Low Molecular Weight Prolamins: Purification of a Component from Barley Endosperm

Gabriel Salcedo,* Rosa Sanchez-Monge, Alejandro Argamenteria, and Cipriano Aragoncillo

The major low molecular weight hordein (LMW hordein-1) from barley has been purified by preparative two-dimensional gel electrophoresis. Both its molecular weight (16 500) and its amino acid composition are very close to those reported for LMW gliadins from wheat and A-secalins from rye. LMW hordein-1 lacks lysine and histidine and has a higher proportion of sulfur amino acids and a lower one of proline than B- and C-hordeins. The values of the compositional difference index determined for binary comparisons between the purified protein and other low molecular weight prolamins are consistent with a high degree of sequence homology between LMW hordein-1 and individual components of LMW gliadins and A-secalins but do not show homology with the sulfur-rich zeins.

The presence of low molecular weight components in hordein preparations (the prolamin fraction of barley endosperm) has been extensively reported (Mesrob et al., 1970; Køie et al., 1976; Shewry et al., 1977). These components, usually called A-hordeins, have molecular weights under 20 000 and amino acid compositions quite different from those of the major barley prolamins (Mesrob et al., 1970; Miflin and Shewry, 1977).

Recently, we have fractionated the A-hordeins, showing that they include two distinct group of proteins. One group consists of polypeptides that are soluble in 0.5 M NaCl and organic solvents, have isoelectric points in the 6–7 pH range, and have higher lysine (>1.5%) and lower glutamic acid (<15%) ratios than typical hordeins. In the Osborne-type extractions, they appear in the salt-soluble fraction and only residually in the prolamin fraction. Consequently, we have considered them as hydrophobic globulins. Components of the second group are not extracted by salt solutions, have high isoelectric points (>pH 9.0), and amino acid compositions within the definition of prolamins, although sulfur amino acids are atypically high. This second group should be considered as low molecular weight hordeins (LMW hordeins) (Salcedo et al., 1980; Aragoncillo et al., 1981).

Low molecular weight prolamins have been also described in other cereal endosperms. We have isolated a group of LMW gliadins in wheat, and the four major components have been purified and characterized (Salcedo et al., 1979; Prada et al., 1982). Recently, Charbonnier et al. (1981) have found prolamins with similar size to the LMW gliadins in rye (A-secalins). The minor sulfur-rich zeins from maize endosperm have also lower molecular weights (M_r under 15 000) than the most abundant zein classes (Gianazza et al., 1977).

We report here the purification and partial characterization of the major LMW hordein and its probable homology with the LMW prolamins previously described in wheat and rye.

MATERIALS AND METHODS

Biological Material. Barley cv. Zephyr (*Hordeum vulgare* L.) was used throughout this study. Kernels were passed twice through a rice mill (25% dry matter loss), and the resulting endosperm was milled through a Culatti mill (fine setting).

Preparative Methods. Flour was delipidated according to Shewry et al. (1978) and chloroform–methanol, 2:1 (v/v), extraction was carried out as described by Rodríguez-Loperena et al. (1975).

The low molecular weight hordeins group from the chloroform–methanol extract (see Figures 1 and 2) was isolated by gel filtration on Sephadex G-100 (to obtain the fraction with molecular weight under 25 000), followed by chromatography on Ultrogel AcA-54 (to subfractionate the M_r <25 000 fraction), as described by Aragoncillo et al. (1981).

The LMW hordein group was further fractionated by preparative two-dimensional electrophoresis using the method of Prada et al. (1982): first dimension, polyacrylamide gel electrophoresis, pH 9 (10% polyacrylamide; 0.8 × 12 cm columns; Tris–glycine buffer, 3 M urea; 7 h; 50 V/cm); second dimension, polyacrylamide gel electrophoresis, pH 3.2 (12% polyacrylamide; 0.8 × 30 × 24 cm slabs; 0.1 M aluminum lactate buffer, 3 M urea; 16 h; 18 V/cm). After electrophoresis slabs were placed in 70%-saturated $(NH_4)_2SO_4$ for 30 min and the proteins detected as opalescent spots. Zones corresponding to component 1 (LMW hordein-1; see Figure 1) were hand dissected, homogenized, dialyzed against 0.1 M acetic acid, filtered through Whatman No. 1 paper, and freeze-dried. The entire purification procedure was carried out at 4 °C.

Analytical Methods. One-dimensional starch gel electrophoresis (pH 3.2) was carried out as described by Aragoncillo et al. (1981). Two-dimensional electrophoresis (polyacrylamide gel electrophoresis, pH 9.0, × starch gel electrophoresis, pH 3.2) was performed by the method of Aragoncillo et al. (1981), based on that used by Mecham et al. (1978). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed according to Laemmli (1970).

