Structure and composition of protein bodies from wild-type and high-lysine barley endosperm

JOHN INGVERSEN

Agricultural Research Department, Danish Atomic Energy Commission Research Establishment Riso, Roskilde, Denmark

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Protein bodies were isolated from 13 and 28 day old endosperms of barley mutant 1508 and its wild type, Bomi barley. The fine structure of the isolated protein bodies was determined by electron microscopy, and the proteins present in the preparations characterized by amino-acid analysis and SDS-polyacrylamide gel electrophoresis. Sections through pellets of isolated protein bodies from both the mutant and the wild type revealed protein body structures corresponding with those observed in sections through the intact starchy endosperms. The majority of the wild-type protein bodies was homogeneous spheres accompanied with a granular component. Particles with the same structure were present in the protein body preparation from the mutant, where, however, the granular component was the most prominent. Amino-acid composition and SDS-polyacrylamide gel electrophoresis of the proteins from the protein body preparation revealed that the wild-type protein bodies contained large amounts of prolamines (the storage protein group which is soluble in 55 % isopropanol) and some glutelins (the storage proteins soluble in dilute alkali), whereas the mutant protein bodies have glutelin as the major component and little prolamines. It is suggested that the homogeneous protein body component represents a storage organelle with a high concentration of prolamines, and the granular component a storage organelle with a high concentration of glutelins.

John Ingversen, Agricultural Research Department, Danish Atomic Energy Commission Research Establishment Riso, DK-4000 Roskilde, Denmark

Genes leading to an increased lysine content of cereal endosperm proteins have been found in maize (Mertz et al. 1964; Nelson et al. 1965), barley (Munck et al. 1970; Ingversen et al. 1973; Doll et al. 1974) and sorghum (Singh and Axtell 1974). Such single gene mutants provide a potentially useful tool in studies on storage protein synthesis and its regulation. The increased lysine content of the endosperm proteins from high-lysine cereals is a result of alterations in storage protein composition and amount of free amino acids (Mertz et al. 1964; Munck et al. 1970; Munck 1972; Ingversen and Koie 1973; Mertz et al. 1974; Brandt 1975). In the case of opaque-2, floury-2 maize and barley mutant 1508 the deposition of lysine-poor prolamines is reduced and compensated for by an increased synthesis of lysine-rich proteins. This change in protein composition is reflected by an altered development and structure of the protein bodies (Wolf et al. 1967; Munck and von Wittstein 1975), which are the principal protein stores of the cereal endosperm.

In an attempt to further characterize the effects of the 1508 mutation on storage protein formation, protein bodies of Bomi barley and its mutant 1508 were isolated at two different stages of endosperm development and their protein composition studies.

Material and methods

Plant material. — Bomi barley (*Hordeum vulgare* L. cv. Bomi) and its high lysine mutant Risø no. 1508 were grown in the field. Spikes were harvested 13 and 28 days after fertilization, frozen and stored at —20°C until analysis.

Analysis of protein bodies. — All procedures were carried out at 0—5°C. Twenty kernels were freed from embryo, husk and pericarp and homo-
genized for 5 min. in a mortar by a gradual addition of 1 + 2 + 2 ml of an extraction buffer consisting of 150 mM TRIS pH 7.5, 10 mM KCl, 1 mM MgCl₂, 1 mM EDTA and 13% sucrose. Starch and cell debris were removed by centrifugation at 900 g for 15 min. The resulting supernatant was layered on the top of a sucrose gradient made up in the following way from the top towards the bottom of the tube: 5 ml 20% sucrose, 20 ml linear sucrose gradient 20–60%, and a cushion of 5 ml 60% sucrose. After centrifugation at 40,000 g for 16 hr the bands had reached equilibrium, and the gradient was eluted by penetrating the bottom of the tube. The OD₅₅₀ profile of the gradient (Fig. 1B, solid line). The high 280 nm absorption of the top of the gradient is due to the sample layer with its high content of dissolved proteins. The isopycnic density of the sedimented particles ranges from 1.21–1.29 g×cc⁻¹, slightly lower than the isopycnic density of 1.26–1.36 g×cc⁻¹ found for protein bodies from mature sunflower cotyledons (Schnarrenberger et al. 1972). The sedimented particles of this band were collected and further purified by pelleting and resuspension in extraction buffer.

Electron microscopy. — The pellet was fixed for two hr in 6% glutaraldehyde, dissolved in 0.05 M phosphate buffer, pH 7.5, and postfixed with 2% osmium tetroxide for two hr, dehydrated through an alcohol series and embedded in Spurr’s low-viscosity epoxy resin. Sections were cut on a Reichert UM-2 microtome, contrasted with uranylacetate and lead citrate and examined in a Siemens Elmiskop I A.

