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Changes in ribosomal proteins of wheat embryos during accelerated ageing of the grain

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Abstract

Electrophoretic separation of ribosomal proteins of wheat embryos from grain analysed immediately after the harvest (control) yielded four anodic and six cathodic fractions. In caryopses subjected to accelerated ageing the ribosomal proteins separated into 5 anodic and 8 cathodic fractions. With the advance of ageing an increase in the amount of low molecular weight molecules was observed in the ribosomes of the embryos. In ribosomes from wheat caryopse embryos (control) 76 individual proteins — 10 acidic and 66 basic ones — were recorded. In the embryos of aged caryopses 53 proteins were found — 16 acidic and 37 basic ones. The mobility of nearly all individual proteins was also changed as compared to that in the control sample. This is additional evidence of the modification of the proteins in the ageing process and leads as consequence to a change in their electric charges.

INTRODUCTION

In up to date investigations concerning processes of seed ageing much attention was devoted to the particular elements of protein biosynthesis and their role in the process of declining viability (Bray and Chow 1976a, b, Hallam et al. 1973, Harrington 1973, Roberts and Osborne 1973, Roberts et al. 1973, Villiers and Edgcumbe 1975). So far no reports are available on changes in ribosomal proteins in the process of ageing. It is only known that during seed ageing the second component of ribosomes — rRNA undergoes many modifications (Bray and Chow 1976b, Kulka 1971, Roberts and Osborne 1973, Wiśniewski and Kulka 1979, Weidner et al. 1980). Therefore an attempt was undertaken to

partly elucidate the eventual dependence between wheat grain viability and quantitative and qualitative changes in the ribosomal proteins of the embryos.

MATERIAL AND METHODS

The experiments were performed with winter wheat grain of the Grana variety cultivated on the experimental plots of the Institute of Plant Biology of the Agricultural-Technical Academy in Olsztyn. The caryopses were harvested in 1977 and 1980. The grain from the 1977 harvest after mild drying was stored for six weeks under laboratory conditions and then viability was determined. Then it was placed in a hygrostat at relative humidity of 75 per cent and stored at room temperature for 36 months. As control served grain of the same variety harvested in 1980. The embryos were prepared out from the wheat caryopses by the method of Johnston and Stern (1957).

Ribosomes were isolated by the method of Golińska and Legocki (1973). About 70 g of embryos were mixed for 60 sec. (6 times for 10 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). pH 8.2 was maintained by means of 1 M Tris. The homogenate was centrifuged twice at 21 000 × g for 15 min. The supernatant was placed on two layers of sucrose (4 ml of 0.5 M sucrose overlaid on 4 ml of 1 M sucrose) prepared on buffer A and centrifuged for 5 h at 135 000 × g. The sediment was delicately suspended in buffer B (50 mM Tris-HCl, pH 7.8 containing 100 mM KCl, 5 mM $MgCl_2$, 5 mM 2-mercaptoethanol and 0.25 M sucrose). The suspension was underlaid with 4 ml 0.5 M sucrose prepared on buffer B and centrifuged for 3 h at 135 000 × g. The resuspended sediment was once more centrifuged under the same conditions as previously. Proteins were isolated from the thus purified ribosomes according to the method of Hardy et al. (1969).

Uni- and two-dimensional electrophoresis of ribosomal proteins was run after Kaltschmidt and Wittmann (1970) in a miniature version. In the first direction 8 per cent acrylamide in 8 M urea was used (pH 8.2). Onto the gel (tubes 2 × 60 mm) 50 μg of protein dissolved in 8 M urea with 0.1 M mercaptoethanol added was placed. Electrophoresis was run for 4 h with current intensity 2 mA per tube. In the second direction 18 per cent acrylamide in 8 M urea (pH 4.0) was applied. Separation was conducted for 12 h at potential difference 80 V and initial voltage (first 30 min) 40 V. The electropherograms were stained with 0.5 per cent amide black and stored in 7 per cent acetic acid.

RESULTS

The wheat caryopses harvested in 1977 after three months of storage under conditions of elevated air humidity attained a corresponding humidity of 15.5 per cent in the environment of 75 per cent relative humidity and under conditions of laboratory storage 13.5 per cent.

Storage under conditions of elevated humidity for 36 months has a significant influence on the caryopses and their germination as compared with the control. A detailed characteristic of germination of the tested caryopses is given in Table 1.

