Cloning and expression of *Xenopus laevis* chaperones Hsp30C and Hsp70 in *Escherichia coli*

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E. coli strains overproducing chaperones Hsp30C and Hsp70 from *X. laevis* were constructed. The entire coding sequence for Hsp30C and Hsp70 was obtained by means of polymerase chain reaction using specific primers and DNA from *X. laevis* liver. The amplified fragments were cloned into the vector pUC57/T and recloned into expression vectors pET28a and pET21b. In the case of Hsp30, amplification mistakes were removed by PCR mutagenesis. The resulting hybrid plasmids were transformed into *E. coli* BL21 (DE3). After 3 h of induction with IPTG the synthesis of Hsp30C and Hsp70 reached 30% of the total cellular protein. Both chaperones were located in the soluble cell fraction.

Key words: chaperone, Hsp30C, Hsp70, PCR, cloning, expression, induction

INTRODUCTION

Molecular chaperones, known as heat shock proteins (Hsp), interact with other proteins to mediate protein folding, refolding, assembly, disassembly and prevent misfolding and aggregation of proteins [1]. The chaperone Hsp70 belongs to the family of highly conserved proteins in the variety of different organisms. Hsp70 is involved in cellular heat shock response and rescues the proper structure of the proteins during the period of stress [2]. Hsp70 is an ATP-dependent chaperone acting together with the co-chaperone Hsp40 (DnaJ) and the nucleotide exchange factor Hsp22 (GrpE) [3].

The small heat shock protein family is quite divergent, except the alpha-crystalline domain [4]. Small Hsp chaperones can form large polymeric structures possibly necessary for their function *in vivo*, including a role as molecular chaperones as well as an involvement in modulation of redox parameters, cellular differentiation or actin capping-decapping activities and conferring resistance to thermal challenge [5, 6]. The latter activity was exhibited on purified Hsp30C protein, which had been recovered as large multimeric complexes interacting with unfolded protein, maintaining it in soluble form and inhibiting its aggregation [6].

In this study we investigate the possibility to overexpress two cloned chaperones, Hsp70 and Hsp30C from *Xenopus laevis*. Our results indicate that successful overexpression (more than 30% from total cellular protein) of these chaperones has been achieved in *E. coli.* The proteins obtained are suitable for further purification and refolding activity testing *in vitro* together with other chaperones.

MATERIALS AND METHODS

All cloning and expression work was performed in *E. coli* strains JM107, RR1 and BL21(DE3)-F^- ompT $r_B^- m_B^-$. Most of cloning procedures were carried out according to Sambrook [7]. All restriction and DNA modifying enzymes, DNA sequencing kit, genomic DNA purification kit were obtained from MBI Fermentas, the pET series plasmids were from Novagen. Expression of genes was achieved by growing appropriate cultures in 4ml or 100 ml LB medium to OD_{600nm} 0.8–1.2 and inducing 2–3 h with IPTG (isopropyl-β-D-thiogalactopyranoside, final concentration 1mM) at 28 °C or 37 °C.

RESULTS AND DISCUSSION

The entire Hsp30C gene of *X. laevis* was obtained by polymerase chain reaction (PCR) using primers complementary to 5'- and 3'- termini of Hsp30C gene (see Table) and DNA isolated from liver of *X. laevis*. After amplification the 644bp fragment was obtained with the cleavage sites for restriction endonucleases NdeI and EcoRI in the ends. This fragment was purified from agarose gel and inserted into the vector pUC57/T designed for direct ligation of

amplified 3'-dA-prolonged DNA fragments. The ligation mixture was transformed into *E. coli* JM107, plasmid DNA from several clones was isolated and digested with NdeI+EcoRI. For verification of Hsp30C gene sequence the automatic sequencing was used, which revealed a discrepancy between the sequence of amplified Hsp30C gene and the known sequence in GenBank database (accession number X57962). To correct the mistake, we applied PCR mutagenesis and after that checked the sequence of a few selected clones by sequencing. The DNA fragment carrying a correct Hsp30C gene sequence was inserted into NdeI-EcoRI sites of the expression plasmid pET28a (Fig. 1) and transformed into *E. coli* RRI. A number of plasmids were purified and digested with endonucleases NdeI+EcoRI. Several selected clones were transformed into the expression host strain *E. coli* BL21 (DE3). After 3 h of induction with 1 mM IPTG, the recombinant protein Hsp30C carrying an N-terminal oligohistidine domain was detected in the bacterial lysate (Fig. 2A). Scanning measurements of Coomassie Brilliant Blue stained SDS-PAGE gels showed chaperone expression in the range of 30–40% of the total cellular protein.

