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# Isolation and characterization of Avenin-like protein type-B from durum wheat

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ABSTRACT

Proteomic techniques were applied for the investigation of "avenin-like protein type-B" in tetraploid wheat. Protein was extracted from endosperm, according to a classical chromatographic procedure and its identity confirmed by liquid chromatography-tandem mass spectrometry analysis. The characterization of avenin-like protein type-B was in good agreement with the gene-derived sequence, with the exception of glutamine as N-terminus. The presence in the glutenin extract and the high number of cysteine residues suggested that avenin-like protein type-B is integrated into gluten polymers via inter-chain disulphide bonds. This raises the question of whether the protein could play a role in determining the functional properties of gluten.



## 1. Introduction

Avenin-like proteins (ALPs) are a small family of wheat storage protein, so named for their weakly similar sequences to avenin proteins of oats. They occur in two isoforms, ALP type-A and ALP type-B. The expression of both classes of cDNA in developing endosperms of wheat and related species was demonstrated by transcriptome profiling. In particular two major (type-A1 and type-B1), and five minor transcripts (type -A2, -A3 and type -B2, -B3, -B4) are known (Kan et al., 2006).

The distinguishing feature of these proteins is that they include high levels of cysteine residues. Each ALP type-A contains a total of 14 cysteine residues (Kan et al., 2006). Because of the high homology sequence similar to that previously reported for a "low molecular weight gliadin" monomer, it may be assumed that cysteine residues of ALP type-A mediate seven intra-chain disulfide bonds (Anderson et al., 2001; Clarke et al., 2003; Salcedo et al., 1979). The molecular mass of ALP type-A is ~18 kDa, corresponding to the amino acid sequence deduced from the ALP gene (Kan et al., 2006). Evidence has been obtained for a protein isolated from wheat grains, on the basis of the partial amino sequence data achieved by mass spectrometry, that it is comparable to the protein sequence of ALP type-A (Dupont et al., 2005). Similarly, five ALPs were identified in the salt-soluble (albumin and globulin) fraction from 36-day-old developing endosperms (Vensel et al., 2005). ALP type-A was detected as putative allergen in wheat-derived foodstuffs based on the N-terminal amino acid sequence of tryptic peptides (De Gregorio et al., 2009). Extraction of ALP type-A monomer under non-reducing conditions means that protein was not engaged in the gluten polymer through S–S bonds, unless they are incorporated by reduction and reoxidation in dough making (Clarke et al., 2003).

In contrast, ALP type-B proteins contain 19 (type-B-1, -3 and -4) or 18 cysteines (type-B-2), and do not correspond to any known protein sequence. They differ from ALP type-A for about a 120-residue insertion, corresponding to a duplicated N-terminal region sequence of mature type-B protein (Kan et al., 2006). The genes of ALP type-B have been characterized in 23 species of Triticeae, including 18 species of Aegilops, barley and diploid, tetraploid and hexaploid forms of wheat (Chen et al., 2008).

Recently, for first time, we detected the presence of ALP type-B in the glutenin (GS) fraction from durum wheat cultivar. The identification was supported by acquiring the sequence of a reasonable number of tryptic peptides and matches between measured and expected MW and p*I* (Mamone et al., 2009). The detection of ALP type-B in the GS fraction and its high content of cysteine residues suggest that it could be integrated via inter-chain disulphide bonds within the GS polymer, possibly contributing to the functional quality of gluten.

*Abbreviations:* 2-DE, two-dimensional gel electrophoresis; ACN, acetonitrile; ALP, avenin-like protein; GS, glutenin; HMW, high molecular weight; HPLC, high-performance liquid chromatography; LMW, low molecular weight; μLC-MS/MS, micro liquid chromatography-tandem mass spectrometry; TFA, trifluoroacetic acid.

