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Changes in ribosomal proteins in wheat embryos in the course of grain development and maturation

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Abstract

It was found, by comparing the densitometric profiles of ribosomal proteins of wheat embryos in milk and full grain ripeness, that in the process of development and ripening of caryopses the percentual proportion of low molecular weight proteins increases at the cost of those of high molecular weight. This concerns both acidic and basic proteins. In electrophoretic separation of ribosomal proteins from embryos of fully ripe seeds by the method of two-dimensional electrophoresis the appearance of three new low molecular weight proteins — an acidic one and two basic ones — was observed. These proteins were not found in the embryos of caryopses of milk ripeness. These results indicate that with development and ripening of wheat caryopses new low molecular weight ribosomal proteins are built into the ribosomes in the embryo. These changes are both quantitative and qualitative.

INTRODUCTION

The process of grain development in cereals can be divided into three distinct stages: the step of endosperm and pro-embryo formation — the phase of green ripeness, the step of actual formation of the embryo and partial accumulation of storage material — the phase of milk ripeness and the step of storage material accumulation proper, loss of water and transition to a state of rest, phase of wax and full morphological ripeness (Pavlov 1967, Grzesiuk 1967, Rejowski 1961a).

The particular steps of caryopse morphogenesis are associated with corresponding physiological and biochemical changes. Therefore the physiological activity of ripening grain undergoes changes as seen among other things in the unequal germination capacity. The biological pro-

perties of ripening seeds affect growth, development and yield of the plants derived from them (Grzesiuk 1961, 1972, Rejowski 1961a, b, Sójka 1961a, b, Kulka 1966).

There are unripe seeds in nearly every bath of grain. This is frequently the result of collecting seeds from unequally ripening plants in the field or plants on which the seeds ripen unequally in the inflorescence.

An essential element deciding of the biological values of the caryopses is the efficiency of the protein biosynthesis system (Heydecker 1972). Ribosomal proteins together with ribonucleic acids form the ribosomal structure and play an important role both in polyribosome formation and in the process of genetic information translation.

It should be mentioned that investigations of ribosomes from developing seeds are but little advanced and mainly concern ribosomal rRNA (Abdul-Baki and Baker 1973, Durre 1975, Donovan 1977, Johari et al. 1977, Weidner and Kulka 1979, 1980).

MATERIAL AND METHODS

As material served winter wheat of the Grana variety cultivated in 1979 on the experimental plots of the Institute of Plant Biology of the Agricultural-Technical Academy in Olsztyn.

Wheat of milk ripeness was collected 17 days after flowering (grain moisture 70%), and wheat of full morphological ripeness 64 days after flowering (grain moisture 24%). The caryopses were removed by hand after two weeks of storage in the ears.

Ribosomes were isolated from the wheat grain embryos by the method of Golińska and Legocki (1973). About 70 g of embryos prepared out by the method of Johnston and Stern (1957) were mixed for 60 sec with 450 ml of buffer A (50 mM Tris-HCl, pH 8.2, 50 mM KCl, 5 mm $MgCl_2$, 5 mM 2 mercaptoethanol, 0.25 M sucrose) with maintenance of pH at 8.2 by means of 1 M Tris. The homogenate was twice centrifuged at 21 000 × g for 15 min. The supernatant was placed on two layers of sucrose (4 ml 0.5 M sucrose with underlying 4 ml of 1 M sucrose) and centrifuged for 5 h at 135 000 × g. The sediment was delicately suspended in buffer B (50 mM Tris-HCl, pH 7.8, 100 mM $MgCl_2$, 5 mM 2-mercaptoethanol, 0.25 M sucrose). The suspension was overlaid on 4 ml 0.5 M sucrose and centrifuged for 3 h at 135 000 × g. Centrifugation was repeated. From the ribosomes thus purified proteins were isolated by the method of Hardy et al. (1969).

Two-dimensional electrophoresis of ribosomal proteins was run by the method of Kaltschmidt and Wittman (1970) in a minia-

ture version with the use in the first direction of 8 per cent acrylamide in 8 M urea (pH 8.2) and in the second direction in 18 per cent acrylamide in 8 M urea (pH 4.0). Electropherograms were stained in 0.5 per cent amide black and stored in 7 per cent acetic acid.