Amino acid composition was determined by following the method of Moore and Stein (1963). Appropriate aliquots were hydrolyzed for 24, 48, and 72 h at 110 ± 1 °C. Performic acid oxidation was carried out according to Hirs (1967). Calculation of the minimum molecular weight, based on the amino acid analysis, and of the set of values best adjusted to it was performed as described by Delaage (1968).

RESULTS AND DISCUSSION

We have previously shown the presence of LMW hordeins in the fraction with molecular weight under 25 000 from the chloroform–methanol, 2:1 (v/v), extract of barley endosperm and their separation from other proteins of this fraction by gel filtration on Ultrogel AcA-54 (Aragoncillo et al., 1981). The LMW hordein group can be fractionated by two-dimensional electrophoresis, polyacrylamide gel electrophoresis, pH 9.0, × starch gel electrophoresis, pH

Departamento de Bioquímica (G.S., R.S.-M., and C.A.) and Departamento de Alimentación Animal (A.A.), E.T.S. Ingenieros Agrónomos, Madrid-3, Spain.

0021-8561/82/1430-1155$01.25/0

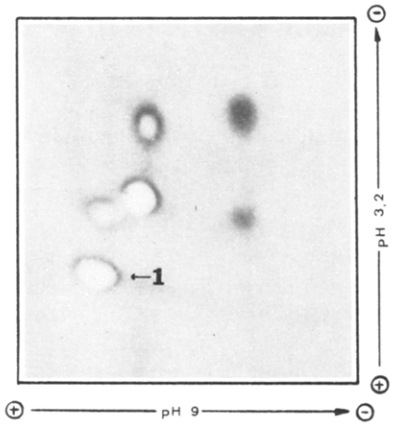

Figure 1. Two-dimensional protein map (polyacrylamide gel electrophoresis, pH 9.0, × starch gel electrophoresis, pH 3.2) of low molecular weight hordeins obtained as described under Materials and Methods (only the map zone corresponding to the main components is shown).

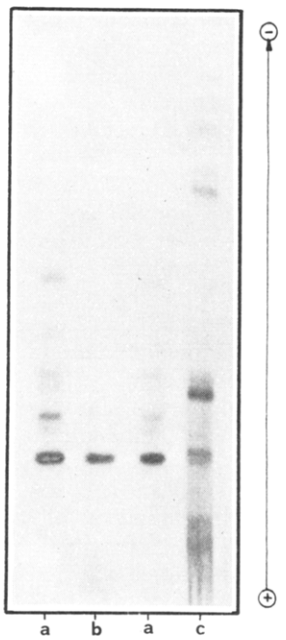

Figure 2. Starch gel electrophoresis at pH 3.2 of the following samples: (a) low molecular weight hordeins; (b) purified LMW hordein-1; (c) chloroform–methanol, 2:1, extract.

3.2, yielding the protein map of Figure 1. The group includes six main components, all with isoelectric points higher than pH 9, as judged by their cathodic mobility at that pH and by their exclusion from pH 3–9 isoelectrofocusing gradients (sample insertion at the acid end).

The major LMW hordein component (numbered 1 in Figure 1) was isolated by preparative two-dimensional electrophoresis. The purified protein runs as a single band on starch gel electrophoresis, pH 3.2 (Figure 2), and NaDodSO$_4$–polyacrylamide gel electrophoresis (not shown).

Molecular weight estimated by NaDodSO$_4$–polyacrylamide gel electrophoresis was 16 500, clearly lower than the values described for B- and C-hordeins [30 000–86 000; see, for example, Shewry et al. (1980)]. LMW hordein-1 is in the same size range reported for LMW gliadins (16 000–19 000; Prada et al., 1982) and A-secalins (16 000; Charbonnier et al., 1981).

The amino acid composition of LMW hordein-1 is presented in Table I. The values are similar to those reported for prolamins, although the major barley prolamins (B- and C-hordeins) have higher levels of glutamic acid plus glutamine and proline and lower of serine, threonine, glycine, alanine, and especially sulfur amino acids (Shewry et al., 1980). The differences in amino acid compositions between LMW gliadins and classical wheat prolamins and between A-secalins and major rye prolamins are similar. The polarity index of the LMW hordein-1, calculated according to Capaldi and Vanderkooi (1972), is low enough to explain its solubility in chloroform–methanol mixtures.

