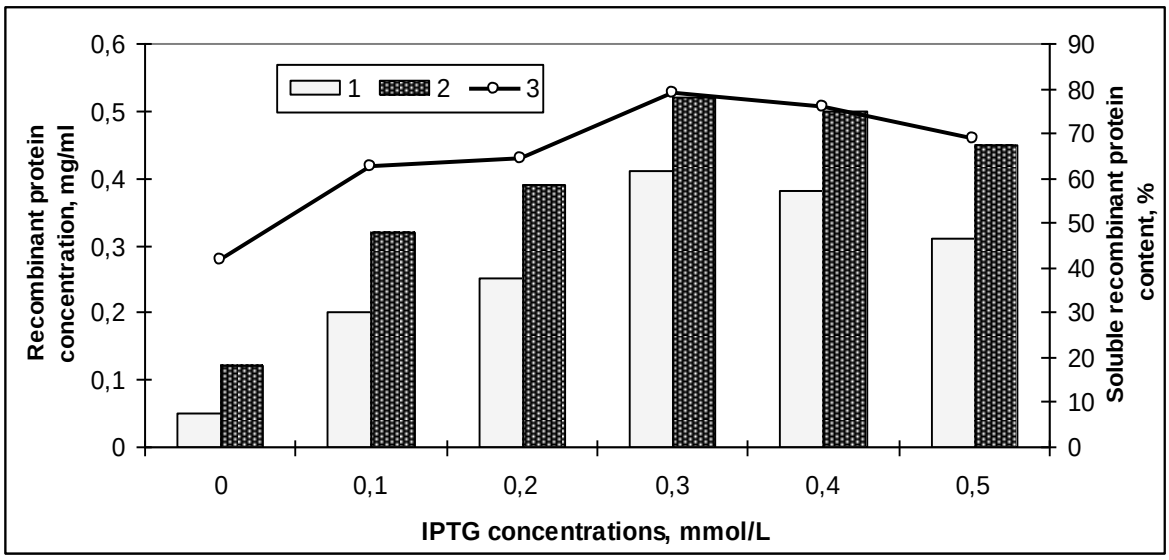

Fig. 3. Dependence of the recombinant protein content in a soluble form on the IPTG concentration.
Note: 1 – total content; 2 – soluble form concentration; 3 – portion of the soluble form.

The following block of the studies was aimed to determine the optimal cultivation time and optimal growth medium. These studies were conducted at a stable cultivation temperature of 37°C and IPTG concentration of 0.3 mM. The results of respective experiments are shown on the Fig. 4. A significant increase in the concentration of the soluble form of recombinant product was observed within the first 3 hours upon addition of the inducer; therefore we can make a conclusion that the optimal cultivation time in this case is 3 hours. The best medium for producer cultivation was LB medium; the worst results were obtained using TB medium. The dynamics of the change of a part of the target product in a soluble form in time (Fig. 5) confirms the correctness of the established optimal temperature for producer cultivation. The absolute content of a soluble fraction of the recombinant protein gradually increases within 1÷3 hours of the cultivation upon inducer addition; however, its portion starts to drop after the 2nd hour of the cultivation. Thus, it is not advisable to carry out the biosynthesis stage for over 3 hours.

