

Novel carrier proteins derived from *Staphylococcus* nuclease for high yield peptide production

SUMMARY

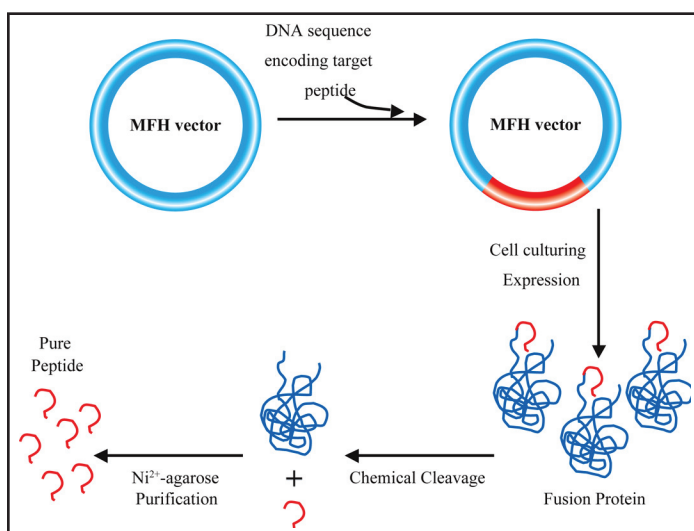
Genome-scale protein-studies and proteomics are increasing the use of peptides. Their synthesis relies mainly on chemical methods that generally suffer from cost disadvantages and low yield for peptides larger than 40 amino acids. This technology overcomes these drawbacks by providing novel carrier proteins for the production of small or large peptides as recombinant fusion proteins. These carriers confer stability and solubility to the peptides as well as simplify and improve their recovery.

APPLICATIONS

- Stable, high-yield and low-cost peptide production of peptides regardless of their size.
- Production of isotopically labeled peptides for NMR spectroscopy.
- Production of equal-molar mixture of various peptides using a single fusion protein.
- Alternative for the production of pharmaceutical peptides, markers for medical imaging and diagnostics.
- Scale up of peptides selected from functional screening of a phage-displayed peptide library.

CONCEPT

Fusion protein constructs are a commonly used strategy to increase the production efficiency of recombinant proteins however this approach has met with limited success for high-yield production of peptides. In this technology, novel carrier proteins were engineered as a series of protein fragments derived from *Staphylococcus* nuclease. These Small Fusion Carriers (SFCs), which confer peptide stability



and solubility, allow highly efficient and low cost production of small or large recombinant peptides (2-100 amino acids residues) using any suitable bacterial expression systems. This high efficiency is achieved through the use of a small carrier protein that allows C- or N-terminal fusions, the formation of inclusion bodies to protect the recombinant peptide from cellular proteases, and the improvement of downstream purification processes (see Figure).

FEATURES AND BENEFITS

Highly efficient peptide production

The SFC fusion approach to peptide production allows maximizing the yield and purity while minimizing the cost. This is achieved by reducing the size of the carrier, enhancing the stability and solubility of the carrier-peptide fusion, and improving carrier removal and peptide purification (yields of >10 mg/L were obtained in LB or M9 medium).

Versatile production system

Recombinant peptides can be produced regardless of their size or amino acid compositions. Increasing the yield of the target peptide is possible through the expression of tandem repeats separated by cleavage sites. As well, this approach may be used to simultaneously produce equal molar amounts of various peptides.

Improved carrier removal and downstream processing

The possibility of introducing any of a variety of proteolytic (e.g. thrombin) and/or chemical (e.g. CNBr) cleavage sites in the SFCs yields a flexible and effective peptide release process. Furthermore, the presence of affinity tags in the carrier protein (e.g., His-tags) reduces the costs and allows easier scale-up as they greatly facilitate the purification of the cleaved target peptide from products harboring the SFCs.

High solubility, no aggregation

In most physiological buffers, the SFC fusion proteins, including the carrier, target peptides, affinity tag(s) and cleavage site(s), are highly soluble (up to about 100 mg/ml) and display high refolding yields (99%) without aggregation.

Ability to produce labeled peptides

The SFC fusion approach allows the production of isotopically labeled peptides at high yield and reasonable cost. Isotopic enrichment of peptides for NMR spectroscopy or other applications can be achieved through double or triple labeling (¹⁵N/¹³C/²H).

PROTECTION STATUS

Staphylococcal nuclease fusion proteins for the production of recombinant peptides (NRC no. 11391).

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