Table I. Amino Acid Composition, Molecular Weight, and Polarity Index of Purified Low Molecular Weight Hordein-1

amino acid	exptl value, mol/100 mol	no. of residues[a]
Lys	0.1	0
His	0.2	0
Arg	2.5	4
Asx	2.2	3
Thr	9.6	14
Ser	12.2	18
Glx	26.2	38
Pro	7.0	10
Gly	6.0	9
Ala	7.2	10
Val	4.4	6
½-Cys	7.1	10
Met	3.6	5
Ile	3.3	5
Leu	4.0	6
Tyr	2.3	3
Phe	2.1	3
residues/mol		144
M_r[b]		15 514
M_r[c]		16 500
polarity index[d]		46.1

[a] Number of residues adjusted by the method of Delaage (1968) to the molecular weight determined by NaDodSO$_4$–polyacrylamide gel electrophoresis. [b] Calculated from the adjusted amino acid composition. [c] Determined by NaDodSO$_4$–polyacrylamide gel electrophoresis. [d] Calculated by addition of the proportion of polar amino acids (Asp, Asn, Glu, Gln, Lys, Arg) plus half the proportion of amino acids of intermediate polarity (Ser, Thr, Tyr, His, Gly) according to Capaldi and Vanderkooi (1972).

The proportions of the different amino acids (moles/100 mol) in LMW hordein-1 are closer to those in the high molecular weight D-hordein, recently described by Miflin et al. (1981), than to those in B- and C-hordeins.

A comparison of LMW hordein-1 with LMW gliadin-2 from wheat (M_r 16 000; Prada et al., 1982) and A$_4$-secalin from rye (M_r 16 000; Charbonnier et al., 1981) is presented in Table II. The index described by Cornish-Bowden (1979), which corrects for differences in size, was used. The values obtained for the binary comparisons of the three LMW prolamins are those expected for proteins closely related, with a high degree of sequence homology. We have also included comparisons with one of the sulfur-rich zeins (M_r 13 500; Gianazza et al., 1977), and in this case, the values do not indicate a relationship (significant amount of common sequences) between the zein and any of the others LMW prolamins. It should be pointed out that the method of comparison does not indicate that sequences are similar when they are not, although it sometimes fails to detect genuine similarities (Cornish-Bowden, 1979).

The purification and partial characterization of the LMW hordein-1 are a new contribution of the definition of low molecular weight prolamins as a group of homologous proteins that is present in different cereal endosperms. Similar group homologies have been already described for the major prolamins, the CM-proteins, and the thionins present in the endosperms of wheat, barley, and

Table II. Values of the Compositional Difference Index Calculated According to Cornish-Bowden (1979) for Binary Comparisons of Some Low Molecular Weight Prolamins[a]

	$S\Delta n$ (0.42N_B)			
	LMW hordein-1	LMW gliadin-2[b]	A_4-secalin[c]	zein 13.5[d]
LMW hordein-1	0	54.3 (60.5)	38.9 (60.5)	322.3 (52.9)
LMW gliadin-2		0	31.1 (61.3)	341.1 (52.9)
A_4-secalin			0	293.7 (52.9)
zein 13.5				0

[a] Compositional difference index: $S\Delta n$. $S\Delta n = {}^1/_2\Sigma(n_{i,A} - n_{i,B})^2 - 0.035(N_A - N_B)^2 + 0.535|N_A - N_B|$, in which $n_{i,A}$ and $n_{i,B}$ are the numbers of amino acid residues of the ith type, N_A is the total number of residues in the bigger protein, and N_B is that of the smaller one. A value for $S\Delta n < 0.42N_B$ indicates a high degree of sequence homology between the two proteins. [b] From Prada et al. (1982). [c] Amino acid analysis from Charbonnier et al. (1981) corrected for $N = 146$ (corresponding to M_r 16 000). [d] Amino acid analysis from Gianazza et al. (1977) corrected for $N = 126$ (corresponding to M_r 13 500).

rye [see Miflin et al. (1981), García-Olmedo et al. (1982), and Salcedo et al. (1982)].

ACKNOWLEDGMENT

We thank Dr. F. García-Olmedo for valuable discussion and J. García-Guijarro for his technical assistance.