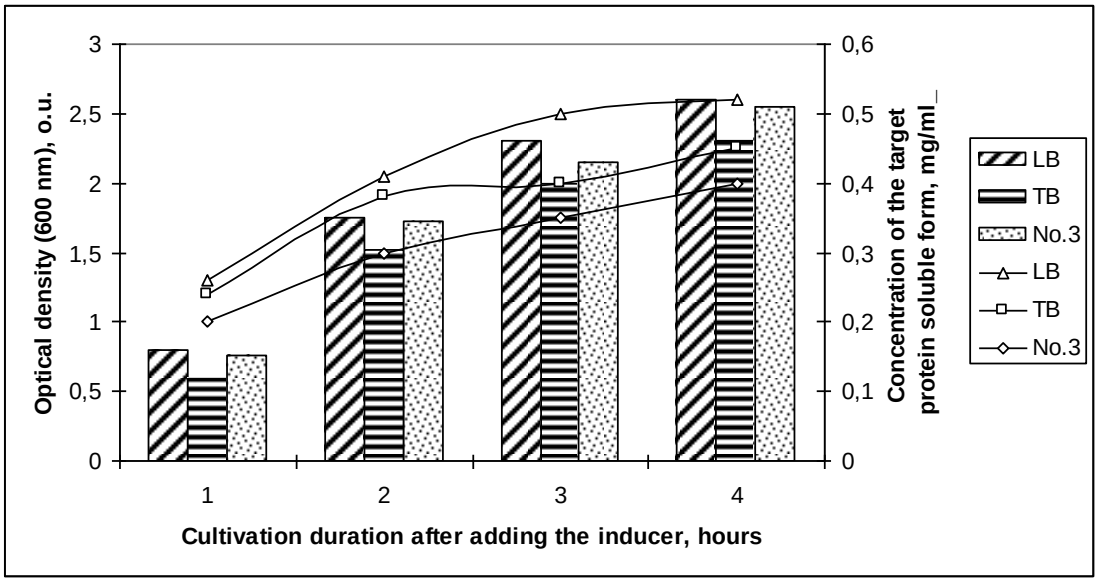

Fig. 4. Determination of the optimal growth medium and cultivation time for the recombinant protein producer strain
Note: histograms – OD_{600} for the growth media LB, TB, and №3; graphs – accumulation dynamics for a soluble form of the target product in the growth media LB, TB, and №3

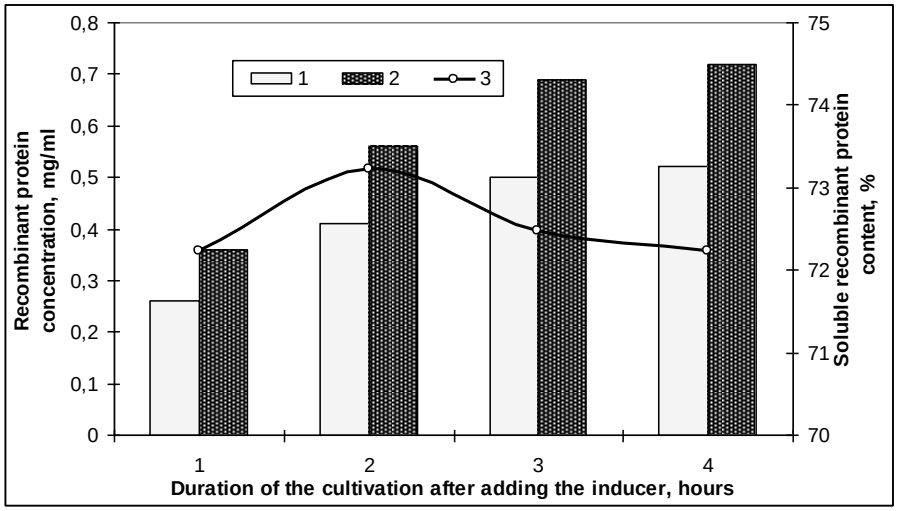

Fig. 5. Dependence of the recombinant protein content in a soluble form on the cultivation time of the producer strain on LB medium

Note: 1 – total content; 2 – soluble form concentration; 3 – portion of the soluble form)

The following block of the experiments was aimed to develope the technologies of the target product extraction and purification. It is necessary to note that the recombinant pET42a/ChtHSP-60 plasmid used in our work assumed obtaining rHSP-60 in the form of a conjugate with GST that implied the use of affinity chromatography on glutathione-sepharose as a basic element for extraction and purification of the target product. Purity control of rHSP-60-GST after the affinity purification procedure was carried out by electrophoresis in PAAG with SDS. The obtained results (Fig. 6) showed that besides a minor block with a MW close to 85 kDa, several additional bands of minor proteins with lower MWs were detected in the preparation after affinity chromatography. Such data also could indicate on the non-specific binding of minor proteins with the affinity column. For verification this assumption, we carried out an additional experiment, which involved the incubation of the affinity sorbent with a solution of tryptone-soluble proteins of a non-transformed *E. coli* strain and electrophoretic analysis of the eluted solution (fig. 6). The obtained results ruled out the non-specific interaction of bacterial proteins with the affinity sorbent. An alternative explanation of the presence of minor protein components in the eluate after affinity chromatography is the partial hydrolysis of the obtained recombinant protein or incomplete rHSP-60-GST translation. A study of the stability of the recombinant protein stored at a temperature of 4°C for 7 days showed no accumulation of the minor protein portion (data are not presented), hence, the most probable explanation of the accumulation of a certain amount of minor proteins is incomplete translation. This assumption was confirmed at the following stages of the work – when assessing the perspective of the use of the obtained recombinant protein for human anti-HSP-60 IgG detection in ELISA. To do this, a fraction of minor proteins was preliminarily separated from the major protein by gel-filtration on Sephadex G-75 (fig. 7).

