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LOW MW GLIADIN-LIKE PROTEINS FROM WHEAT ENDOSPERM

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Abstract—A new group of hydrophobic endosperm proteins from *Triticum aestivum* has been characterized. It consists of 10 components with MWs in the range of 17000–19000, which have a similar range of electrophoretic mobilities at pH 3.2 as the classical gliadins. However, they have a higher proportion of sulphur amino acids and lower levels of glutamine and proline than the gliadins.

INTRODUCTION

The term gliadins was originally used to designate the prolamine fraction from wheat endosperm, a mixture of protein components that is extracted with 70% ethanol [1]. Some components of this extract were thought to be albumin-like or globulin-like contaminants, so more restricted definitions of gliadins have been proposed based on additional criteria, such as solubility in other solvents [2, 3], MW range [4] or electrophoretic mobilities [5, 6]. However, it has been shown that at least one group of the suspected contaminants [7, 8], the CM proteins, actually consisted of proteo-lipid-like hydrophobic components [9–12]. We now report a new group of wheat endosperm proteins which could be classified as gliadins according to their solubility and their electrophoretic mobility but which are of lower MW and different amino acid composition than previously described gliadins, with the possible exception of one protein component purified by Ewart [13].

RESULTS

Extraction and fractionation

The proteins under study can be extracted from delipidated flour with $CHCl_3$–MeOH 2:1 or with 70% EtOH, but are not extracted with other $CHCl_3$–MeOH mixtures (1:7 or 7:1) or with water. As previously described [11], these extracts yield 3 protein fractions after gel filtration on Sephadex G-100. The third fraction, which is under 25000 MW, can be further fractionated by starch-gel electrophoresis at pH 3.2, yielding a group of components with higher mobility, which have been previously designated CM proteins, and a second group with lower mobility, which was previously thought to consist of gliadin contaminants [12]. A number of components can be detected in this group by appropriate staining conditions (Fig. 1): 6–8 in hexaploid cultivars (*T. aestivum* cvs Candeal de Castilla and Chinese Spring) and 4–6 in tetraploid cultivars (*T. turgidum* cvs Ledesma and Senatore Capelli).

A two-dimensional electrophoretic method (pH 9 × pH 3.2) was developed in order to avoid the interference of gliadins, which are also present in crude extracts (70% EtOH or $CHCl_3$–MeOH, 2:1). The fractionation of different samples using this method is shown in Fig. 2.

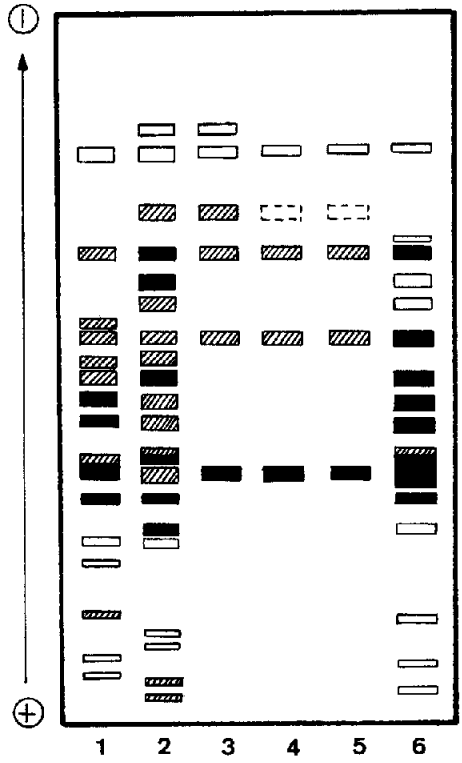

Fig. 1. Starch gel electrophoresis (aluminium lactate buffer 0.1 M, pH 3.2, 3 M urea; 15 hr run at 12 V/cm) of the following samples: chloroform–methanol 2:1 (v/v) extract from (1) cv Senatore Capelli (*T. turgidum*); (2) cv Candeal (*T. aestivum*); (6) cv Ledesma (*T. turgidum*); (3), (4) and (5) fractions of MW under 25 000 from chloroform–methanol 2:1 extracts from the cvs Candeal, Senatore Capelli and Ledesma, respectively. Gels were stained with 0.6% nigrosine in MeOH–H_2O–HOAc, 5:5:1, for 16 hr.

