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Heterologous expression and protein engineering of wheat gluten proteins

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Abstract

A range of systems are available for the production of recombinant wheat gluten proteins, from simple and widely used systems based on *Escherichia coli* to more sophisticated eukaryotic systems in yeasts or cultured insect cells. The characteristics of these systems are summarised and their advantages and disadvantages for application to wheat gluten proteins discussed. We then review the applications of heterologous expression systems to the synthesis and characterisation of wheat gluten proteins, including the production of wild type and mutant proteins for structure–function studies. We also discuss the use of heterologous expression to establish model systems including perfect repeat peptides based on motifs present in gliadins and glutenin subunits and 'analogue glutenin proteins' based on C hordein of barley. It is concluded that the pET series of vectors and *E. coli* are suitable for most applications, providing high-level expression and being rapid and easy to use.
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1. Introduction

The heterologous expression of proteins and protein engineering are often considered to be almost synonymous but in fact they are quite different in concept.

Heterologous expression refers to the expression of a protein in an organism different to that from which it originates. In practical terms, this is usually a microorganism or cell type which can be readily grown in culture, with high cell densities facilitating the production of high yields of recombinant (i.e. expressed) proteins. However, expression systems are not limited to cells or organisms that can be cultured and in some cases it may be advantageous to use whole eukaryotic organisms such as plants as hosts. The most widely used microorganism is *Escherichia coli* but yeasts (*Saccharomyces cerevisiae*, *Pichia pastoris*) and filamentous fungi are also used. The characteristics of these systems and their relative advantages and disadvantages for the expression of gluten proteins will be discussed in detail in Section 2.

Abbreviations: ANG, analogue glutenin proteins; CD, circular dichroism; ES-MS, electrospray mass spectrometry; FT-IR spectroscopy, Fourier-transform infra-red spectroscopy; MALDI-tof MS, matrix assisted laser description ionisation-time of flight; Q-tof MS, quadruple-time of flight mass spectrometry.

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Perhaps, the widest use of heterologous expression systems is to allow the production of single homogeneous proteins. In some cases, the protein encoded by a gene or cDNA has not been characterised, or even identified, at the protein level. A heterologously expressed protein after purification can be used to assay for biological activity in vitro or to raise antibodies that can, in turn, be used to determine its location and amount in vivo. In the case of cereal prolamins, the identification of individual proteins may not pose a problem but the purification of a single component from a highly complex mixture of related proteins frequently does. Hence, heterologous expression is most frequently used to produce single pure components for use in structure–function studies.

Protein engineering is used to define structure–function (or structure–functionality) relationships by creating and characterising mutant forms of a protein. In the case of peptides and small proteins, the mutant forms can be created chemically by peptide synthesis. However, this becomes increasingly difficult and expensive as the length of the polypeptide chain increases and heterologous expression is the preferred route, with mutations being introduced into the DNA encoding the protein using standard genetic engineering technology. The wild type and mutant proteins are then expressed in an appropriate system, purified and characterised.

Analysis of the recombinant proteins can provide information on the molecular basis for the functional properties of cereal grain proteins, which may have direct applications in developing optimal conditions for grain processing. However,

in many cases, it will be necessary to effect improvements via plant breeding, including wide crossing to introduce novel proteins from related species, or by genetic engineering. These approaches are expensive and time consuming and breeders are therefore reluctant to embark on new projects unless there is a sound scientific basis and reasonable chance of success. In such cases, heterologous expression and protein engineering can be used to allow the functional properties of individual proteins to be determined as a prelude to their selection or introgression by plant breeding, or introduction using genetic engineering technology.

This review focuses on the application of heterologous expression systems to study the structures, interactions and functional and biological properties of wheat gluten proteins. However, before discussing this work in detail we will briefly describe the characteristics of the most widely used expression systems, focusing on specific factors which determine their appropriateness for expressing gluten proteins.

2. Characteristics of expression systems

Three types of expression systems are widely used for heterologous protein production, exploiting *E. coli*, yeasts and cultured insect cells as hosts. Their characteristics and advantages/disadvantages are summarised in Table 1 and discussed briefly below.

