LIPID BINDING PROTEINS FROM THE ENDOSPERMS OF WHEAT AND OATS

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Abstract—A protein, designated lipid binding protein (LBP), has been purified from the petrol extracts of wheat and oat endosperms by hydrochloric acid precipitation in a non-polar medium and preparative electrophoresis. The purified LBP appeared to be homogeneous both by electrophoresis in sodium dodecyl sulphate-polyacrylamide (SDS-PAGE) gels (MW *ca* 14 500) and by electrophoresis (PAGE) at pH 3.2. The amino acid composition indicates a high degree of homology between the LBPs from the two sources, as judged by the indexes of Cornish-Bowden and of Harris and Teller. As in the case of thionin, a previously characterized polypeptide from the ether extract, LBP becomes ether-insoluble, chloroform-soluble by precipitation with acetone, and solubility in ether is restored by binding of digalactosyl diglyceride to the chloroform-soluble form.

INTRODUCTION

The presence of proteins in the petrol (or diethyl ether) extracts from the endosperms of wheat and other Gramineae has been known for a long time [1–7], but most of the research on these proteins has been devoted to the thionins (for a review see ref. [8]), while the characterization of other protein components of these extracts has been neglected. We report here the isolation of a second type of ether-extractable protein from the endosperms of wheat (*Triticum aestivum* L.) and oats (*Avena sativa* L.), for which the designation lipid binding protein (LBP) is proposed.

RESULTS AND DISCUSSION

Solubility properties and purification

Experiments similar to those previously reported for wheat thionins [9] were performed in the case of the LBPs. A preliminary investigation of its solubility in organic solvents was carried out. All protein present in the petrol extract precipitated after the addition of 9 volumes of acetone. The precipitate was thus rendered insoluble in petrol but was still soluble in chloroform. Addition of digalactosyldiglyceride (DGDG) to the chloroform-soluble form led to the recovery of solubility in petrol for both thionins and LBPs. The LBPs co-precipitated with thionin in a lipid-free form upon the addition of 3 volumes of 1 M hydrochloric acid in ethanol. The lipid-free form did not become soluble in petrol upon the addition of DGDG. The characterization of the other lipid components required for solubility in petrol was not pursued further. In all these qualitative solubility experiments, LBPs showed the same properties as those previously reported for the thionins [9]. As judged from the preparative electrophoresis elution profiles (see Fig. 1), LBP represents between 30 and 50% of the protein extracted with petrol, which is rather variable among cultivars and can be as high as 1% of total protein [10]. In the case of thionins, we have shown that only about 10% of it is extracted with petrol [7]. Unfortunately, we have not yet developed a method to determine the total content of LBP in cereal endosperm and it is plausible that LBP is not quantitatively extracted with petrol.

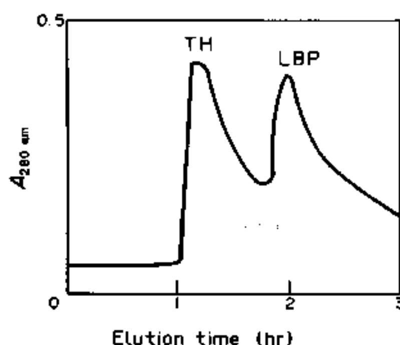

Fig. 1. Purification of the lipid binding protein (LBP) by preparative electrophoresis in a 10% polyacrylamide column (10 × 1.6 cm). A lipid-free protein preparation from the petrol extract of wheat endosperm was applied. The peak corresponding to LBP eluted after that of thionin (TH).

Purification of the protein was carried out by preparative electrophoresis of the ethanolic-HCl precipitates (Fig. 1). The purified proteins appeared to be homogeneous by electrophoresis in SDS-PAGE and in PAGE at pH 3.2 (Fig. 2). The apparent MW by SDS-PAGE was *ca* 14 500 for both proteins, but it should be pointed out that some proteins with hydrophobic domains are known to deviate from the mobility in SDS-PAGE that would correspond to their MW [11, 12].

