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RELATIONSHIPS AMONG LOW MW HYDROPHOBIC PROTEINS FROM WHEAT ENDOSPERM

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Abstract—Low MW proteins extractable with chloroform–methanol mixtures from wheat endosperm have been purified from different *Triticum* species and partially characterized. Their amino acid composition and MWs are consistent with previous genetic evidence concerning relationships among these proteins: proteins CM1 and CM2 are homoeologous (ancestral homologues); proteins CM3 and CM3′ are allelic variants; proteins 16 and 17 are homoeologous.

INTRODUCTION

The presence in wheat endosperm of proteins extractable with chloroform–methanol mixtures was first reported by Meredith *et al.* [1]. They studied the solubility of the extracted proteins, in aqueous and non-aqueous solvent mixtures, and fractionated them by changing the proportions of chloroform and methanol [2–4]. Rohrlich and Niederauer [5] isolated from the same extract a lipoprotein component that seemed to be homogeneous when subjected to paper electrophoresis.

Proteins extracted with chloroform–methanol (2:1) were fractionated by starch-gel electrophoresis (pH 3.2), yielding two groups of proteins [6]. The group with the lower electrophoretic mobility seemed to consist of classical gliadins and that with the higher mobility corresponded to the so designated CM-proteins. In allohexaploid wheat (*Triticum aestivum* L., genomes AABBDD), 3 main bands, designated CM1, CM2 and CM3, were present, whereas in allotetraploid wheat (*Triticum turgidum* Desf., genomes AABB) only bands CM2 and CM3 were detected. García-Olmedo and Carbonero [7] purified and partially characterized CM1 and CM2, showing that they were controlled by homoeologous chromosomes (ancestral homologues) 7D and 7B respectively. These proteins, as well as CM3, were also purified by Redman and Ewart [8] and by Aragoncillo [9]. The latter also purified protein CM3′, which is a variant of CM3 present in a few cultivars of *T. turgidum*.

Rodríguez-Loperena *et al.* [10] and Aragoncillo *et al.* [11] have further investigated these proteins in different genetic stocks using combined electrofocusing and electrophoresis for their fractionation. They deduced from chemical and genetic evidence the following relationships between electrophoretic bands and two-dimensional map components (spots): CM1 equivalent to spots 3 and 4 in *T. aestivum*, CM2 equivalent to spots 8 and 9 in *T. aestivum* and in *T. turgidum*, CM3 equivalent to spots 11, 12 and 13 in *T. aestivum* and to spots 12–13 in *T. turgidum*, and CM3′ equivalent to spots 12′ and 13′ in *T. turgidum*. They suspected that the pairs 3–4, 8–9, 12–13 and 12′–13′ could be fractionation artifacts, each of them representing a single protein. Two more components of the chloroform–methanol extract, designated 16 and 17, were detected and their genetic control assigned to chromosomes 4A and 4D respectively.

All these proteins, which are also soluble in 70% ethanol and have a MW under 25000, have been sometimes classified as albumins and globulins [12, 13, etc.]. However, it is becoming increasingly evident that they can not be included in any of the main Osborne solubility classes.

We report here the small-scale purifications of proteins CM1, CM2, CM3, 16 and 17 from *T. aestivum* and CM2, CM3, CM3′ and 16 from *T. turgidum*. The proteins have been partially characterized in order to establish their possible relationships.

RESULTS AND DISCUSSION

Extractability of proteins by different chloroform–methanol mixtures

The extractability of this group of proteins by different chloroform–methanol mixtures was studied in a semi-quantitative way. These observations are summarized in Table 1. All proteins investigated were extracted with about equal efficiency by the 2:1 and 1:2 mixtures. Proteins CM1 and CM2 were also efficiently extracted by mixtures with a lower proportion of chloroform. Only proteins CM3 and CM3′ are extractable by mixtures with a high proportion of chloroform (7:1). Finally, proteins 16 and 17 were poorly extracted by mixtures outside the 2:1 to 1:2 range.

