Plant Science, 41 (1985) 41—48
Elsevier Scientific Publishers Ireland Ltd.

IMMUNOLOGICAL RELATIONS BETWEEN GLUTELIN-2 AND LOW MOLECULAR WEIGHT ZEIN-2 PROTEINS FROM MAIZE (*ZEA MAYS* L) ENDOSPERM

M.D. LUDEVID*, J.A. MARTÍNEZ-IZQUIERDO, M. ARMENGOL, M. TORRENT, P. PUIGDOMÈNECH and J. PALAU

Institut de Biologia de Barcelona CSIC, Jordi Girona Salgado, 18—26, 08034 Barcelona (Spain)

(Received April 10th, 1985)
(Revision received June 25th, 1985)
(Accepted June 25th, 1985)

The glutelin-2 (G2) fraction from maize endosperm has been purified and antibodies obtained against its main polypeptide component (28 kilodalton (kD) G2). These antibodies have been used to establish the relationship between 28 kD G2 and zein-2 (Z2) low molecular weight polypeptides (14 kD Z2, 10 kD Z2) that were also purified. We have observed by immunoblotting that the anti-glutelin-2 sera crossreact with one of Z2 polypeptides (14 kD Z2). The specificity of immunoreactions has been determined by the enzyme-linked immunosorbent assay (ELISA). Affinity-purified antibodies recovered from 28 kD G2 and 14 kD Z2 immunoblotting bands cross-react with both polypeptides, suggesting the presence of common antigenic determinants. Both the immunological data and the high cysteine content indicate that they are related polypeptides that might have common functions inside the storage reservoirs of maize endosperm cells.

Key words: endosperm proteins; glutelins; immunoblotting; zeins, *Zea mays* L.

Introduction

The main protein fractions in maize endosperm are zeins (about 50% of the whole protein), and glutelins (about 35—40%). These two fractions have been defined according to their solubility in 70% ethanol and alkali solution, respectively [1]. As seen by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, the zein-1 fraction (Z1) contains three proteins of an apparent molecular weight of 19, 21.5 and 22 [2]. Z2, extracted with 70% ethanol in the presence of a reducing agent [3], contains two polypeptides of 14 and 10 kD in addition to some of the Z1 proteins. G2 is a subfraction (around 15% of total protein) of the large group of glutelins and it is composed of a major polypeptide of about 28 kD and a minor one of 58 kD [4,5].

Maize protein bodies are considered the storage site of all major zein polypeptides [6] and recently it has been revealed that G2 polypeptides are specifically associated with protein bodies [5]. Homologies seen from DNA sequences suggest that some of the polypeptides found in the protein bodies have structural similarities. However, studies on the primary structure of maize endosperm proteins have been difficult due to the complexity of this family of proteins and to their poor solubility in aqueous solvents. The sequence of some high molecular weight zein polypeptides has been deduced from cDNA sequences [7,8]. The partial sequence of G2 protein has also been published [9]. An alternative approach for comparing structural features of these proteins is by means of immunological methods. Since antigenic pro-

*To whom reprint requests should be sent.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; G2, glutelin-2 (extracted with 50 mM sodium borate pH 8.0, 0.6% 2-mercaptoethanol, 0.2 M NaCl); kD, kilodalton; SDS, sodium dodecyl sulfate; Z1, zein-1 (extracted with 70% ethanol); Z2, zein-2 (extracted with 70% ethanol, 0.6% 2-mercaptoethanol).

0168-9452/85/$03.30

perties of proteins depend on their primary and tertiary structure, antigenic crossreactions might reflect sequence homologies and/or conformational similarities. In this way, antigenic relations among different cereal prolamins have been described [10].

In the present report we show antigenic homologies between different components of Z1, Z2 and G2 protein fractions. These data further define these proteins in terms of functional classification and the understanding of the structural relations within protein bodies.