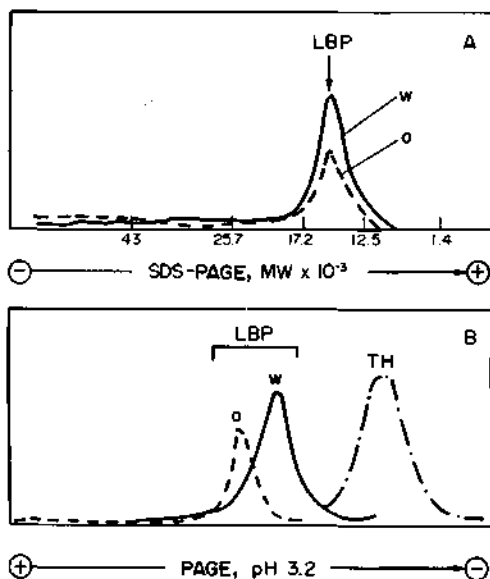

Fig. 2. Densitometric tracings of electrophoretic bands corresponding to purified lipid binding proteins (LBPs) from wheat (w) and oats (o). (A) SDS-PAGE; MW standards were ovalbumin (43 000), chymotrypsinogen (25 700), myoglobin (17 200), cytochrome *c* (12 500), bacitracin (1400) from Serva AG. (B) PAGE, pH 3.2; a wheat thionin preparation (TH) was included in the gel.

Amino acid composition and possible relationship to previously described proteins

The amino acid compositions and apparent MWs of the purified proteins are presented in Table 1. The number of moles of each amino acid that corresponds to the apparent MW was adjusted by the method of Delaage [15]. The amino acid compositions of the LBPs purified from wheat and oats are those expected of homologous proteins, according to both of the compositional indexes proposed by Cornish-Bowden [16] and Harris and Teller [13], respectively (Table 2). In fact, the divergence between the amino acid compositions of the wheat and the oats protein is almost as low as that found between two purification batches of the same protein (unpublished results). Although for each LBP a single electrophoretic band was observed, it is possible that each preparation could include more than one genetic variant because of the hexaploid nature of both wheat and oats.

A survey of the literature was carried out to check whether similar purified proteins or crude protein fractions had been previously reported. Two such fractions were found in wheat and oats, respectively: (i) Frazier *et al.* [14] obtained a supernatant fraction, after 20% ammonium sulphate precipitation of the acetic acid extract of wheat gluten, which contained one major and several minor protein components by SDS-PAGE. This fraction, which has been claimed to represent up to 10% of gluten, was designated ligolin because of its ability to ligate radionuclide-labelled glyceroltriolein during dough-mixing [14]. (ii) Kim *et al.* [17] obtained a fraction, designated avenin-C, by gel filtration of a crude avenin preparation from oat endosperm. This fraction presented one major and one minor protein component by electrophoresis in starch gels at pH 3.2 [17].

The amino acid compositions of ligolin and avenin-C are presented in Table 1 and their comparisons with both LBPs are shown in Table 2. Both compositional indexes suggest that the major component of the avenin-C fraction is probably identical to the LBP from oats, the small divergence in amino acid composition being

Table 1. Amino acid compositions and MWs of purified lipid binding proteins (LBPs) from wheat and oats

	Mol of amino acid/10^3 mol				Mol of amino acid/mol of protein*			
Amino acid	Wheat LBP	Oat LBP	Ligolin [14]	Avenin-C [17]	Wheat LBP	Oat LBP	Ligolin	Avenin-C
Arg	73.8	61.2	57.5	64	10	8	8	8
His	12.4	18.6	17.6	19.5	2	2	2	3
Lys	76.8	71.5	43.7	34	10	9	6	4
Phe	37.2	28.4	38.5	29	5	4	5	4
Tyr	15.9	17.2	28.8	13.5	2	2	4	2
Leu	62.0	67.9	84.0	91	8	9	12	12
Ile	44.3	44.1	40.6	49	6	6	6	6
Met	10.0	21.5	0	36	1	3	0	5
Cys	76.6	59.0	31.2	81	10	8	4	11
Val	51.4	49.0	64.5	36	7	6	9	5
Ala	44.6	56.7	89.5	47	6	7	13	6
Gly	99.2	107.8	101.5	98	13	14	14	13
Pro	57.2	59.6	62.5	66	8	8	9	9
Glu	151.8	172.2	128.1	187	20	22	18	24
Ser	70.3	53.9	72.5	43	9	7	10	6
Thr	41.3	42.3	52.6	42	5	6	7	5
Asp	75.6	69.1	82.5	61	10	9	12	8
No. of residues					132	130	139	131
MW*					14590	14271	14905	14503

*Calculated according to Delaage [15] as the best adjustment to the apparent MW obtained by SDS-PAGE for LBP.

