Short Communication

Comparison of Plant Cytoplasmic Ribosomal Proteins by Two-Dimensional Polyacrylamide Gel Electrophoresis

Received for publication August 16, 1973 and in revised form October 15, 1973

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ABSTRACT

The electrophoretic mobilities of the cytoplasmic ribosomal proteins of several species of plants were compared using two-dimensional electrophoresis. The total number of proteins as well as the number of acidic and basic proteins in individual species varied markedly. Of the species examined, Triticum aestivum had the highest number of basic cytoplasmic ribosomal proteins and Hordeum vulgare had less than half as many. However, marked similarities were noted in the electrophoretic mobilities of many of the proteins, especially for wheat, rye, and barley and for peas and beans. There was a statistically significant positive correlation between the numbers of basic proteins in the species and their chromosome number.

It has been shown that the complex of ribosomal proteins prepared from various species of bacteria (8, 9), animals (3, 15), and higher plants (1, 2, 4, 11, 12, 14) differ. Previous studies in our laboratory (5) employing two-dimensional electrophoresis to compare proteins of 70S and 80S wheat leaf ribosomes revealed that the proteins of chloroplast and cytoplasmic ribosomes differed in numbers, in their relative amounts, and in electrophoretic mobilities. The cytoplasmic ribosomes contained more proteins than have been reported for animals, and the chloroplast ribosomes contained more than have been reported for prokaryotes. Both classes of ribosomes contained a relatively large number of proteins that migrated towards the anode.

In view of these findings, we set out to extend the work by comparing the ribosomal proteins of several species of higher plants. This report deals with the 80S cytoplasmic ribosomes of these species.

MATERIALS AND METHODS

Ribosomes from green leaves of different plant species were prepared by the method reported earlier (7). Leaves of 4.5-day-old seedlings of Triticum aestivum cv. Manitou, Triticum durum cv. Hercules, Hordeum vulgare cv. Gateway, Secale cereale cv. Prolific, and primary leaves of 10-day-old Phaseolus vulgaris cv. Black wax and Pisum sativum cv. Homesteader were used for isolation of the cytoplasmic ribosomes. Spinacia oleracea cv. Bloomsdale was supplied fresh by a local market gardener. For separation of 70S and 80S ribosomes from the same plant material in large quantities, a 7 to 35% (w/v) convex sucrose density gradient centrifugation was carried out in a Beckman Ti 14 zonal rotor (6). The Mg concentrations of the isolation media as well as of the zonal buffers were adjusted to provide optimal yield of ribosomes from the individual plant species. The concentrations were 1 mM for barley, 5 mM for beans, peas, and spinach, and 10 mM for rye and wheat.

Proteins were extracted from the cytoplasmic ribosomes with 66% acetic acid in the presence of 30 mM MgCl₂. After lyophilization, the ribosomal proteins were dissolved in the electrophoresis buffer containing 6 M urea, and the protein concentration was determined by the method of Lowry et al. (10). For separation of the ribosomal proteins, the modified method of Kaltschmidt and Wittmann (8) was used. However, the protein samples were applied to two separate gels in the first dimension and were electrophoresed towards the cathode and the anode separately (5). Methyl green and bromophenol blue were coelectrophoresed with the basic and acidic proteins, respectively, to enable runs with uniform migration distances in the first dimension. The second dimension electrophoresis was carried out as described earlier (5), but methylene blue was used as a marker.

RESULTS AND DISCUSSION

The ratio of 70S to 80S ribosomes differed with the species. The amount of 70S relative to 80S was about 20% for the four cereals but was less for beans, peas, and spinach. Prior to protein extraction, the purity of each 80S ribosome fraction was determined by linear sucrose density gradient centrifugation or by analytical ultracentrifugation. The 80S ribosomes were devoid of 70S contamination.

Although only one variety of each species was examined in this study, in another investigation in our laboratory in which two varieties of rye of widely different genetic backgrounds were compared it was found that the number and patterns of their 80S ribosomal proteins were similar.

The proteins were considered to be of ribosomal origin, since washing the ribosomes with NH₄Cl before extraction of the proteins did not alter the electrophoretic patterns or number of proteins obtained for any species. However, it should be noted that this might not have ensured that all the proteins were of ribosomal origin, just as the extraction procedure...
Fig. 1. Two-dimensional polyacrylamide gel electrophoresis of cytoplasmic ribosomal proteins from several plant species. Electrophoresis in the first dimension was in gels (13.5 × 0.6 cm) of 4% acrylamide, 0.125% methylene bisacrylamide and electrophoresis buffer containing 6 M urea, 0.15 M borate, 6.5 mM Na_{2}EDTA, and 0.12 M tris, pH 8.6. Approximately 0.5 mg of each protein sample and 10 μl of 0.5% methyl green were layered for the basic proteins (anode to cathode) and about 1.0 mg of each sample and 10 μl of 0.5% bromophenol blue was layered for separation of the acidic proteins (cathode to anode). Electrophoresis was at 4 ma per tube till the slower moving band of methyl green (for basic proteins) or the band for bromophenol blue (acidic proteins) had reached the bottom of the tube (17-22 hr). The gels were equilibrated in 8 M urea in acetate buffer, pH 4.6, for 20 min. before laying them on top of the second dimensional gel (14.0 × 14.0 × 0.6 cm) composed of 18% acrylamide and 0.5% methylene bis-acrylamide. A piece of plastic 3 mm² was inserted into the second dimension gel before polymerization to mark the end of the first dimension gel. After polymerization and removal of the plastic a drop of methylene blue was applied to the hole. For electrophoresis in this dimension (anode to cathode) 0.18 M glycine-acetate buffer, pH 4.6, was used and it was run at 120 v with the current limited to 220 ma till the dye reached 1 cm from the bottom of the gel (18–22 hr). All electrophoreses were at 4°C. The gels were stained in 0.5% amido black in 5% acetic acid for 1 hr and were destained electrophoretically in 3% acetic acid. Photographs of the second dimensional electrophoresis of each species after separate anionic and cationic first dimensional electrophoresis were combined to give a composite photograph for each species.
Table I. Number of 80S Ribosomal Proteins in Several Species of Plants as Determined by Two-Dimensional Electrophoresis and Their Correlation with Chromosome Number