Materials and methods

Plant material and protein extraction

Double hybrid maize E-10 (*Zea mays* L.) seeds were harvested at maturity and stored at −70°C. Kernels were de-embryonated and the endosperms ground to a flour. Z1, Z2 and G2 protein fractions were extracted with 70% ethanol; 70% ethanol, 0.6% 2-mercaptoethanol and 50 mM sodium borate (pH 8.), 0.2 M NaCl, 0.6% 2-mercaptoethanol, respectively, as previously described [1]. Total endosperm proteins were extracted by homogenizing 25 mg of flour in 0.5 ml 50 mM Tris-HCl (pH 6.8), 5% 2-mercaptoethanol, 5% SDS and further extraction for 1 h at room temperature.

Purification of low molecular weight Z2 and G2 polypeptides

Ten kilodalton and fourteen kilodalton polypeptides from Z2 protein fraction (10 kD Z2 and 14 kD Z2, respectively) were purified by preparative, electrophoresis by means of a 1100 PG (BRL) apparatus. Z2 (4 mg) dissolved in 0.5 ml SDS-sample buffer was loaded on a 7 × 1-cm rod gel formed by 6 cm of 15% acrylamide running gel and 1 cm of 6% acrylamide stacking gel. The electrophoresis was run according to the buffer system of Laemmli [11] at 5.5 mA for 18 h. Fractions (1 ml) were collected with a 7 ml/h flux-elution of running buffer. In order to check the purity of the samples, 100-μl aliquots were evaporated to dryness, resuspended in sample buffer and loaded on a 15% polyacrylamide slab gel according to Laemmli [11] with an acrylamide/biscarylamide ratio of 150:1. The proteins were stained using the silver stain method [12].

The 14 kD Z2 polypeptide was also prepared by gel filtration chromatography in Sephacryl S-200 [13]. Z2 (40 mg) dissolved in 8 ml of solvent A (70% ethanol, 2% SDS, 2% 2-mercaptoethanol) were passed through a 150 × 2.5 cm column packed using the same solvent. For immunological and amino acid analyses the fractions containing the 14 kD polypeptide were pooled, treated with proprionic acid (25% v/v) in order to extract SDS, dialyzed against water and lyophilized.

The 28 kD polypeptide (28 kD G2) was purified from the G2 protein fraction by ion-exchange chromatography on a DEAE-cellulose column (120 × 2.5 cm) as previously described by Vitale et al. [4]. The 28 kD G2 protein was eluted with 50 mM sodium borate buffer (pH 8.0), 0.5% 2-mercaptoethanol while the high molecular weight polypeptide (58 kD G2) was eluted with a 0.1—1 M NaCl linear gradient in the same buffer. The fractions containing the 28 kD G2 polypeptide were pooled, dialyzed against water plus 0.5% 2-mercaptoethanol and lyophilized.

Amino acid analyses were performed after 24 h hydrolyses at 110°C by 6 N HCl in a Beckman model 119 C automatic Autoanalyzer. For cysteine determinations all purified proteins were oxidized with performic acid prior hydrolysis.

Immunological methods

Antibodies against G2 and Z1 were raised in New Zealand white and brown rabbits according to Dierks-Ventling and Cozens [10]. The animals were injected intracutaneously and intramuscularly at 10-day intervals with 2.5 mg of antigen dissolved in 1 ml of 6 M urea—10 mM 2-mercaptoethanol and emulsified with an equal volume of Freund's complete adjuvant (Gibco). Injections of 2.5 mg of the antigen were given regularly

once a month. Eight days after the fourth injection, 10—15 ml of blood was collected and sera were prepared and stored in small aliquots at −20°C. The titre of antisera was followed by passive haemaglutination. Immunoblotting against different endosperm protein fractions was carried out in order to test the specificity of the sera. G2, Z1 and Z2 fractions were submitted to analysis by SDS-polyacrylamide gel electrophoresis and they were directly transferred to nitrocellulose sheets (BA 85, Schleicher & Schuell) by means of a Trans-Blot device (BioRad) as described elsewhere [5]. A fluorescein-labelled swine anti-rabbit IgG (Dako-immunoglobulins) was used for the antibody detection. The incubated filters were placed under 254 nm illumination and photographed with a MP-4 Polaroid Land Camera.