RESULTS

Acidic ribosome proteins isolated from embryos of grains collected at milk ripeness and full ripeness could be divided by unidimensional electrophoresis into four main fractions with a similar profile. On the other hand, the basic proteins migrating to the cathode separated into six main fractions with different profiles. Distinct quantitative differences were found in the proportions of the particular fractions. The percentual participation of acidic ribosomal proteins with high molecular weight migrating to the anode (fractions 1 and 2) in embryos of caryopses at milk ripeness was 86.8 per cent and at full ripeness 63.5 per

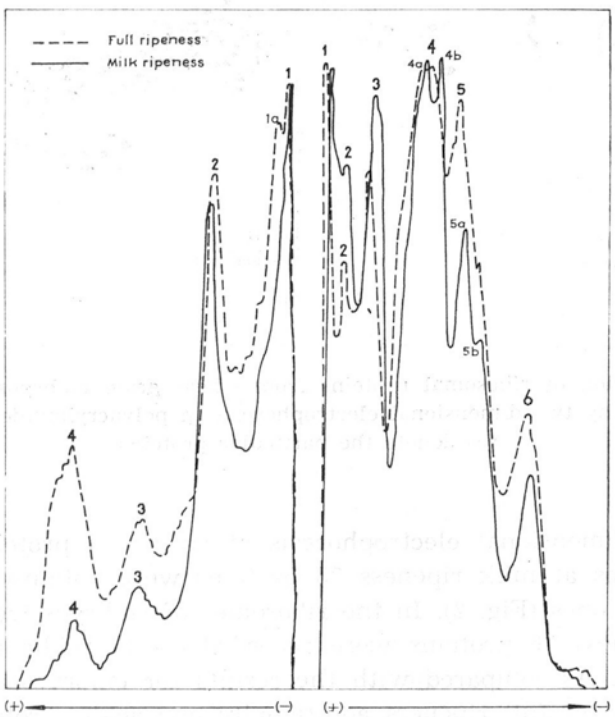

Fig. 1. Separation of ribosomal proteins from wheat grain embryos at milk and full ripeness, achieved by unidimensional electrophoresis in polyacrylamide gel. On the left separation of acidic proteins (migrating to anode) and on the right separation of basic proteins (migrating to cathode). 1-6 — protein fraction numbers

cent, whereas the sum of acidic fractions of low molecular weight proteins (fractions 3 and 4) constituted at milk ripeness 13.2 per cent and at full ripeness 36.5 per cent (Fig. 1).

Similar tendencies were noted in the separation of ribosomal basic proteins, although the differences were less pronounced. For instance the proportion of the low molecular weight fraction 5 in total basic ribosomal proteins obtained from embryos of caryopses at milk ripeness was 20.5 per cent, whereas at full ripeness it amounted to 23.6 per cent, and the corresponding values for fraction 6 were 9 and 14.1 per cent (Fig. 1).

Summing up the above results we may conclude that in the process of development and ripening of wheat embryos the percentual proportion of low molecular weight proteins increases in the ribosomes. The changes discussed concern both acidic and basic ribosomal proteins.

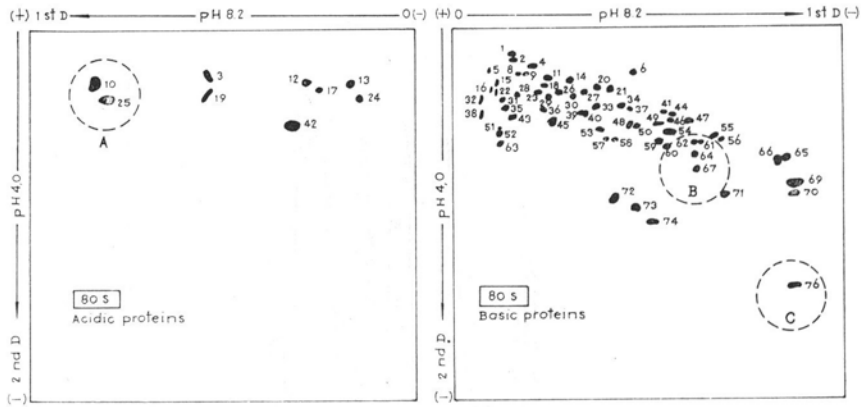

Fig. 2. Separation of ribosomal proteins from wheat grain embryos of milk ripeness, achieved by two-dimensional electrophoresis in polyacrylamide gel. The figures denote the particular proteins

By two-dimensional electrophoresis of ribosomal proteins of wheat grain embryos at milk ripeness 73 proteins were obtained — 9 acidic and 64 basic ones (Fig. 2). In the ribosomes of embryos from caryopses of full ripeness 76 proteins were recorded — 10 acidic and 66 basic ones (Fig. 3). As compared with the results for embryos at milk ripeness, in those of full ripeness additionally one acidic protein (point A, Figs. 2 and 3) and two basic ones (points B and C, Figs. 2 and 3) were found. It is supposed that the cause of those changes is rather enhanced synthesis of ribosomal low molecular weight proteins in the end period of ripening than degradation of high molecular weight proteins.

