STUDIES ON CORN PROTEINS. X. POLYPEPTIDE MOLECULAR-WEIGHT DISTRIBUTION IN LANDRY-MOUREAUX FRACTIONS OF NORMAL AND MUTANT ENDOSPERMS[1]

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ABSTRACT

The endosperm proteins of normal corn inbred Oh 43 and mutants o_2, fl_2, fl_2o_2, bt_2, and bt_2o_2, as well as normal corn inbred W22 and its mutant o_7, were separated into fractions by the Landry-Moureaux method. Based on molecular weights determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, fraction I (saline-soluble) had major polypeptides with average molecular weights of 58,000, 24,500, 22,000, and 13,400 daltons. Fraction II (zein), with the exception of bt_2o_2, contained major polypeptides with average molecular weights of 25,000 and 21,800 daltons. Fraction III (zein-like) had major polypeptides with average molecular weights of 26,000, 23,000, and 18,000 daltons, and fraction IV (glutelin-like) had major polypeptides with average molecular weights of 61,000, 58,000, 25,700, and 19,000 daltons. Fraction V (true glutelin) polypeptides did not separate clearly on the gel. The 25,000 dalton component of fraction II in o_2 and fl_2o_2 is reduced below that in normal, fl_2, and o_7. The 44,000 dalton component of fraction II is a unique component of fl_2 and fl_2o_2, as is the 14,000 dalton component of fraction III in o_2 and o_7.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis can be used with considerable reliability for determining the approximate molecular weights of polypeptide chains in a wide variety of proteins (1). Paulis *et al.* (2) recently reported studies on normal, o_2, and fl_2 endosperms using this method. Molecular weights of corn protein subunits were determined on albumins, globulins, zein, and alcohol-soluble and -insoluble glutelins. They used 5% acrylamide gels and found one broad band with a molecular weight of 22,000 daltons in the zein fraction from normal, o_2, and fl_2 endosperms. We report here the use of this technique to study the nature of the proteins in the Landry-Moureaux (LM) fractions in mature single and double endosperm mutants of corn. We used 10% acrylamide gels which were less porous and permitted separation of the zein into two distinct bands with molecular weights of 21,800 and 25,000 daltons. This also permitted better separation of the alcohol-soluble glutelins. However, it did not permit detection of proteins with molecular weights above 100,000 as found by Paulis and coworkers (2).

We have already reported the distribution of proteins between LM fractions in mature endosperm (3,4) and the amino acid composition of these protein fractions (5). We have also reported the distribution of the protein fractions in the developing endosperm (6). The two normal and five high-lysine mutants used in these studies, namely Oh 43 normal (Oh 43+), *floury*-2 (fl_2), *opaque*-2 (o_2), *brittle*-2 (bt_2), *floury*-2 *opaque*-2 (fl_2o_2), *brittle*-2 *opaque*-2 (bt_2o_2), W22 normal

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(+), and W22 *opaque*-7 (W22 o_7) have been described in a previous publication (4).

MATERIALS AND METHODS

SDS-Polyacrylamide Gel Electrophoresis

Landry-Moureaux fractions obtained from 5 g of ground, defatted endosperm (4) were dialyzed against distilled water at 4° C for 48 hr. The dialyzed fractions were then lyophilized and their nitrogen determined using micro-Kjeldahl.

Depending on the amount of protein (N × 6.25) in the lyophilized fractions, solutions were prepared at an equal protein level (0.4–0.5 mg/ml) by adding a solution containing 1% sodium dodecyl sulfate (SDS), and 1% 2-mercaptoethanol (2ME), in 0.1*M* sodium phosphate buffer pH 7.0. The protein solutions were incubated for 3 hr at 37° C, and dialyzed overnight at room temperature against 0.01*M* sodium phosphate buffer pH 7.0 containing 0.1% SDS and 0.1% 2ME. The dialyzed protein solutions were centrifuged to remove any undissolved material.

A 10% acrylamide solution with 0.27% methylene bisacrylamide cross linker was used for all the fractions except in fraction V (8.5%). The electrophoresis was carried out in glass tubes (10 cm and i.d. 5 mm) using 0.1*M* sodium phosphate buffer, pH 7.1, and 0.1% SDS at a constant current of 8 mA/gel with positive electrode in the lower chamber (1). Bromophenol blue in glycerol was used as a marker dye.

The gels were stained with coomassie brilliant blue for 1 hr at room temperature and destained overnight with a solution of 7.5% acetic acid and 5.0% methanol in water.

