Biochem. J. (1968) **106**, 689
Printed in Great Britain

Chloroplast and Cytoplasmic Low-Molecular-Weight Ribonucleic Acid Components of the Leaf of *Vicia faba* L.

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(*Received* 29 *August* 1967)

1. A method for the extraction of plant nucleic acids and their separation on methylated-serum-albumin–kieselguhr columns is described. It is demonstrated that the characteristics of the elution profiles of material from the same source are consistently reproducible. 2. Major dissimilarities were found in the elution profiles of nucleic acids from root and from leaves of *Vicia faba* L. These dissimilarities were confirmed by polyacrylamide-gel electrophoresis. 3. Four distinct types of low-molecular-weight RNA were demonstrated to be present in leaves, clearly distinguished by their behaviour when chromatographed on methylated-serum-albumin–kieselguhr columns. (*a*) Both cytoplasmic and chloroplast ribosomes contained a low-molecular-weight RNA, and these components were distinct from each other. (*b*) The chloroplast possessed a unique 'soluble' RNA (i.e. RNA that is not precipitated by centrifugal forces that sediment ribosomes) which was not present in the rest of the cell. (*c*) A soluble component, probably transfer RNA, was found in both the chloroplasts and in the cytoplasm. 4. The components distinguishable by methylated-serum-albumin–kieselguhr column chromatography could not be distinguished by sucrose-density-gradient centrifugation.

The demonstration of the central role of the nucleic acids in the regulation of cellular activities has stimulated numerous investigations of their nature in plants. Of specific interest is the chloroplast, an organelle that is now thought to be semi-autonomous. Recent critical investigations suggest that the chloroplasts of the mature leaf possess all the biochemical components of a classical genetical system, i.e. DNA (for a summary see Kirk & Tilney-Bassett, 1967), RNA (Jacobson, Swift & Bogorad, 1963) and a protein-synthesizing system (Sissakian, Philippovich, Svetailo & Aliyev, 1965; Spencer, 1965). There is also evidence for a chloroplast DNA-dependent RNA polymerase (Kirk, 1964*a*,*b*), and light-stimulated DNA synthesis has been demonstrated in developing leaves (Bogorad, 1967).

The further characterization of the chloroplast system requires a comparison of the nucleic acids of green and non-green tissue and of the nucleus, cytoplasm and chloroplasts of green cells. Several differences in the chemical and physical properties of nuclear and chloroplast DNA, and also in cytoplasmic and chloroplast ribosomes in leaves of higher plants, are reported and discussed below.

However, apart from the report published while this work was in progress by Wollgiehn, Ruess & Munsche (1966) that the pattern of elution profiles from methylated-serum-albumin–kieselguhr columns is different for chloroplast and cytoplasmic ribosomes, no investigation of the different low-molecular-weight RNA components in leaves has been made. A comparison of the low-molecular-weight RNA components from different parts of the same plant and from leaves of different species is reported in the present paper.

In work concerned with the characterization of plant nucleic acids (Ingle, Key & Holm, 1965; Chroboczek & Cherry, 1966; Waters & Dure, 1966; Wollgiehn *et al.* 1966), a popular method involves the fractionation of the samples on methylated-serum-albumin–kieselguhr columns, since a clear separation of the major types of nucleic acid can be achieved with this technique. However, the quality of the separations is very variable, depending on the precise method used. Before a comparative study of nucleic acids can be made it is therefore necessary to establish to what extent differences between elution profiles of different samples are valid or are merely a consequence of small variations in technique. In the present paper evidence for the validity and reproducibility of the elution profiles from methylated-serum-albumin–kieselguhr columns is given, and the use of such

column chromatography in the study of the intracellular localization of RNA components of *Vicia faba* leaves is reported. A preliminary note of some of the results of this investigation has been published (Dyer & Leech, 1967).

METHODS

Extraction of nucleic acids. The method of Kirby (1965) was slightly modified for the isolation of the nucleic acid. Plant tissue was homogenized in an Omni-Mixer at full speed for 30sec. at room temperature in a mixture of a 6% (w/v) sodium 4-aminosalicylate solution and 80% (w/w) aqueous phenol (1–5g. of tissue to 10ml. of sodium 4-aminosalicylate and 20ml. of phenol). The homogenate was immediately filtered through two layers of muslin and the filtrate centrifuged at 2000*g* for 10min. at 0°. The upper aqueous phase was removed with a Pasteur pipette and the nucleic acids in it were precipitated by addition of 2vol. of ice-cold ethanol and storage at −20° for 4hr. The precipitate was collected by centrifugation and washed twice by resuspending it in 10ml. of ice-cold ethanol–water–10% (w/v) NaCl (25:10:1, by vol.). Modifications of this method were tried as follows: (1) bentonite (1mg./ml.) was added to the sodium 4-aminosalicylate solution; (2) 8-hydroxyquinoline (0·1%) was added to the phenol; (3) the 4-aminosalicylate was replaced by sodium dodecyl sulphate (1%, w/v); (4) the material was extracted for a longer period (15min.). None of the changes improved nucleic acid extraction, and they were not adopted.