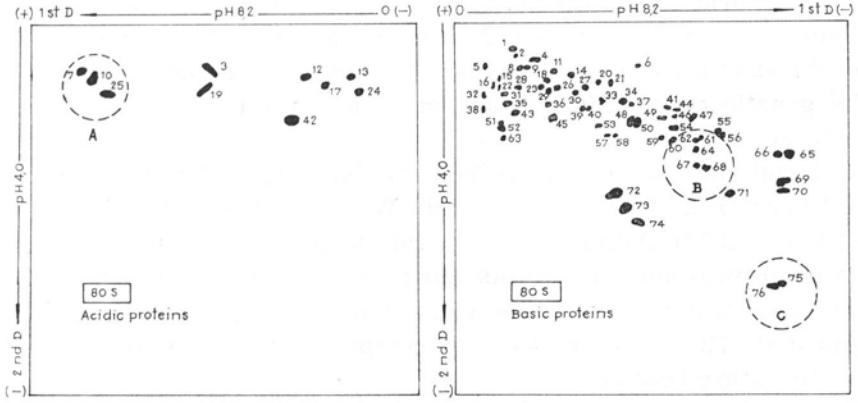

Fig. 3. Separation of ribosomal proteins from wheat grain embryos at full ripeness, achieved by two-dimensional electrophoresis in polyacrylamide gel. The figures denote the particular proteins

DISCUSSION

The amount of rRNA in the grain increases in the first half of the period of grain formation, then it diminishes up to the phase of full maturity (W e i d n e r and K u l k a 1979). Cereal varieties with a high protein content (e.g. wheat) synthesize during development larger amounts of rRNA as compared with the cereals of low protein content (D o n o v a n 1977). It should be stressed that the same relation is observed during germination of high- and low-protein content wheat varieties (C h i n g and R y n d 1978). It was also observed that the ratio of rRNA to tRNA in developing sorgho seed does not undergo major changes (J o h a r i et al. 1977). In other investigations it has been, however, demonstrated that the relative rRNA content in total RNA (expressed as RNA %) decreases throughout the whole development period (W e i d n e r and K u l k a 1979).

During the second half of the caryopse development period progressing rRNA degradation was observed in the endosperm, most intensive in the end period of caryopse ripening (W e i d n e r and K u l k a 1979). This process is accompanied by advancing ageing of the endosperm, leading to degradation of at least part of the ribosomes. In the starch part of the endosperm, however, attempts at detecting ribonucleoprotein ribosome-like particles have so far been unsuccessful (A b d u l - B a k i and B a k e r 1973). The cells of the aleurone layer in the embryo then preserve fully active ribosomes with unchanged structure (A b d u l - B a k i and B a k e r 1973, D u r r e 1975). It should be added that synthesis and accumulation of rRNA continue in cereal embryos almost to the end of ripening of caryopses (C h a n g C h o n g 1963, D u f f u s and R o s i e 1975, D u r r e 1975, J o h a r i et al. 1977, W e i d n e r and K u l k a 1979, 1980).

In the world literature no data are available on ribosomal proteins developing in cereal caryopses. Neither is the mechanism of this process fully elucidated, in spite of great advances in the study of translation of the genetic code. This mechanism is only rather precisely described in bacteria.

Bacterial ribosomal subunits 30S and 50S contain 21 and 34 proteins, respectively (Kaltschmidt and Wittmann 1970, Woll and Stöffler 1974). Although in general outline the course of translation is similar in pro- and eukaryonts, the results of up to date investigations indicate that this process in the cells of higher organisms is much more complicated. This may be seen by comparing the composition of pro- and eukaryotic ribosomes.

The total number of proteins isolated from eukaryotic ribosomes derived from various sources is similar but not identical. Ribosomes isolated from the yeast *Saccharomyces cerevisiae* have 34 proteins in the 40S subunit and 42 in the 60S subunit (Grankowski et al. 1976). The number of proteins in HeLa cells is 35 and 47, respectively (Schiffmann and Horak 1978).

Sikorski et al. (1979) revealed 79 ribosomal proteins from wheat embryos. In the subunit 40S 35 proteins were recorded and in the 60S subunit 44 proteins. Similarly as in our investigation results (Fig. 3) 10 acidic proteins were obtained migrating to the anode (three from subunit 40S and seven from subunit 60S).

Two proteins denoted by Sikorski et al. (1979) as L43 and L44 are fast-migrating, and under the conditions of the method of Kaltschmidt and Wittmann (1970) applied by us they pass to the vessel with cathodic buffer. These proteins may be observed on the electropherogram when the time of electrophoresis is shortened during separation in the second direction (from 12 to 8 h).

The number of 76 ribosomal proteins obtained by the routine method from wheat caryopse embryos of full maturity (Fig. 3) differs from the results of Sikorski et al. (1979) by one basic protein.

