TWO DIMENSIONAL ACRYLAMIDE GEL ELECTROPHORESIS OF WHEAT LEAF CYTOPLASMIC AND CHLOROPLAST RIBOSOMAL PROTEINS

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1. Introduction

Cytoplasmic and chloroplast ribosomes from the same plant species have been shown by acrylamide gel electrophoresis to differ greatly in protein composition [1–6]. The limited resolving power of single-dimension electrophoresis did not, however, allow determination of the total number of proteins in each of the ribosome species. Two-dimensional acrylamide gel electrophoresis, which has been used to separate the ribosomal proteins of *E. coli* [7,8], has recently been used to determine the approximate number of proteins in ribosomes of higher animals [9,10]. So far similar analysis of plant ribosomal proteins have not been published.

This paper reports the separation of the ribosomal proteins from cytoplasmic and chloroplast ribosomes of wheat leaf by two-dimensional acrylamide gel electrophoresis. The cytoplasmic and chloroplast ribosome proteins were almost all different, with the cytoplasmic ribosomes containing more proteins. Both chloroplast 70 S and cytoplasmic 80 S wheat ribosomes contained more proteins than have been reported in *E. coli* 70 S ribosomes.

2. Materials and methods

Wheat leaf ribosomes were prepared by a modification of the method reported earlier [11]. Wheat leaves (*Triticum vulgare* cv. Manitou, 4.5 days old) were ground in the presence of Triton X-100 and sodium deoxycholate, followed by centrifugation of the ribosomes and separation of the resulting ribosome mixture on a zonal rotor. Before the ribosome mixture was layered onto the zonal rotor it was passed through a "Bio-Gel A–0.5 M Agarose" column equilibrated with 0.5 M NH_4Cl in zonal buffer (10 mM tricine, 10 mM $MgCl_2$, 4 mM 2-mercaptoethanol, pH 7.5) to remove surface bound protein contaminants.

Wheat leaf ribosomal proteins from both 70 S chloroplast and 80 S cytoplasmic ribosomes were extracted with 66% acetic acid. The precipitated RNA was removed by centrifugation (25,000 *g*, 15 min) and the supernatant lyophilized. After lyophilization, the proteins were dissolved in the first dimensional electrophoresis buffer of Kaltschmidt and Wittmann [7] for application to the electrophoresis gel. Their procedure was modified so that the sample was not sandwiched in the center of the first dimensional gel. Instead, protein samples were applied to the tops of two separate 12.0 × 0.6 cm gels. One was run from anode to cathode; the other was run from cathode to anode. This allowed a greater separation in the first dimension. First dimension electrophoresis was for 17 hr at 120 V. The second dimension gels (18% acrylamide, pH 4.6) were 14.0 cm square × 0.6 cm thick and electrophoresis in this direction was for 18 hr with the voltage limited to 120 V and the current limited to 220 mA per slab or less. All electrophoreses were done at 4°. Whenever comparisons were made between different protein samples, the two samples were electrophoresed simultaneously in the same electrophoresis apparatus in both the first and second dimension.