The ELISA technique used was essentially according to Craig et al. [14]. Purified polypeptides (1.5—200 ng) dissolved in 20 μl of the appropriate buffer (50 mM sodium borate (pH 8.5), 0.2 M NaCl, 0.6% 2-mercaptoethanol for G2 and 70% ethanol, 2% 2-mercaptoethanol for Z2) were placed in wells of microtiter polyvinyl plates (Dynatech Laboratories, Inc.); 180 μl of coating buffer (0.1 M sodium carbonate (pH 9.6), 0.2% NaN_3) were added and the antigens were allowed to be adsorbed for 2 h at room temperature and overnight at 4°C in a wet chamber. Following the adsorption period the wells were processed as described [14] and developed using goat anti-rabbit IgG alkaline phosphatase (EC 3.1.3.1) conjugate (Sigma). *p*-Nitrophenyl phosphate was used as the alkaline phosphatase substrate and the reaction was stopped after 15 min by adding 100 μl of 2 M NaOH. The absorbance of each well was recorded at 405 nm. Controls were done with non-immunized rabbit sera.

In order to purify antibodies against a specific electrophoretic band total maize endosprem proteins extracted were loaded on a preparative slab gel (20 cm × 1.5 mm), submitted to SDS-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose sheets. Vertical 8 mm wide nitrocellulose strips were incubated with antisera and fluorescein-labelled swine anti-rabbit IgG to identify the position of the antigens. Horizontal strips corresponding to the position of the 28 kD G2 and 14 kD Z2 polypeptides were cut out and incubated with anti-G2 serum. For elution of the bound antibodies the nitrocellulose strips were washed with buffer and incubated for 5 min at room temperature with 0.2 M glycine—HCl (pH 2.8) [15]. The eluates were immediately adjusted to pH 7.5 (with 2 M NaOH) and 3% bovine serum albumin. The antibodies prepared in this way were used for immunoblotting studies as described above. Controls were carried out in order to show that the antigens were not eluted with the antibodies.

Results

Specificity of the anti-G2 antisera

The G2 fraction used for the present study was prepared essentially according to the Landry and Moureaux [1] procedure. The extraction yielded a protein fraction that shows two main bands by SDS gel electrophoresis, a broad and diffuse band running at a position corresponding approximately to a molecular weight of 28 kD (28 kD G2) and a second one of 58 kD (58 kD G2). The two proteins were fractionated by the use of ionic exchange chromatography [4]. The electrophoretic analysis of the fractions produced is presented in Fig. 1, gel A, and the amino acid analysis of the two protein fractions is shown in Table I. The difference in amino acid composition between these two proteins is clearly different indicating that they correspond to two distinct polypeptides. The two polypeptides present in the G2 fraction have a high proportion of cysteine (6.2% for 28 kD G2 and 9% for 58 kD G2).

Antibodies were elicited in rabbits against purified 28 kD G2 and total G2. The titre of the sera was determined by passive haemaglutination giving dilution values around 1/256. These values did not vary significantly