The Gram-negative bacterium *E. coli* is the most widely used microorganism for heterologous protein expression and many workers only consider other systems if yields from *E. coli* are inadequate or if the protein produced is biologically inactive. Expression systems developed from *E. coli* benefit from its well-characterised genetics and a large number of vectors and host strains are now available commercially allowing the precise system to be optimised for individual proteins.

The copy numbers of *E. coli* expression vectors depend on their plasmid of origin with most vectors being derived from low copy number plasmids. For example, the widely used pET vectors (Studier and Moffatt, 1986) are based on the pBR322 plasmid which is present at 15–60 copies/cell. However, high copy number plasmids such as pUC (>200 copies/cell) have been used to construct vectors, such as pLEX (Mieschendahl et al., 1986), for high-level protein expression (see excellent reviews by Jonasson et al., 2002; Sørensen and Mortensen, 2005).

Unicellular eukaryotic yeasts have been used for large-scale production of recombinant proteins since the early 1980s. Our detailed knowledge of yeast genetics and physiology makes these organisms particularly suitable for development as a eukaryotic expression system. In particular, yeast cells recognise eukaryotic translation, processing and modification signals and so perform eukaryotic processing steps on the polypeptides expressed. Most early studies used bakers' yeast (*S. cerevisiae*) (reviewed by Hinnen et al., 1994) but an increasing number of alternative systems have been developed to avoid specific limitations of this species. They include the methylotrophic yeast species *Hansenula polymorpha*, *P. pastoris* and *Candida boidinii* (Faber et al., 1995; Gellissen, 2000; Gellissen and Melber, 1996; Gellissen et al., 1992) and the budding yeast *Kluyveromyces lactis* (Bergkamp et al., 1992).

The methylotrophic yeast species share a highly inducible methanol utilisation pathway and can therefore grow on methanol as a sole carbon and energy source. *P. pastoris* has proved to be an excellent host for protein production and has become increasingly popular due to a wide choice of *host* strains and vectors and its ability to be cultured in high density fermenters (Cereghino and Cregg, 2000; Daly and Hearn, 2005). However, so far *Pichia* has not been used for expression of wheat gluten proteins.

Most yeast expression vectors can either exist autonomously in the cell or become integrated into the bacterial genome (and hence are often termed episomes rather than plasmids). They may be present either in low copy number (1–2) (such as YRp and YCp), or in high copy number (YEp), with the latter (maintaining about 30–100 copies per cell) being based on the 2 μm plasmid.

Protein expression in insect cells was first reported in the early 1980s (Smith et al., 1983) using baculovirus as an expression vector. Baculoviruses constitute one of the most diverse groups of arthropod viruses with the best studied member of the family being *Autographa californica*, a nuclear polyhedrosis virus (AcMNPV) with a double stranded, circular DNA genome. The most commonly used insect host cell lines were originally derived from pupal ovarian tissue of *Spodoptera frugiperda* (the fall armyworm, a tropical lepidopteran species). The baculovirus system has been widely used and has signaficant advantages including the capacity to accept large inserts of DNA and the production of high yields of recombinant protein (for recent reviews see Ikonomou et al., 2003; Kost and Condreay, 1999; Philipps et al., 2005; Possee, 1997). However, the large size of the AcMNPV genome means that it is not possible to construct the viral expression vector using standard recombinant DNA technology with restriction enzymes. Instead, the foreign gene is inserted into a transfer vector flanked by viral sequences and used to co-infect cultured insect cells together with wild type AcMNPV. Recombination between the transfer vector and wild type virus occurs leading to the production of recombinant virus, which can be isolated and used to infect fresh insect cells for protein production. Thus, the production of the recombinant virus is a more time consuming and technically demanding process than the construction of vectors for *E. coli* and yeast expression. Furthermore, the multiplication of the recombinant AcMNPV, the determination of the virus titre and the optimisation of conditions are all time consuming. Nevertheless, once established the baculovirus system can give spectacularly high yields of recombinant proteins.

Despite these clear advantages, there has only been one report of the expression of a wheat gluten protein using a baculovirus system (Thompson et al., 1994).

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