Methylated-serum-albumin–kieselguhr chromatography. The methylated-serum-albumin–kieselguhr columns were prepared as described by Mandell & Hershey (1960), with the improvements suggested by Sueoka & Yamane (1962) and Yamane & Sueoka (1963). The columns were 1·8cm. in diameter and about 5cm. in height. A maximum of 2mg. of nucleic acid was applied in 40ml. of starting buffer (0·3M-NaCl–50mM-sodium phosphate buffer, pH6·7). The columns were then washed with the starting buffer until the u.v. absorption of the effluent at 254mμ was constant. The nucleic acids were eluted with a 200ml. linear gradient of NaCl (0·3–1·15M) in 50mM-sodium phosphate buffer, pH6·7, at a flow rate of 1ml./min., maintained constant by means of a peristaltic pump. The column effluent was continuously monitored at 254mμ with a u.v. analyser constructed on the principles described by Begg (1961), and the elution profiles of the nucleic acid were recorded on a 5mv Honeywell recorder with an 11in. span and a chart speed of 1in./7·5min. The output signal of the u.v. analyser was approximately linear up to an extinction value of 0·6. The salt concentration was determined by measuring the refractive index of the column eluate at regular intervals during elution. Spectra of nucleic acid-containing solutions from the columns were determined with an Optica CF4 DRNI spectrophotometer with nucleic acid-free column eluate as a solvent reference solution.

Co-chromatography of nucleic acid fractions from organelles with total leaf nucleic acid was accomplished as follows. The column eluate containing the nucleic acid from the organelles was diluted with an equal volume of phosphate buffer to lower the NaCl concentration, and the nucleic acid was then readsorbed on a fresh column. A suitable quantity (about 1mg.) of total leaf nucleic acid was next absorbed on to the same column, and the elution was carried out as usual.

Polyacrylamide-gel electrophoresis. RNA for electrophoresis was recovered from the column eluates and freed from phosphate by adding an equal volume of ethanol and passing the mixture through a 5g. cellulose column (Whatman CF11) previously equilibrated with a solvent consisting of equal volumes of ethanol and 0·1M-NaCl. The column was washed with 75ml. of solvent, and after the eluate had drained to the column surface the RNA that was adsorbed on the cellulose was eluted with 0·1M-NaCl. The RNA was then precipitated overnight at −20° by the addition of 2vol. of ethanol. Electrophoresis was carried out as described by Richards, Coll & Gratzer (1965), and the gels were scanned at 450mμ with the SP.590 scanning accessory to a Unicam SP.500 spectrophotometer.

Preparation of subcellular components. Structurally intact chloroplasts were isolated from destarched leaves of *Vicia faba* (var. Sutton) from plants between 2 and 3 weeks old by the method of Leech (1964). The grinding medium was 0·4M-sucrose in phosphate buffer (67mM, pH7·3). The bacterial surface viable count of chloroplast preparations was not more than 5×10^3/ml. after incubation on full nutrient agar at 26° for 48hr.

Cytoplasmic ribosomes from leaves were sedimented by centrifugation at 105000*g* for 6hr. of the supernatant that remained after sedimentation of larger subcellular particles at 40000*g* for 15min. After the precipitation of nuclei, chloroplasts, chloroplast fragments, mitochondria and cell-wall debris (40000*g*), purified bentonite (1mg./ml., prepared as described by Petermann & Pavlovec, 1963), 2-mercaptoethanol (final concentration 6mM) and $MgCl_2$ (final concentration 5mM) were added to the medium. Although considerable precautions were taken in the preparation of 'cytoplasmic' ribosomes to avoid rupture of the larger organelles, contamination of the cytoplasmic ribosomal fraction with some of the ribosomes from broken organelles cannot be avoided. This fraction will therefore be referred to as 'cytoplasmic'. The medium in which 'cytoplasmic' ribosomes were prepared consisted of 0·4M-sucrose and 50mM-tris buffer, pH7·4. Root ribosomes were prepared in a similar way except that the $MgCl_2$ was included from the start. Chloroplast ribosomes were derived exclusively from intact chloroplasts that were osmotically ruptured by suspending them in a medium containing 50mM-tris buffer, pH7·4, and 5mM-$MgCl_2$. Before centrifuging, sucrose (0·4M) was added. Lamellae were sedimented at 40000*g* for 6hr.

Preparation of aminoacyl-RNA. Intact chloroplasts were lysed in a solution containing: 10mM-tris–HCl, pH7·8; 14mM-magnesium acetate; 60mM-KCl; 6mM-2-mercaptoethanol. To this, 0·5ml. of M-tris–HCl, pH7·8, 0·1ml. of 0·14M-magnesium acetate, 0·2ml. of 20mM-ATP, 0·6ml. of 75mM-phosphoenolpyruvate, 0·05mg. of pyruvate kinase (Sigma Chemical Co., St Louis, Mo., U.S.A.), 2μl. of 2-mercaptoethanol and 0·1ml. of radioactive algal-protein hydrolysate (5μc, batch 28; The Radiochemical Centre, Amersham, Bucks.) were added. After incubation at 37° for 15min., an equal volume of phenol was added and the nucleic acid in the aqueous phase precipitated and chromatographed on a methylated-serum-albumin–kieselguhr column. Each low-molecular-weight fraction was dried separately on to a planchet and its radioactivity determined with a Nuclear–Chicago D47 gas-flow counter. For

determination of background radioactivity, appropriate samples of column effluent in which there was no nucleic acid were collected, dried and counted.

