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Published in:
FEBS Letters

DOI:
10.1016/0014-5793(88)80300-4

Publication date:
1988

Volume 236, number 1, 132–134 FEB 06186 August 1988

Purification of fusion proteins expressed by pEX3 and a truncated pEX3 derivative

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Received 10 June 1988; revised version received 27 June 1988

A derivative of the pEX3 expression vector was constructed that codes for the first 407 amino acids of the 1051 amino acids of the pEX3 fusion protein. The amount of truncated fusion protein (40 mg/g cells), obtained by expression in *E. coli*, was similar to that produced by the original pEX3 vector. The truncated fusion protein was purified more easily from *E. coli* contaminants than the original fusion protein by washing with 2 M urea and 0.5% Triton X-100.

pEX fusion protein; Truncated form; Protein purification

1. INTRODUCTION

Recombinant β-galactosidase fusion proteins, produced in *E. coli*, are widely used for different purposes. They are used as immunogen to elicit antibodies to the foreign part of the fusion protein [1–5], or as an agent to stimulate T-cell proliferation [6]. Furthermore, they are used in probing the topology of a foreign protein [7–10], or as a tag to detect minor proteins in *E. coli* [11].

In general, the fusion proteins contain nearly the complete β-galactosidase sequence. Alternatively, truncated forms of β-galactosidase have been used to produce human proinsulin [12]. An example of the first class of fusion proteins is the pEX fusion protein [13]. The pEX vector codes for a protein of 1051 amino acids which by itself is a fusion of 3 protein fragments, viz. cro, lacI and lacZ. The pEX fusion protein forms insoluble aggregates in the cells and is expressed to about 25% of total cell protein [13]. DNA fragments up to 400 bp can be cloned into the pEX plasmids at the C-terminus of the cro-lacI-lacZ sequence without substantial loss of efficiency of expression of the fusion protein [13].

Partial purification of the fusion protein can be achieved by the collection of the insoluble material after cell lysis. Further purification of this fusion protein in sufficient quantities is hampered by its insolubility. Chromatographic procedures can be applied after solubilization of the fusion protein by boiling in SDS to disrupt aggregates of *E. coli* proteins and the fusion protein. Contaminating proteins from *E. coli* may interfere when fusion proteins are used in immunological assays and have e.g. a non-specific effect on T-cell proliferations [6]. Therefore, it would be advantageous to produce a fusion protein that associates to a lesser extent with *E. coli* contaminants and therefore would be much easier to purify.

In the present study we report the construction of a pEX derivative that codes for a truncated fusion protein of 407 amino acids and its purification is compared with that of the original pEX fusion protein.

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2. MATERIALS AND METHODS

2.1. *Construction of pEX3407*

Recombinant DNA techniques were performed according to Maniatis et al. [14]. Expression plasmid pEX3, kindly provided

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by Dr K.K. Stanley [13], was digested with *Eco*RV and *Sma*I and the large fragment was isolated by gel electrophoresis. This fragment was self-ligated and transformed into *E. coli* pop 2136. Ampicillin-resistant colonies were screened for expression of a 50 kDa fusion protein. Overnight cultures, grown at 30°C, were induced at 42°C for 90 min, cells were harvested and aliquots were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gel according to Laemmli [15]. The resulting clone was designated as pEX3407.

2.2. *Purification of fusion proteins*

100 ml cultures of pEX3 and pEX3407 were grown at 30°C to $A_{600} = 0.3$ and then shifted to 42°C for 3 h to induce production of the fusion protein. Cells were harvested and lysed by addition of lysozyme (0.8 g/g cells) and subsequently deoxycholate (4 mg/g cells) as described by Marston [16]. DNA was broken by sonication and the cell lysate was centrifuged at 12000 × *g* for 15 min at 4°C. The pellet containing the inclusion bodies was resuspended in 9 vols (v/w) 2 M urea in lysis buffer (50 mM Tris-HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl) and incubated for 5 min at room temperature. The suspension was centrifuged at 12000 × *g* for 5 min at 4°C and the pellet was resuspended in 9 vols of lysis buffer, containing 0.5% (v/v) Triton X-100 and 10 mM EDTA. After incubation for 5 min at room temperature, the suspension was centrifuged as described above and the pellet was washed twice with 9 vols of water and subsequently lyophilized. Samples were analyzed by SDS-PAGE.

3. RESULTS AND DISCUSSION

3.1. *Construction of pEX3407*

In fig.1 the construction of pEX3407 is shown. The pEX3 vector was digested with *Eco*RV and *Sma*I and the large fragment was self-ligated. The resulting plasmid, designated as pEX3407, codes for a truncated form of the cro-lacI-lacZ fusion protein of pEX3. The coding region terminates at amino acid 407 and is followed by the remainder of the linker region of pEX3 in the same reading frame as in pEX3. The *Sma*I site in the linker region was deleted by the cloning procedure. The truncated pEX3407 fusion protein is expressed at about the same high levels as the pEX3 fusion protein, and the recovery of both fusion proteins was similar, approx. 4 mg fusion protein per 0.1 g cells (wet wt). The pEX3407 fusion protein has a relative molecular mass of 50 kDa, as compared to the original pEX3 fusion protein with a molecular mass of 116 kDa.