Table 1

Germination of wheat caryopses in different age

Swelling and germination conditions	Sample of grain	Dry weight of 100 embryos, g	Increment of dry weight of embryos, %	Dry weight of endosperm, g/100 caryopses	Decrement of dry weight of endosperm, %
12 h, 0°C	A	0.121	—	2.997	—
	B	0.119	—	2.980	—
24 h, 22°C	A	0.146	20.7	2.872	4.2
	B	0.123	3.3	2.920	2.0
48 h, 22°C	A	0.197	62.8	2.732	8.8
	B	0.133	11.8	2.868	3.8

A — grain harvested in 1980, analysed after 3 months of storage under laboratory conditions, germination ability = 96%.

B — grain harvested in 1977, analysed after 36 months of storage in a hygrostat at humidity of 75%, germination ability = 5%.

Electrophoretic separation of ribosomal proteins from wheat grain embryos analysed immediately after harvest (control) yielded four anodic and six cathodic fractions. In the caryopses subjected to accelerated ageing the ribosomal proteins from the embryos separated into five anodic and eight cathodic fractions (Fig. 1). These results prove that during ageing of wheat caryopses significant changes occur in the ribosomal proteins of the embryo. It may be said in general that with the advancing process of ageing the amount of low molecular weight proteins increases in the ribosome at the cost of the high molecular weight ones (probably by way of their degradation). This is true both for acidic (appearance of a 5th fraction) and basic proteins migrating to the cathode (high proportion of the sum of fractions 6, 7 and 8).

Separation of ribosomal proteins from wheat grain embryos analysed immediately after the harvest (control sample) by two-dimensional electrophoresis yielded 76 individual proteins — 10 acidic and 66 basic ones (Fig. 2).

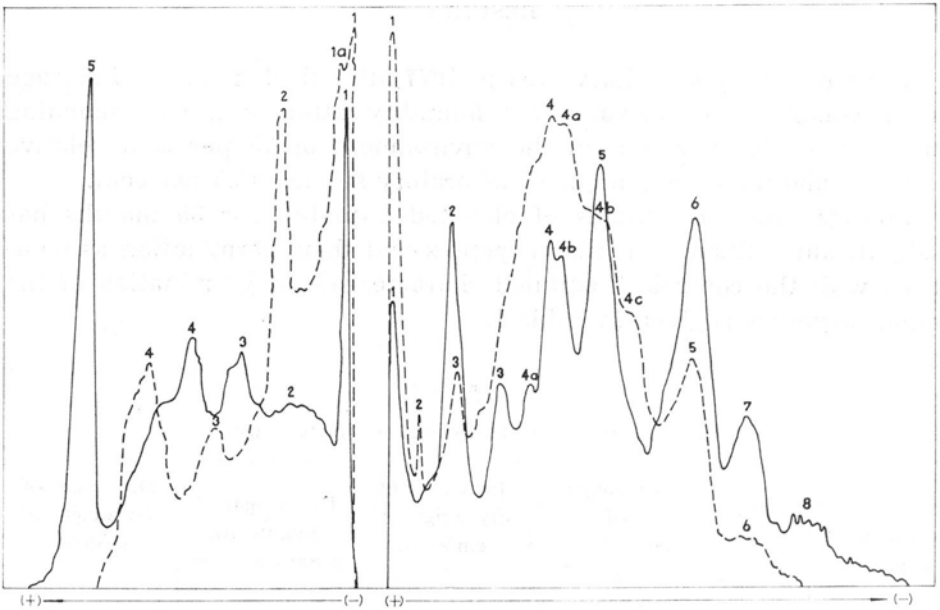

Fig. 1. Separation of ribosomal proteins from embryos of wheat grain harvested in 1977 and analysed after 3 years of storage in elevated air humidity (———) and of grain harvested in 1980 (— — —) control. Ribosomal proteins were separated by unidimentional electrophoresis on polyacrylamide gel. On the left separation of acidic proteins (migrating to anode) and on the right of basic proteins (migrating to cathode). 1-8 numbers of protein fractions