RESULTS

Elution profiles of nucleic acids from various tissues and species. The elution profile from a methylated-serum-albumin–kieselguhr column of broad-bean leaf nucleic acid is shown in Fig. 1. The leaf nucleic acid may be separated into three main subfractions. In order of elution they are designated here as low-molecular-weight RNA, DNA and high-molecular-weight RNA. Within the low-molecular-weight RNA further subfractions may be distinguished. The first and main fraction (*A*) is followed by a second (*B*), which is evident in Fig. 1 as a shoulder to the first peak. After the second peak two components (*C* and *D*) are eluted, which appear as discrete peaks in the profile. The DNA fraction is also subfractionated, but the components are not easily distinguishable in the extinction profile. In order of elution, the two high-molecular-weight RNA fractions were confirmed to be light and heavy ribosomal components by individual analyses on sucrose density gradients. The u.v. spectrum (from 220 to 320mμ) of each fraction was consistent with its being a nucleic acid. In Fig. 2 is shown a characteristic u.v. extinction curve for total leaf low-molecular-weight RNA.

To demonstrate the consistency of the features of the elution profile of the broad-bean nucleic acid, three equal samples of the same total nucleic acid sample from broad-bean leaf were chromatographed; the resultant elution profiles are shown in Fig. 3. Separate columns were used on consecutive days and the experiment was carried out 1 year after the sample was prepared the profile of which is shown in Fig. 1. The profiles are very similar, the main difference being the degree to which the second (*B*) low-molecular-weight RNA fraction is a discrete peak rather than a shoulder of the first peak. The third and fourth low-molecular-weight fractions (*C* and *D*), however, are very distinct in all the separations. A feature found on some occasions (Fig. 3c) is the presence of a small subfraction just preceding the bulk of the first fraction and another eluting between the low-molecular-weight RNA and DNA. Elution of the DNA and of the high-molecular-weight ribosomal RNA varies little.

Since most features in the elution profiles of the broad-bean leaf nucleic acid are constant, it is justifiable to compare them with elution profiles of the nucleic acid prepared from other parts of the same type of plant, or from different species, prepared and chromatographed in exactly the same way.

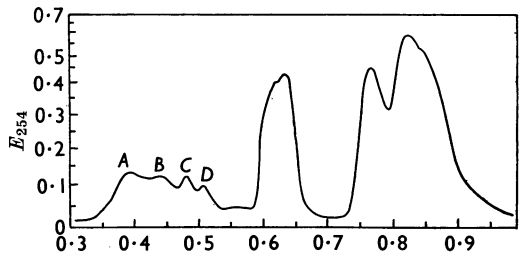

Fig. 1. Elution profile from the methylated-serum-albumin–kieselguhr column of total nucleic acid of *Vicia faba* leaf. Nucleic acid extraction and chromatography are described in the Methods section.

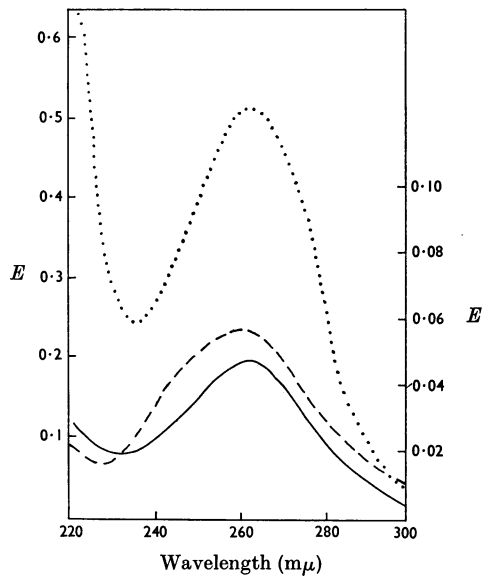

Fig. 2. Extinction curves. ——, Total leaf low-molecular-weight RNA; – – – –, chloroplast low-molecular-weight ribosomal RNA; ····, chloroplast soluble RNA. The spectra of the total leaf RNA and the chloroplast ribosomal RNA were determined in the eluate after their fractionation on methylated-serum-albumin–kieselguhr columns. Eluates derived from the columns before the elution of the nucleic acids were used as solvent reference solutions. The chloroplast soluble RNA was dissolved in 50mM-sodium phosphate buffer, pH 6·7, containing 0·3M-NaCl. The extinction values on the left-hand axis refer to the chloroplast soluble RNA and those on the right to the low-molecular-weight RNA of the total leaf and of the chloroplast ribosomes.

A comparison is made in Fig. 4 between the elution profiles of nucleic acids from broad-bean root, flower and etiolated leaves. A comparison with elution profiles from the green leaf (Fig. 1)

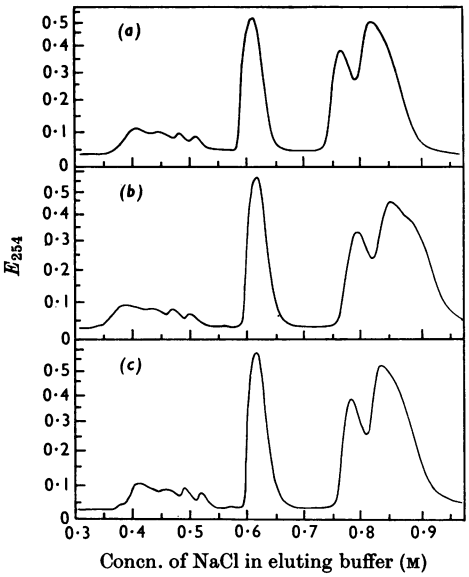

Fig. 3. Elution profiles of three equal portions of the sample of the total nucleic acids from broad-bean leaf chromatographed on separate columns on separate days.

