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# HETEROLOGOUS EXPRESSION OF GRANZYME H IN *ESCHERICHIA COLI*

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**ABSTRACT.** Granzyme H belongs to the family of serine proteases, which plays an important role in fighting infections as part of the natural immune system. This enzyme is produced by natural killer cells and the T lymphocytes, inducing apoptosis in target cells. The precise role of the enzyme is not yet clarified; its crystal structure is unknown. The sequence encoding Granzyme H was cloned from human lymphocytes for heterologous expression. The assembled pET17cGraH expression vector contains the sequence encoding granzyme H under the control of a strong inducible promoter. Protein production was carried out in *E. coli Bl21(DE3)plysS* cultures.

***Keywords:*** *apoptosis, Granzyme H, heterologous expression, serine proteases*

## INTRODUCTION

The human Granzyme family is constituted of five members: Granzyme A, B, K, M, and H are present in placental mammals, being crucial components of the immune response by elimination of host cells infected by intracellular pathogens.

Granyzmes are released by cytotoxic T lymphocytes and by resting natural killer cells. The enzymes secreted by these two cell-types are granule associated enzymes, so called “granzymes” [1]. These cytotoxic granules contain a protein named perforin, which forms pores on the surface of cell membranes, and also contain serin proteases responsible for the breakdown of proteins.

Granzyme B and H are evolutionary strongly related, showing a 71% structural homology in their amino acid composition, also being located on the same chromosome. Sedelies et al found that Granzyme H is frequently more abundant than Granzyme B in NK cells, fact that proves a

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role for Granzyme H in complementing the pro-apoptotic function of Granzyme B in human NK cells [2]. Despite the high-leveled similarity in amino acid composition, a study demonstrated that Granzyme H is capable of inducing cell death in a caspase-independent manor [3].

Granzyme H is a specific human enzyme, a chimotripsin-like serine protease which's coding sequence is located on chromosome 14. According to Andrade et al., Granzyme H was reported to proteolitically inactivate adenoviral proteins necessary for replication [4].

The aim of this study is to express the recombinant Granzyme H in a prokaryotic system in order to study the expression conditions and quantity of the produced protein for further applications: determination of enzymatic properties and crystallization.

## RESULTS AND DISCUSSION

As resulted from **Figure 1.**, we obtained a significant amount of insoluble protein with a molecular weight of 33kDa, corresponding to the molecular weight of Granzyme H. It can be observed that 4 h of induction does not increase significantly the amount of the expressed protein in comparison with 1.5 h induction. This means that the induction time should not exceed 2 h, but variation of temperature may help to produce soluble proteins.

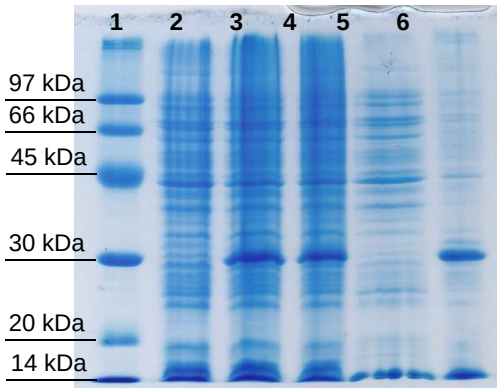


**Figure 1.** Expression of Granzyme H.

Lane 1: Molecular weight marker (Amersham); lane 2: Cell pellet before induction; lane 3: cell pellet after 1.5 h of induction; lane 4: cell pellet after 4 h of induction; lane 5: soluble proteins after sonication; lane 6: insoluble proteins (inclusion bodies) after sonication.

## CONCLUSIONS

Following the transformation of E. coli Bl21(DE3)plysS host strain with pET17c-GraH vector we succeeded in the expression of Granzyme H.

The expressed protein can be found in significant quantity, but in insoluble form. Several conditions need to be improved, for example by lowering the expression temperature at 20 $^{o}$C, which may increase the produced soluble protein level [5].

An advantage of the insoluble protein production is the formation of relatively pure inclusion body aggregates, from which Granzyme H can be easily purified.

## EXPERIMENTAL SECTION

### *Plasmids and bacterial strains*

The cloning and expression of the recombinant Granzyme H was carried out in the pET system from Novagen, a system which is operating under control of the bacteriophage T7 promoter.

The coding sequence of the Granzyme H was cloned from human lymphocytes and subcloned in a pET17c vector, resulting in the pET17c-GraH vector. *E. coli Bl21(DE3)plysS* (Stratagene) strain was used (genotype: $F^{-}$, *ompT*, $hsdS_B$ ($r_B^{-}$, $m_B^{-}$), *gal dcm*, *λ(DE3)*, *pLysS*, $Cm^r$), as a high-stringency protease deficient expression host.

### *Protein expression*

Electrocompetent BL21(DE3)pLysS cells were obtained following a protocoll described in the operator's manual (Xcell GenePulser, BioRad). Pulsing was carried out conform the manufacturer's manual. After electroporation, cell suspension supplemented with SOC media (2% bactotryptone, 0.5% yeast extract, 0.05% NaCl, 0.01 M $MgCl_2$, 0.01 M $MgSO_4$, 0.02 M glucose, pH7, reagents from Merck) was incubated 3 h at 37 $^{0}$C, and plated on LB agar containing 100 µg/ml Ampicillin. The plates were incubated overnight at 37 $^{0}$C

A single colony was inoculated in 2 ml LB broth with100 µg/ml Ampicillin and incubated overnight at 37 ℃ at 250 rpm. To prevent leaky protein expression, 1 % glucose was added to the overnight culture. Glucose was removed by centrifugation. After centrifugation cells were diluted to a final volume of 200 ml. They were incubated with shaking (250 rpm) at 37 ℃, until they reached the OD600 to 0.4-0.6 value, indicating the logarithmic growth phase.

At this point, an end-concentration of 0.5 mM IPTG (isopropyl-thio-galactopyranoside, Sigma) was added to the culture, allowing the T7 RNA polymerase expression and target gene-expression. The culture was further incubated for 4 h in the same conditions. 1 ml samples were taken before

the induction and during the expression period, each hour. Samples for electrophoresis were centrifuged and stored at -20°C.

Cell disruption was conducted by resuspension in 20 ml of distilled water supplemented with 0.25 g/ml lysozyme and freezing at -80 $^{o}$C for 2 h. To completely disassociate the cellular components, cell suspensions were melted and sonicated (sonicator Dr.Hierschler) 5 cycles of 10 sec at 70% amplitude, with 10sec pause between the cycles. This procedure was accomplished on ice to prevent excess heat formation and denaturation of the protein.

Insoluble proteins accumulated in inclusion bodies were obtained by centrifugation for 15 min at 4℃, 14000 rpm.

Sample preparation for electrophoresis was conducted according to Laemmli [4], and the produced proteins separated on a 15% SDS-PAGE were visualized by Coomassie staining.

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