Method For High Level Production Of Recombinant Protein

Tech ID: 19155 / UC Case 2009-147-0

BACKGROUND

The expression of a cloned gene to isolate large quantities of its protein product demands a highly efficient expression system in which protein can be purified to homogeneity, especially for crystallographic and therapeutic purposes. It is often difficult to achieve high-level expression of biologically active recombinant proteins from eukaryotes. Several systems have emerged that involve fusing the gene of interest downstream of a second gene to produce a fusion protein. A major drawback of this approach is the covalent linkage of the two proteins, where the presence of the fusion partner may prevent or interfere with subsequent use of the desired protein. To overcome this problem, a protease recognition site can be constructed between the two fused proteins; however, this involves altering the N terminus of the desired product and resulting in the expression of an unauthentic protein. Furthermore, cleavage of the fusion protein is rarely complete, causing a reduction in protein yield, and it may also occur nonspecifically within the fused protein. Ubiquitin (Ub), the fusion partner, is a small eukaryotic protein that offers a natural yield enhancement, and uniquely, allows the Ub moiety to be removed by highly specific proteases known as deubiquitylating enzymes. A related system using WT ubiquitin for the production of soluble proteins has been reported. However, in many cases it is desirable to have the protein produced in an insoluble form for 1) reduced toxicity, 2) protection from proteolysis, and 3) ultimately higher yield.

TECHNOLOGY DESCRIPTION

Scientists at the UC San Diego have designed a recombinant protein expression system that delivers significantly higher yields than previously reported systems and includes an easy means of purification. It allows the production of a variety of proteins and peptides with authentic N termini for a range of downstream applications. Higher yields are obtained by redesigned protein core amino acids because of reduced toxicity and proteolysis due to the "tuned" stability of the ubiquitin fusion. They are expressed in inclusion bodies that enable the proteins to be refolded and efficiently cleaved from the fusion by ubiquitinase. This results in higher expression levels than comparable systems that express soluble proteins. Ultimately, the present technology can be useful for the production of both soluble and membrane proteins.

APPLICATIONS

- The present invention provides methods for delivering higher yields than the Baker system.
- The invention is useful for the production of both soluble and membrane proteins.
- The technology produces proteins and peptides with authentic N termini for a range of downstream applications.
- Commercial applications include reagent uses, such as:
  - Production of proteins in their native form
  - Single or multiple amino acid mutants
  - Isotopically labeled reagents
  - Fluorescent or biotinylated reagents
  - Reagents for protein therapeutics
  - Animal testing reagents and/or components of kits

STATE OF DEVELOPMENT

A U.S. patent application has been filed.

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OTHER INFORMATION

CATEGORIZED AS

- **Biotechnology**
  - Proteomics
- **Medical**
  - Research Tools
- **Research Tools**
  - Expression System
  - Protein Synthesis

RELATED CASES

2009-147-0

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