Table 2. Comparison of purified lipid binding proteins (LBPs) with the previously reported ligolin [14] and avenin-C [17] fractions, using the indexes of Harris and Teller (ref. [13]; upper half) and Cornish-Bowden (ref. [16]; lower half), modified as indicated in Experimental

	Wheat LBP	Ligolin	Oat LBP	Avenin-C
Wheat LBP	—	1.20	0.58	1.11
Ligolin	1.34	—	1.15	1.56
Oat LBP	0.27	1.13	—	0.84
Avenin-C	0.99	1.72	0.54	—

probably due to the minor component in the avenin-C preparation. It is also likely that the LBP from wheat is the major component of the ligolin fraction, but the compositional indexes are less conclusive in this case, possibly because of a greater proportion of minor contaminants in ligolin as compared with avenin-C.

EXPERIMENTAL

Biological material. An experimental stock (JVT1) of oats, *Avena sativa* L., and wheat cultivar Aragón 03, *Triticum aestivum* L., were from INIA (Madrid, Spain). Endosperms were first obtained after a short treatment in a Culatti mill and then milled by a longer treatment in the same mill (fine setting).

Purification procedures. Lipid-free proteins from the petrol extract of wheat were obtained by precipitation with 3 vols. of 1 M HCl in EtOH as previously described [10]. In the case of oats, the proteins were first precipitated with 6 vols. of cold Me_2CO and the ppt. was treated once with 3 vols. of 1 M HCl in EtOH/vol. of petrol (bp 50–70°), twice with cold EtOH, twice with petrol, and then air-dried. The proteins reported here (LBPs) were purified from these crude fractions by prep. electrophoresis on 10% polyacrylamide columns (1.5 × 10 cm) in 0.1 M HOAc buffer, pH 2.9, as described for thionin purification [18].

Reconstitution experiments. The petrol extract was concd to a final vol. of 1 ml/g of original flour. Nine vols. of Me_2CO were added and the ppt. formed was recovered by centrifugation and dissolved in 1 vol. $CHCl_3$. Reconstitution of the petrol-soluble form was achieved by adding digalactosyldiglyceride (DGDG) in 1 vol. $CHCl_3$ and drying *in vacuo*. DGDG was prepared as described [9]; the amount obtained from 2 g flour was used to reconstitute the amount of Me_2CO ppt. obtained from 1 g flour. Proteins were analysed in supernatants and ppts. by electrophoresis after delipidation with 1 M HCl + EtOH–Et_2O (3:1).

Analytical procedures. Gels for PAGE at pH 3.2 were polymerized in H_2O and then incubated in aluminium lactate buffer, pH 3.2, and 2% mercaptoethanol overnight. The SDS-PAGE method used was a modification of that described in ref. [19]. Staining of proteins was according to ref. [20]. Densitometric tracings were obtained with a Joyce–Loebl densitometer.

Amino acid analyses and performic acid oxidation were carried out by published procedures. Samples were hydrolysed in a thermoblock at 110 ± 1° for 24, 48 and 72 hr. A JEOL JLC-6AH autoanalyser was used. The number of moles of each amino acid corresponding to 1 mol of protein was adjusted according to ref. [15].

Indexes of composition divergence. The indexes proposed in refs. [13] and [16] were used with certain modifications. The first index is based on the amino acid composition, expressed in residues/10^3 residues, and does not take into account the size of the proteins being compared. According to ref. [21], values for this index under 70 indicate a high degree of homology between the proteins being compared, and in a high proportion of the comparisons with values between 70 and 90 there is sequence homology. In Table 2 we use a modification of this index which consists of dividing the index by 70 so that values below 1 indicate homology, and values between 1 and 1.28 indicate probable homology. A similar modification of the index has been used previously [22]. This index takes into account the size of the proteins, so in the case of ligolin and avenin-C we have used the amino acid compositions corresponding to the adjustments obtained by the method of ref. [15] which were closer to the apparent MWs of the LBPs (Table 1). The modified index used is $S\Delta Nr = S\Delta N/0.42N$, where $S\Delta N$ is the index proposed in ref. [16] and N is the number of amino acid residues of the smaller protein of the two being compared. Pairs of proteins with $S\Delta N < 0.42N$ (equivalent $S\Delta Nr < 1$) have a 95% probability of having sequence homology and if $0.42N < S\Delta N < 0.93N$ (equivalent to $1 < S\Delta Nr < 2.2$) the proteins have sequence homology in *ca* 90% of the cases [16]. Out of several thousand comparisons in which $S\Delta Nr < 1$, only one pair of proteins was found not to have sequence homology [23].

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