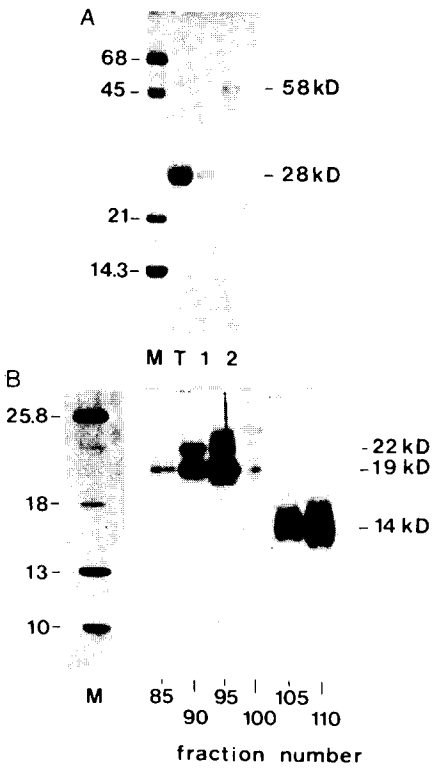

Fig. 1. Preparation of 28 kD G2, 58 kD G2 and 14 kD Z2 polypeptides. Electrophoretic analysis of: A, ion exchange chromatography (DEAE-cellulose) of G2 protein fraction: (T) total G2 protein fraction; (1) protein eluted with 0.05 M sodium borate (pH 8.0), 0.6% 2-mercaptoethanol (28 kD G2); (2) protein eluted with 0.05 M sodium borate (pH 8.0), 0.2 M NaCl, 0.6% 2-mercaptoethanol (58 kD G2). B, Sephacryl S-200 gel filtration of Z2 protein fraction in 70% ethanol, 2% SDS, 2% 2-mercaptoethanol. (M) Molecular weight ($\times 10^{-3}$) markers.

when rabbits of different races were used or when comparing the sera raised using either purified 28 kD G2 or whole G2.

The specificity of the antisera in relation with different endosperm fractions was tested by immunoblotting. The result of this experiment using serum obtained with purified 28 kD G2 is shown in Fig. 2A. Three endosperm protein fractions were tested: Z1, extracted with 70% ethanol (showing the typical 19—21.5 kD pattern), Z2 extracted in the presence of a reducing agent and showing two bands at 14 kD and 10 kD in addition to the Z1 polypeptides and whole G2 prepared as described above. The immunoblot shows that anti-G2 sera do not react with Z1, give a strong reaction with 28 kD G2 and also react with 58 kD G2 and some polypeptides of the Z2 fraction. The positive reaction for 28 kD G2 and 58 kD G2 was observed using both the antisera raised with purified 28 kD G2 and with total G2 fraction. Two polypeptide bands react with the anti-G2 antisera in the Z2 fraction. One of them runs in the same position of 28 kD G2 and indeed some contamination from this fraction is usually seen when the Z2 fraction is analyzed by electrophoresis (see Fig. 2B and Fig. 3). The second immunosensitive band corresponds to the 14 kD Z2, this crossreaction was reproducible for all sera and Z2 samples tested.

It was convenient to test whether the lack of reaction of the anti-G2 serum with Z1 was due to an artifact produced by the method of detection used to specific properties of Z1. For this purpose a control serum was raised in rabbits against Z1 proteins. This serum was used to test blots containing similar samples as in previous experiments. The result is shown in Fig. 2C. This figure shows that the serum raised against Z1 is not specific for Z1 since it weakly reacts with other polypeptides including G2. However it shows that Z1 polypeptides may react with antibodies in immunoblots confirming that the lack of reaction with the anti-G2 antisera was not an artifact due to the technique.

Reaction of anti-G2 sera against purified endosperm polypeptides

The different antigenicities observed between the Z2 and G2 polypeptides with the anti-G2 serum prompted us to quantitize the crossreaction observed. For this purpose, two low molecular weight polypeptides (14 kd Z2 and 10 kD Z2) of the Z2 fraction, and the two polypeptides (28 kD G2 and 58 kD G2) of the G2 fraction were purified. One of the Z2 polypeptides (14 kD Z2) shows cross-reaction with the anti-G2 serum while the other one

Table I. Amino acid composition of Z1, Z2 and G2 protein fractions and purified polypeptides.