Electrophoresis. — The pellets were suspended in 100 µl TRIS/borate, pH 8.9, (125 mM TRIS, 20 mM boric acid), 2% sodiumdodecylsulfate (SDS), 1% β-mercaptoethanol and incubated at 50°C for 1 hr. Twenty-five µl of the resulting solution were applied to 7.5% polyacrylamide gels prepared with TRIS/borate buffer, pH 8.9, containing 1% SDS. A constant current of 2.5 mA was used for 105 min. The proteins were stained with Coumassie blue.

Amino-acid analysis. — Automatic amino-acid analysis took place after hydrolysis in vacuum. Nitrogen determinations were done by micro Kjeldahl on the lyophilized pellets.

Results and discussion

Protein bodies (aleuron grains, cf. Frey Wyssling and Muhlethaler 1965, p. 159) from sunflower cotyledons have been prepared from cell homogenates by isopycnic density gradient centrifugation (Schnarrenberger et al. 1972). When homogenates of wild-type barley endosperm harvested 28 days after fertilization were subjected to gradient centrifugation a broad opalescent band was present between the center and the bottom of the gradient. This band appeared as the major peak in the OD₅₅₀ profile of the gradient (Fig. 1B). The high 280 nm absorption of the top of the gradient is due to the sample layer with its high content of dissolved proteins. The isopycnic density of the sedimented particles ranges from 1.21–1.29 g×cc⁻¹, slightly lower than the isopycnic density of 1.26–1.36 g×cc⁻¹ found for protein bodies from mature sunflower cotyledons (Schnarrenberger et al. 1972). The sedimented particles of this band were collected and further purified by pelleting and resuspension in extraction buffer.

Fig. 2A shows a section through a portion of cells from a 28-day old Bomi barley endosperm with an aggregate of protein bodies inside a vacuole. The developing protein bodies in the starchy endosperm of barley consist of two components, the homogeneously structured spheres (a) and a granular component (b) in which the homogeneous spheres are embedded (Munck and von Wettstein 1975; von Wettstein pers. comm.). The homogeneous spheres correspond to what has been called protein bodies in maize (Khoo and Wolf 1970) and wheat (Buttrose 1963). Fig. 2B, which is a section through the purified pellets reveals that the preparation of the isolated protein bodies contains both components, the homogeneously structured spheres (a) and the granular component (b). The components of this preparation have an appearance that is similar to the protein bodies isolated by Ory and Henningsen (1969) in barley and by Adams and Novellie (1975) in sorghum. The protein bodies of the starchy endosperm cells are morphologically very different from the aleuron grains of the aleuron cells in the cereals (Jochsen et al. 1971) or the aleuron grains of dicotyledonous seeds. Aleuron grains containing the phytate-rich globooids and the carbohydrate-rich crystalloids have been isolated from aleurone layers of rice (Tanaka et al. 1973) and distinguished from the protein bodies of the starchy endosperm. Since the latter comprise the vast majority when the whole endosperm is ground up it is not surprising that aleuron
grains were not detected in the present preparations.

The isopycnic density of the isolated protein bodies from the 28-day old mutant 1508 endosperm was 1.13-1.24 g×cc⁻¹, i.e. lower than that of Bomi protein bodies (Fig. 1B, broken line). A section through a portion of a cell from a mutant 1508 endosperm (Fig. 2C) shows the protein bodies to consist mainly of a granular component (b) in which few homogeneous spheres (a) are embedded (MUNCK and VON WETTSTEIN 1975; VON WETTSTEIN, pers.comm.). The protein bodies have thus a very different morphology than those of the wild type. The section through the pellet of the isolated protein bodies from the mutant (Fig. 2D) reveals that the morphology has been preserved during isolation. Homogeneous spheres are embedded in the granular component. The strong osmiophily of the homogeneous component in Figure 2C is observed in some fixations and not in others and may be caused by different degrees of compaction during protein body formation (VON WETTSTEIN, pers.comm.).

Protein bodies were also isolated from 13-day old endosperms (Fig. 1A). At this early stage of development both wild-type and mutant endosperm homogenates contained protein bodies with isopycnic densities ranging from 1.13-1.24 g×cc⁻¹.

The nitrogen content of the protein body preparations from the 28-day old endosperms was 7.4% for the mutant as compared to 11% for the wild type. The low value for mutant 1508 signifies a high content of non-protein material in the preparation.