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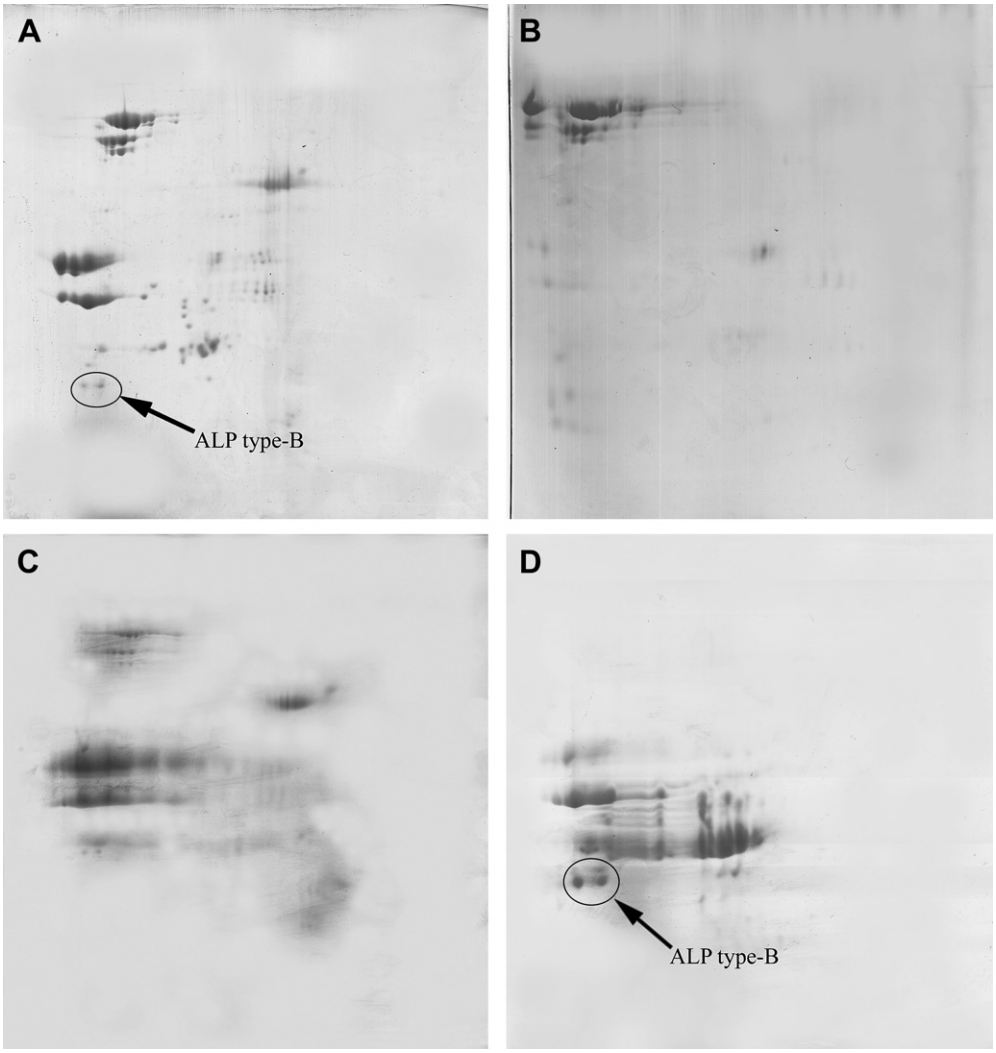


**Fig. 1.** 2-DE separation of reduced and alkylated GS. A: total GS extract with 50% propan-1-ol; B, C, D: GS extract with 60%, 70%, 85% propanol-1-ol concentration respectively. Arrow indicates the spot containing ALP type-B protein.

To facilitate understanding of the biochemical and physical functionality of ALP type-B, and to establish its role in wheat quality, we focused our attention on the protein characterization. Herein, we report a procedure for ALP type-B protein characterization from a common Italian durum wheat cultivar, including protein fractionation, chromatographic purification and tryptic peptide identification by μLC-MS/MS analysis.

## 2. Experiments

### 2.1. Chemicals and reagents

Trypsin, dithiothreitol, iodoacetamide, 4-vinylpyridine, Tris–HCl, guanidine chloride, ammonium bicarbonate and HPLC solvents were purchased from Sigma (St. Louis, Missouri, USA). Reagent and strips for electrophoresis analysis were from GE Healthcare (Amersham Biosciences, Uppsala, Sweden).

### 2.2. Protein extraction

Flour of the wheat cultivar Svevo (*Triticum durum*) was used. Selective extraction of GS with 50%, 60%, 70% and 85% was performed according to Masci et al. (2002). Pellets containing GS subunits were dried down in a Savant Speed-Vac (Savant, Farmingdale, NY, USA) concentrator and stored at −20 °C.

### 2.3. Two-dimensional gel electrophoresis (2-DE) analysis

Protein samples were dissolved in 0.3 M Tris–HCl, pH 8.0, containing 6 M guanidine/HCl and 5 mM dithiothreitol at 37 °C for 2 h. The resulting solution was carboxymethylated by reaction with 55 mM iodoacetamide at room temperature for 45 min in the dark. The sample was freed from low molecular weight compounds by filtration through a PD-10 desalting column in 5% acetic acid and lyophilized. Finally proteins were separated by 2-DE and single spot identification was carried out as described by Mamone et al. (2009).

### 2.4. High-performance liquid chromatography (HPLC) analysis

Liquid chromatography was performed using an HPLC Agilent 1100 series (Palo Alto, CA, USA). The unalkylated GS fraction extract with 85% propan-1-ol was filtered through the 0.45 μm filters (Agilent) and injected onto a Vydac (Hesperia,CA, USA) C8 narrow-bore column (208TP52, 25×0.46 cm i.d., 5 mm film thickness) with a flow rate of 1 ml/min. Solvent A was 0.1% trifluoroacetic acid (TFA) in water and solvent B was 0.1% TFA in acetonitrile (ACN). Proteins