Phytochemistry, Vol. 21, No. 2, pp. 339–343, 1982.
Printed in Great Britain.

0031-9422/82/020339-05 $03.00/0

COMPARISON OF CYTOPLASMIC RIBOSOMAL PROTEINS OF GATEWAY BARLEY AND ITS MUTANT

KIRPA R. KOUNDAL* and SAUL ZALIK

Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada

(*Received* 6 *February* 1981)

Key Word Index—*Hordeum vulgare*; Gramineae; barley; electrophoresis; ribosomes; ribosomal proteins.

Abstract—The proteins of the cytoplasmic ribosomes isolated from dry embryos of Gateway barley and its virescens mutant were compared by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE). The monosomes of both the lines gave similar patterns with 60 basic proteins. Upon dissociation of the monosomes, for the mutant, the basic proteins of the large subunits migrated more slowly than those of the normal and lacked three proteins but had three additional spots. Also, the proteins of the small subunits differed. The mutant lacked three of the proteins present in the normal but had three additional spots. Therefore, the large and small subunits contained a total of 34 and 41 basic proteins, respectively, in both the lines. There were several spots with identical electrophoretic mobilities in the small and large subunits of these two lines.

INTRODUCTION

Comparative biochemical investigations of Gateway barley and its virescens mutant have revealed several characteristic differences. Although the mutation is due to a single nuclear gene [1,2] that manifests itself by low pigment levels, deficiency of some chloroplast proteins and poorly developed plastids, which with time corrected themselves [3,4]. It has been implied that the production of crystalline prolamellar bodies in etioplasts is related to the presence of ribosomes [5,6] and studies from many laboratories have established that both chloroplastic and cytoplasmic ribosomes are involved in the synthesis of chloroplast lamellar proteins and of ribulose-1,5-bisphosphate carboxylase oxygenase [7–9]. Since the virescens mutant of Gateway is a single gene recessive nuclear mutant [2], it is possible that the symptoms of mutant are related to the deficiency or defect in structure and function of the cytoplasmic ribosomes. Thus, we have earlier examined the characteristics of ribosomes and ribosomal subunits [10] and in this paper, we report the studies on the ribosomal proteins from the wild type and its mutant.

RESULTS AND DISCUSSION

2D-PAGE of ribosomal proteins from barley embryos proteins of monosomes

At least three separate ribosomal preparations were made for each of the normal and mutant barley embryos and separate PAGE runs were carried out for each preparation. The protein patterns of individual runs were similar and were, therefore, used to compare ribosomal proteins of the normal and mutant on the basis of their mobilities. Each of the stained spots was assigned a number depending upon the mobility of the individual proteins in second dimension [11].

As seen from Fig. 1, the 80S protein pattern of normal (A) and mutant (B) were similar. Both resolved into 60 basic ribosomal proteins. There have been a number of reports which pointed out that different ribosomal proteins may show different intensities upon staining. Since ribosomal proteins of *E. coli* have been found in less than one copy per particle [14, 15], the mere occurrence of the faint spots were regarded as indicating the presence of ribosomal proteins. The majority of proteins of these monosomes gave complicated patterns, therefore these monosomes were dissociated and proteins of their subunits were compared.

Proteins of small subunits

Although the conditions of PAGE were standardized as much as possible in terms of gel concentration, amount of protein applied, electrophoresis buffer, the duration of run, the current applied and the temperature, it can be seen that the patterns of the proteins (Fig. 2) of the 40S ribosomal subunits of the normal and mutant were different. Both the normal and mutant contained 34 basic proteins but the mutant lacked proteins 26, 29 and 30 and had three additional spots a, b and c. Proteins of small subunits of both normal and mutant appeared as bands upon PAGE and this may have been due to overloading. Sherton and Wool [12] reported band formation upon PAGE of ribosomal proteins.