3. Results and discussion

Approximately 85 spots were found when 80 S

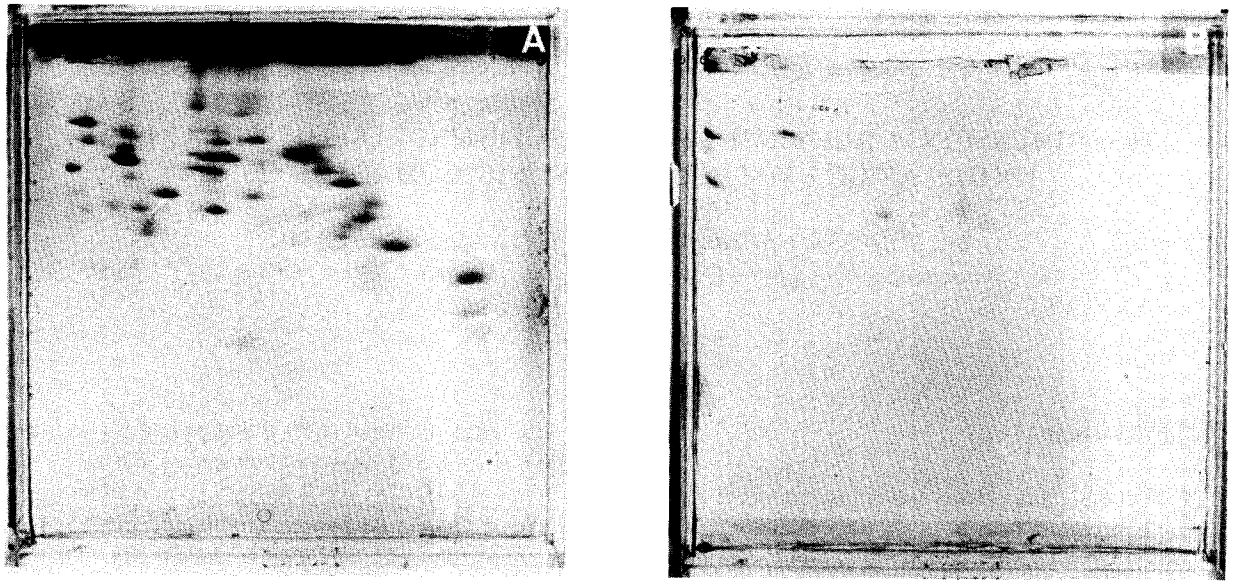

Fig. 1. Two dimensional polyacrylamide gel electrophoresis of proteins from wheat cytoplasmic ribosomes according to the method of Kaltschmidt and Wittmann [8]. Electrophoresis in the first dimension was carried out in gels (12.0 × 0.6 cm) containing 4% acrylamide, 0.125% methylene bis-acrylamide and electrophoresis buffer containing 6 M urea, 0.15 M boric acid 6.5 mM Na_2 EDTA and 0.12 M Tris pH 8.6. 200 μg of protein from each sample were layered. After 17 hr run at 120 V the gel was equilibrated in buffer pH 4.6 for 20 min before applying to the top of the second dimensional gel (14.0 × 0.6 cm) containing 18% acrylamide and 0.5% methylene bis-acrylamide. For both anode and cathode 0.18 M glycine and acetic acid buffer pH 4.6 was used. Electrophoresis in this direction was for 18 hr. The gels were stained in 0.5% amido black in 5% acetic acid and destained electrophoretically in 3% acetic acid. A) Proteins electrophoresed from anode toward cathode. B) Proteins electrophoresed from cathode toward anode.

wheat leaf ribosomal protein was electrophoresed. Of these 69 had moved toward the cathode in the first dimension and only 16 moved toward the anode (fig. 1A and B). The total number of 80 S ribosomal proteins of wheat thus seems to be greater than have been reported for 80 S ribosomes from animals [9, 10] where approx. 75 protein spots were found. While the number of spots observed varied slightly among different ribosome preparations there were significantly more proteins present in these ribosomes than have been reported for prokaryotic ones [7,8]. In contrast to the findings with animal ribosomes the 80 S wheat ribosomes contained about 16 proteins which moved toward the anode at pH 8.6.

The chloroplast ribosomes yielded about 75 spots on two-dimensional electrophoresis (fig. 2). This is in marked contrast to the prokaryotic 70 S ribosomes which only contain about 55 proteins [8]. Approx. 26 of the proteins migrated toward the anode during the first dimensional electrophoresis (fig. 2B) while about 50 moved toward the cathode (fig. 2A).

Since the intensity of some protein spots from both 80 S and 70 S ribosomes varied to some extent with preparations, it is possible that some of them were products of aggregation and oxidation or other artifacts. Even if some of the spots were artifacts the chloroplast ribosomes contained more proteins than prokaryotic ribosomes and wheat cytoplasmic ribosomes contained even more.

Composite drawings have been made which allow easier comparison of the protein patterns of the total 70 S and 80 S ribosome proteins (fig. 3). The patterns obtained from second dimensional electrophoresis after both anionic and cationic first dimensional separations have been combined into a single drawing for each ribosome species. The darker staining spots are shown as filled circles while the lighter spots are shown as open circles. A comparison of figs. 3A and 3B shows that few of the proteins of the 70 S and 80 S ribosomes when electrophoresed simultaneously migrated to