<table>
<thead>
<tr>
<th>Species</th>
<th>Variety</th>
<th>Chromosome No.</th>
<th>Acidic Proteins</th>
<th>Basic Proteins</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triticum aestivum</td>
<td>Manitou</td>
<td>n</td>
<td>17</td>
<td>69</td>
<td>86</td>
</tr>
<tr>
<td>Triticum durum</td>
<td>Hercules</td>
<td>21</td>
<td>21</td>
<td>54</td>
<td>75</td>
</tr>
<tr>
<td>Hordeum vulgare</td>
<td>Gateway</td>
<td>7</td>
<td>13</td>
<td>31</td>
<td>44</td>
</tr>
<tr>
<td>Secale cereale</td>
<td>Prolific</td>
<td>7</td>
<td>20</td>
<td>47</td>
<td>67</td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>Black Wax</td>
<td>11</td>
<td>8</td>
<td>54</td>
<td>62</td>
</tr>
<tr>
<td>Pisum sativum</td>
<td>Homesteader</td>
<td>7</td>
<td>12</td>
<td>48</td>
<td>60</td>
</tr>
<tr>
<td>Spinacia oleracea</td>
<td>Bloomsdale</td>
<td>6</td>
<td>21</td>
<td>49</td>
<td>70</td>
</tr>
</tbody>
</table>

Simple correlation coefficient between chromosome number and number of proteins

-0.07 +0.82 +0.73

1 Significant at the 5% level.

might not have ensured that all the ribosomal proteins were extracted (13).

At least two separate ribosomal preparations of each species and not less than three electrophoretic runs for each preparation were made. For the individual species, the protein patterns were virtually identical for all the runs. Despite the attempts to standardize all the procedures by quantitation and the use of tracking dyes, small differences in the relative intensity of spots and their migration distances were noted. As may be seen from Table I and Figure 1, Manitou had the largest number of ribosomal proteins and barley the least. Because of the change in duration of the electrophoretic runs, the protein pattern was somewhat different, but the number of basic and acidic proteins found in the cytoplasmic ribosomal proteins of Manitou wheat were the same as reported by us previously (5).

Coelectrophoresis of marker dye with the proteins of each species in both dimensions permitted fairly uniform migration distances and made it possible to compare the proteins on the basis of their mobilities. The comparison was aided by superimposing a grid over the photographs and comparing all the species in terms of the number and pattern of their proteins within various regions of the grid. From Table I, it is seen that Manitou had more basic proteins than the other species and barley had the least. Examination of all the basic proteins between the origin and column B, that is, all the proteins that migrated slowly in the first dimension, showed that Manitou, Hercules, rye, and spinach had nearly the same number in this region. However, their mobilities varied (Fig. 1). Barley, beans, and peas had fewer proteins in this region. The protein pattern of rye resembled Manitou more than any other species. Considering the area between columns B and D, Manitou had more proteins than any other species, and barley had the least. A number of the proteins of each species had identical mobilities, but others differed. The greatest similarity appeared in peas and beans. Between columns D and F, the highest number of protein spots were again found in Manitou and the least in barley. The protein patterns of peas and beans in this region were alike, and those of barley and rye were also similar. For convenience, all the proteins in the area between columns F and J were considered together. Clearly, barley and rye had fewer spots than any of the other species. Although the numbers and mobilities of the proteins varied to some extent with the species, there was a similarity in their pattern. Beans, peas, and spinach were most alike and barley and rye were similar.

As was previously reported for Manitou wheat (5), all of the species examined contained acidic ribosomal proteins but the intensities of the spots varied considerably (Fig. 1). Beans had fewer acidic proteins than the other species (Table I). Examination of the area between the origin and column B showed that rye had the most spots and beans the least. With the exception of spinach, a relatively large number of the acidic proteins migrated slowly in the first dimension. Some similarity in the protein pattern of this region was noted for Manitou and rye, but comparison of the acidic proteins was difficult because of the differences in intensity of the spots. No proteins were detected for beans and peas in the area between columns B‘ and D’, but the protein patterns for the other species differed. Likewise, the protein pattern seen for each species in the area between columns D‘ to 11 was different.

Although the statistically significant positive correlation between the number of chromosomes in these species and their number of basic proteins (Table I) is extremely interesting, it would be speculative to attribute any biological significance to this without more extensive investigation.

Acknowledgment—We thank B. Zytaruk for technical assistance.

LITERATURE CITED