The Molecular Integrity of Chloroplast Ribosomal Ribonucleic Acid

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Instability of chloroplast rRNA has been observed with essentially all chloroplast RNA preparations. This paper describes experiments that show that, under normal conditions of preparation and fractionation, only the heavy chloroplast component (mol.wt. 1.1×10^6) is unstable, the light chloroplast rRNA (mol.wt. 0.56×10^6) and the cytoplasmic rRNA species (mol.wt. 1.3×10^6 and 0.70×10^6) being stable. The stability of the 1.1×10^6 -mol. wt. molecule varies with different plant species, as also does the size and the number of fragments produced. Cleavages in three particular regions of the molecule are very frequent within the range of tissues studied. The 1.1×10^6 -mol.wt. rRNA is, however, stabilized by the presence of Mg^{2+} during the preparation and fractionation of the RNA.

It has been established that chloroplasts contain rRNA species (Stutz & Noll, 1967) that differ in size from the cytoplasmic components (Loening & Ingle, 1967). The two chloroplast rRNA species (mol.wt. 1.1×10^6 and 0.56×10^6) are not, however, present on fractionation in a 1:1 molecular ratio of heavy to light components, as expected for rRNA, and as usually observed for the cytoplasmic components (mol.wt. 1.3×10^6 and 0.70×10^6) (Loening & Ingle, 1967). In fact, the results of analysis of chloroplast rRNA from spinach, tobacco, radish, pea and tomato leaves led to the suggestion that chloroplast ribosomes contained only one species of RNA, the light component (Spencer & Whitfield, 1966). The low recovery of the 1.1×10^6 -mol.wt. rRNA together with the presence of additional smaller components, suggested degradation of the heavy chloroplast rRNA. Studies using total RNA prepared from radish cotyledons indicated that in this tissue, under the conditions of preparation, the 1.1×10^6 -mol.wt. rRNA was breaking into two components, with mol.wt. of 0.4×10^6 and 0.7×10^6 , the latter having the same mobility on electrophoresis as the cytoplasmic 0.7×10^6 -mol.wt. rRNA (Ingle, 1968). After correction for such breakdown of the 1.1×10^6 -mol.wt. rRNA, estimated from the amount of the 0.4×10^6 -mol.wt. rRNA present, the calculated ratios of the amounts of the rRNA species were reasonably close to the ratio of their molecular weights. Newly synthesized chloroplast rRNA, as measured by the incorporation of a radioactive precursor, was stable under conditions in which the older, accumulated, RNA was

unstable (Ingle, 1968). This suggested that the newly synthesized 1.1×10^6 -mol.wt. rRNA was stable, but that with time, something happened which resulted in the 1.1×10^6 -mol.wt. rRNA being broken down into the two smaller pieces.

This paper describes experiments that show conclusively that, under normal conditions, only the 1.1×10^6 -mol.wt. rRNA is unstable, that the stability of 1.1×10^6 -mol.wt. rRNA varies with different plant species and that the size and number of fragments produced varies with different plant species. It is also shown that the 1.1×10^6 -mol.wt. rRNA may be stabilized by the presence of bivalent cations e.g. Mg^{2+} or Ca^{2+} .

MATERIALS AND METHODS

Plant tissue. Radish seedlings (*Raphanus sativus* L. var. Cherrybelle) were grown under a 16h photoperiod of 1600 ft-candles at 22.5°C as described by Ingle, Joy & Hageman (1966). Cotyledons were used after 5-7 days growth. Spinach (*Spinacia oleracea*), broad bean (*Vicia faba*), French bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), maize (*Zea mays*), swisschard (*Beta vulgaris*, var. cicla) and *Pinus sylvestris* were grown under normal greenhouse conditions, and the young leaves were used for preparation of chloroplasts and nucleic acid. Sterile conditions were not employed, since the bacterial contamination level present on the leaf material was such that the amount of bacterial RNA was 0.1 to 0.01% of the chloroplast rRNA (see Ingle, 1968). *Chlamydomonas reinhardtii* was grown in 1% peptone in tap water, and *Euglena gracilis* var. *saccarophila*, a bleached mutant, was grown heterotrophically on 0.1% sodium acetate, 0.1% beef extract, 0.2% yeast extract, 0.2% Bactotryptone and 0.001% $CaCl_2$.