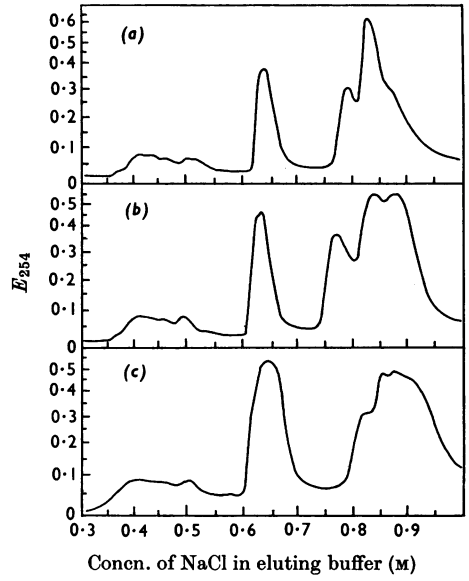

Fig. 5. Elution profiles of total nucleic acid from leaves of three species of angiosperms. (*a*) *Nicotiana tabacum*; (*b*) *Pisum sativum*; (*c*) *Zea mays*.

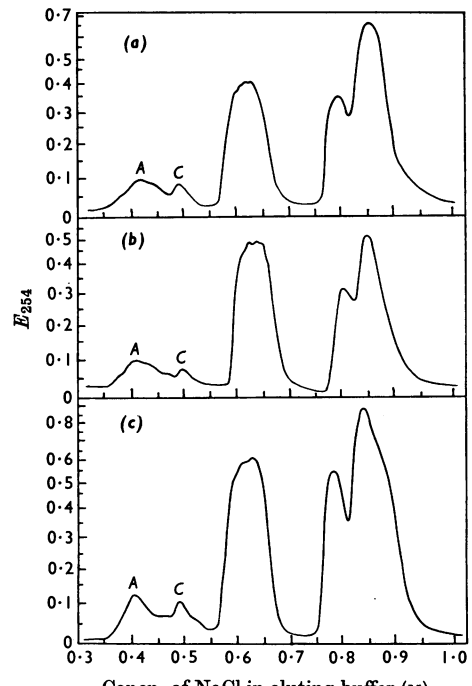

Fig. 4. Elution profiles of total nucleic acids of three different tissues of *Vicia faba* L. (*a*) Whole root; (*b*) whole flower; (*c*) etiolated leaf.

shows that the most obvious difference in the non-green tissues is the smaller number of low-molecular-weight fractions that can be discerned. Although the first low-molecular-weight RNA fraction (*A*) is found in all three tissues, the second (*B*) is either present in a much smaller amount or is completely absent. In addition, instead of the prominent third and fourth subfractions that can be distinguished in the normal leaf, only one such component occurs in the other tissues, although there is a trace of the second component in etiolated leaves. A difference that is not quite so clearly defined is in the relative amounts of heavy and light ribosomal RNA; proportionately more of the heavy RNA is present in non-green tissues than in the green leaves.

Minor dissimilarities are found between the nucleic acids from the leaves of different types of plant. In Fig. 5 the profiles of three different angiosperms are shown. Although their overall characteristics are similar, they differ in several details. The most significant of these is that in tobacco the low-molecular-weight peaks corresponding to the third and fourth of the broad bean are less clearly separated and in the pea there is only one peak in this region, which is considerably more prominent than in either broad bean or tobacco. The high-molecular-weight ribosomal RNA fractions from the different plants also differ in their chromatographic properties. The light and heavy components are separated in tobacco (as they are in

the broad bean), but in the pea the heavy ribosomal RNA is occasionally further subfractionated (Fig. 5*b*). This feature, although not consistently reproducible, clearly demonstrates that the heavy ribosomal fraction is heterogeneous. Of the profiles, the most distinctive is that of the monocotyledon, *Zea mays* (sweet corn). Only two low-molecular-weight fractions are clearly visible, but there is a partial subfractionation of the second. The high-molecular-weight ribosomal components are also not as clearly fractionated into light and heavy components as they are in the profiles of nucleic acid from the other plants.

Reliability of the elution profiles. The reproducibility of the elution profiles from methylated-serum-albumin–kieselguhr column chromatography of nucleic acids prepared from samples of the same plant material on several different occasions is remarkably good (Fig. 3). This suggests that the consistent differences found between profiles of nucleic acids from different species (Fig. 5) and from different tissues of the same species (Fig. 4) are indeed significant. In the comparison of green and non-green tissue the differences are particularly marked in the region of low-molecular-weight RNA.

Before further characterization and intracellular-localization studies of these low-molecular-weight components, it is necessary to establish beyond all reasonable doubt that they are not artifacts due to bacterial contamination or to the action of native ribonuclease in the preparations.

The distinct chloroplast components could not be artifacts due to bacterial contamination because surface viable counts on complete nutrient agar at 26° for 48hr. gave a value of not more than 10^3/ml. (corresponding to about 0·15mg. of chlorophyll). This count did not increase if the chloroplasts were incubated for 3hr. at 30° before analysis for bacterial contaminants.