Table 1. Relative yield* of proteins extracted by chloroform–methanol mixtures

Protein	$CHCl_3$	7:1	$CHCl_3$–MeOH ratio (v/v) 5:1	2:1	1:2	1:5	1:7	MeOH
CM1	—	—	tr	++	++	++	++	tr
CM2	—	—	tr	++	++	++	++	tr
CM3	—	+	+	++	++	+	+	tr
CM3′	—	+	+	++	++	+	+	tr
16	—	—	—	++	++	+	+	—
17	—	—	—	++	++	+	—	—

* ++, yield with $CHCl_3$–MeOH (2:1); +, protein present at lower level; tr, trace; —, protein absent.

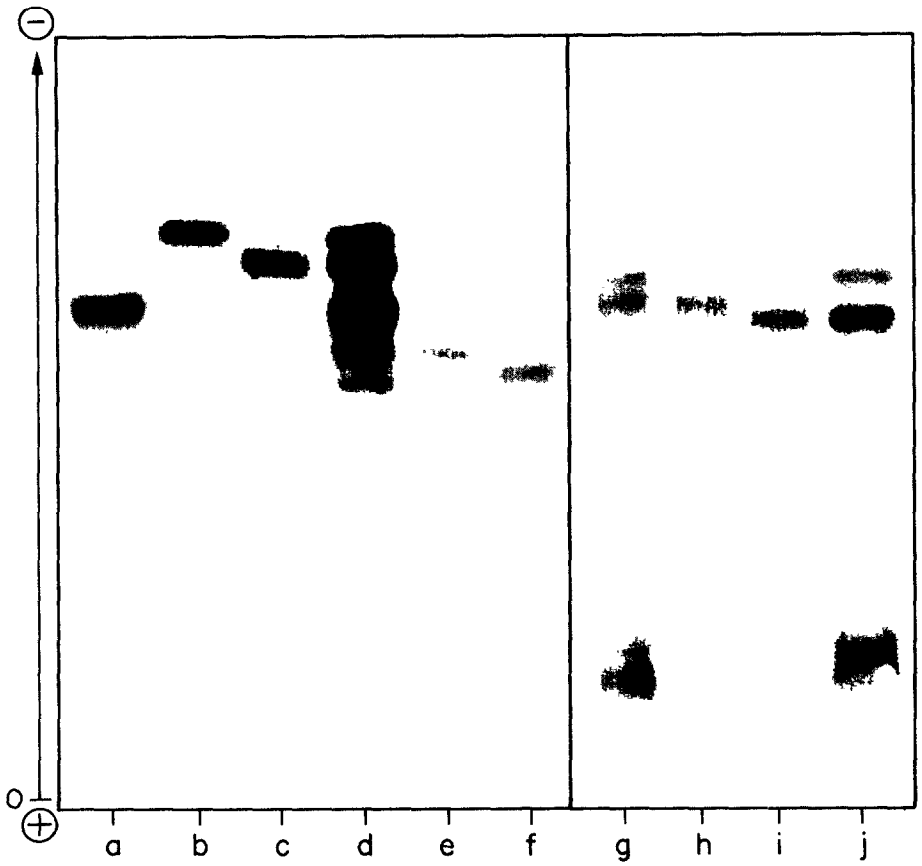

Fig. 1. Starch gel electrophoresis (0.1 M aluminium lactate buffer, 3 M urea, pH 3.2; 2 5 hr run at 20 V/cm and 5°; gels were stained with 0.5% Nigrosine in MeOH–H_2O–GOAc, 5:5:1, for 16 hr) of the following samples: (a) purified CM3; (b) purified CM1; (c) purified CM2; (d) $CHCl_3$–MeOH 2:1 extract from cv. Candeal (*T. aestivum*); (e) purified 16; (f) purified 17; (g) $CHCl_3$–MeOH (2:1) extract from cv. Ledesma (*T. turgidum*); (h) purified CM3′, (i) purified CM3; (j) $CHCl_3$–MeOH (2:1) extract from cv. Senatore Capelli (*T. turgidum*). Samples (a), (b), (c), (e) and (f) are from cv. Candeal, (h) from cv. Ledesma, and (i) from cv. Senatore Capelli.