Proteins of large subunits

As with the small subunits, the proteins of the mutant 60S migrated more slowly than those of the normal subunits in the second dimension. Both normal and mutant contained 41 basic proteins (Fig. 3) of which a few were highly stained. The mutant lacked proteins 4, 5 and 13 but had three additional proteins a, b and c.

In comparing the protein patterns of the small and large subunits, it can be seen that the proteins of the 40S differ considerably from proteins of their respective 60S subunits in number, mobility and band formation. These

* Present address: Water Technology Centre, Indian Agricultural Research Institute, New Delhi 110012, India.

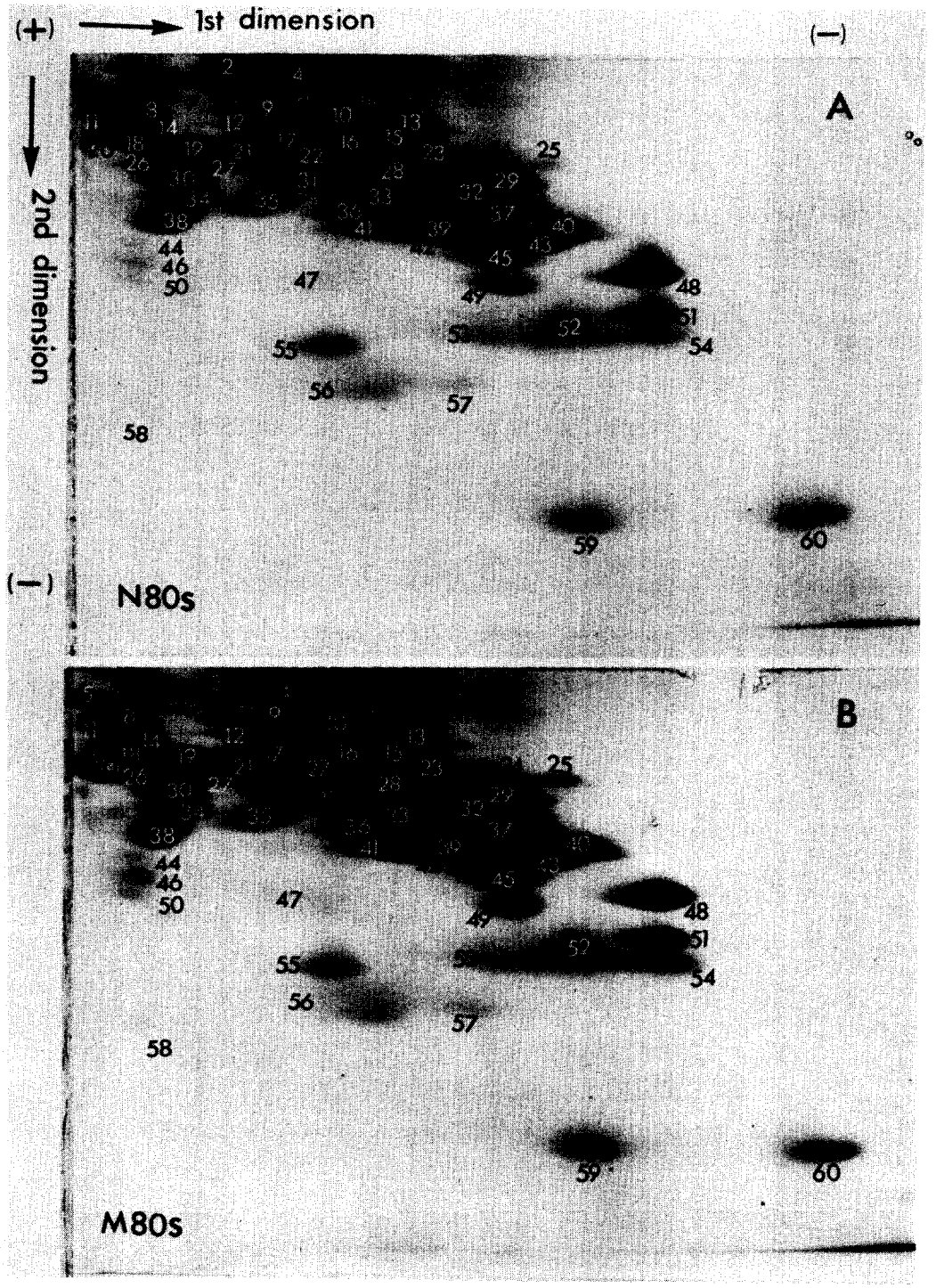

Fig. 1. Two-dimensional gel electrophoretograms of the proteins from cytoplasmic ribosomes from embryos of Gateway barley (A) and its mutant (B).