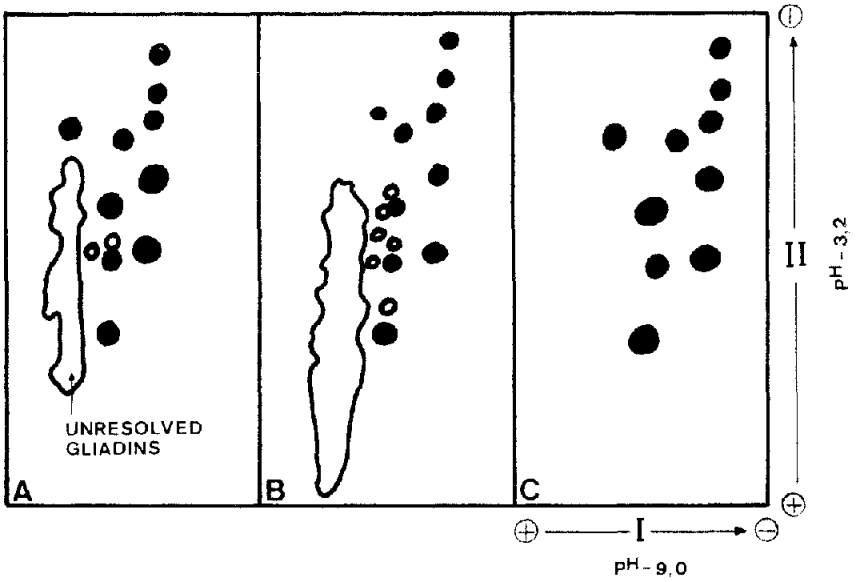

Fig. 2. Maps obtained by two-dimensional electrophoresis (Tris glycine buffer pH 9.0, acrylamide 10%; 2.5 hr at 50 V/cm × aluminium lactate buffer pH 3.2, starch 12.5%; 15 hr run at 12 V/cm) of the following extracts from cv Candeal (*T. aestivum*): (A) chloroform–methanol 2:1 (v/v); (B) ethanol 70%; (C) fraction of MW under 25000 from chloroform–methanol extracts. Black spots represent components with MW under 25000; open ones represent those with MW above 25000. Gels were stained with 0.6% nigrosine in MeOH–H_2O–HOAc, 5:5:1, for 16 hr.

The two-dimensional map of the fraction under 25000 MW from the $CHCl_3$–MeOH extract consists of 10 components, which are also resolved in the crude $CHCl_3$–MeOH and in the 70% EtOH extracts of both *T. aestivum* cultivars. The isoelectric points of all components of this group are high as evidenced by their migration to the cathode at pH 9.

A preparation of 'classical gliadins' obtained by sequential solvent extraction according to Beckwith *et al.* [14] contained all the above mentioned components, but in a much smaller proportion than in the crude $CHCl_3$–MeOH extract. As in the case of CM proteins [11], these proteins are soluble in water after gel filtration in acid buffer plus urea, dialysis and freeze-drying, without losing their solubility in $CHCl_3$–MeOH and in 70% EtOH.

Preparative electrophoresis, MW and amino acid composition

This group of proteins was separated on a preparative scale from other components of the $CHCl_3$–MeOH extract in two steps; first from glutenins and gliadins by gel filtration, and then from CM proteins by preparative electrophoresis on a polyacrylamide column at pH 9. The preparative procedure was monitored by analytical one- and two-dimensional electrophoresis. The preparation thus obtained was subjected to electrophoresis in sodium dodecyl sulphate together with pure protein standards of known MW. The proteins banded in the 17000–19000 MW range.