In addition it is also extremely unlikely that these low-molecular-weight components are the results of ribonuclease degradation of high-molecular-weight RNA. Ribonuclease activity characteristically gives rise to a range of RNA components intermediate in size between high-molecular-weight and low-molecular-weight RNA species, and not to a limited number of components with similar physical properties as was consistently found in the present investigation. Each nucleic acid fraction was isolated on a minimum of three occasions, and on chromatography gave consistently similar profiles. In addition, if the low-molecular-weight components were produced by the action of ribonuclease on high-molecular-weight RNA it would be expected that the proportion of low-molecular-weight components would increase with time, would be lowered by the presence of ribonuclease inhibitors and would also be affected by the particular method of extraction used. None of these characteristic manifestations of ribonuclease activity was found. In Fig. 6(*b*) is shown an elution profile in which ribonuclease degradation has taken place (RNA from cytoplasmic ribosomes that had been centrifuged for 1½hr. and then for 18hr. in the absence of bentonite and 2-mercaptoethanol); it is entirely different from any of the other elution profiles shown here, e.g. Fig. 6(*c*). Also, to account for the characteristic additional leaf components, a specific leaf ribonuclease giving rise to distinct low-molecular-weight components would have to be postulated. It would seem reasonable on the basis of this evidence to preclude the possibility that the low-molecular-weight components described here are produced as a result of ribonuclease activity.

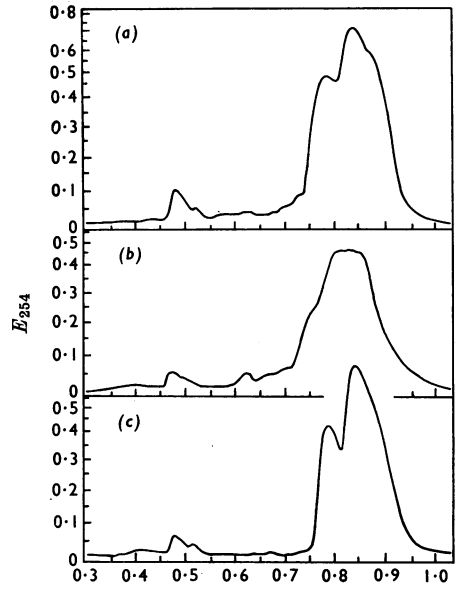

Fig. 6. Elution profiles of the nucleic acids of 'cytoplasmic' ribosomal RNA prepared in the absence and presence of a ribonuclease inhibitor. Preparative methods are described in the text, except that high-speed centrifugation was for (*a*) 1½hr. without bentonite and 2-mercaptoethanol, (*b*) 18hr. without bentonite and 2-mercaptoethanol and (*c*) 5hr. with bentonite and 2-mercaptoethanol.

Elution profiles of nucleic acids from intact chloroplasts. Since the outstanding difference between root and leaf tissues is the presence of chloroplasts in the leaves, the possibility that the chloroplast low-molecular-weight RNA components are unlike those of the rest of the cell was next investigated.

In Fig. 7 the elution profiles of the nucleic acids of broad-bean leaves (Fig. 7*b*) and of intact chloroplasts (Fig. 7*a*) are compared; clearly they differ

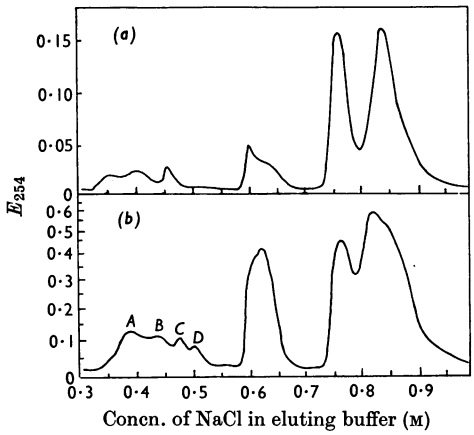

Fig. 7. Elution profiles from methylated-serum-albumin–kieselguhr columns of the total nucleic acids of (*a*) isolated intact chloroplasts and (*b*) whole leaves of *Vicia faba* L. Chloroplast isolation and nucleic acid extraction are described in the Methods section.

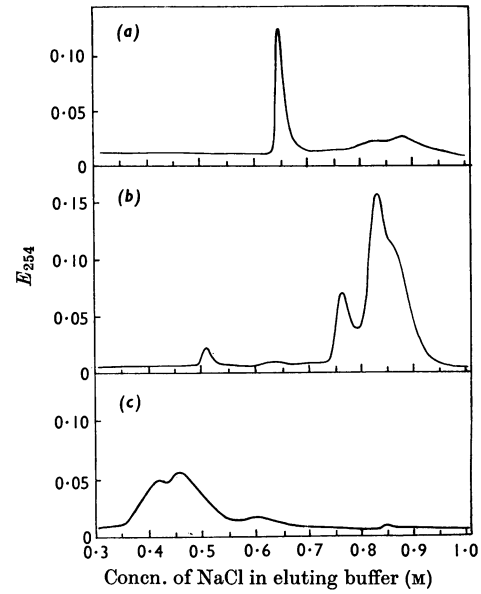

Fig. 8. Elution profiles of the total nucleic acids from different fractions of the chloroplast: (*a*) lamellae; (*b*) chloroplast ribosomes; (*c*) supernatant after ribosomal precipitation. Chloroplasts were isolated according to Leech (1964) and broken osmotically in cold 20mM-tris buffer, pH 7·4, for 30min. (*a*) Lamellae were sedimented by centrifugation at 40000*g* for 10min. and washed three times in tris buffer. (*b*) Chloroplast ribosomes were sedimented from the 40000*g* supernatant at 105000*g* for 5hr. (*c*) Supernatant was from the 105000*g* spin.

markedly from one another. Four low-molecular-weight fractions can be distinguished in the total leaf, but only three are apparent in the chloroplast. On comparison of the profiles, the first chloroplast component appears to correspond to the first of the leaf components, the second to the second, and the third of the chloroplast components to either the third or fourth of the leaf components. Also, the chloroplast DNA is a relatively smaller proportion of the combined nucleic acid complement than in the total leaf, and is eluted in a different and characteristic manner. Finally, the heavy and light ribosomal RNA fractions differ greatly in their relative amounts and in the distinction of their chromatographic separation in the whole leaf and in the chloroplast.