To assess the solubility of the resulting protein, the cultures were harvested within 3 h after induction with IPTG at 37 °C, the cells were lysed by sonication and the lysates separated into soluble and insoluble fractions by centrifugation. Figure 2B shows that almost all induced chaperone was located in the soluble cell fraction.

It is very convenient to express the target gene in the plasmid pET28, because the resulting protein can be purified in one step by means of metal chelate affinity chromatography. The recombinant protein will contain the N-terminal stretch of 6 histidine residues. To express the native chaperone we used the plasmid pET21b. In this case Hsp30C accumulated up to 20–30% of the total soluble cell protein (data not shown).

For the cloning and expression of Hsp70 we applied the same approach as for Hsp30C. The first step was amplification of the gene encoding sequence, ligation of the resulting 1945 bp fragment into vector pUC57/T and transformation of *E. coli* JM107 cells. To avoid amplification mistakes, the clones obtained were screened using restriction endonucleases

Table. **Primers used for PCR**

Primer	Sequence 5'→ 3'
30N (5'-terminus of hsp30C)	CATATGTTTCCTCTCAGCCTCGTAC
30C (3'-terminus of hsp30C)	GAATTCAGTCCACTTTTTGGTCTCC
70N (5'-terminus of hsp70)	CATATGGCAACCAAAGGAGTCGCA
70C (3'-terminus of hsp70)	AAGCTTAGTCAACTTCTTCTATAGT
Asp-131 (for mutagenesis of hsp30C)	GATGGAAACTACTTCCATGAATAC
Glu-130C (for mutagenesis of hsp30C)	CTCAGTGTCACTTTTCCGCTCATG

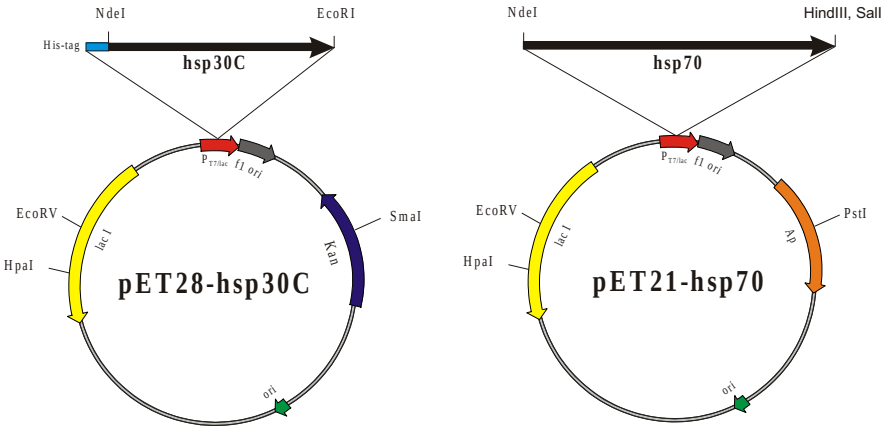

Fig. 1. Maps of Hsp30C and Hsp70 expression plasmids

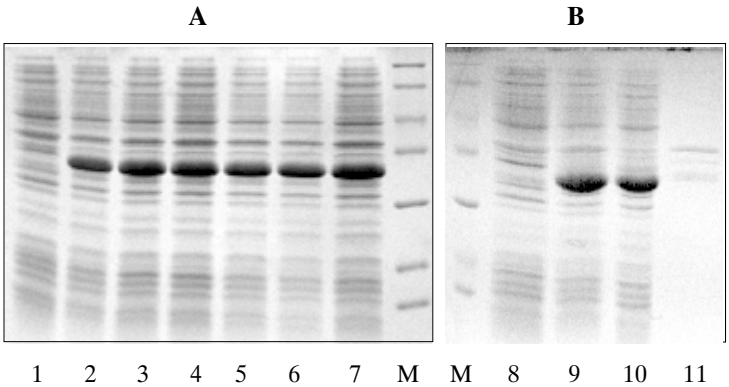

Fig. 2. Analysis of Hsp30C protein expression in *E. coli* BL (DE3) pET28-hsp30C (**A**) and solubility (**B**) by 15% SDS-PAGE stained with Coomassie Brilliant Blue.
Lanes:
1, 8 – total cellular protein before induction;
2–7, 9 – total cellular protein after 3 h induction with 1mM IPTG;
10 – soluble cellular protein after induction;
11 – insoluble cellular protein after induction;
M – MW markers (116,0; 66,0; 45,0; 35,0; 25,0; 18,4; 14,4 kDa)