Amino acid	Z1	Z2	14 kD Z2	10 kd Z2	G2	28 kD	58 kd G2
Lysine	0.3	0.1	0.7	1.6	0.6	Traces	3.0
Histidine	1.0	0.9	1.2	2.0	6.8	7.2	5.6
Arginine	1.0	1.5	2.0	1.3	2.3	2.1	2.2
Aspartic acid	5.0	5.1	2.7	5.6	1.5	Traces	6.7
Threonine	2.8	3.1	3.5	4.5	4.3	4.5	4.0
Serine	6.4	6.3	6.5	7.6	4.5	4.0	7.5
Glutamic acid	20.5	19.2	19.0	15.3	17.1	16.1	21.8
Proline	10.8	11.0	12.6	11.8	21.6	25.6	7.1
Glycine	2.0	4.8	11.0	9.1	7.5	7.0	9.5
Alanine	13.3	12.7	10.0	9.0	5.4	5.2	6.3
Cysteine	0.4	2.5	5.5	ND	6.8	6.2	9.0
Valine	3.5	3.1	3.0	3.8	6.7	7.1	5.0
Methionine	1.0	6.2	4.0	10.4	0.6	0.5	0.6
Isoleucine	3.6	2.1	2.0	3.1	2.4	2.0	3.0
Leucine	19.1	14.7	9.7	10.6	8.7	9.4	5.4
Tyrosine	3.3	2.9	5.4	1.8	1.5	1.5	0.6
Phenylalanine	5.6	3.6	1.0	3.1	1.3	1.2	2.0

[a] Expressed as moles of amino acid/100 mol of amino acids analyzed. Trp has not been determined.
[b] The extent of amidation of aspartic acid glutamic acid was not determined.
[c] Determined as cysteic acid.

(10 kD Z2) does not show any, as seen by immunoblotting (see Fig. 2A). Two methods were used for the purification of low molecular weight Z2 polypeptides, gel filtration chromatography in the presence of SDS for the purification of 14 kD Z2 and preparative electrophoresis for the purification of 10 kD Z2. The result of the first method carried out according to Abe et al. [13] is given in Fig. 1, gel B. Preparative polyacrylamide gel electrophoresis gave the protein profile shown in Fig. 3 where the UV-absorption profile and the electrophoretic analysis of several fractions are presented. The purity of the 10 kD Z2 was judged acceptable for applying immunological methods. In agreement with Essen et al. [16], it is also interesting to note that the presence of 28 kD G2 in the Z2 fraction is confirmed.

ELISA tests were used in order to compare the antigenicity of the different purified polypeptides. The results of the reaction of an anti-28 kD G2 with 28 kD and 58 kD G2 are presented in Fig. 4A and the reaction of the serum with 28 kD G2, 14 kD and 10 kD Z2

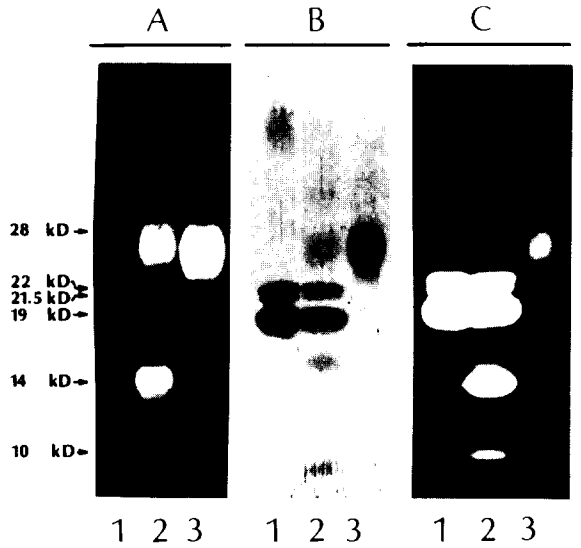

Fig. 2. Reactions of different endosperm proteins with anti-G2 and anti-Z2 sera assayed by immunoblot. Z1(1), Z2(2) and G2(3) fractions were analyzed by SDS-polyacrylamide gel electrophoresis and either stained with silver stain (B) or transferred to nitrocellulose filters. Duplicate filters were incubated with anti-G2 (A) or anti-Z1 (C) sera and developed with fluorescein-labelled swine anti-rabbit IgG. In (A), (B) and (C) the same samples but different electrophoretic runs were used.