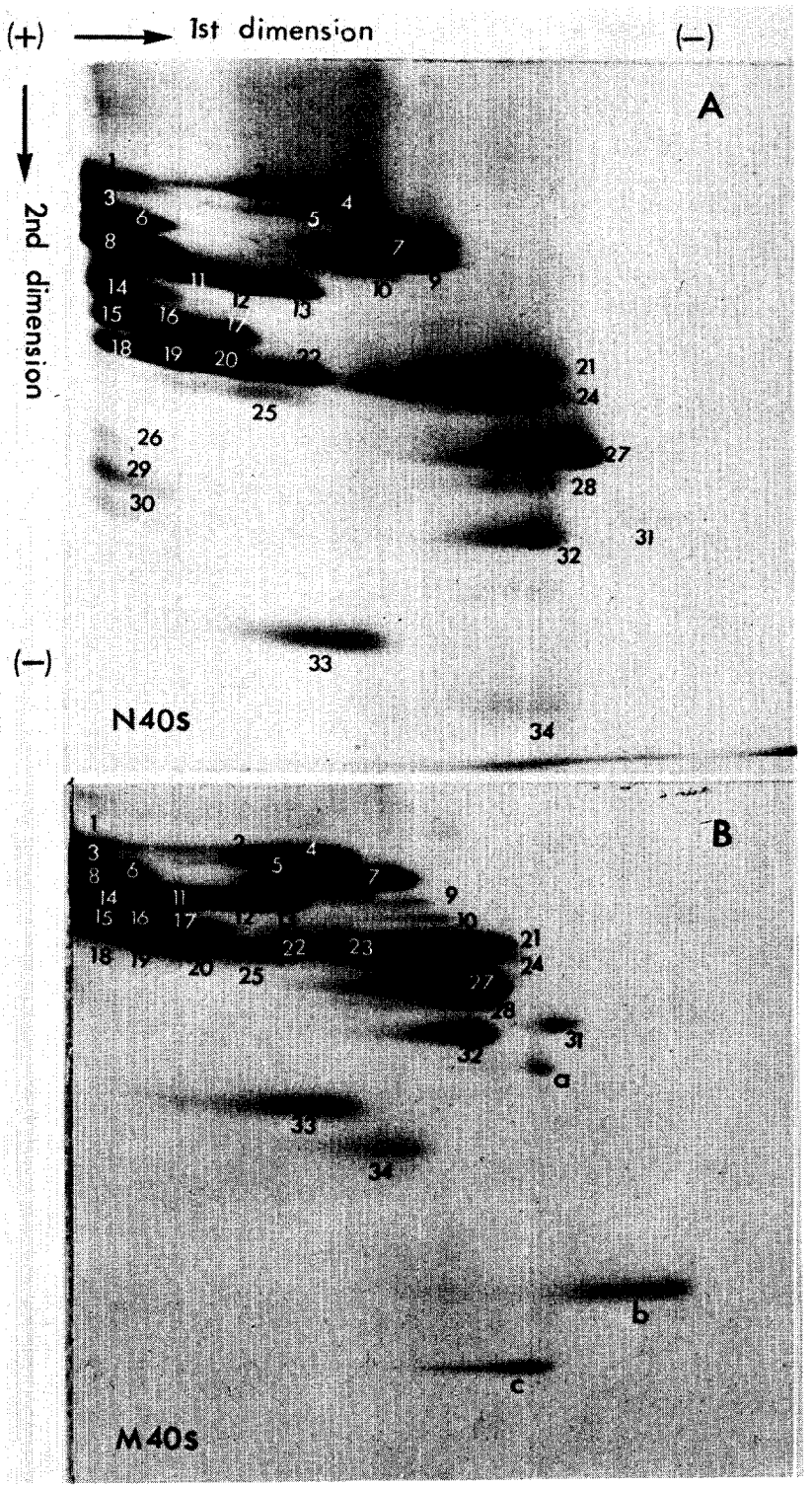

Fig. 2. Two-dimensional gel electrophoretograms of the proteins of the 40S subunits of cytoplasmic ribosomes from embryos of Gateway barley (A) and its mutant (B).