Purification of proteins

Purification of the proteins under study was undertaken at a small scale (1–3 mg) by preparative electrofocusing, followed by preparative electrophoresis in the case of CM3′, starting from the fraction under MW 25000 of the chloroform–methanol (2:1) extract, which was obtained by gel filtration of the crude extract in Sephadex G-100 [10]. The purified proteins were electrophoretically homogeneous, as shown in Fig. 1.

To investigate whether the pairs of spots (3–4, 8–9, 12–13, 12′–13′), observed by Rodríguez-Loperena *et al.* [10] in the two-dimensional maps (electrofocusing × electrophoresis), represented actual heterogeneity or were fractionation artifacts, the separation was carried out using ampholines of narrower pH ranges (pH 6–8 and pH 7–9, instead of pH 5–8). Each of the pairs appeared as single spots by the new separation procedures, both when whole extract or when purified protein were fractionated. So we conclude from this and out previous results [10] that each of the pairs corresponds to a single protein.

Relationships among purified proteins

The amino acid composition and the MW of the purified proteins are presented in Table 2. The half-cystine values were greater than 5 moles/100 moles of amino acids for all the CM-proteins, but accurate figure are not reported because no performic acid oxidized samples were analysed.

The homeology (ancestral homology) of proteins CM1 and CM2 was demonstrated by García-Olmedo and Carbonero [7] and confirmed by Redman and Ewart [8]. Our present results are in agreement with these reports and also demonstrate that CM2 proteins from *T. aestivum* and from *T. turgidum* are practically identical with regard to amino acid composition.

The amino acid composition reported here for CM3 from *T. aestivum* is in general agreement with that reported by Redman and Ewart [8] and differs from that reported by Aragoncillo [9], because the preparation then analysed included protein 11, which is encoded by chromosome 7D.

It has been previously shown [11] that, in *T. aestivum*, there are two genes coding for proteins with the same mobility and isoelectric point as protein CM3. These are located in chromosome 4A and in an undetermined D genome chromosome. The fact that CM3 proteins isolated from *T. aestivum* (genomes ABD) and *T. turgidum* (AB) have the same MW and amino acid composition indicate that the two genes located in the A and D genomes must code for the same or a very similar protein.

Table 2. Amino acid composition (mol/100 mol of amino acids analysed) and MW of purified proteins

Amino acid†	*T. aestivum* cv. Candeal					*T. turgidum* cv. S. Capelli			*T. turgidum* cv. Ledesma		
	CM1	CM2	CM3	16	17	CM2	CM3	16	CM2	CM3′	16
Lys	3.2	3.1	2.6	2.4	2.9	3.1	2.3	1.9	3.0	2.2	3.0
His	2.9	2.8	1.8	1.4	1.8	2.9	1.5	0.7	2.9	1.5	2.2
Arg	7.5	7.3	6.6	6.1	4.8	7.4	5.4	7.0	6.9	7.3	6.4
Asx	6.8	9.1	6.5	7.4	7.3	9.4	6.8	8.0	9.8	6.5	7.7
Thr	4.6	6.1	4.6	6.4	6.4	6.8	5.7	7.4	7.1	4.9	6.3
Ser	8.0	5.2	6.4	7.0	6.7	4.6	7.2	6.2	4.4	7.3	6.4
Glx	14.5	11.9	13.1	20.3	18.7	9.5	13.0	16.4	9.0	12.5	19.4
Pro	11.6	12.2	11.8	12.2	15.2	11.3	11.8	10.5	12.4	11.4	9.9
Gly	8.4	10.2	8.4	7.0	8.4	12.0	7.9	7.8	10.6	7.9	8.5
Ala	5.4	5.0	6.1	3.7	4.3	5.7	7.5	5.7	5.9	7.4	4.4
Val	6.6	8.5	7.9	4.2	4.0	8.9	7.0	5.0	9.8	7.1	4.6
Met	0.5	1.1	0.8	1.4	1.6	1.4	1.0	3.3	2.1	1.0	1.9
Ile	3.9	3.1	5.0	4.0	3.7	2.9	3.7	5.0	2.5	4.2	3.9
Leu	9.2	7.7	10.8	10.4	9.2	7.4	11.1	9.3	6.5	11.3	10.5
Tyr	3.6	4.2	4.1	3.4	2.7	4.6	4.4	3.8	5.1	3.8	2.6
Phe	3.2	2.4	3.5	2.6	2.2	2.2	3.6	2.1	2.1	3.7	2.1
SDS MW‡	12,600	12,450	13,000	11,800	11,850	12,100	13,000	—	11,900	13,300	12,300

† Cys/2 and Trp were not analysed; ‡ MWs, determined by SDS electrophoresis as described in ref. [14].

