PRODUCTION OF β-GALACTOSIDASE FUSED PROTEIN A AND THE PURIFICATION OF IT IN AN AQUEOUS TWO-PHASE SYSTEM

Andres Veide, Lars Strandberg, Halldis Hellebust and Sven-Olof Enfors

Department of Biochemistry and Biotechnology
The Royal Institute of Technology
S-100 44 Stockholm, Sweden

We have studied the production of the genetically fused protein Staphylococcal protein A/E. coli β-galactosidase (SpA-βgal). The cultivation conditions were found to have a profound effect on the overall productivity of SpA-βgal. Biologically active SpA-βgal was produced corresponding to 12% of the cell dry weight. The purification of SpA-βgal was based on the partitioning properties of β-galactosidase during extraction in an aqueous two-phase system consisting of poly(ethylene glycol) 4000 and potassium phosphate. Stability studies of SpA-βgal revealed that the β-galactosidase moiety remained intact both with respect to size and enzyme activity, while the SpA moiety was completely degraded by E. coli proteases.

INTRODUCTION

There is a growing interest in the development of processes for the isolation and purification of proteins. A reason for this is the fast growth of the recombinant DNA (rDNA) technology allowing bacteria to overproduce native, as well as foreign proteins. The rDNA technology can also be used to facilitate the purification of proteins. For example, the fusion of human Insulin-Like Growth Factor I (IGF-I) to the IgG-binding protein A was utilised to design a large scale process for the production of IGF-I with E. coli [1].

We have studied the production of the genetically fused protein Staphylococcal protein A/E. coli β-galactosidase (SpA-βgal). There are few data concerning large-scale cultivation of rDNA products under controlled conditions [2]. Therefore the influence of different cultivation conditions on the production of SpA-βgal with an E. coli strain was investigated [4].

A critical step in the purification of intracellular proteins is the primary purification, which includes removal of bacterial cell debris. A continuous process for large-scale isolation of β-galactosidase from a suspension of disintegrated E. coli cells has been developed. In poly(ethylene glycol) (PEG) 4000/potassium phosphate aqueous two-phase systems (pH 7), all cell debris, the major part of the unwanted proteins, and nucleic acids were partitioned to the denser salt-rich phase. Since
β-galactosidase had an extreme partition coefficient, compared to the average E. coli proteins, β-galactosidase could be collected in a small PEG-rich top phase without sacrificing too much of the recovery [3]. Based on the partitioning properties of β-galactosidase in the PEG 4000/potassium phosphate aqueous system, a similar purification procedure was investigated for SpA-βgal.

CULTIVATION

We have studied the influence of different cultivation conditions on the production of SpA-βgal with E. coli RRI lacZ ΔM15 (pRIT1, pNF2690) [4]. The strain was developed by Uhlen and co-workers [5]. The fused protein has the enzymatic capacity of β-galactosidase as well as the IgG-binding capacity of protein A [5,6]. The production has been studied both under the uncontrolled conditions in shake flask cultures and under controlled fermenter conditions. The amount of produced, biologically active SpA-βgal was strongly dependent on the cultivation conditions.

The expression of SpA-βgal is under the control of the lambda phage promoter PR and its temperature sensitive repressor cl 857 [5]. The construction allows for the possibility to control the expression by the culture temperature and thereby to separate the growth phase from the production phase. The production is so intensive that no cell growth occurs. At 30°C the repressor is active and the cells can grow and at 42°C the repressor is inactive and SpA-βgal is produced. The experimental protocol for the examinations of the influence of the cultivation conditions has been described in detail elsewhere [4].

We have found that when cultivated in shake flasks the level of SpA-βgal raised during the first few hours after temperature induction, but thereafter declined to a zero level. Electrophoresis showed that after two hours in the production phase, approximately 50% of the produced SpA-βgal existed in an insoluble, inactive form in the cells, and, after 7 hours, all of the product existed in this form, as so-called inclusion bodies. This inactivation could have been caused by the falling pH in the shake flask culture or by some action of the acetic acid that was produced. Undissociated acetic acid has the ability to penetrate the cell wall and cell membrane and dissociate within the cells and thereby lowering the intracellular pH [7]. The inactivation could also have been caused by some other conditions, for example, by a lowered intracellular redox potential caused by oxygen starvation.

![Graph showing specific activity of SpA-βgal and cell growth](image)

*Fig. 1* Specific activity of SpA-βgal (U/mg cell dry weight) and cell growth (OD610) as a function of time after induction, determined as described elsewhere [4]. Shaded symbols indicate fermenter culture induced at a low cell density and open symbols induction at a high cell density.