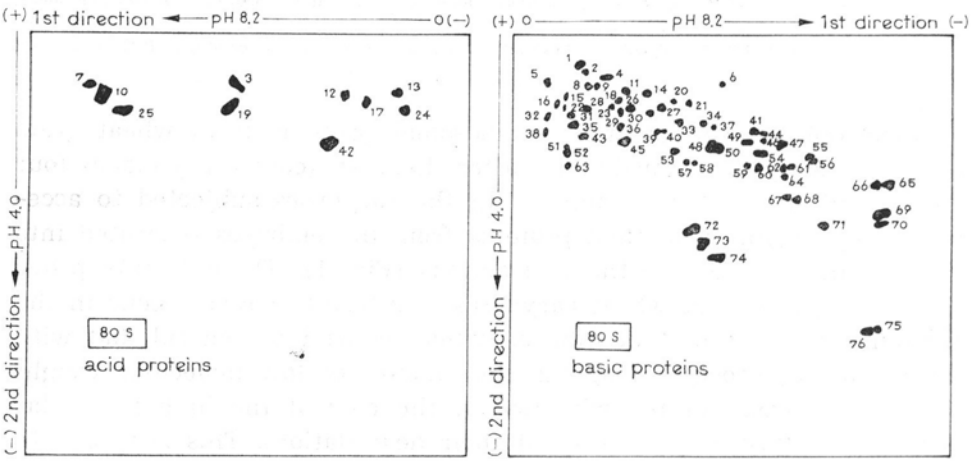

Fig. 2. Separation of ribosomal proteins from embryos of wheat grain harvested in 1980 and analysed immediately after the harvest (control). The ribosomal proteins were separated by two-dimensional electrophoresis in polyacrylamide gel, according to Kaltschmidt and Wittmann (1970). On the left separation of acidic proteins (migrating to anode) and on the right of basic proteins (migrating to cathode). 1-76 numbers of particular proteins

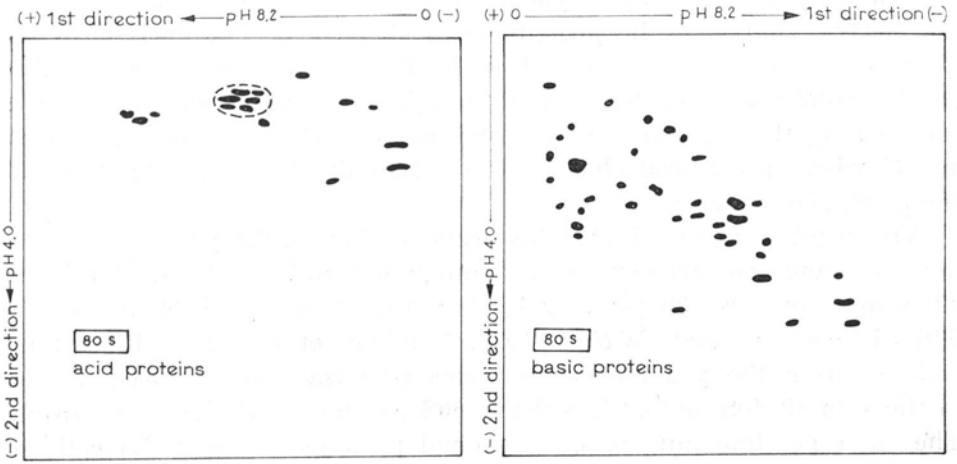

Fig. 3. Separation of ribosomal proteins from embryos of wheat harvested in 1977 and analysed after 3 years of storage under elevated air moisture conditions. The ribosomal proteins were separated by two-dimensional electrophoresis in polyacrylamide gel according to Kaltschmidt and Wittmann (1970). On the left separation of acidic proteins (migrating to anode) and on the right of basic proteins (migrating to cathode). The dashed line separates the group of proteins not observed in the control sample

In embryos from ageing caryopses 53 individual proteins were found — 16 acidic and 37 basic ones (Fig. 3). The appearance of a new group of six is observed in the acidic ribosomal proteins from ageing grains as compared with the control. The migration velocity of this group indicates that they belong to fraction 4 (on the densitometric diagram — Fig. 1). The remaining acidic proteins and the basic ones showed a changed electrophoretic mobility. Therefore this group of proteins cannot be considered as identical with those appearing in the electrophoretic picture after separation of ribosomal proteins from embryos of the control grain.