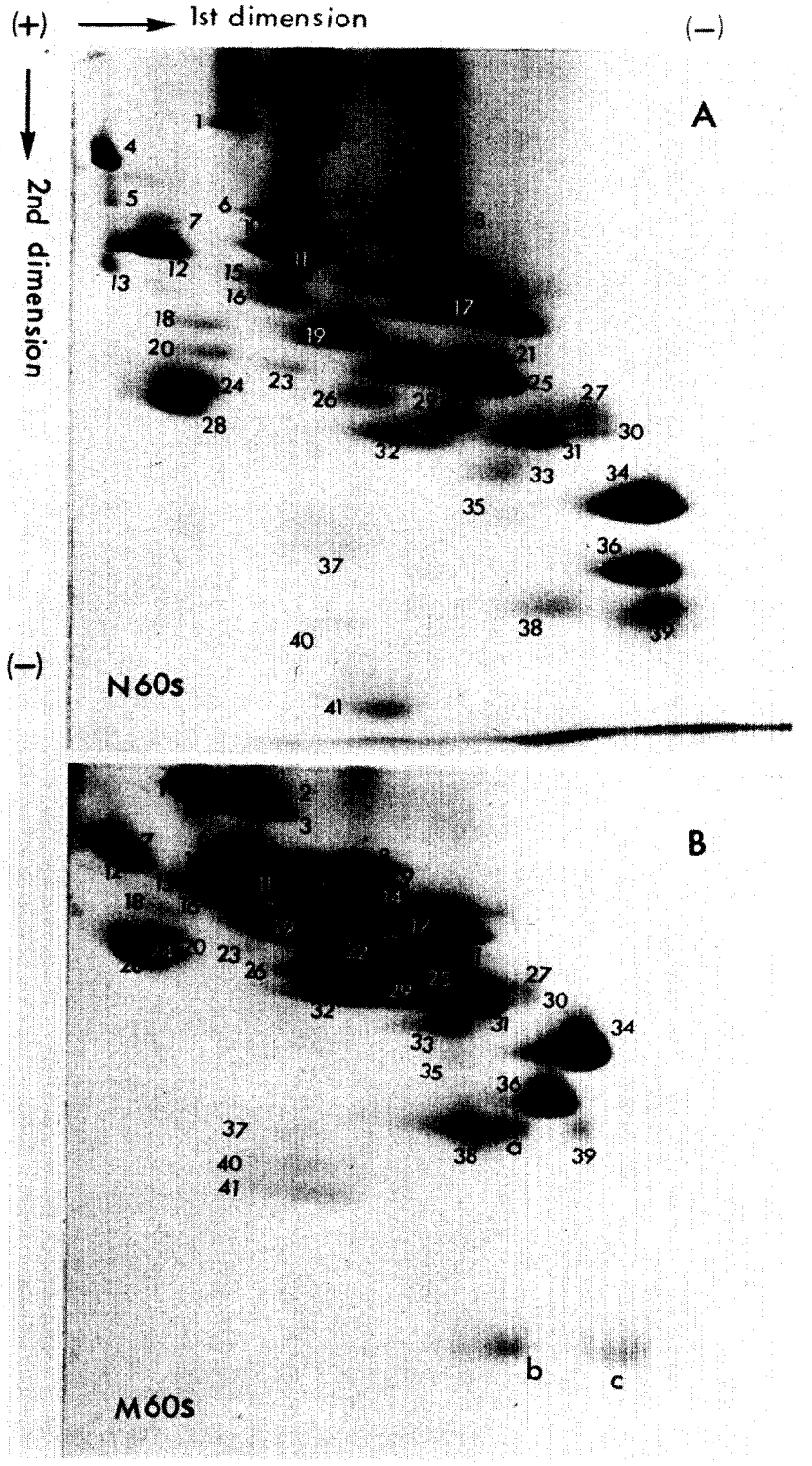

Fig. 3. Two-dimensional gel electrophoretograms of the proteins of the 60S subunit of cytoplasmic ribosomes from embryos of Gateway barley (A) and its mutant (B).

differences were observed for both the normal and mutant subunits. There were spots with identical PAGE mobilities in the small and large subunits but it is difficult to decide whether these represent identical proteins occurring in both the subunits or whether they represent proteins with identical migration. The summation of the basic proteins of both small and large subunits gave 75 proteins while monosomes had only 60 protein spots for both the normal and mutant. This may have been due to poor resolution of a large number of proteins present in the monomeric ribosomes or due to identical mobilities of proteins from the two subunits. Nagabhusan *et al.* [13] reported 31 cytoplasmic ribosomal protein from 4.5 old leaves of Gateway barley and Gualerzi *et al.* [14] observed 32–40 proteins in small subunits and 44–55 proteins in large subunits of pea seedlings. The lower number of proteins obtained [13] might have been due to the lower Mg^{2+} concentration used for the extraction of ribosomal proteins since a high level of Mg^{2+} ion (0.1 M) has been found to increase the yield of ribosomal proteins [15].

It is difficult to explain the loss of three proteins in each of the ribosomal subunits of the mutant. The organization of different proteins in the ribosomes is very complicated [16] therefore, it may be possible that some proteins may be substituted by others for particular functions. Regarding the additional proteins found in the mutant, these