The average amino acid composition of these proteins is presented in Table 1. This analysis indicates that the hydrophobic properties of these proteins are intrinsic (not due to lipid ligands) because *ca* 54% of the amino acid residues are non-polar. The fact that their isoelectric points are above pH 9 means that most of the Glx residues (21.3/100) are glutamine.

Table 1. Amino acid composition (mol/100 mol of amino acid analysed) of low MW gliadin-like proteins

Amino acid*		Amino acid	
Lys	1.0	Ala	7.4
His	0.8	Val	5.7
Arg	4.1	1/2 Cys	8.5
Asx	3.1	Met	3.4
Thr	7.5	Ile	5.1
Ser	8.2	Leu	4.9
Glx	21.3	Tyr	2.5
Pro	6.3	Phe	1.9
Gly	8.1		

* Trp was not analysed.

DISCUSSION

It is evident that, because of their solubility in 70% EtOH and their electrophoretic mobilities, these proteins have been included in gliadin preparations obtained by different authors [15–17]. It is surprising that they have not been previously detected, especially when sodium dodecyl sulphate electrophoresis has been carried out with the preparations. Bietz and Wall [15] found some components under 36000 MW: one at 25600 and other at 11400, the latter probably corresponding to CM proteins. However, they did not detect components with MW around 20000, probably because a lower proportion of these proteins was present in their preparation and because they stain poorly with Coomassie blue.

The amino acid composition of this group of proteins, their MW range and their electrophoretic mobilities are compatible with the inclusion in this group of the protein purified by Ewart [13].

With respect to gliadins, these proteins have a higher content of cysteine, threonine, glycine, alanine and especially, methionine; as well as a lower proportion of proline, leucine and glutamine, although the latter is still the most abundant amino acid [1, 13]. In comparison with glutenins, similar differences are observed for cysteine, alanine, methionine and glutamine [1]. The CM proteins have a higher content of basic amino acids and a lower content of glutamine and methionine [9, 12].

On the basis of the above observations, it is difficult to fit this group of proteins into any of the main protein classes. Perhaps the operative designation of low MW gliadins would be the most appropriate.

EXPERIMENTAL

Biological material. Flours of 65% extraction from *T. aestivum* cvs Candeal de Castilla and Chinese Spring and *T. turgidum* cvs Senatore Capelli and Ledesma were used in this study.

Extraction and gel filtration. The $CHCl_3$–MeOH (2:1) and the 70% EtOH extracts, both at analytical and preparative scales, were obtained as previously described [11, 18]. Classical gliadins were obtained according to ref. [19] as modified in ref. [14].

Analytical methods. One-dimensional starch-gel electrophoresis was carried out in lactic acid–aluminium lactate buffer, pH 3.2, 3 M urea for 15 hr at 12 V/cm or alternatively, in Tris–glycine buffer, pH 9, 3 M urea, for 7 hr at 18 V/cm. Two-dimensional electrophoresis combined the above conditions: the 1st dimension being carried out in 10% polyacrylamide gel columns of 2 × 70 mm at pH 9 for 2.5 hr at 50 V/cm.

Amino acid analysis was carried out essentially according to ref. [20], and performic acid oxidation according to ref. [21]. The samples were hydrolysed for 24 hr at 110 ± 1°.

MWs were determined by electrophoresis in SDS according to ref. [22].

Preparative methods. Isolation of the group of proteins under study was carried out from the gel-filtration fraction under 25000 MW, by preparative electrophoresis in Tris–glycine buffer, pH 9 in a 1.7 × 13 cm 10% polyacrylamide column. Recovery of the proteins was performed by the method of ref. [23] with modifications. The protein bands were visualized by exposing the gels to 30% TCA for 1 hr and then they were hand dissected and homogenized in 2 M urea. The homogenates were dialysed against 0.1 M HOAc for 48 hr. The polyacrylamide was separated by filtration on Whatman No. 1 paper and the filtrates were directly freeze-dried.

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