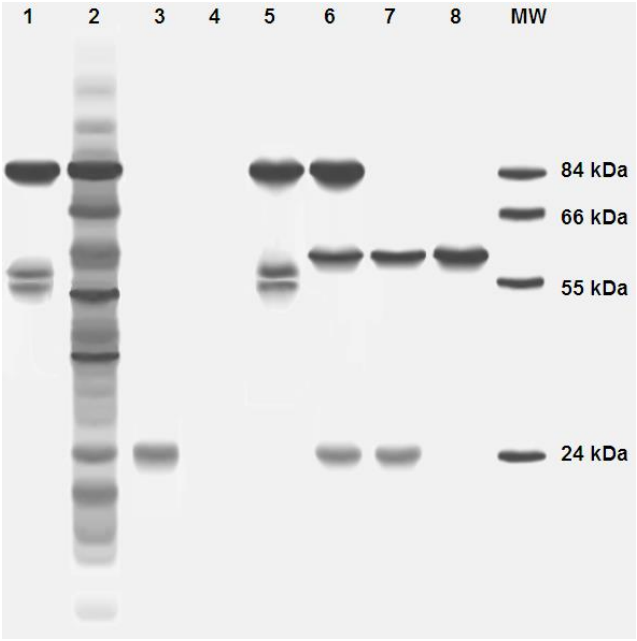

Fig. 6. Electrophoregram of preparations during purification of recombinant protein

Note: 1 – rHSP-60-GST before chromatographic purification, 2 – cells in 2 hours after expression induction, 3 – GST; 4 – proteins from lysates of non induced cells which were adsorbed on the affine sorbent; 5 – proteins from lysates of induced cells which were adsorbed on the affine sorbent; 6, 7 – cleavage of rHSP-60-GST, proteins after 1 h and 6 h, respectively; 8 – reference preparation of rHSP-60; MW – molecular weight markers.

When constructing the pET42a/ChtHSP-60 plasmid, we introduced a nucleotide sequence coding the amino acid sequence, which is a specific site for the recognition of the factor Xa. The activated coagulation factor Xa (Stuart-Prower factor) is a serine proteinase, which specifically recognizes and hydrolyzes the amino acid sequence Ile-Glu (Asp)-Gly-Arg-↓-X (X ≠ Pro) (Ludeman et al, 2003). Taking into account that factor Xa recognition site is absent in the amino acid sequence of *Ch. trachomatis* HSP-60 (GenBank: AAS19618.1), there is a possibility for enzymatic cleavage of the synthetized recombinant rHSP-60-GST conjugate and extraction of rHSP-60. Hydrolysis of the recombinant conjugate was carried out on an affinity sorbent: factor Xa was added to glutathione-sepharose suspension with immobilized rHSP-60-GST protein conjugate. During this procedure, *Ch. trachomatis* HSP-60 passed into solution and eluted from the column. Enzyme separation was done by ultracentrifugation with the use of the commercial set Ultracel YM-50.

Within the framework of additional experiments, we determined optimal conditions for the recombinant protein proteolysis. The proteolytic enzyme was used at a concentration of 10 µg per 1 mg of the rHSP-60-GST conjugate. At the same time, at a temperature of 37°C, which was reported by other authors (Nagai and Thogersen, 1987; Schagger and von Jagow, 1987), we observed an insignificant accumulation of non-specific hydrolysis products (the possibility of non-specific protein cleavage was reported in the information provided by the enzyme manufacturer). When the enzymatic reaction temperature was decreased to room temperature, it was possible to neutralize the non-specific proteolysis. When studying the degree of the recombinant conjugate hydrolysis in time, it was found that full rHSP-60-GST cleavage occurred within 6 hours (Fig. 6): after 6 hours of the proteolytic cleavage, a band corresponding to MW of the recombinant conjugate disappeared – only two bands corrresponding to rHSP-60 and GST proteins, were recorded on the electrophoregram. Output of rHSP-60 after the protein conjugate proteolysis composed 59% of the quantity of the protein immobilized on the affinity sorbent that composed 84% of the theoretical output.