may be weakly bound to the normal ribosomal subunits and thus washed off during the preparation procedure but in the mutant it is possible that due to mutation these proteins remain attached to the particle. It is difficult to say whether they are true ribosomal proteins or not. Since these differences were not evident from protein patterns of the monosomes, the possibility cannot be ruled out that during the dissociation procedure alterations occurred in a few related proteins of the two subunits and accounted for the differences in mobility.

Despite these differences between the respective subunits, both homologous and heterologous subunits of two lines were able to reassociate when mixed in a ratio of 1:1 A_{260} units to form active monosomes [10] which showed that the ribosomal subunits contained a complement of proteins sufficient to support [^{14}C]phenylalanine incorporation activity. It is thus suggested that additional studies with the chloroplast and cytoplasmic ribosomal proteins of barley seedlings and its mutant would be useful in obtaining further information, using a more sensitive technique for exact comparison.

EXPERIMENTAL

Isolation of ribosomes and ribosomal subunits. Ribosomes and ribosomal subunits from embryos of Gateway barley and its mutant were isolated by the method reported earlier [10].

Isolation of ribosomal proteins. Proteins were extracted from the ribosomes and ribosomal subunits with 66% HOAc in the presence of 0.1 M $MgCl_2$. After lyophilization, the ribosomal proteins were dissolved in the first-dimensional PAGE buffer containing 6 M urea and protein concn was determined by the method of ref. [17].