The u.v. extinction spectra of chloroplast low-molecular-weight ribosomal RNA and chloroplast soluble RNA are shown in Fig. 2.

Location of chloroplast nucleic acids. The characteristic intrachloroplast distribution of the chloroplast nucleic acids is shown in Fig. 8; the chloroplasts were fractionated by differential ultracentrifugation into lamellae (Fig. 8*a*), ribosomes (Fig. 8*b*) and soluble fractions (Fig. 8*c*) (see the Methods section). All the DNA remained firmly bound to the lamellae, but nearly all the RNA could be removed from the lamellae by thoroughly washing them. As well as the light and heavy high-molecular-weight ribosomal RNA, the third low-molecular-weight chloroplast component was found exclusively in the ribosomal fraction. This could be conclusively shown when all the ribosomes had been sedimented. The 90min. at 105000*g* frequently allowed for sedimentation of ribosomes by other workers was insufficient for chloroplast ribosomes and therefore the suspensions were centrifuged for 5hr. at this speed. Both the first and second low-molecular-weight RNA fractions remained in the supernatant 'soluble' fractions after this centrifugation.

The marked difference between the soluble RNA of the chloroplast and that of the cytoplasm is shown in Fig. 9. Although two broad peaks are evident in the elution profiles of the soluble RNA of the chloroplasts there is only one in the cytoplasm. (Some fractionation of the second-eluting chloroplast-specific component had been observed on other occasions.) The first-eluting component, which has chromatographic properties in common with those of the cytoplasmic soluble RNA and which is possibly common to both chloroplasts and cytoplasm, is likely to be the 'transfer' RNA of which there are a large number of species.

Characterization of chloroplast low-molecular-weight RNA. To characterize further the soluble components of the chloroplast low-molecular-weight

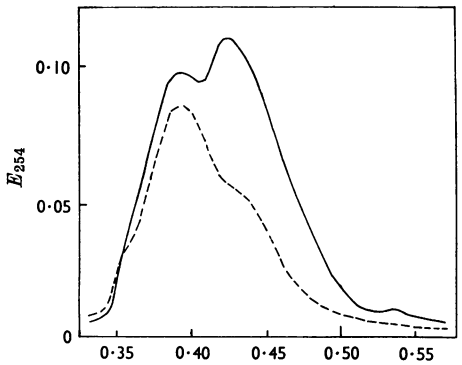

Fig. 9. Profiles of soluble RNA fraction (eluted between 0·35 M- and 0·55 M-NaCl) from chloroplasts (——) and from the cytoplasm (– – – –) of the broad-bean leaf (*Vicia faba*).

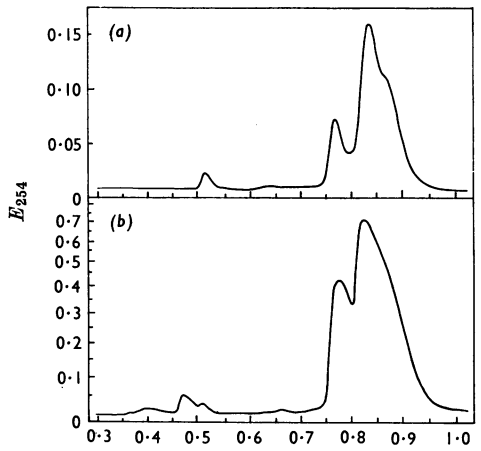

Fig. 10. Elution profiles of total RNA derived from (*a*) chloroplast ribosomes and (*b*) 'cytoplasmic' ribosomes.

RNA, ^{14}C-labelled aminoacyl RNA was prepared. Intact chloroplasts were lysed in 5 ml. of a solution containing 10 mM-tris–hydrochloric acid, pH 7·8, 14 mM-magnesium acetate, 60 mM-potassium chloride and 6 mM-2-mercaptoethanol. The lysed chloroplasts were incubated in the presence of a radioactive protein hydrolysate as described in the Methods section, and the nucleic acid was isolated and chromatographed on a methylated-serum-albumin–kieselguhr column.

The relative radioactivity due to ^{14}C-labelled aminoacyl-RNA formation in the three chloroplast low-molecular-weight fractions in order of elution was 168, 39 and 67 counts/min. respectively (background 24 counts/min.). Thus the most extensive amino acid reaction occurs with the first fraction, suggesting that the majority of the transfer RNA is in this fraction.

In Fig. 10 it is seen that low-molecular-weight RNA is also associated with the cytoplasmic ribosomes, but a small amount of soluble RNA also remains attached to these ribosomes, in contrast with the chloroplast ribosomes. This soluble RNA is eluted just before a distinct low-molecular-weight component of the type found in the chloroplast ribosomes. Contamination of the preparation by chloroplast ribosomes is indicated by the appearance in the profile (Fig. 10*b*) of a small peak just following that of the low-molecular-weight RNA of the cytoplasmic ribosomes.

