A possible osmodependent protease in \textit{Escherichia coli}

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Abstract: When a strain of \textit{Escherichia coli}, expressing a hybrid protein GalK-\(\beta\)-Gal, is shifted to high osmolarity, the \(\beta\)-galactosidase activity strongly decreases within 20 min of shock. The loss of \(\beta\)-galactosidase activity results from degradation of the hybrid protein under osmotic stress. The results raise the possibility that osmotic stress induces a specific osmodependent protease.

Key words: Osmolarity; Proteolysis; \textit{Escherichia coli}

Introduction

In \textit{Escherichia coli}, as in eukaryotic cells, proteins with a highly abnormal conformation are rapidly degraded. Such abnormal proteins can be generated: (i) by incorporation of amino acid analogs, such as canavanine, instead of a normal residue [1]; (ii) by premature termination of polypeptide chains caused by incorporation of puromycin [1,2]; and (iii) by mutation, as for the product of the \textit{lacZX90} allele [2]. Foreign proteins cloned in \textit{E. coli} are subject to intracellular degradation, e.g. human tissue plasminogen activator (TPA) which fails to form the proper disulfide bonds and accumulates to a low level because it is degraded [1]. Many \(\beta\)-galactosidase fusion proteins are also degraded either in yeast or in \textit{E. coli} [3]; the rate of proteolysis of such proteins is highly dependent on the amino-terminal residue [3]. An initial step in the degradation of all these abnormal proteins seems to be catalyzed in vivo by La protease, the product of \textit{lon} gene [4].

Under stressful conditions, such as heat shock, many proteins are denatured and seem to be readily proteolyzed. Under the same conditions, many proteins are induced; among them the chaperone proteins DnaK, DnaJ and GroEL and two types of proteases, the La protease [5] and the product of the \textit{clpP} gene [6] which functionally binds to the heat shock cognate (hsc)-like ClpA protein [7] and, possibly, to the heat shock protein ClpB [8].

The accumulation of abnormal proteins has been found to induce the heat shock response and to stimulate proteolysis; conversely, the pro-
teolysis of abnormal proteins is decreased in the presence of mutations in some heat shock genes [2].

These findings suggest that the increase in proteases can help cells to degrade abnormal proteins generated upon exposure to environmental stress. Moreover, the presentation of abnormal or heat-denatured proteins to the proteases could require their recognition and, possibly, their unfolding by the heat-shock chaperones [2,9].

Here we report on the degradation of a hybrid fusion protein under osmotic stress, which was found to increase the cell content of at least one stress protein, chaperone DnaK [10]. The data suggest the presence of osmodependent proteolytic activity in E. coli cells.

Materials and Methods

Bacteria

E. coli PB103 (F− dadR1 trpE61 trpA62) was grown at 37°C in minimal medium [10] with tryptophan and glucose as carbon source. AB1157 was also used, as a control (data not shown) under the same conditions.

Plasmids and ftsZ::lacZ fusions

Plasmids pCX38, pCX32 and pCX25 were originally constructed by Wang, de Boer and Rothfield to study the expression of ftsZ gene from its different promoters via a fusion with lacZ. Three restriction fragments from the fts-ZAQ region of E. coli were then cloned in a low-copy-number vector, pFZY1, with the mp18 multiple restriction site linker of M13 inserted upstream of a promotorless β-galactosidase-encoding lacZ gene [11].

Plasmid pFZY1 carries successive fragments originating from different vectors [12]. A part of the galK gene including the galK leader region and a maximum of 52 bp of the coding region have been added to create a region of transcript immediately 5’ to the translation start point, allowing translation to initiate at a constant rate. This fragment was linked to the lac operon which was cut from another vector, resulting in an undetermined length of the galK-encoding region directly linked to the 7th codon of the lacZ gene [12]. Nevertheless, the activity of the altered lacZ gene was not changed. Therefore, the pFZY1 vector encodes a hybrid protein, GalK/β-Gal, free of galactokinase activity.

The control strain AB1157 harbors phage λJFL100, which carries the lacZ gene lacking its own promotor and fused to promotors p3p4 of the ftsZ gene (a fusion constructed by J.F. Lutkenhaus). This fusion expresses a complete, non-hybrid β-galactosidase (kindly supplied by A. Robin and R. D’Ari).