3.2. *Purification of fusion proteins*

Cells expressing pEX3 and pEX3407 fusion proteins were lysed with lysozyme and deoxycholate. The pEX3 fusion protein and the truncated

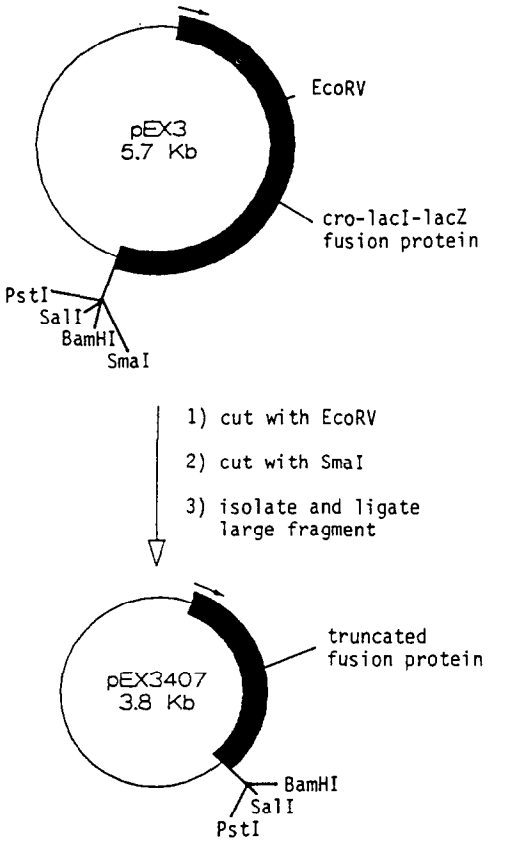

Fig.1. Construction of pEX3407. pEX3 was digested with *Eco*RV and *Sma*I and the large fragment was self-ligated to give pEX3407. The coding region of the cro-lacI-lacZ fusion protein terminates at amino acid 407. The remaining unique restriction sites in the cloning linker are shown. The arrow indicates the start and direction of transcription.

pEX3407 fusion protein exist as insoluble inclusion bodies in the bacterial cells. After centrifugation of the cell lysate, both fusion proteins are present in the pellet and not in the supernatant fraction. This is illustrated in fig.2, in which lanes 3 and 6 (pEX3 fusion protein and pEX3407 fusion protein, respectively) show the analysis of proteins present in the pellets and lanes 2 and 5 (pEX3 fusion protein and pEX3407 fusion protein, respectively) the composition of the supernatants. The pellet obtained after centrifugation of the lysate for cells expressing the pEX3407 fusion protein is less contaminated with *E. coli* proteins than the pellet containing the pEX3 fusion protein. An additional amount of contaminating proteins was removed by

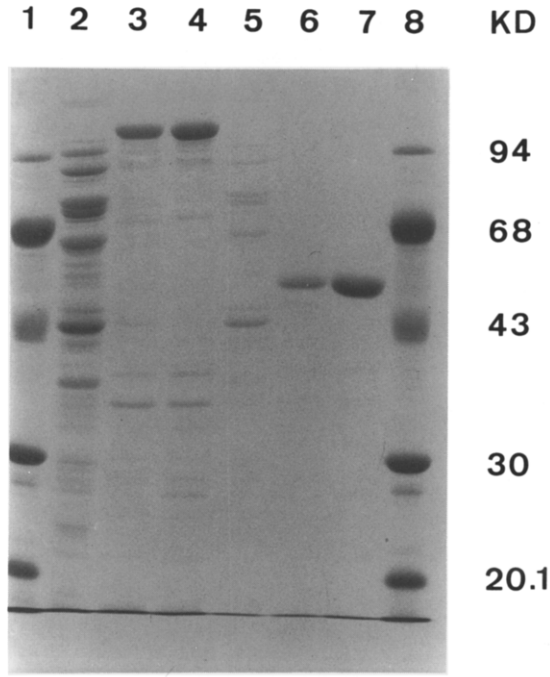

Fig.2. Purification steps of fusion proteins of pEX3 and pEX3407 cells analyzed by SDS-PAGE. Proteins were subjected to SDS-PAGE on a 10% gel under reducing conditions. Proteins were stained with Coomassie brilliant blue R-250. Molecular mass markers (lanes 1 and 8). Supernatants obtained after centrifugation of the lysate of pEX3 (lane 2) and pEX3407 (lane 5) cells. Pellets obtained after centrifugation of the lysate of pEX3 (lane 3) and pEX3407 (lane 6) cells. Pellets after washing of pEX3 (lane 4) and pEX3407 (lane 7) fusion proteins, with 2 M urea and 0.5% Triton X-100.

washing with 2 M urea and 0.5% Triton X-100, resulting in approx. 95% pure pEX3407 fusion protein (fig.2, lane 7). In contrast to this, the original pEX3 fusion protein is still contaminated with substantial amounts of *E. coli* proteins (fig.2, lane 4). The use of truncated β-galactosidase fusion proteins of 590 and 450 amino acids [12] did not result in more purified preparations after precipitation of the fusion protein from a 7 M guanidine-HCl cell extract, as compared to the original β-galactosidase fusion protein of 1007 amino acids. This difference is probably due to the fact that the truncated cro-lacI-lacZ fusion protein described here is basically a protein other than the truncated β-galactosidase described by Guo et al. [12].

The truncated pEX3 derivative has been successfully used to clone large gene fragments of glycoprotein D of herpes simplex virus type 1 (HSV-1 gD, up to 972 bp) without loss of efficiency of expression. These relatively large recombinant gD-fusion proteins were isolated by the procedure described here for the pEX3407 fusion protein giving similar results and were used for immunization of rabbits (Kocken, C.H.M., unpublished).

The truncated pEX derivative may be useful for expression of other proteins, particularly for the production of specific antibodies and in those cases where impure preparations would interfere with immunological assays.

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