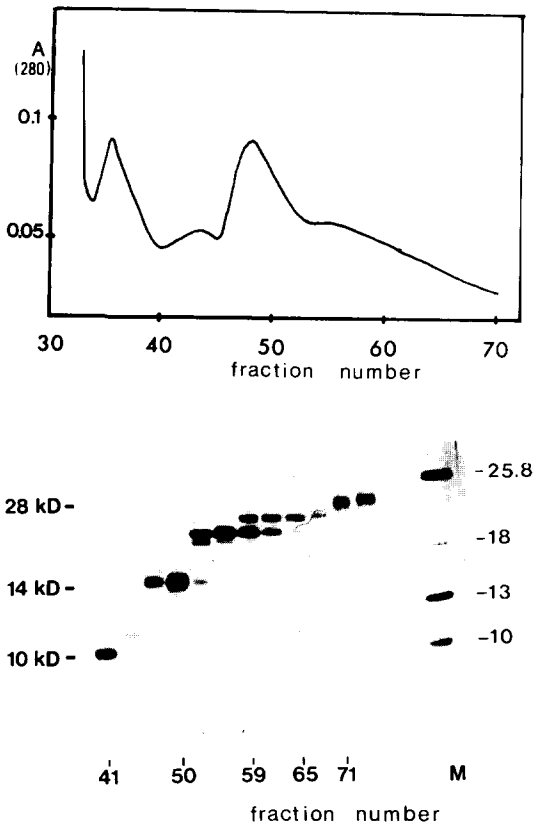

Fig. 3. Purification of low molecular weight Z2 polypeptides. Elution profile of preparative SDS-polyacrylamide gel electrophoresis (7 X 1 cm rod gel) of 4 mg Z2 and the corresponding electrophoretic analysis of different eluted fractions. (M) molecular weight ($\times 10^{-3}$) markers.

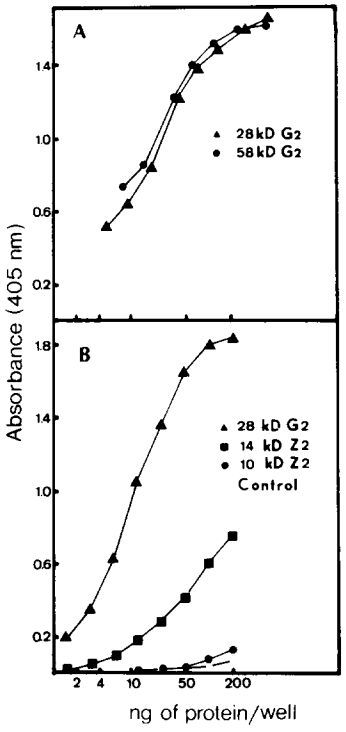

Fig. 4. Specificity of antisera against 28 kD G2 assayed by ELISA. A, represents the 28 kD G2 antiserum reaction with the two G2 protein fraction polypeptides. B, represents the 28 kD G2 antiserum reaction with 28 kD G2 and Z2 low molecular weight polypeptides. Two sera with different titre were used in the two figures. Non-immune serum was used as control experiment.

are presented in Fig. 4B. The results show that the antigenicity of the two polypeptides composing the G2 protein fraction is very similar and that 14 kD Z2 reacts with a lesser intensity than 28 kD G2 with anti-G2 serum. The 10 kD polypeptide has only a residual reaction, quantitatively similar to the reaction using non-immune serum, in accordance with what was observed by immunoblotting.