The finding of both qualitative and quantitative differences in ribosomal proteins during ontogenetic development of wheat caryopses may indicate a change in their function in this process. These changes must affect the biological value of the caryopses collected at various ripeness stages. When discussing changes in ribosomal proteins the ribosomal RNA should be kept in mind. The participation of this component in translation of the genetic code is no less essential than that of ribosomal proteins.

In spite of the distinct qualitative difference found in the ribosomal proteins of the wheat embryo at various ripeness stages closer interpretation of this finding is difficult. As long as the function of the individual proteins is not known these difficulties will persist.

REFERENCES

Abdul-Baki A. A., Baker J. E., 1973. Seed Sci. Technol. 1: 89-126.
Chang Chong W., 1963. Nature 198: 1167-1169.
Ching T. M., Rynd L., 1978. Plant Physiol. 62: 666-870.
Donovan G. R., 1977. Cereal Chem. 54: 638-656.
Duffus C. M., Rosie R., 1975. Phytochemistry 14: 319-323.
Durre L. S., 1975. Ann. Rev. Plant Physiol. 26: 259-278.
Golińska B., Legocki A. B., 1973. Biochim. Biophys. Acta. 324: 156-170.
Grankowski N., Kudlicki W., Paleń E., Gąsior E., 1976. Acta Biochim. Polon. 23: 341-352.
Grzesiuk S., 1961. Zesz. Nauk. WSR Olsztyn 11: 3-127.
Grzesiuk S., 1967. Fizjologia nasion. PWRiL, Warszawa.
Grzesiuk S., 1972. Zesz. Probl. Post. Nauk Rol. 125: 401-425.
Hardy J. S., Kurland G. G., Voynow P., Mora G., 1969. Biochemistry 8: 2897-2905.
Heydecker W., 1972. Vigour. In: Viability of seeds. Roberts H. A. (ed.). Chapman and Hall Ltd, pp. 205-280.
Johari R. P., Mehta S. L., Nalik M. S., 1977. Phytochem. 16: 19-24.
Johnston F. B., Stern H., 1957. Nature 1970: 160-161.
Kaltschmidt E., Wittmann H. G., 1970. Anal. Biochem. 36: 401-412.
Kulka K., 1966. Acta Soc. Bot. Pol. 35: 17-24.
Pavlov A. N., 1967. Nakopleniye bielka w ziernie pshenicy i kukuruzhy. Izd. Nauka, Moskwa.
Rejowski A., 1961a. Rocz. Nauk Rol. Ser. A 85: 293-305.
Rejowski A., 1961b. Rocz. Nauk Rol. Ser. A 85: 37-110.
Schiffmann D., Horak J., 1978. Eur. J. Biochem. 82: 91-102.
Sójka E., 1961a. Hod. Rośl. Aklim. Nas. 5: 689-703.
Sójka E., 1961b. Hod. Rośl. Aklim. Nas. 5: 705-720.
Sikorski M. M., Przybył D., Legocki A. B., Kudlicki W., Gąsior E., Zalac J., Borkowski T., 1979. Plant Sci. Lett. 15: 387-397.
Weidner S., Kulka K., 1979. Acta Soc. Bot. Pol. 48: 501-510.
Weidner S., Kulka K., 1980. Acta Soc. Bot. Pol. 49: 423-433.
Wool J. G., Stöffler G., 1974. In: Ribosomes. Nomura M., Tissieres A. and Lengyel P. (eds.), Cold Spring Harbor Laboratory, New York, pp. 417-460.

Zmiany w białkach rybosomalnych zarodków pszenicy podczas rozwoju i dojrzewania ziarna

Streszczenie

Porównując profile densytometryczne białek rybosomalnych zarodków pszenicy o dojrzałości mlecznej oraz pełnej stwierdzono, że w procesie rozwoju i dojrzewania ziarniaków wzrasta procentowy udział frakcji białek drobnocząsteczkowych kosztem frakcji białek wysokocząsteczkowych. Dotyczy to zarówno białek kwaśnych jak i zasadowych. W rozdziałach elektroforetycznych białek rybosomalnych zarod-

ków dojrzałości pełnej metodą elektroforezy dwukierunkowej zaobserwowano pojawienie się trzech nowych drobnocząsteczkowych białek, jednego kwaśnego i dwóch zasadowych. Białek tych nie stwierdzono w zarodkach ziarniaków o dojrzałości mlecznej. Uzyskane wyniki świadczą o tym, że wraz z rozwojem i dojrzewaniem ziarniaków pszenicy, do rybosomów w zarodkach dobudowywane są nowe drobnocząsteczkowe białka rybosomalne. Zmiany te mają zarówno charakter ilościowy jak i jakościowy.