β-Galactosidase assay

β-Galactosidase activity was assayed as described [13]. Samples were centrifuged before the A420 was measured.

Immunoblotting of β-galactosidase

Immunoblotting of β-galactosidase was carried out as described [10] with anti-β-galactosidase monoclonal antibodies (purchased from Boehringer).

Results

Osmotic stress

As shown elsewhere [14], upon increase of the medium osmolarity, cells enter a stress period, successively involving, immediate cell plasmolysis, then partial deplasmolysis (partial restoration of the cytoplasmic volume essentially as a consequence of K+ uptake). After deplasmolysis cell growth recovers, although the doubling time is increased. These three phases can be characterized by typical absorbance variations, as shown in Fig. 1.

The β-galactosidase activity expressed from plasmid pCX38 decreases under osmotic shock

Plasmid pCX38 harbors all six promotors of ftsZ gene. When cells of E. coli PB103 transformed with pCX38 were grown in a 300 mosM medium, the rate of β-galactosidase activity (in other words the rate of ftsZ promotors transcription) was proportional to the rate of the cell mass.
increase, as shown in Fig. 1. Strikingly, within 10–15 min after the osmolarity of the medium had been raised to 1500 mosM by addition of 0.6 M NaCl, the β-galactosidase activity dropped to 20–30% of the initial value. Enzyme activity increased again when deplasmolysis was almost complete and growth was recovered. The rate of β-galactosidase activity, at steady state at high osmolarity, was even increased relative to the rate of overall protein synthesis. Similar results were obtained with PB103 transformed with plasmids pCX32 and pCX25, expressing the lacZ gene from ftsZ promoters p1p2 and p3p4p5p6, respectively.

Strain AB1157, with another fusion of ftsZ promoters::lacZ which expressed a genuine β-galactosidase and not a hybrid protein, was also tested under the same conditions (see Materials and Methods). In this case, the enzyme activity was constant during the stress period; after growth recovery at high osmolarity, the rate of β-galactosidase activity was also higher than the rate of growth (manuscript in preparation).

All these results suggest that the decrease under upshock of β-galactosidase activity in the strain transformed with pCX38 could be accounted for the hybrid conformation of the protein.

The hybrid β-galactosidase is degraded under shock

The decrease of β-galactosidase activity in cells of PB103 harboring pCX38 correlated with degradation, as shown by the analysis of β-galactosidase content by immunoblotting with anti-β-galactosidase antibodies. The time course of enzyme degradation shown in Fig. 2 was compatible with that of the decrease of the activity (Fig. 1).

In contrast, when the chromosomal lac operon of PB103 cells free of pCX38 was induced with IPTG, neither the decrease of enzyme activity under stress nor its degradation could be observed.
Discussion

These data suggest that, under osmotic stress, *E. coli* either expresses a specific protease or increases the expression/activity of a protease allowing it to degrade a hybrid, possibly unfolded, protein.

It has been demonstrated that the presence of abnormal proteins may induce the synthesis of some heat shock proteins, e.g. Lon protease [2]. Abnormal protein production can result from mutations, synthetic errors, or from the exposure to unfavorable conditions such as high temperature. Abnormal proteins are often unfolded and consequently vulnerable to proteolysis.

The \( \beta \)-galactosidase expressed from plasmids pCX32, pCX38 and pCX25 is made up of a fragment of galactokinase K linked to a truncated \( \beta \)-galactosidase. Therefore, it is conceivable that such a hybrid polypeptide can unfold more easily when cells are exposed to an environmental stress.

The degradation process of hybrid \( \beta \)-galactosidase appears to be specifically dependent on the osmotic stress; indeed, it is turned on when plasmolysis starts and turned off when deplasmolysis is almost ended. Moreover, this proteolysis appears to be different from a general stress protease, such as La or Clp proteases, since the hybrid protein was not significantly degraded under heat shock. It is possible, therefore, that osmotic stress triggers the expression of a specific, osmodependent, protease able to degrade proteins that became unfolded under shock. Osmotic upshock increases the cellular content of the chaperone protein DnaK [10]. DnaK and the unknown, osmodependent, protease could cooperate in the degradation of abnormal proteins in a similar manner as was proposed for DnaK and La protease [2,9] or, possibly, for ClpP and ClpB [15].

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References


