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Short communication

Optimization of Bacteria-based Lysates used in cell-based and cell-free Protein Synthesis

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Abstract

Compared to cell-based protein synthesis, cell-free synthesis has a number of advantages, e.g. the short time from ORF to the translated protein. Compared to batch approaches, also the output (mg protein/ml) can be significanty higher. Both approaches need lysis of bacteria. If reliable, defined, reconstituted and very pure components are not available for cell-free protein synthesis, there is a need for optimized lysates. The present communication describes "Induced Lysis" optimization of such a lysate ("Induced Lysis").

Keywords: Optimization, Bacteria-based Lysates, Cell-based Protein Synthesis, Cell-free Protein Synthesis

Introduction

Synthesis of products such as proteins for research and commercial use can be achieved by application of convential cell-based methods (the bacteria contain the genetic information for the product) and by cell-free techniques. In this case, the bacterial cells do not contain the genetic information for the product. The cell-free version has been developed during the last decade, and it might turn out in the future as the approach of choice not only for research but also for commercial use. For both variants, lysis of the bacteria is needed to get access to the product. Usually, lysis is achieved by mechanical shear forces, by sonication, by homogenization by pressue release, by application of enzymes (Lysozym) or other chemicals. The present communication describes a new variant of lysis, the "Induced Lysis". A major role in this variant plays the bacterial elongation factor EF-Tu.

In a bacterial cell, protein synthesis needs a concerted cooperation of all components of the synthesis system including elongation factor EF-Tu ("Elongation Factor Thermo unstable"). In protein synthesis EF-Tu is responsible for catalyzing the binding of an aminoacyl-tRNA ("charged tRNA") to the ribosome, directed by a 3-nucleotide sequence (codon) in a mRNA. mRNA is transcribed from a template-DNA (the gene coding for the protein that will be synthesized as the product). For cell-free synthesis the enzymes needed for this transcription are present in the lysate. For the initiation of translation leading to synthesis of the protein product by cell-free protein synthesis the following ingredients have to be added to the lysate: the template-DNA, ATP, GTP (for the function of EF-Tu) and all amino acids needed for the product.

EF-Tu is present in a bacterial cell in an amount that is much higher than that needed in protein synthesis [1, 2]. Speculations on additional functions of EF-Tu led, during the past two decades, to the assumption that EF-Tu might play a role as a component of a bacterial cytoskeleton [3-9]. A few years ago, this assumption was confirmed. It was shown that EF-Tu modulates filament formation of actin-like MreB protein [10] and interacts and colocalizes with this protein [11]. MreB is a protein making up one of the major cytoskeletal structures in todays bacteria [12,13]. An exception is the wall-less bacterium Mycoplasma pneumoniae; it was reported to lack MreB, but nevertheless it has a very sophisticated cytoskeleton [4,7]. Aspects of evolution of the cytoskeleton are discussed. It was postulated that the "primordial"cytoskeleton did consist of EF-Tu, and it was speculated that EF-Tu, in the very early time of evolution, had only a function in protein synthesis. Only later it did gain an additional function as a component of a cytoskeleton [9]. EF-Tu is a very "old" and highly conserved protein; its structural properties are very similar in all bacterial strains.

Results and Proposal

During studies on presence and localization of EF-Tu in bacterial cells, using electron microscopic techniques including immunogold-labelling, it turned out by investigations of ultrathin sections and negatively-stained samples that the gold label was not only distributed all over the interior of the cells (a "cell-wide web") but also aligned as a kind of lining at the inside of the cytoplasmic membrane. The EF-Tu molecules were shown to form fibrils that could also be isolated from lysed cells [9]; in vitro formation of fibrils was demonstrated [9]. On the basis of findings on the structural orgaization of EF-Tu [14] a model was developed that demonstrated that domain 3 of one EF-Tu does fit into a cleft in domain 2 of a neighboring EF-Tu molecule, and so on (fibril formation) [9]. "Truncated" EF-Tu molecules, consisting only of domain 3, were in vitro mixed with intact EF-Tu molecules. The result was that fibril formation did no longer take

place [9]. A system was deviced that allows, after growth of a culture of cells with intact EF-Tu in the fermenter, to switch on synthesis of "truncated" EF-Tu (s. above). This is achied by combining the DNA sequence coding for domain 3 (s. above) with a DNA sequence that allows protein synthesis at slightly elevated temperature with preservation of correct protein folding. Result: within a period of one to two hours, the cells in the fermenter started lysis [15]. This system is based on the following features: in a vector (a plasmid) a stretch of DNA is inserted that codes for domain 3 of EF-Tu only and that can be translated, under a special condition, in the bacterial cells containing the naturally present gene coding for the full-size EF-Tu protein. This special condition is an elevated temperature (only 2 to 3 degrees) of the content in the fermenter. This temperature shift is applied when, in the case of cell-based protein synthesis, the bacteria have synthesized the optimal amount of the product. Hence, initiation of translation of the gene coding for domain 3 will lead to more and more numbers of domain 3 protein in the bacterial cell. Domain 3 protein will now compete with full-size EF-Tu for binding sites in the – always- growing EF-Tu-based cytoskeleton. As soon as a sufficient number of domain 3 protein is inserted in the growing cytoskeleton, the cytoskeleton will more and more loos its stability: fibrills can no longer be formed. The result is that after two to three hours nearly all bacteria present in the fermenter will be lysed. In the case of cell-based protein synthesis lysis with conventional means is no longer needed. In the case of cell-free protein synthesis an optimized lysate is available.

The lysate has very special properties. In contrast to results of convential lysis procedures which end up by formation of very small particulate matter, "induced lysis" as described above has, as a result, preservation of complex structures similar to those present in the cell prior to lysis, including ribosomes, free EF-Tu and EF-Tu transiently bound to the - disruped - cytoskeleton. The reason appears to be that, as the first steps of induced lysis, the bacterial cell wall and the cytoplasmic membrane get lost, and the cell content gets free [9]. Depending on the duration of induced lysis, the various particles in this mixrure of particulate matter will also be more and more reduced in size, caused by loss of stability of the EF-Tu-containing cytoskeleton.

A lysate with these properties may contain a rather well preserved cell-free system for operation as protein-synthesizing means with ribosomes bound to EF-Tu that is either organized in fibrilles or free in the cytosol/cytogel. It might be very interesting to measure the efficiency of protein synthesis when different lysis sytsems (s. above) are used. Such a measurement could be simply done with an SDS gel electrophoresis of a mixture of all synthesized proteins, obtained by appropriate centrifugation of the lysate. The protein of choice that was the goal of the protein synthesis would also be present in the mixture, and its purification can start.

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