To assess the perspectives of the use of the obtained rHSP-60 for the creation of highly sensitive and specific means for *in vitro* diagnostics, we carried out comparative studies of the activity of the obtained *Ch. trachomatis* HSP-60 as a part of immunosorbent in ELISA for detecting corresponding specific antibodies of the IgG class in human serums.

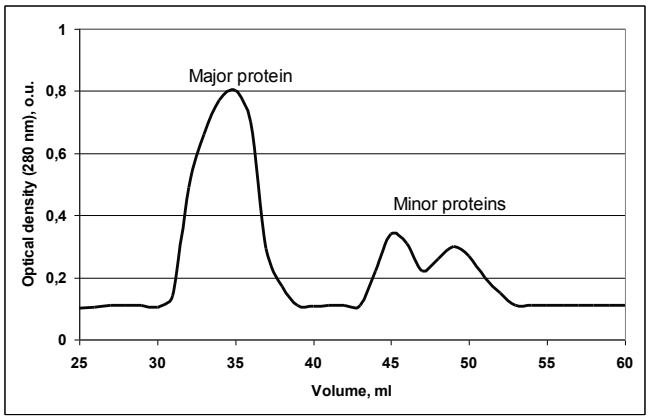

Fig. 7. Gel-filtration of rHSP-60-GST conjugate on Sephadex G-75

During preliminary stages, we created an evaluation serum panel (10 samples), including samples preliminarily tested in commercial immunoenzyme sets for the content of IgG-antibody to the major protein of the *Ch. trachomatis* outer membrane and IgG-antibodies to *Ch. trachomatis* HSP-60.

As immobilization antigens in plate wells we used: the obtained rHSP-60 and its protein conjugate rHSP-60-GST, commercial recombinant *Ch. trachomatis* HSP-60 protein (Bioclone Inc, USA) and GST. Additionally, we carried out an assessment of the immunological activity of minor protein fractions contained in the eluate after the affinity chromatography on glutathione-sepharose. The experiment results for the group of positive serums were assessed based on the positivity index (PI) – a ratio of the average arithmetic value of optical density in ELISA for positive and negative (cut off) serums. Fig. 8 shows the results of this study block: using the same recombinant protein concentration in the immunosorbent, for our obtained rHSP-60, we obtained a 30% higher PI compared to the analogue commercial protein. Of interest were the results of a comparison of the immunological activity of our obtained rHSP-60 and its conjugate with GST – positivity indices using these two proteins in the immunosorbent composition were comparable – 2.2 and 2.4, respectively. At the same time, no interaction between serum immunoglobulins and GST enzyme was detected. Such data indicate on absolute possibility of the use of the GST-conjugated HSP-60 protein for immunodiagnostic purposes. These experiments also confirm our previous assumptions on the nature of minor proteins contained in the eluate after the affinity chromatography on glutathione-sepharose: presence of insignificant immunological activity (PI = 1.12) unambiguously evidences that these proteins are products of the incomplete translation of the recombinant protein.

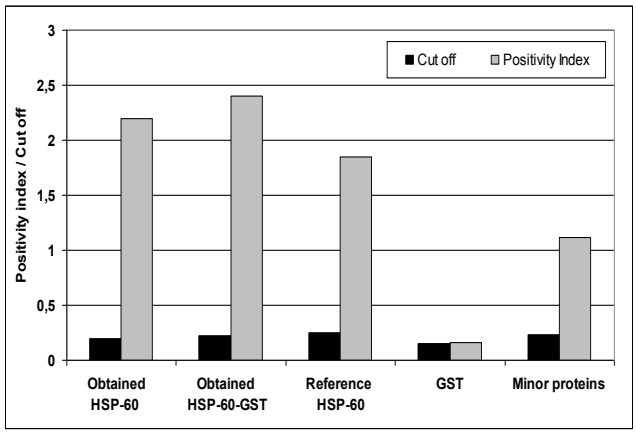

Fig. 8. Comparative evaluation of the immunological activity of Ch. trachomatis rHSP-60 and rHSP-60-GST conjugate

Conclusions.