Our unpublished evidence indicates that proteins CM3 and CM3′ from *T. turgidum* are inherited as allelic varients at a single locus. The close similarity of their amino acid composition supports their allelic relationship.

The purification, amino acid composition and MWs of proteins 16 and 17 are reported here for the first time. Previous genetic and chemical evidence indicated their possible homology [10, 11]. The present results support this relationship and they also suggest a homology of protein 16 from *T. aestivum* and that from *T. turgidum*.

Glutamic acid and proline are the amino acids present in a greater proportion in all these proteins. Another common feature is the high proportion of non-polar amino acids (49–59%, not counting half-cystine and tryptophan), which probably explains their solubility in organic solvents.

It is difficult to classify these proteins into any of the solubility classes of Osborne. Their amino acid composition is closer to the so called low glutamic acid glutenins [15] than to the other classes. In fact, protein 17 has an aminoacid composition which is quite close to that of fraction P of Mita and Yonezawa [16]. On the basis of the peculiar solubility properties of CM-proteins, which can be made water soluble without losing their solubility in chloroform–methanol mixtures, they were classified as a proteolipids [8, 10].

EXPERIMENTAL

Flours of *ca* 65% extraction from *Triticum aestivum* cv. Candeal and *T. turgidum* cvs. S. Capelli (CM3 phenotype) and Ledesma (CM3′ phenotype) were used in this study.

Analytical procedures. Combined electrofocusing and electrophoresis was carried out by a previously described [10] modification of the method of ref. [17]. Ampholine range was changed when needed. Amino acid analysis of purified proteins were performed essentially according to ref. [18]. MWS were determined by SDS electrophoresis as described in ref. [14].

Protein extraction. For the extractability expts, 20 g of flour, previously extracted with petrol (bp 50–70°), were placed in a glass column (3.2 × 15 cm) and extracted with 10 vol of the appropriate solvent mixture. The solvents were evaporated *in vacuo* and a known amount of the extract was fractionated by combined electrofocusing × electrophoresis. The relative yield of the different components was determined semi-quantitatively by densitometry (Chromoscan, Joyce & Loebl; 620 nm filter).

Preparative methods. Crude $CHCl_3$–MeOH (2:1) extracts from the different flours were prepared and fractionated by gel filtration in Sephadex G-100 as previously described [10]. The peak under MW 25000 was collected in each case, dialysed against H_2O for 48 hr and freeze-dried.

The freeze-dried material was fractionated by a preparative electrofocusing method similar to that of ref. [19]: 30 mg of the protein mixture were dissolved in 1 ml of 9 M urea and inserted on top of a 1.7 × 13 cm electrofocusing column (7.5% acrylamide gel, ampholines pH 5–8). The voltage was gradually increased up to 470, (keeping current under 10 mA) and kept at that value for 7 hr. The separation was carried out at 4°. The gel was then immersed in 30% TCA for 1 hr. Focused bands, which were visible after this treatment, were dissected by hand and homogenized in 2 M urea. The homogenates were dialysed against 2 M urea for 24 hr and against 0.1 M HCO_2H for 48 hr. Acrylamide was separated by filtration through Whatman No. 1 and the filtrates freeze-dried. Ampholines remaining in the preparation were extracted with Me_2CO after TCA precipitation of the protein.

In the case of protein CM3′, further purification by preparative electrophoresis was required. This was carried out on a 1.7 × 8 cm column (7.5% acrylamide, aluminium lactate buffer 0.1 M, pH 3.2, 3 M urea), using an electroelution device described in ref. [20].

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