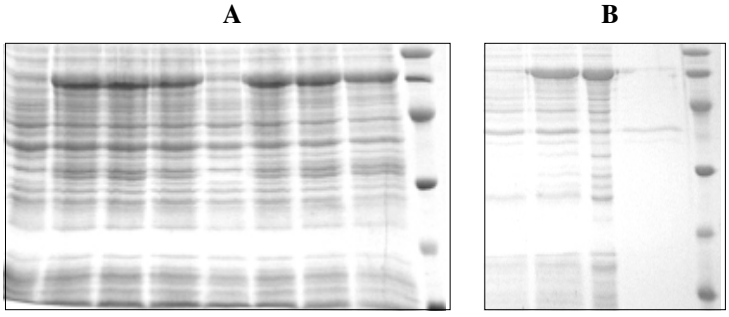

Fig. 3. Analysis of Hsp70 protein expression in *E. coli* BL (DE3) pET28-hsp70 (**A**) and solubility (**B**) by 12% SDS-PAGE stained with Coomassie Brilliant Blue.
Lanes:
1, 5, 9 – total cellular protein before induction;
2–4, 6–8, 10 – total cellular protein after 3h induction with 1mM IPTG;
11 – soluble cellular protein after induction;
12 – insoluble cellular protein after induction;
M – MW markers (94,0; 67,0; 45,0; 30,0; 20,1; 14,4 kDa).
Lanes:
1–8 – induction at 37 °C;
9–12 – induction at 28 °C

BamHI, EcoRI, HindIII, NdeI, PvuII, XbaI. First of all were selected the plasmids yielding appropriate restriction pattern corresponding to that of the known sequence of Stump et al., [8] and from Genbank database (accession number L36924). A few of them were partially sequenced. Two checked plasmids were incubated with endonucleases AatI+SalI, the fragment containing a chaperone gene was purified from agarose gel and partially digested with endonuclease NdeI. A 1945 bp fragment was isolated and inserted into NdeI-SalI sites of the expression plasmid pET28a. After transformation of *E. coli* RRI strain with ligation mixture, several clones were checked according to the known restriction pattern with restriction endonucleases BamHI, HindIII, NdeI, SalI. A few selected plasmids were transformed into *E. coli* BL21 (DE3). Induction and Hsp70 protein solubility results showed that after inducion at 28 °C almost all chaperones have been found in the soluble cell fraction and the expression level reached 30% of the total cell protein (Fig. 3).

The Hsp70 gene was also expressed by using the vector pET21b (Fig. 1). The solubility and expression level corresponded to those obtained in pET28a (data not shown).

In conclusion, we can state that the expression level obtained (more than 30% from total cellular protein) and the solubility of Hsp70 and Hsp30C chaperones in *E. coli* after induction provides a possibility of successive purification procedures and refolding activity testing *in vitro* together with the other chaperones.

References

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XENOPUS LAEVIS ŠAPERONŲ HSP30C IR HSP70 KLONAVIMAS IR EKSPRESIJA _ESCHERICHIA COLI_

S a n t r a u k a

Sukonstruoti *E. coli* kamienai, produkuojantys *X. laevis* šaperonus Hsp30C ir Hsp70. Šaperonus koduojanti seka buvo gauta polimerazinėje grandininėje reakcijoje panaudojus specifinius pradmenis ir DNR, išskirtą iš *X. laevis* kepenų. Amplifikuoti fragmentai buvo įklonuoti į vektorių pUC57/T ir perklonuoti į ekspresijos vektorius pET28a ir pET21b. Hsp30C atveju amplifikacijos klaidos buvo ištaisytos panaudojus PGR mutagenezę. Gautos hibridinės plazmidės buvo įvestos į *E. coli* BL21(DE3) kamieną. Po 3 valandų indukcijos su IPTG šaperonų Hsp30C ir Hsp70 sintezė siekė 30% viso ląstelės baltymų kiekio. Abu šaperonai kaupėsi tirpioje ląstelės frakcijoje.