The proteins used as molecular-weight (mol wt) markers were: lysozyme (14,300); β-lactoglobulin (18,400); trypsin (23,000); pepsin (35,000), and ovalbumin (43,000). All conditions of incubation, dialysis, and electrophoresis

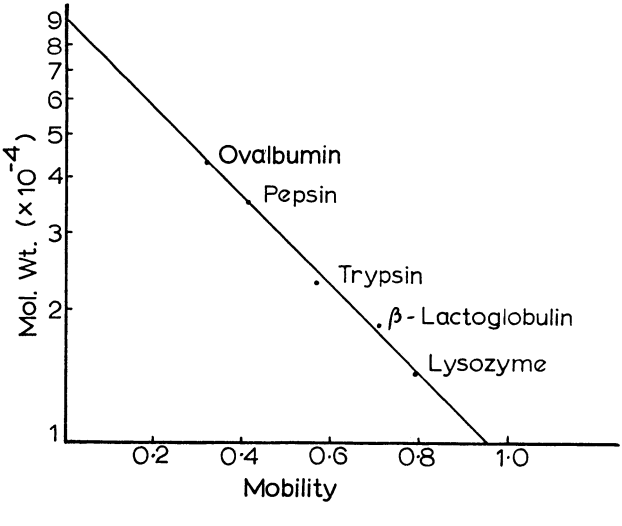

Fig. 1. Reference mobility as related to molecular weight of standard purified proteins.

were kept identical in corn and marker proteins. Figure 1 shows the mobility and log molecular weight curve obtained with marker proteins, used in the calculation of the molecular weight of corn fraction polypeptides.

RESULTS AND DISCUSSION

Figure 2 shows the acrylamide gel patterns for fraction I polypeptide chains. The darkest staining bands in most of the normals and the mutants had average (calculated) molecular weights of 58,000, 24,500, 22,000, and 13,400 daltons. Faint to strong bands were also observed in many of the patterns at 56,400, 53,000, 36,400, 22,000, 10,900, and 10,000 daltons. This fraction contains the polypeptides of albumins and globulins of the endosperm.

Figure 3 shows the acrylamide gel patterns for fraction II (true zein) polypeptide chains. The darkest staining bands in most of the normal and mutants had average (calculated) molecular weights of 25,000 (24,600–25,700), and 21,800 (21,200–22,000) daltons. These two characteristic zein bands are completely absent in the bt_2o_2 double mutant. The small amount of zein or fraction II isolated from the double mutant (2.9% of the total nitrogen (4)) contains only reduced polypeptides with an average molecular weight of 10,000 daltons. This 10,000-dalton band is not found in the other normal and single mutant fraction II samples and therefore cannot be considered to be a normal constituent of the zein fraction. The 25,000-dalton component is markedly reduced in amount in o_2 zein and this effect carries over to its double mutant with fl_2. Thus, o_2 appears to be unique in this respect. A 44,000-dalton component is

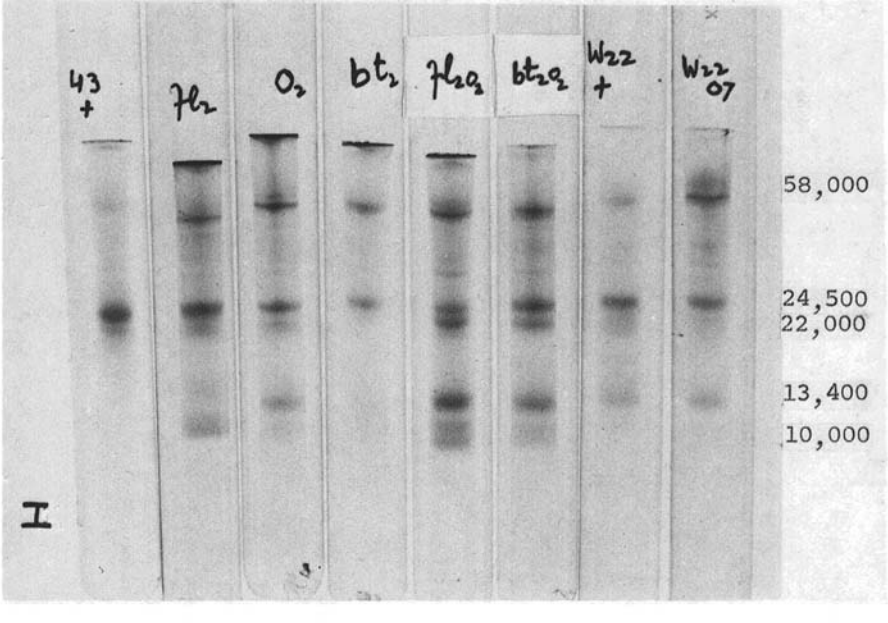

Fig. 2. Acrylamide gel patterns of protein polypeptides derived from Fraction I of corn endosperms.