The low-molecular-weight RNA components from cytoplasmic and chloroplast ribosomes were shown to be different by co-chromatographing them with total leaf nucleic acid (Fig. 9). When the low-molecular-weight ribosomal component of the cytoplasm is co-chromatographed with total leaf nucleic acid the amount of the third low-molecular-weight fraction is increased (Fig. 11*a*). In contrast, addition of the low-molecular-weight chloroplast ribosomal component increases the amount of the fourth low-molecular-weight fraction in the total leaf sample (Fig. 11*b*). Addition of both together to the total leaf fraction gives increases in two regions of the profile. Confirmation that the low-molecular-weight ribosomal component is of cytoplasmic origin is derived by co-chromatographing the low-molecular-weight ribosomal RNA of root tissue with total leaf nucleic acid. Again the third low-molecular-weight component is increased (Fig. 11*c*).

The two additional low-molecular-weight RNA components identified above in the elution profiles of total nucleic acids from isolated chloroplasts of *Vicia faba* must definitely be located within the chloroplast. The chloroplast suspensions used here have previously been shown by electron microscopy (Leech, 1964) to consist almost entirely of intact chloroplasts; 90% of the profiles in such preparations are of membrane-bounded chloroplasts, 5% of chloroplast fragments and 5% of mitochondria. One low-molecular-weight RNA component is located in the chloroplast ribosomes (Fig. 8*b*) and co-chromatography shows that it is distinct from the similar but not identical low-molecular-weight RNA component of the 'cytoplasmic' ribosomes. The other distinct low-molecular-weight RNA is a 'soluble' component. To make sure that the fractionation of the low-molecular-weight RNA was not the result of some peculiar feature of the methylated - serum - albumin – kieselguhr column separation, and to try to improve the resolution of the fractions, the low-molecular-weight RNA of roots and of leaves were recovered from the columns

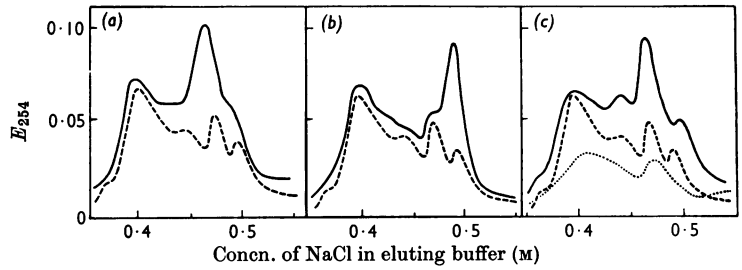

Fig. 11. Profiles of the total leaf low-molecular-weight RNA (eluted between 0·35 M- and 0·55 M-NaCl) with (——) and without (– – – –) the addition of low-molecular-weight RNA of isolated ribosomes from different sources: (*a*) ribosomes from the 'cytoplasmic' fraction of leaf (*Vicia faba*); (*b*) ribosomes from intact chloroplast (*Vicia faba*); (*c*) ribosomes from root (*Vicia faba*). The dotted line (····) in (*c*) is the elution profile of the total low-molecular-weight RNA of the root.

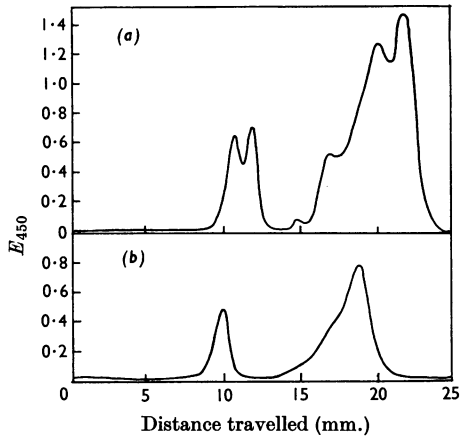

Fig. 12. Densitometer traces of polyacrylamide-gel electrophoretograms, in 10% gels, of low-molecular weight-RNA from broad bean: (*a*) leaf; (*b*) root. The material was prepared and electrophoresis carried out as described in the text.

and subjected to polyacrylamide-gel electrophoresis (Fig. 12). There are only two clearly discernible fractions in electrophoretograms of low-molecular-weight RNA from the root: one component of low mobility and the other of higher mobility. In the leaf electrophoretograms there are two slow-moving components that can be resolved, and these correspond to the third and fourth column fractions; the RNA of greater mobility is also subfractionated. The additional components in the latter fraction (compared with the root nucleic acid) appear to originate from the chloroplast. Thus polyacrylamide-gel electrophoresis confirms the demonstration by methylated-serum-albumin–kieselguhr column chromatography that a greater number of low-molecular-weight RNA components are present in the leaf than in the root of *Vicia faba* L.

The low-molecular-weight components partially resolved on the methylated-serum-albumin–kieselguhr columns and on electrophoresis cannot, however, be resolved by conventional sucrose-density-gradient centrifugation. All low-molecular-weight RNA components are found exclusively in the slowest-sedimenting of the three fractions distinguishable in the gradients. This would suggest that the methylated-serum-albumin–kieselguhr column chromatography gives more definitive separation of the low-molecular-weight RNA components than does sucrose-density-gradient centrifugation.

Base-ratio analysis of the two new low-molecular-weight RNA chloroplast components is at present not possible because of the extremely low concentration of each component within the chloroplasts and because of difficulties of preparing in bulk intact chloroplasts, freed completely from cytoplasmic contamination. [The relative size of the extinction values for each component can be clearly seen in the elution profiles. Intact chloroplasts contain between 3 and 5% total RNA by weight (Kirk & Tilney-Bassett, 1967) and each of the low-molecular-weight components represents about one-fiftieth of the total RNA].