Antigenic homology between 28 kD G2 and 14 kD Z2 polypeptides

Finally, it was of interest to know whether the antibodies reacting with 28 kD G2 were the same as those reacting with the 14 kD Z2 polypeptide. To do so, antibodies reacting with the 28 kD G2 and with the 14 kD Z2 polypeptides were eluted from a nitrocellulose strip and incubated with a nitrocellulose filter containing the two polypeptides. The results are shown in Fig. 5 and it appears that at least a subpopulation of antibodies reacting with each one of the two polypeptides tested can react with the other one. The antibody elution was controlled and two treatments were assayed. The HCl/Gly pH 2.4 procedure as described in Materials and methods promotes the total elution of antibodies (lane 2) whereas using the sodium thiocyanate procedure described by Krone et al. [17] only

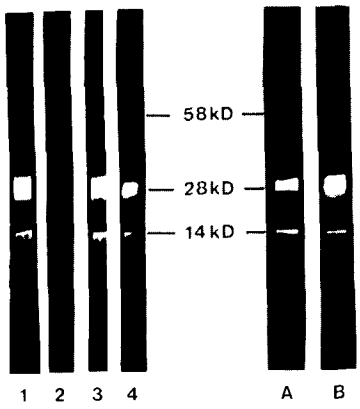

Fig. 5. Crossreaction of whole endosperm proteins with antibodies eluted from specific reactive electrophoretic bands. Total endosperm proteins were separated by electrophoresis and transferred to nitrocellulose filters. Vertical strips were cut out and reacted against antibodies eluted from 14 kD Z2 band (A) or 28 kD G2 band (B) by treatment with 0.2 M glycine—HCl (pH 2.8). Control strips: incubated with whole anti-G2 serum (1); incubated with whole anti-G2 serum and treated with 0.2 M glycine—HCl (pH 2.8) in order to check the elution of the antibodies (2); treated as in (2) and incubated afterwards with anti-G2 serum as a control of the non-elution of the antigen (3); and incubated with whole anti-G2 serum and treated with 3 M KSCN (4), where only partial antibody elution was observed.

partial elution was observed. The lack of antigen leakage after HCl/Gly treatment was tested by nitrocellulose strip reincubation with anti-G2 serum (Fig. 5, lane 3). The fluorescent signal obtained by reincubation of the eluted filter was similar to the specific antibody reactivity observed without treatment (lane 1).

Discussion

In the present paper we have studied the immunological crossreactivity between different proteins from maize endosperm. The 14 kD polypeptide from Z2 protein fraction crossreacts with antisera raised against the 28 kD polypeptide from G2, but no reaction was observed with Z1 or 10 kD Z2 proteins. Immunological homologies between these two proteins are not due to the presence of two different antibodies in serum. As described previously, antibodies eluted from specific electrophoretic bands of 14 kD Z2 and 28 kD G2, incubated with anti-28 kD G2 sera, crossreact with both of them. It is evident that these proteins have common antigenic determinants, a result that probably indicates homologies in the sequence. These data and the finding that the apparent molecular weight of 14 kD Z2 is half that of 28 kD G2 suggests either a post-translational processing of 28 kD G2 or a double chain formation by disulfide bridging. However, the overall amino acid composition of both polypeptides and the fact that the electrophoresis is carried out after treatment with 5% 2-mercaptoethanol as well as the reduction-alkylation treatments of G2 (data not shown) never yields a 14 kD Z2 protein discards the hypothesis involving disulfide bond formation. On the other hand, results from our laboratory [18] showing that in vitro translation of endosperm mRNAs selected by hybridization with specific clones produce either the 28 kD G2 or the 14 kD Z2 polypeptides may indicate that the presence of this protein is not due to post-translational processing of 28 kD G2. From these data we can clearly conclude that these are two different but related proteins having different amino acid composition but common antigenic determinants.