LITERATURE CITED

Aragoncillo, C.; Sanchez-Monge, R.; Salcedo, G. *J. Exp. Bot.* **1981**, *32*, 1279–1286.
Capaldi, R. A.; Vanderkooi, G. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 930–932.
Charbonnier, L.; Terce-Laforgue, T.; Mosse, J. *J. Agric. Food Chem.* **1981**, *29*, 968–973.
Cornish-Bowden, A. *J. Theor. Biol.* **1979**, *76*, 369–386.
Delaage, M. *Biochim. Biophys. Acta* **1968**, *168*, 573–575.
García-Olmedo, F.; Carbonero, P.; Jones, B. L. *Adv. Cereal Sci. Technol.* **1982**, *5*, 1.
Gianazza, E.; Viglienghi, V.; Righetti, P. G.; Salamini, F.; Soave, C. *Phytochemistry* **1977**, *16*, 315–317.
Hirs, C. H. W. *Methods Enzymol.* **1967**, *11*, 197.
Køie, B.; Ingversen, J.; Andersen, A. J.; Doll, H.; Eggum, B. O. "Evaluation of Seed Protein Alterations by Mutation Breeding"; International Atomic Energy Agency: Vienna, Austria, 1976; p 55.
Laemmli, U. K. *Nature (London)* **1970**, *227*, 680–685.
Mecham, D. K.; Kasarda, D. D.; Qualset, C. O. *Biochem. Genet.* **1978**, *16*, 831–853.
Mesrob, B.; Petrova, M.; Ivanov, Ch. *Biochim. Biophys. Acta* **1970**, *200*, 459–465.
Miflin, B. J.; Field, J. M.; Shewry, P. R. *Proc. Int. Seed Protein Symp.* **1981**, in press.
Miflin, B. J.; Shewry, P. R. "Techniques for the Separation of Barley and Maize Seed Proteins; EEC: Luxembourg, 1977; p 13.
Moore, S.; Stein, W. H. *Methods Enzymol.* **1963**, *6*, 819.
Prada, J.; Sanchez-Monge, R.; Salcedo, G.; Aragoncillo, C. *Plant Sci. Lett.* **1982**, in press.
Rodríguez-Loperena, M. A.; Aragoncillo, C.; Carbonero, P.; García-Olmedo, F. *Phytochemistry* **1975**, *14*, 1219–1223.
Salcedo, G.; Prada, J.; Aragoncillo, C. *Phytochemistry* **1979**, *18*, 725–727.
Salcedo, G.; Sanchez-Monge, R.; Aragoncillo, C. *J. Exp. Bot.* **1982**, in press.
Salcedo, G.; Sanchez-Monge, R.; Argamentería, A.; Aragoncillo, C. *Plant Sci. Lett.* **1980**, *19*, 109–119.
Shewry, P. R.; Field, J. M.; Kirkman, M. A.; Faulks, A. J.; Miflin, B. J. *J. Exp. Bot.* **1980**, *31*, 393–407.
Shewry, P. R.; Hill, J. M.; Pratt, H. M.; Leggatt, M. M.; Miflin, B. J. *J. Exp. Bot.* **1978**, *29*, 677–692.
Shewry, P. R.; Pratt, H. M.; Charlton, M. J.; Miflin, B. J. *J. Exp. Bot.* **1977**, *28*, 597–606.

Received for review February 9, 1982. Accepted June 28, 1982. This work was supported by a grant from the Fundación Ramón Areces.

Purification and Characterization of a Type-1 Lipoxygenase from Pea Seeds

Patricia A. Reynolds and Barbara P. Klein*

A type-1 lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) was purified from pea seeds by a combination of ammonium sulfate fractionation, gel filtration, ion-exchange chromatography, and preparative isoelectric focusing in a granulated gel. Lipoxygenase-1 was very unstable, especially at pH values below 6, and extensive loss of enzyme activity occurred during preparative isoelectric focusing. Partially purified lipoxygenase-1 focused into enzyme-active bands at pH 4.05 and 4.20. This preparation effectively catalyzed the oxidation of linoleate, linolenate, methyl linoleate, and trilinolein substrates but exhibited much lower activity than type-2 pea lipoxygenases. Highest activity occurred with linoleic acid, with maximum activity in the 9.0–10.0 range and an apparent K_m of 0.20 mM at pH 9.0. Apparent molecular weights of 64 000 and 65 000 were obtained by gel filtration chromatography and sodium dodecyl sulfate–polyacrylamide gel electrophoresis of the isoenzyme with pI = 4.05, respectively. Like soybean lipoxygenase-1, pea lipoxygenase-1 was not as effective in carotene cooxidation as the type-2 enzymes, and production of 280 nm absorbing compounds occurred only after the system became anaerobic. Lipoxygenase-1 was ineffective in bleaching chlorophyll.

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.13.11.12) is present in a wide variety of plants, especially legumes, and is believed to be one of the main oxidative catalysts in vegetables. The primary physiological role of the lipoxygenase reaction in plants is unclear, although it has been associated with ripening, abscission, and germination (Axelrod, 1974; Veldink et al., 1977; Pattee, 1979).

Lipoxygenase catalyzes the oxidation of unsaturated

Department of Foods and Nutrition, University of Illinois, Urbana, Illinois 61801.

0021-8561/82/1430-1157$01.25/0