DISCUSSION

The two additional low-molecular-weight RNA components described here have not been reported previously from elution profiles of nucleic acids from isolated chloroplasts. However, there have been a number of reports of the occurrence in animal cells and bacteria of low-molecular-weight RNA with chromatographic properties similar to those of the low-molecular-weight ribosomal components described here (Galibert, Lelong, Larsen & Boiron, 1966; Watson & Ralph, 1966). This RNA has been

studied in some detail and is now thought to be a structural component of ribosomes, since it is synthesized at the same rate as ribosomal RNA of high molecular weight and is present in approximately equimolar proportions with this (Bachvarov & Tongur, 1966; Galibert *et al.* 1966; Jackson, 1966). Although this RNA is similar in base composition and size to the transfer RNA (their relative sedimentation coefficients are 5s and 4s), it contains a relatively small amount of pseudouridylic acid and no methylated bases, has no amino acid-acceptor activity and is found associated exclusively with the ribosomes.

In numerous studies of plant nucleic acids in which methylated - serum - albumin – kieselguhr columns have been used (e.g. Ingle *et al.* 1965; Chroboczek & Cherry, 1966), a low-molecular-weight RNA component is distinguishable in the elution profiles and is referred to as a 'soluble RNA'. It is very likely to be equivalent to the low-molecular-weight ribosomal RNA reported in the present paper. Chroboczek & Cherry (1966) claim that this RNA remains in the supernatant after ribosome precipitation but clearly, from the results reported here, it does not. Our findings are supported by those of Wollgiehn *et al.* (1966), who also report a low-molecular-weight RNA associated with plant ribosomes, but they were unable to establish whether or not it had also occurred in the 'soluble' fraction.

The evidence presented here that the chloroplasts from the leaves of *Vicia faba* possess a low-molecular-weight ribosomal RNA and a soluble RNA distinct from those found in other parts of the cell would support the view that chloroplasts are semi-autonomous. Evidence is accumulating that other nucleic acid components of the chloroplast also differ from those of the rest of the cell (for a summary see Kirk & Tilney-Bassett, 1967). Differences in chemical and physical properties of nuclear and chloroplast DNA in leaves of higher plants have frequently been reported (Kirk, 1963; Chun, Vaughan & Rich, 1963; Green & Gordon, 1965, 1966; Kislev, Swift & Bogorad, 1966). In addition, leaf chloroplast ribosomes appear to differ from cytoplasmic ribosomes in size (Jacobson *et al.* 1963; Gunning, 1965), sedimentation coefficients (Lyttleton, 1962; Sissakian *et al.* 1965; Clark, Matthews & Ralph, 1965; Spencer, 1965; Boardman, Francki & Wildman, 1965; Stutz & Noll, 1967) and the sedimentation coefficients of their sub-units (Svetailo, Philippovich & Sissakian, 1967).

In leaves of several species two classes of ribosome can be distinguished with sedimentation coefficients of about 80s and about 70s respectively, the smaller class of particles apparently being restricted to the chloroplast. The actual S values vary in different reports, and this may reflect technical or real differences in S values of different species. Spencer & Whitfield (1966) reported a further difference between chloroplast and cytoplasmic ribosomes in spinach; they detected two high-molecular-weight RNA components (sedimenting at 16s and 23s) in the 'cytoplasmic' ribosomes but only one RNA component (16s) in chloroplast ribosomes. In contrast, Pollard, Stemler & Blaydes (1966) found both 18s and 28s RNA components in both types of ribosome. The work of Stutz & Noll (1967) clearly indicates the presence of both 16s and 23s RNA in bean chloroplast ribosomes; this RNA could not be distinguished on sedimentation properties from bacterial ribosomal RNA. RNA of cytoplasmic ribosomal origin, however, had S values of 16s and 25s, which correspond closely to the 16·5s and 24·7s given by Click & Tint (1967) for ribosomal RNA from etiolated pea shoots and potato tubers. We have also found two different high-molecular-weight RNA components (Fig. 4) in both chloroplast and 'cytoplasmic' ribosomes from *Vicia faba* leaves. The reason for the disparity between the results of Spencer & Whitfield (1966) and those of other workers may reflect some specific features in their extraction of RNA from spinach leaf chloroplast ribosomes since Wollgiehn *et al.* (1966), in contrast, found two distinct high-molecular-weight ribosomal RNA components from this source. Although they have been sought, differences in base ratios of RNA of chloroplast and 'cytoplasmic' ribosomes of the order of those reported for *Euglena* (Brawerman, 1963) have not been found for leaves (Spencer & Whitfield, 1966; Wollgiehn *et al.* 1966; Pollard *et al.* 1966). It may be that chloroplast and cytoplasmic ribosomes are more similar in higher plants than in algae, but there is also a possibility that real differences may be obscured because of the constant presence of chloroplast ribosomes in the 'cytoplasmic' ribosomal fraction from leaves. The presence of similar but certainly not identical low-molecular-weight RNA components in chloroplast and cytoplasmic ribosomes reported here is therefore noteworthy.

From the algae *Euglena*, *Acetabularia* and *Chlamydomonas* there is also considerable evidence that differences exist between chloroplast and cytoplasmic nucleic acids (Gibor, 1966; Schiff & Epstein, 1966; Sager & Ishida, 1963; Eisenstadt & Brawerman, 1964; Brawerman, Pogo & Chargaff, 1962).

The nucleic acids of the chloroplast would therefore appear to differ in several respects from those of the rest of the cell, but the interrelationships of the different components during the differentiation and functional life of the cell await investigation.

We are very grateful to Miss M. Anne O. George and to Miss C. E. Poole for their skilled technical assistance during this work.

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