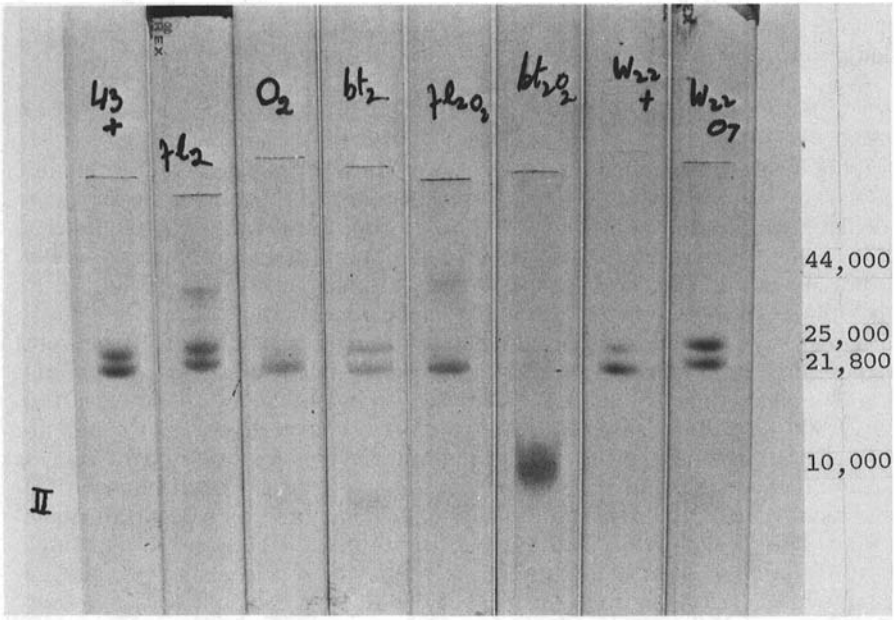

Fig. 3. Acrylamide gel patterns of protein polypeptides derived from Fraction II of corn endosperms.

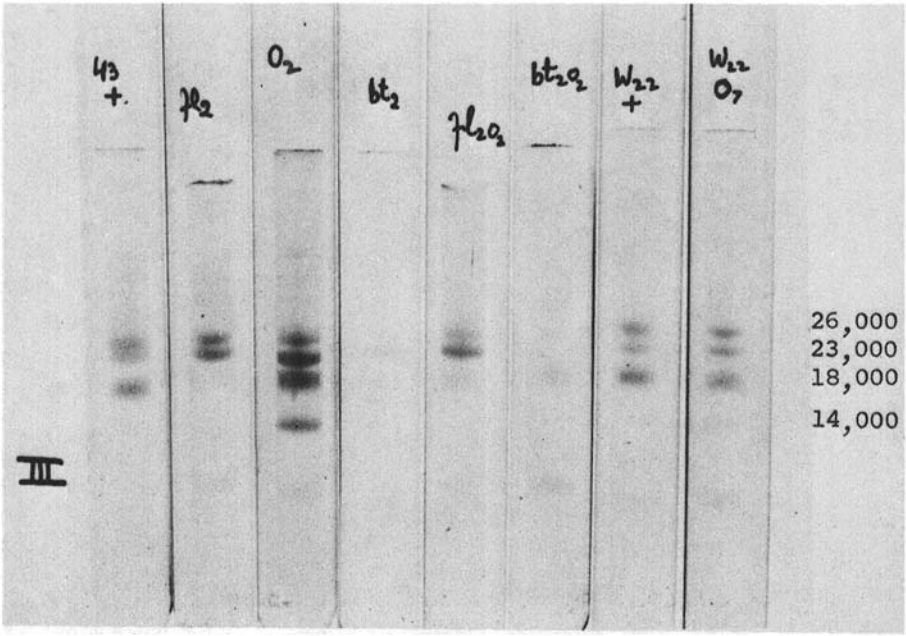

Fig. 4. Acrylamide gel patterns of protein polypeptides derived from Fraction III of corn endosperms.

seen in the fl_2 and its double mutant with o_2, which appears to be a unique component of fl_2.

Figure 4 shows the acrylamide gel patterns for fraction III (zein-like) polypeptide chains. The darkest staining bands in most of the normals and mutants had average (calculated) molecular weights of 26,000 (26,000–26,400) and 23,000 (22,500–24,000) daltons. These are similar to, but not overlapping with, the two major bands in fraction II. Faint to strong bands were also observed in some of the normals and mutants with average (calculated) polypeptide molecular weights of 61,000, 46,100, 42,300, 21,000, 18,000, and 14,000 daltons. The 14,000 dalton component appears to be unique to o_2 where it is present in large amount, and to o_7 where it is present in a trace amount.