PAGE of ribosomal proteins. (i) First-dimensional PAGE. For the separation of the ribosomal proteins, the method of ref. [18] as modified in ref. [19] was used. First-dimension PAGE was carried out in 4% acrylamide gel in 15 × 0.6 cm (i.d.) glass tubes. Pre-electrophoresis was done at 4 mA/tube for 1 hr using 6 M urea, 0.15 M borate, 6.5 mM EDTA and 0.12 M trizma (pH 8.6). Then 0.5 mg of ribosomal protein sample (*ca* 300 μl) in 10% sucrose was placed on top of the gels and methyl green was used as a tracking dye. PAGE was carried out at 5 mA/tube towards the cathode for 15–16 hr. The gels were equilibrated in urea–acetate buffer (6 M urea, 0.012 N KOH, pH 4.6) for 20 min before laying them on top of the second-dimensional gel.

(ii) Second-dimensional PAGE. The second-dimensional PAGE was carried out in 18% gel in the same apparatus as previously described in ref. [19]. Immediately after equilibrating, the 1-dimensional (1-D) gel was laid horizontally on the 2-dimensional (2-D) gel and additional 2-D gel was poured over the 1-D gel. The gel soln was kept in ice before pouring and the apparatus was kept at 4° for 1 hr prior to pouring to avoid rapid polymerization of the polyacrylamide. Methylene blue was used as a tracking dye. PAGE was carried towards the cathode at 120 V with the current limited to 220 mA at 4° for 18 hr.

Staining and destaining. After electrophoresis, the gel slabs were stained with 0.1% Coomassie Brilliant Blue R 250 in destaining solvent for 3 hr with occasional agitation. The gel slabs then destained electrophoretically by applying 220 mA current for 6 hr in a destaining apparatus containing destaining solvent (10% MeOH and 10% HOAc).

Acknowledgements—We are grateful to Mr. Barry Zytruk for technical assistance. K. R. Koundal was the recipient of a National Overseas Scholarship from the Government of India. The investigation was supported by a grant from the National Research Council of Canada.

REFERENCES

1. Walker, G. W. R., Dietrich, J., Miller, R. and Kasha, K. (1963) *Can. J. Genet. Cytol.* **5**, 200.
2. Stephansen, K. and Zalik, S. (1971) *Can. J. Botany* **49**, 49.
3. MacLachlan, S. and Zalik, S. (1963) *Can. J. Botany* **41**, 1053.
4. Jhamb, S. and Zalik, S. (1973) *Can. J. Botany* **51**, 2147.
5. Gunning, B. E. S. and Jagoe, M. P. (1967) in *Biochemistry of Chloroplasts* (Goodwin, T. W., ed.) p. 665. Academic Press, New York.
6. Wellburn, A. R., Quail, P. H. and Gunning, B. E. S. (1977) *Planta* **134**, 45.
7. Eytan, G. and Ohad, I. (1970) *J. Biol. Chem.* **247**, 122.
8. Bar-Nun, S. and Ohad, I. (1977) *Plant Physiol.* **59**, 161.
9. Ellis, R. J. (1979) *Trends Biochem. Sci.* **4**, 241.
10. Koundal, K. R., Cohan, A. S. and Zalik, S. (1980) *Physiol. Plant.* **50**, 194.
11. Kaltschmidt, E. and Wittman, H. G. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1276.
12. Sherton, C. C. and Wool, I. G. (1972) *J. Biol. Chem.* **247**, 4460.
13. Nagabhushan, N., Gulyas, A. and Zalik, S. (1974) *Plant Physiol.* **53**, 516.
14. Gualerzi, C., Janda, H. G., Passow, H. and Stoffler, G. (1974) *J. Biol. Chem.* **249**, 3347.
15. Hardy, S. J. S., Kurland, C. G., Voynow, P. and Mura, G. (1969) *Biochemistry* **8**, 2897.
16. Nomura, M. (1973) *Science* **179**, 864.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
18. Kaltschmidt, E. and Wittman, H. G. (1970) *Analyt. Biochem.* **36**, 401.
19. Jones, B. L., Nagabhushan, N., Gulyas, A. and Zalik, S. (1972) *FEBS Letters* **23**, 67.