The amino-acid composition of the proteins in

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Fig. 1. Isolation of protein bodies by isopycnic centrifugation in sucrose gradients. An endosperm cell homogenate from barley mutant 1508 (- - - - -) and from wild type (-----) were prepared 13 days (A) and 28 days (B) after fertilization. The homogenate was placed on top of a 20-60 % (w/w) sucrose gradient (o—o). The gradients were eluted after centrifugation at 40,000 g for 16 hr and the optical density of the fractions determined at = 280 nm.
Fig. 2A – D. Comparison of the fine structure of the protein bodies in the endosperm of wild type barley (A) and its high lysine mutant 1508 (C) with that of the isolated protein bodies from wild type (B) and the mutant (D). In the wild type (A) the protein bodies consist of a homogeneous component (a), surrounded by a granular component (b). These two types of
structures can also be recognized in the pellet of the isolated protein bodies (B). In the mutant (C) few spheres of the homogeneous component are embedded in the dominating granular component (b). This protein body organization can be recognized in the isolated protein bodies of the mutant (D). Cytoplasm with ribosomes is indicated by (c). × 30,000.
Table 1. Amino-acid composition of proteins in preparations of protein bodies from 28-day old endosperms from the barley mutant 1508 and the wild type

| Amino acid | Mutant 1508 | Wild type |
|------------|-------------|-----------|
| Lys        | 5.90        | 2.58      |
| His        | 3.61        | 2.29      |
| NH₃        | 2.78        | 3.66      |
| Arg        | 7.21        | 4.28      |
| Asp        | 8.06        | 3.70      |
| Thr        | 5.76        | 3.28      |
| Ser        | 6.41        | 4.76      |
| Glu        | 24.28       | 36.91     |
| Pro        | 9.64        | 17.84     |
| Gly        | 6.67        | 3.15      |
| Ala        | 5.80        | 3.07      |
| Cys        | 1.15        | 1.67      |
| Val        | 6.87        | 5.37      |
| Met        | 2.11        | 1.52      |
| Ile        | 4.48        | 4.27      |
| Leu        | 7.99        | 8.17      |
| Tyr        | 5.14        | 6.08      |
| Phe        | 5.48        | 6.92      |

The individual proteins were deposited with almost identical intensity during the period studied, i.e. from 13 to 28 days after fertilization. Fig. 3 depicts representative gels of the proteins extracted from the fractions of Fig. 1B. Coelec-
trophoresis of protein body proteins and prol-
amines extracted from mature kernels with 55% isopropanol revealed that both the wild type and the mutants contained polypeptides in the protein bodies with the same electrophoretic mobility as the major bands of the prolamines. These poly-
peptides were, however, much less prominent in the extracts of the mutant protein bodies. It has earlier been demonstrated by immunoelec-
trophoresis of extracts from isolated barley protein bodies that those contain prolamines (TRONIER et al. 1971). The present investigation confirms this conclusion. Furthermore, the altered mor-
phology of the protein bodies in mutant 1508, characterized by the paucity of the homogeneous component is likely to be related to the reduction in the major prolamine bands and the recognition of bands not visible in the gel patterns of the extracts from wild-type protein bodies.

No new bands appear when total extracts of reduced endosperm proteins of the mutant are subjected to SDS-polyacrylamide gel electrophoresis, but the relative amounts of the different endosperm proteins are altered. Since the albu-
mins and globulins were already extracted when the tissue was homogenized a major portion of the proteins associated with the mutant protein bodies must belong to the glutelins (the storage protein group soluble in dilute alkali).

The above-mentioned results on amino-acid composition indicated the protein bodies of both the wild type and the mutant to contain prolam-
ines and other proteins. It is, therefore, apparent that the wild-type protein bodies contain large amounts of prolamines and some glutelins, whereas the mutant protein bodies have glutelin as a major component and little prolamines. The morphological difference between wild type and mutant intimates that the homogeneous component represents a storage organelle with a high concentration of prolamines and the granular component a storage organelle with high concentra-
tion of glutelins.

CHRISTIANSON et al. (1974) have isolated protein bodies by zonal centrifugation from wild-type maize and its high-lysine mutant opaque-2 and characterized them by scanning electron micros-

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Fig. 3. Comparison of the protein band patterns after electrophoresis of extracts from mutant (A) and wild type (B) protein bodies on SDS-polyacrylamide gels. Proteins were extracted from the gradient fractions shown in Fig. 1B. A typical prolamine band pattern is found in fractions 6 to 10 for the wild type proteins (B). The mutant proteins of fractions 4 to 8 gave an altered pattern with a pronounced relative deficiency of some of the most prominent bands of the wild type. The arrows indicate the prolamine bands, i.e. the polypeptides present in a alcoholic extract of a mature wild type endosperm.

The opaque-2 starchy endosperm appears to contain fewer and smaller protein bodies than the wild-type endosperm (Wolf et al. 1967; Christianson et al. 1974). It will be of interest to compare the fine structure and protein composition of the small opaque-2 protein bodies with those of the wild-type maize protein bodies. This should give additional information as to whether or not the opaque-2 gene is homologous to the 1508 gene. So far I have been unable to demonstrate the presence of an insoluble protein in my gradient either by OD$_{280}$ elution profile or by electrophoresis. More studies are, however, necessary to insure that a glutelin-2 like protein is absent in mature barley endosperm. Some proteins associated with the opaque-2 protein bodies are soluble in 70% ethanol, which establishes their prolamine character (Christianson et al. 1974). Using a similar extraction procedure it was impossible to extract measurable amounts of prolamines from mutant 1508 protein bodies.

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