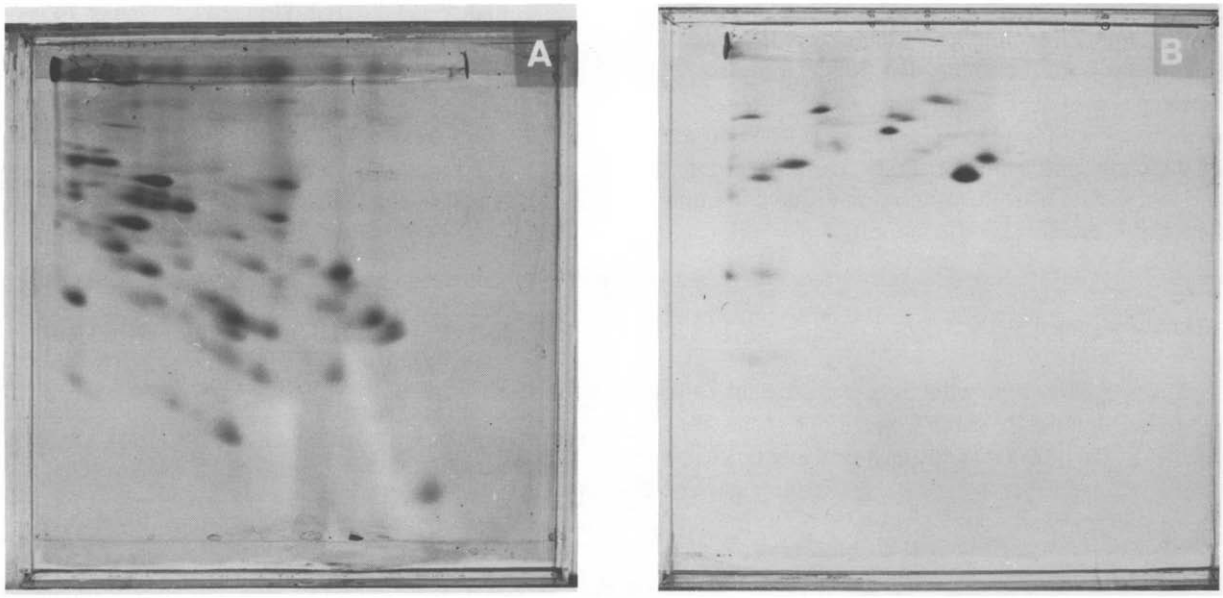

Fig. 2. Ribosomal proteins of wheat chloroplast ribosomes (for details see fig. 1). A) Proteins electrophoresed from anode toward cathode. B) Proteins electrophoresed from cathode toward anode.

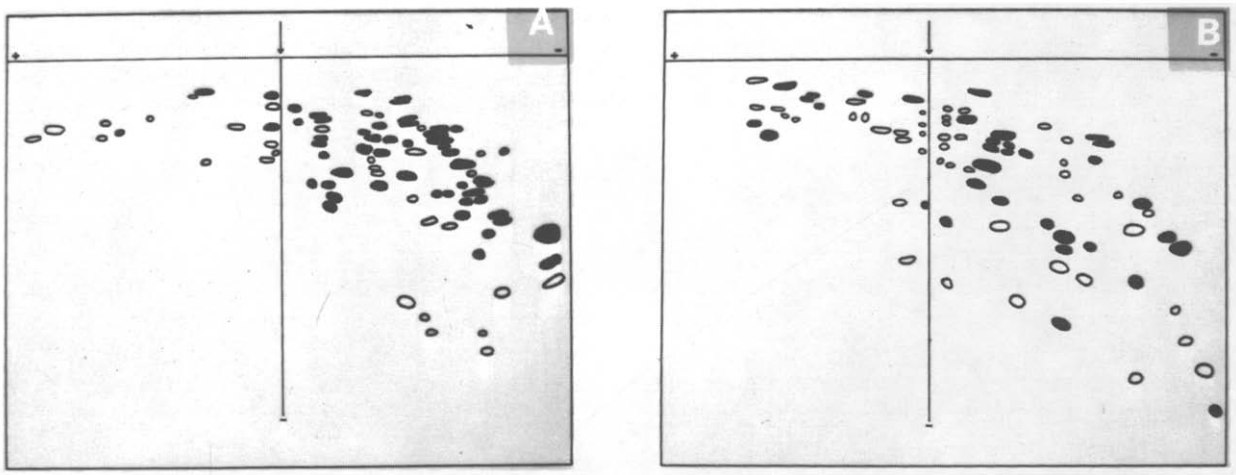

Fig. 3. Composite drawings of the second dimensional electrophoresis pattern of each ribosome species. A) Total proteins of 80 S cytoplasmic ribosomes from wheat leaf (drawn from fig. 1 A and B), B) Total proteins of 70 S chloroplast ribosomes (drawn from fig. 2 A and B). The darker staining spots are shown as filled circles while the lighter spots are shown as open circles.

identical or nearly identical positions in the second dimensional gel. Of the proteins which migrated toward the anode in the first dimension only two or three of the darker spots moved to positions which are near enough on the gel that there is a possibility they occur in both ribosome species. While the number of proteins migrating toward the cathode in the first dimension gel makes comparison difficult, it is obvious that the majority of the proteins of the two ribosomes are quite different.

In summary, use of the modification of the two dimensional electrophoresis method [7,8] allowed greater separation in the first dimension. Cytoplasmic wheat leaf ribosomes were found to contain

more proteins than have been reported for animals [9,10] and unlike those of animals 16 proteins migrated toward the anode. The 70 S chloroplast ribosomes contained significantly more proteins than have been found in prokaryotes [8] and more of these moved toward the anode. The proteins of the two species of wheat ribosomes differed in numbers and in electrophoretic mobility.

Acknowledgements

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