Preparation of total nucleic acid. Total nucleic acid was prepared by homogenizing 1g of tissue in a pestle and

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mortar with 5 ml of detergent medium [1% tri-isopropyl-naphthalene sulphonate, 6% *p*-aminosalicylate, 50 mM-NaCl (or KCl), 10 mM-tris-HCl, pH 7.4]; EDTA (sodium salt) (10 mM) or MgCl_2 (10 mM) was added to the detergent medium as indicated in the text. The homogenate was shaken with an equal volume of phenol mixture (phenol containing 10% *m*-cresol and 0.1% 8-hydroxyquinoline, saturated with 10 mM-tris-HCl, pH 7.4). After centrifugation the lower phenol layer was removed, the aqueous layer plus the interface was made to 0.5 M-NaCl, and re-extracted with a further volume of phenol mixture. The upper aqueous phase was removed after centrifugation and re-extracted with an equal volume of phenol mixture, and then the nucleic acids were precipitated from the final aqueous phase by the addition of 2 vol. of ethanol and storage at 0°C for 16 h. The precipitate was washed once in 80% ethanol containing 0.5% sodium dodecyl sulphate, and then reprecipitated from 0.15 M-potassium acetate-0.5% sodium dodecyl sulphate.

Preparation of chloroplast RNA. Chloroplasts were isolated by using a modification of the method of Cockburn, Walker & Baldry (1968). Plants were destarched in the dark for 24–48 h before chloroplast isolation. Laminæ (25 g) were homogenized for 3–5 s in a Polytron (Northern Media Supply Co.) or 'VirTis' homogenizer containing 100 ml of ice-cold medium [0.33 M-sorbitol, 5 mM- MgCl_2 , 1 mM-NaCl, 50 mM-Hepes (*N*-2-hydroxyethylpiperazine-*N'*-ylethane-2-sulphonic acid), 2 mM-sodium isoascorbate adjusted to pH 6.5 at 2°C]. The homogenate was squeezed through two layers of muslin, filtered through a further eight layers and centrifuged at 2°C from rest to 4000 *g* to rest in approximately 90 s. The supernatant was decanted and the pellet gently resuspended by using a glass rod and a small piece of absorbent cotton wool in 5 ml of ice-cold resuspending medium (0.33 M-sorbitol, 1 mM-dithioerythritol, 50 mM-Hepes, 10 mM- MgCl_2 , pH 7.8, at 20°C). The rapid centrifugation step was repeated and the resulting chloroplast pellet was resuspended and solubilized with Triton X-100 (2% final concentration in 30 mM-KCl, 10 mM-tris-HCl, pH 7.4, 10 mM- MgCl_2). After centrifugation at 10000 *g* for 10 min at 0°C, nucleic acid was prepared from the supernatant by shaking it with an equal volume of the double-strength detergent mixture, followed by phenol deproteinization. EDTA or Mg^{2+} was included in the preparation as described in the text. The ethanol precipitate was washed with 80% ethanol containing 0.5% sodium dodecyl sulphate and was fractionated without further reprecipitation.

Fractionation of the RNA. The RNA preparations were fractionated by polyacrylamide-gel electrophoresis (Loening, 1967). Two electrophoresis buffers were used, one containing EDTA [36 mM- NaH_2PO_4 , 30 mM-tris, 1 mM-EDTA (sodium salt), 0.2% sodium dodecyl sulphate] and the other magnesium [30 mM-tris, 16 mM-HCl, 0.1 mM-EDTA (sodium salt), 2 mM-magnesium acetate, 0.1% sodium dodecyl sulphate]. The final nucleic acid preparation was dissolved in one of these buffers, containing 5% (w/v) sucrose, to give a final RNA concentration of approx. 1 mg/ml. The nucleic acid sample (10–25 μg) was applied to a 2.4% gel and fractionated by electrophoresis for 3–4 h at 50 V (EDTA buffer, 6 mA/8 cm gel; Mg^{2+} buffer, 3 mA/gel). The gels were scanned at 265 nm in a Joyce-Loebl Chromoscan. The RNA components are referred to in terms of their molecular weights (Bishop, Claybrook

& Spiegelman, 1967; Loening, 1968). All molecular weights were derived from the EDTA electrophoresis with HeLa rRNA as the standard (mol.wt. 1.75×10^6 and 0.70×10^6). Although the molecular weight of the heavy chloroplast rRNA varied from 1.01×10^6 – 1.12×10^6 with the range of plants studied (see Fig. 7), for simplicity in the text this component is called the 1.1×10^6 -mol.wt. rRNA. To study the stability of a particular RNA species in the two buffer systems, the component was localized from a 1:1 scan of the gel, that region of the gel was then excised and placed on top of a second gel for electrophoresis in the other buffer.

RESULTS

The range of stability of the 1.1×10^6 -mol.wt. rRNA is indicated with the plant species shown in Fig. 1. With RNA prepared from seedlings of *Pinus*

Fig. 1. Fractionation of total RNA from pine, spinach and swisschard. Total nucleic acid was prepared and fractionated in the presence of EDTA, as described in the Materials and Methods section. (a) *Pinus*; (b) spinach; (c) swisschard.

