

# Investigation of the starch-binding properties of wheat friabilin

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**ABSTRACT** The genes of two wheat proteins related to the texture of the grain, puroindoline A and grain softness protein 1, were cloned and expressed in *Escherichia coli*. Starch binding properties of the heterologously expressed proteins were studied *in vitro*. Both proteins bind to starch similarly to nativ friabilin prepared from soft wheat flour, and thus they are likely to be properly folded.

Acta Biol Szeged 49(1-2):29-30 (2005)

## KEY WORDS

puroindoline  
grain softness protein  
starch  
heterologous expression

The most important trait that determines how a particular wheat cultivar is processed is grain hardness (texture). Grain hardness forms the fundamental basis of commercial differentiation. It is possible to differentiate 'soft' and 'hard' hexaploid wheats and 'very hard' durum wheat as three distinct qualitative classes. Texture determines flour particle size, starch damage, water absorption and milling yield. Therefore, grain hardness is an indicator of the suitability of particular flour for a particular product. To the grower, texture is important as generally higher premiums are paid for the harder wheats (Morris 2002).

The hardness of the grain is determined by the adhesion of the endosperm's protein matrix to the starch granules. Due to strong adhesion, starch granules fragmentize during milling of hard wheat. This fragmentation is called starch damage. Starch damage is the most important factor in determining water absorption of flour. It also determines the amount of carbohydrates available to yeasts for fermentative activity. Therefore it affects positively gas production, loaf volume and, as a result, baking quality.

The fraction of basic proteins with an apparent molecular weight around 15 kDa in wheat flour is called friabilin, due to the relationship between their amount present and the softness (friability) of the grain. Friabilin extracted from wheat has been proven to bind to the surface of starch granules *in vitro* (Bloch et al. 2001). Friabilin was shown to contain three major polypeptides: puroindoline A, puroindoline B (Morris et al. 1994) and grain softness protein 1 (Rahman et al. 1994). Presence of both puroindoline A and B is necessary for the soft phenotype (Morris 2002). Puroindolines bind polar lipids (Dubriel et al. 1997) similar to those present on the surface of starch granules *in vivo*. However, there are no available information on how puroindolines prevent adhesion between starch granules and matrix protein (glutenin, gliadin, etc.). It is also unclear, why are both puroindoline A and B necessary for the grain to be soft.

Our aim is to clarify the function of friabilins in the wheat grain texture by studying the interactions between starch granules, friabilin components and matrix proteins of the endosperm. The high similarity amongst the friabilin components makes it difficult to purify the individual components from wheat flour; therefore we decided to produce them in a heterologous system.

## Materials and Methods

### Plant material

Starch binding experiments have been performed on starch columns prepared from bread wheat cultivars Riband (soft), Mercia (hard) and from durum wheat cultivar Martondur. Wheat friabilin was prepared from soft wheat cultivar Riband, using a method adapted from (Blochet et al. 1993).

### Heterologous expression of friabilin proteins

Puroindoline-a gene was amplified from a cDNA library. The signal peptide was replaced for a methionine. The PCR fragment was cloned into pET11d expression vector and checked by DNA sequencing. The clone was called PA pET11d.

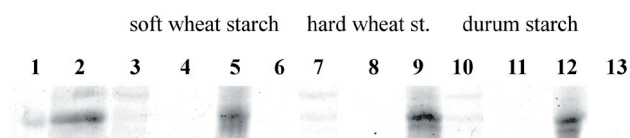
Part of a cDNA clone for GSP-1 (Grain Softness Protein) gene was amplified for cloning and expression. Oligonucleotide 1 contained a sequence to replace the signal peptide and the N-terminal peptide sequence by a codon for Met. The PCR fragment was cloned into pET17b expression vector and checked by DNA sequencing. The construct was called GSPP.

The proteins were expressed in Origami B (DE3) as host cell.

### SDS-PAGE

After overnight expression, bacterial cultures were centrifuged. For SDS-PAGE and Western blotting, pellets were resuspended in loading dye (2% [w/v] SDS, 100mM DTT, 50mM Tris-HCl (pH 6.8), 10% [v/v] glycerol, 0.002% [w/v]

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**Figure 1.** Binding of GSPP to wheat starch granules. Lane 1: molecular weight standard, lane 2: GSPP expressed in *E. coli*, lanes 3, 7, 10: flow through, lanes 4, 8, 11: washing, lanes 5, 9, 12: elution by SDS, lanes 6, 13: elution from pure starch.

bromophenol blue). After heating at 100°C for 2 minutes, the solubilized proteins were separated on 15% Laemmli gels. Fermentas Protein Ladder or Fermentas Prestained Protein Ladder was used as molecular weight standard.

### Starch binding experiments

For starch binding, bacterial pellets were resuspended in 8 M urea, 50 mM Tris-HCl (pH 6.8) and incubated for 4 hours at room temperature with constant agitation. Samples were dialysed against 50mM Tris-HCl (pH 6.8) at 4°C. The dialysed samples were used promptly for starch binding experiments. Starch binding experiments were performed using a method adapted from (Bloch et al. 2001).

### Results and Discussion

PA and GSPP proteins expressed in bacteria were identified by Western blot analysis using an antiserum raised against purified wheat friabilin. PA and GSPP proteins extracted by urea or by SDS have an apparent molecular weight of approx. 15kDa (under reducing conditions). The molecular weight calculated from the DNA sequences is 14kDa for both PA and GSPP. The difference between the calculated and apparent molecular weights of PA and GSPP might be caused by the large number of basic amino acid residues present in these polypeptides. A similar difference between the calculated and apparent molecular weights was reported for native wheat friabilin (Gautier et al. 1994). GSPP protein was also analysed by MALDI-TOF. The results confirm that the expressed protein is identical to the designed polypeptide.

Starch binding properties of the expressed proteins were tested on three different types of starch purified from wheat cultivars Riband (soft), Mercia (hard) and Martondur (durum). Friabilin extracted from wheat flour was reported to bind to starch granules purified from soft wheat cultivar Riband and to starch granules purified from hard wheat cultivar Mercia (Bloch et al. 2001). Therefore, a wheat friabilin preparation was used as positive control in the binding experiments.

Our results show that all the three expressed polypeptides are capable of binding to the three types of starch columns (Fig. 1), similarly to native friabilin prepared from flour.

The method developed in this work is suitable for testing the functionality of different friabilin components and their modified derivatives.

### Acknowledgements

M. Gárdonyi has received a postdoctoral fellowship from OTKA (D048401).

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