DISCUSSION

As another alternative mechanism of the changes arising in the enzymes of ageing organisms is more and more frequently suggested the occurrence of posttranslation protein modifications. One of the best known modifications which may be significant in the process of ageing of proteins is deamidation of the asparaginyl and glutaminyl rests (McKerrow 1979, Robinson and Rudd 1974, Charache et al. 1977). The problem of proteolytic splitting off of the end groups of amino acids is also taken into account (Kahn et al. 1977). Both deamidation of the asparagyl and glutamyl rests and proteolysis of the end amino acids as well as other kinds of modification such as phos-

phorylation, acetylation, adenylation or glycosylation cause a change in the electric charge of the protein. Such changes in ribosomal proteins of ageing embryos as compared with the control are visible in the results obtained in protein separation. Most pronounced changes are revealed by the method of two-dimensional electrophoresis (cf. Figs. 2 and 3) where a changed electrophoretic mobility is noted in nearly all the particular proteins.

The total number of proteins isolated from eukaryotic ribosomes derived from various sources is similar but not identical. Rat liver ribosomes comprise 30 proteins in the subunits 40S and 39 in subunit 60S (Sherton and Wool 1972, Welfe et al. 1971). Ribosomes isolated from the yeast *Saccharomyces cerevisiae* contain 34 proteins in the subunit 40S and 42 in subunit 60S (Grankowski et al. 1976). The corresponding number of ribosomal proteins in the HeLa cells is 35 and 47 (Schiffmann and Horak 1978).

Sikorski et al. (1979), when analysing ribosomal proteins from wheat embryos, recorded a total of 79 proteins: in subunit 40S — 35 and in subunit 60S — 44 proteins. Similarly as in the present study (Fig. 2) 10 acidic proteins migrating to the anode (3 from subunit 40S and 7 from subunit 60S) were detected. Two of the 79 proteins obtained by the above mentioned authors are fast migrating ones and are not noticeable on the electropherogram obtained by the routine method of Kaltschmidt and Wittmann (1970). These proteins can be observed when the duration of electrophoresis in the second direction is shortened. In the present investigations 76 individual proteins (Fig. 2) were recorded in ribosomes of the wheat embryo in the control sample. As already mentioned, Sikorski et al. (1979) found in both ribosomal subunits from wheat embryos 77 individual proteins (plus two fast migrating ones). It is possible that presently 76 and not 77 proteins were found because whole ribosomes 80S were analysed instead of their subunits.

The finding of both qualitative and quantitative differences in ribosomal proteins and of completely changed electrophoretic mobility of the individual proteins points to serious changes occurring in the ribosome during the ageing process. These changes must depress the biological value of the caryopses. The observed unfavourable modifications within the ribosome may make initiation of protein synthesis in the embryo cells impossible and in this way prevent seed germination.

The results presently obtained lead also to the conclusion that in long stored grain the ribosomal proteins are a labile component.

This is confirmed by analysis of the electrophoretic picture of ribosomal proteins obtained from embryos of grains with a low germination capacity and control grains. The reduced mass of ribosomes in old rye grain has been reported earlier by Osborne et al. (1971). It is,

however, difficult to predict the effect in the process of translation of the splitting off of several or a dozen or so proteins from the ribosome since so far the functions of the individual ribosomal proteins in the process of translation are not known.

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Zmiany w białkach rybosomalnych zarodków pszenicy podczas przyspieszonego starzenia się ziarna

Streszczenie

W wyniku rozdziału elektroforetycznego białek rybosomalnych zarodków ziarna analizowanego bezpośrednio po zbiorze (kontrola) uzyskano cztery frakcje anodowe oraz sześć frakcji katodowych. W ziarniakach poddanych przyspieszonemu procesowi starzenia, białka rybosomalne rozdzielały się na pięć frakcji anodowych

i osiem frakcji katodowych. Wraz z postępującym procesem starzenia stwierdzono w rybosomach zarodka wzrost zawartości białek niskocząsteczkowych.

W rybosomach zarodków ziarniaków pszenicy (kontrola) stwierdzono 76 indywidualnych białek — 10 kwaśnych i 66 zasadowych. W zarodkach ziarniaków zestarzałych stwierdzono 53 białka — 16 kwaśnych i 37 zasadowych, oraz zmienioną ruchliwość nieomal wszystkich indywidualnych białek w stosunku do próby kontrolnej. Świadczy to dodatkowo o modyfikacji badanych białek w procesie starzenia, czego konsekwencją jest zmiana ich ładunków elektrycznych.