Subcellular localization of glutelin-2 in maize (*Zea mays L.*) endosperm

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Abstract

Accumulation of the 28 KD protein of the glutelin- (G2) fraction was followed in developing maize endosperm, using sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and peak integration of scanned gels. 28 KD glutelin-2 could already be observed from 15 days after pollination and its accumulates reached a plateau during the second half of the development period. The process of biosynthesis of 28 KD glutelin-2 and zeins occurs in a parallel way. Subcellular fractions obtained from linear sucrose gradient centrifugation of developing maize endosperms were analyzed by SDS-PAGE and immunoblotting using a serum reacting against glutelin-2 and 14 KD Z2. Glutelin-2 was found to be present in the protein bodies when subcellular fractionation was carried out without dithiothreitol (DTT). The presence of a reducing agent causes the elution of glutelin-2 from protein bodies. Immunocytochemical labelling using the protein A-colloidal gold technique in protein bodies incubated with anti-G2 IgG revealed that G2 is located mainly in the periphery of protein bodies. These results are interpreted as indicating a structural role for glutelins in protein bodies.

Introduction

Recent studies have yielded a great deal of information on cereal storage proteins. In maize the attention has been primarily focussed on zeins, the major protein fraction (65%) of the endosperm. Zeins are proteins soluble in organic solvents such as 70% ethanol and several polypeptides having molecular weights from 10 to 22 KD have been described from electrophoretical studies (8). During endosperm development, zeins accumulate from around 15 days after pollination and all polypeptides of this fraction are synthesized by membrane-bound polysomes and stored in protein bodies (1) where these proteins are exclusively found (12). The genes coding for some of zein polypeptides have been cloned (9) and it has been shown that they represent a multigene family of proteins scattered along the genome (15, 20).

Glutelins are the second largest group of seed proteins (around 35 to 45%) in maize endosperm. They have not received as much attention as zeins, partly because they constitute a rather heterogeneous group. However a glutelin subfraction called glutelin-2 (G2), prepared by extraction of defatted flour in alkali solution in the presence of a reducing agent, is a better defined group of polypeptides, accounting for around 15% of total endosperm protein. It is composed of a major polypeptide around 28 KD and a second component of 58 KD.

The function of G2 in maize endosperm is not clear neither is its relation with zeins. While several authors found only zeins in protein bodies (2, 13, 21), Vitale et al. (22) recently found a 28 KD non-zein protein in this subcellular fraction. The purpose of the present article is to get information about G2 that could contribute to understand their function in maize endosperm. Two kinds of experiments have been carried out, first the study of the rate of synthesis of the glutelin-2 component of
28 KD during maturation of endosperm and second, localization of these proteins in subcellular fractions by use of immunological methods.

Material and methods

Measurement of protein accumulation

Endosperms (ten seeds in each case) from 15, 18, 21, 26, 32 and 44 days after pollination (DAP) and mature double hybrid E-10 maize were used in this study. All series of endosperms were homogenized in a Virtis 45 homogenizer in the presence of acetone/hexane 51:49 at 4 °C. Total proteins were extracted from 25 mg of air dried flour with 0.5 ml sample buffer (0.25 M Tris-HCl pH 6.8, 5% sodium dodecyl sulphate (SDS), 5% 2-mercaptoethanol) for 2 h at room temperature and centrifuged for 10 min at 8 000 g. Aliquots of the supernatants were directly loaded on SDS-PAGE (10) gel slabs containing 15% acrylamide and a 150/1 acrylamide/bis-acrylamide relation. Silver stain (14) was used to detect proteins in the gel. The gels were scanned using a Chromoscan 3 Joyce Loebl densitometer. The values of peak integration were multiplied by the endosperm dry weight and plotted against days after pollination. The results are average values of three different experiments.

Subcellular fractionation

Five g of dissected endosperms from 21 DAP E-10 maize grains stored at -70 °C were ground in a mortar in the presence of 5 ml of buffer A (100 mM Tris-HCl, pH 8.0, 50 mM KCl, 1 mM EDTA, 10 mM MgCl2) containing 10% sucrose and filtered through a nylon cloth and centrifuged for 500 g. Aliquots of the supernatants were directly loaded on SDS-PAGE (10) gel slabs containing 15% acrylamide and a 150/1 acrylamide/bis-acrylamide relation. Silver stain (14) was used to detect proteins in the gel. The gels were scanned using a Chromoscan 3 Joyce Loebl densitometer. The values of peak integration were multiplied by the endosperm dry weight and plotted against days after pollination. The results are average values of three different experiments.

Antisera

Antisera against purified G2 were raised in rabbits as described previously (3). The IgG fraction was obtained by DEAE-cellulose chromatography (Whatman DE-52) and it was lyophilized, dissolved in PBS buffer (10 mM sodium phosphate, 0.15 M NaCl pH 7.3) and stored at -20 °C. Nonimmune sera were employed in control experiments. Antiserum against G2 reacts with G2 proteins: 28 KD and 58 KD, and with a 14 KD maize endosperm protein from the zein fraction as judged by protein blotting. Antiserum against G2 shows no reaction with high molecular weight zein polypeptides.

Immunoblotting

Following the SDS-PAGE, the separated proteins were electrophoretically transferred for 2 h at 60 V and 10 °C to nitrocellulose sheets as described previously (17) by using a BioRad Trans-Blot device. The sheets, preincubated in PBS buffer with 0.05% Nonidet P-40, 0.02% sodium azide, 3% bovine serum albumin, were incubated at 37 °C overnight with anti-G2 serum (dilution 1:50) and extensively washed with 1 M NaCl in PBS. A fluorescein-labelled pig anti-rabbit IgG (Dako-immunoglobulins) was used for the antibody detection.

Electron microscopy and immunocytochemical labelling

Protein body fractions isolated in the presence and in the absence of 1 mM DTT were fixed for 1 h with 3% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.4, and postfixed with 1% OsO4 in the same buffer. Fixed samples were then washed in cacodylate buffer, dehydrated in a graded series of acetone and embedded in Araldite-Epon. Thin sections were stained with uranyl acetate and lead citrate and viewed with a Philips EM 301 electron microscope.

The preparation of the protein A-gold complex (pAg) and subsequent labelling were carried out following the procedure described by Slot & Geuze (19) with minor modifications. The protein body fraction was fixed by immersion in 0.3% glutaraldehyde in PBS for 5 min at room temperature. The preparation was then neutralized with 0.2 M NH4Cl in PBS for 10 min, carefully washed with PBS and