1. We have developed and justified optimal technological parameters for obtaining and purification of the recombinant *Ch. trachomatis* HSP-60. It has been shown that the recombinant protein accumulates in *E. coli* BL21 (DE3) strain cells both in a soluble form and in inclusion bodies. We determined optimal cultivation conditions for the producer-strain for maximum accumulation of the soluble form of the target product (79%): LB medium, stable temperature of 37°C, IPTG concentration 0.3 mM, and cultivation time 3 hours.

2. The developed method for the target product purification is based on the use of the affinity chromatography on glutathione-sepharose (obtaining the rHSP-60-GST protein conjugate) and enzymatic hydrolysis of the GST-containing protein using the coagulation factor Xa (obtaining rHSP-60) as well as gel-filtration on Sephadex G-75 for final purification.

3. It has been proven that the obtained rHSP-60 preparations as well as its GST-conjugate are highly effective when used in the immunosorbent composition in ELISA for the detection of specific IgG-antibodies that makes possible their use for the development of highly specific and sensitive immunodiagnostic methods.

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БІОТЕХНОЛОГІЯ ОТРИМАННЯ РЕКОМБІНАНТНОГО БІЛКА ТЕПЛОВОГО ШОКУ (HSP-60) *CHLAMYDIA TRACHOMATIS* ТА ОЦІНКА ПЕРСПЕКТИВ ЙОГО ВИКОРИСТАННЯ У СЕРОЛОГІЧНІЙ ДІАГНОСТИЦІ

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Виявлення гуморальної імунної відповіді на білок теплового шоку масою 60 кДа Chlamydia trachomatis (HSP-60) має велике діагностичне значення, адже свідчить про ймовірний аутоімунний процес та ризик патології репродуктивної системи у жінок. Для розробки високоінформативного імуноферментного набору для виявлення анти-HSP-60 антитіл необхідно мати у розпорядженні відповідні біологічні компоненти, передусім очищений білок HSP-60. Найбільш сучасним є отримання білків-антигенів за допомогою методів генної інженерії та молекулярної біотехнології, що забезпечує не тільки стандартизацію отримуваного білка, але й дає можливість розробляти прогресивні технології виділення та очищення останнього. Проведено науково-методичну розробку біотехнології отримання рекомбінантного HSP-60 (rHSP-60) із накопиченням останнього у цитоплазмі клітин E. coli у розчинному вигляді: встановлені оптимальні параметри культивування продуценту (поживне середовище, температура, концентрація індуктора експресії та тривалість біосинтезу) забезпечують переважне (79%) накопичення цільового білка у розчинній формі. Технологія очистки rHSP-60 базується на використанні афінної хроматографії на глутатіон-сефарозі та ферментативного гідролізу GST-вмісного білку із використанням фактора згортання крові Ха, а також гель-фільтрації на Sephadex G-75 для доочищення. Для оцінки перспектив використання отриманого rHSP-60 нами були проведені порівняльні дослідження активності отриманого HSP-60 Ch. trachomatis як частини імуносорбенту у ІФА для виявлення відповідних специфічних антитіл класу IgG у сироватках крові людини. У якості антигенів для іммобілізації у лунках планшету використовували: власно отримані rHSP-60 та його білковий кон'югат rHSP-60-GST, комерційний аналог та GST. Додатково проводили оцінку імунологічної активності фракції мінорних білків, що містилися у елюаті після афінної хроматографії на глутатіон-сефарозі. Результати експериментів для групи позитивних сироваток оцінювали за індексом позитивності – співвідношенням середнього арифметичного значення оптичної густини у ІФА для позитивних та негативних (cut off) сироваток. Для отриманого rHSP-60 було отримано індекс позитивності на 30% вищій у порівняння із аналогічним комерційним протеїном. Порівняння імунологічної активності отриманого rHSP-60 та його кон'югату із GST засвідчили, що індекси позитивності при використанні двох даних білків у складі імуносорбенту були зіставними – 2,2 та 2,4, відповідно. Було доведено, що отримані нами препарати rHSP-60, а також його GST-кон'югату є високоактивними при їх використанні у складі імуносорбенту у імуноферментному аналізі для виявлення специфічних IgG-антитіл.

Ключові слова: білок теплового шоку, Chlamydia trachomatis, рекомбінантний білок, біосинтез, хроматографічна очистка.

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