It is important to point out that the amino acid compositions of 14 kD Z2 and 28 kD G2 polypeptides present similarities with other cereal storage proteins [19], i.e. high Gl content and absence of basic amino acids. However, the Cys level differs from that of the major zein polypeptides [20]. Z1 peptides form a multigene protein family [21] and they are deposited in protein bodies formed in the rough endoplasmic reticulum [6]. Recent results using immunolgoical detection methods on purified maize endosperm protein bodies showed that G2 and 14 kD Z2 proteins also accumulate in these organelles and that such proteins are eluted from protein bodies

when using a reducing agent [5]. From our studies of the immunological crossreactivity between Z2 and G2 proteins, we propose that the 14 kD Z2, 28 kD G2 and 58 kD G2 belong to the same family of proteins. Their deposition within the protein bodies seems to indicate that they are a minor group of storage proteins distinct from zeins in the maize endosperm, that may also play a role on protein body structure.

Acknowledgements

We are grateful to Miss Jenny Colom and J.A. Subirana (Departamento de Química Macromolecular, ETSII, Barcelona) for their help in the amino acid analyses. This research was supported by grants from Fundación Ramón Areces, Comisión Asesora de Investigación Científica y Técnica and CIRIT from the Generalitat de Catalunya.

References

1 J. Landry and T. Moreaux, Bull. Soc. Chim. Biol., 52 (1970) 1021.
2 P.S. Misra and E.T. Mertz, Cereal Chem., 53 (1976) 705.
3 C. Soave, P.G. Righetti, C. Lorenzone, E. Gentinetta and F. Salamini, Maydica, 21 (1976) 61.
4 A. Vitale, E. Smaniotto, T. Longhi and E. Galante, J. Exp. Bot., 33 (1982) 439.
5 M.D. Ludevid, M. Torrent, J.A. Martinez-Izquierdo, P. Puigdomènech and J. Palau, Plant Mol. Biol., 3 (1984) 227.
6 B. Burr and F.A. Burr, Proc. Natl. Acad. Sci. U.S.A., 73 (1976) 515.
7 D. Geraghty, M. Peifer, I. Rubenstein and J. Messing, Nucl. Acids Res., 9 (1981) 5163.
8 D. Geraghty, J. Messing and I. Rubenstein, EMBO J., 1 (1982) 1329.
9 A. Essen, J.A. Bietz, J.W. Paulis and J.S. Wall, Nature, 296 (1982) 678.
10 C. Dierks-Ventling and K. Cozens, FEBS Lett., 142 (1982) 147.
11 U.K. Laemmli, Nature, 227 (1970) 680.
12 J.H. Morrisey, Anal. Biochem., 117 (1981) 307.
13 M. Abe, S. Arai, H. Kato and M. Fujimaki, Agric. Biol. Chem., 45 (1981) 1467.
14 S. Craig, A. Millerd and D.J. Goodchild, Aust. J. Plant Physiol., 7 (1980) 339.
15 N. Duran, M. Bach, P. Puigdomènech and J. Palau, Molec. Immunol., 21 (1984) 731.
16 A. Essen, J.A. Bietz, J.W. Paulis and J.S. Wall, Cereal Chem., 58 (1981) 534.
17 G. Krohne, R. Stick, J.A. Kleinschmidt, R. Moll, W.W. Franke and P. Hansen, J. Cell Biol., 94 (1982) 749.
18 S. Prat, J. Cortadas, P. Puigdomènech and J. Palau, Nucl. Acids Res., 13 (1985) 1493.
19 B.J. Miflin, J.M. Field and P.R. Shewry, Seed proteins, in: J. Danssant, J. Mossé and J. Vaughan (Eds.), Phytochemistry Society of Europe Symposia Series, Vol. 20, Academic Press, New York, 1983, p. 255.
20 P.G. Righetti and E. Gianazza, Planta, 136 (1977) 115.
21 K. Pedersen, J. Devereaux, D.R. Wilson, E. Sheldon and B.A. Larkins, Cell, 29 (1982) 1015.