Figure 5 shows the acrylamide gel patterns for fraction IV (glutelin-like) polypeptide chains. The darkest staining bands in all samples had an average (calculated) polypeptide molecular weight of 25,700 (24,700–26,800) daltons. This overlaps with one of the major bands in fraction III. Faint to strong bands are also observed in some of the normals and mutants with average (calculated) polypeptide molecular weights of 61,000, 60,000, 58,000, 54,400, 47,000, 40,000, 38,000, 19,000, 13,400, and 11,400 daltons. Fraction V polypeptides did not separate clearly (Fig. 6).

Isolation of the individual bands in fractions I to IV and determination of the amino acids in these bands would be helpful in determining the relationship of the polypeptide fragments in the different fractions. Since we have found that the LM fractions II to V resemble four alkylated-reduced fractions isolated by Paulis

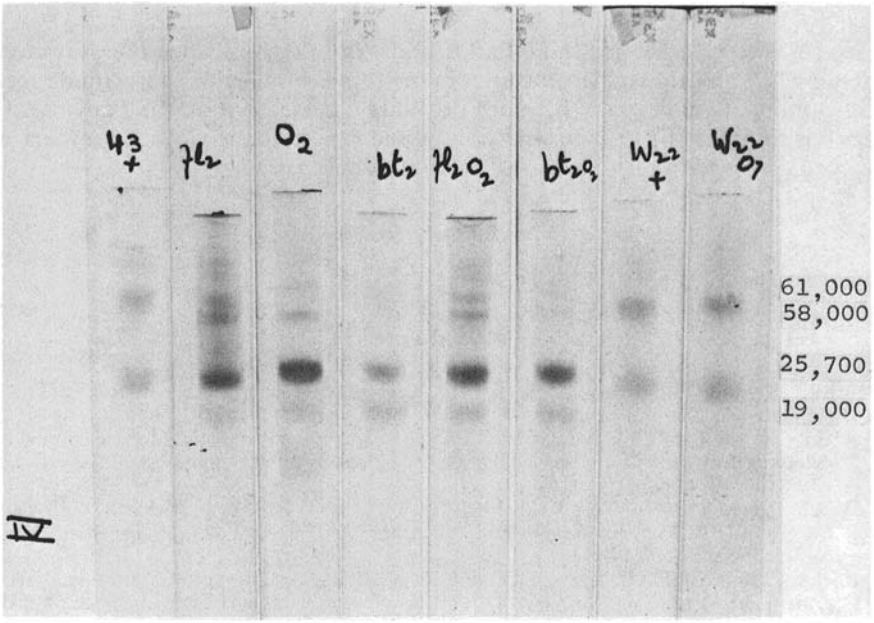

Fig. 5. Acrylamide gel patterns of protein polypeptides derived from Fraction IV of corn endosperms.

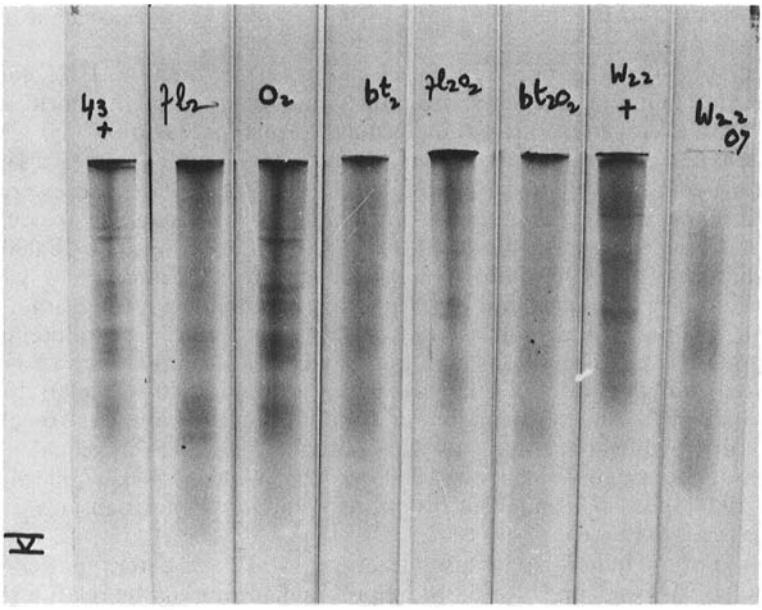

Fig. 6. Acrylamide gel patterns of protein polypeptides derived from Fraction V of corn endosperms.

et al. (2), Misra *et al.* (5), and Paulis and Wall (7), and since the alkylated fractions are more stable during physical measurements, acrylamide gel electrophoresis studies may be more profitably carried out on the Paulis-Wall fractions to obtain further information on the nature of the various proteins in the corn endosperm of normal and mutant genotypes.

Acknowledgment

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