STUDIES ON CORN PROTEINS. X. POLYPEPTIDE
MOLECULAR-WEIGHT DISTRIBUTION IN
LANDRY-MOUREAUX FRACTIONS OF
NORMAL AND MUTANT ENDSOMERS

P. S. MISRA, E. T. MERTZ, Department of Biochemistry, and D. V. GLOVER, Department of
Agronomy, Purdue University, Lafayette, IN 47907

ABSTRACT

Cereal Chemistry 53(5): 705-711

The endosperm proteins of normal corn
inbred Oh 43 and mutants o2, fl2, fl2o2, bt2, and
bt2o2, as well as normal corn inbred W22 and
its mutant o1, 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 26,000, 23,000, and 18,000 daltons,
and fraction IV (glutelin-like) had major
polypeptides with average molecular weights of
21,000, 18,000, 16,000, and 13,000 daltons. Fraction II (zein), with the exception of
bt2o2, 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
21,000, 18,000, 16,000, and 13,000 daltons. Fraction V (true glutelin) polypeptides did not
separate clearly on the gel. The 25,000 dalton
component of fraction II in o2 and fl2o2 is
reduced below that in normal, fl2, and o1. The
44,000 dalton component of fraction II is a
unique component of fl2 and fl2o2, as is the
14,000 dalton component of fraction III in o2
and o1.

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, o2, and fl2 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, o2, and fl2 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 (fl2), opaque-2 (o2),
brittle-2 (bt2), floury-2 opaque-2 (fl2o2), brittle-2 opaque-2 (bt2o2), W22 normal

1Journal Paper 5939. Purdue Agricultural Experiment Station. Supported by the Agency for International
Development under contract "Inheritance and Improvement of Protein Quality and Content in Maize." Reprint
requests should be directed to E. T. Mertz. Present address of P. S. Misra: National Botanic Gardens, Lucknow,
India.

Copyright © 1976 American Association of Cereal Chemists, Inc., 3340 Pilot Knob Road, St. Paul,
Minnesota 55121. All rights reserved.
(+), and W22 opaque-7 (W22 o7) 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

![Graph](image-url)

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 bt2o2 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 o2 zein and this effect carries over to its double mutant with fl2. Thus, o2 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 \( f_l \) and its double mutant with \( o_2 \), which appears to be a unique component of \( f_l \).

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

We thank Chi-Wan Chen for technical assistance.

Literature Cited


[Received July 7, 1975. Accepted November 13, 1975]