Fig. 2. Effect of Mg^{2+} and EDTA on the fractionation of total RNA from radish cotyledons. (a) Total nucleic acid was prepared and fractionated in the presence of EDTA. (b) Fractionation of the EDTA preparation by magnesium electrophoresis. (c) Fractionation of a magnesium preparation by magnesium electrophoresis.

sylvestris the ratios of the cytoplasmic (mol.wt. $1.3 \times 10^6/0.7 \times 10^6$) and chloroplast (mol.wt. $1.1 \times 10^6/0.56 \times 10^6$) rRNA species were 2.05 and 1.90 respectively, indicating little or no breakdown of the 1.1×10^6 -mol.wt. component. In the case of spinach the ratio of the chloroplast RNA species was 1.18, suggesting that about half of the 1.1×10^6 -mol.wt. rRNA had broken down. Many green tissues give fractionation profiles similar to that of

Fig. 3. Stability of radish chloroplast RNA on magnesium and EDTA fractionation. RNA was prepared from radish cotyledon chloroplasts in the presence of Mg^{2+} . (a) Fractionation by magnesium electrophoresis. (b) Fractionation by EDTA electrophoresis. (c) EDTA electrophoresis of the 1.04×10^6 -mol.wt. rRNA from (a). (d) EDTA electrophoresis of the 0.56×10^6 -mol.wt. rRNA from (a).

spinach. Very little 1.1×10^6 -mol.wt rRNA was present in the fractionation of swisschard RNA, the chloroplast ratio being 0.2. The presence of

nuclease inhibitors during the preparation of total nucleic acid did not improve the yield of 1.1×10^6 -mol.wt. rRNA; in fact bentonite decreased the yield of this component. The bentonite was, however, suspended in EDTA solution, and EDTA without bentonite similarly gave a low yield of 1.1×10^6 -mol.wt. rRNA. Replacement of the EDTA in the detergent mixture with Mg^{2+} increased the amount of the 1.1×10^6 -mol.wt. rRNA. This indication of the importance of Mg^{2+} necessitated a reconsideration of the fractionation procedure, since the electrophoresis buffer contained EDTA. More 1.1×10^6 -mol.wt. rRNA was present when total nucleic acid was fractionated in a magnesium buffer. When RNA was extracted from radish cotyledons in the presence of EDTA, and fractionated in the EDTA buffer, the ratio of the chloroplast components was 0.50 (Fig. 2a). When the same preparation was fractionated in the magnesium buffer more 1.1×10^6 -mol.wt. rRNA was present, the ratio being 0.94. When RNA was extracted in the presence of Mg^{2+} , and then fractionated in the magnesium buffer, the ratio of the chloroplast RNA species was 2.02. It was, therefore, possible to stabilize the chloroplast 1.1×10^6 -mol.wt. rRNA by having Mg^{2+} present during the preparation and fractionation of the nucleic acid.

The fractionation of magnesium preparations of RNA by magnesium-gel electrophoresis was not, however, very satisfactory. The magnesium buffer gave less resolution than the EDTA buffer, and the magnesium preparations contained more non-nucleic acid material than EDTA preparations. Together, these two factors prevented reasonable loading of the gel. The problem of magnesium stability of the 1.1×10^6 -mol.wt. rRNA was therefore studied by using RNA preparations derived from purified chloroplasts. Such preparations were free from contamination and were consequently better resolved. When RNA was prepared from radish chloroplasts in the presence of Mg^{2+} , and fractionated in the magnesium buffer, the chloroplast RNA species were present in a ratio of 2:1 and the cytoplasmic components were present as minor contaminants (Fig. 3a). Fractionation of the same RNA preparation in the EDTA buffer resulted in the almost complete loss of the 1.1×10^6 -mol.wt. rRNA, with increases in the amount of 0.70×10^6 and 0.43×10^6 -mol.wt. rRNA. To confirm

Fig. 4. Stability of broad-bean chloroplast RNA on magnesium and EDTA fractionation. RNA was prepared from broad-bean leaf chloroplasts in the presence of Mg^{2+} (a) Fractionation by magnesium electrophoresis. (b) Fractionation by EDTA electrophoresis. (c) EDTA electrophoresis of the 1.08×10^6 -mol.wt. rRNA from (a). (d) EDTA electrophoresis of the 0.57×10^6 -mol.wt. rRNA from (a).

that the 1.1×10^6 -mol.wt. rRNA was breaking into these two fragments, the 1.1×10^6 -mol.wt. rRNA peak from a magnesium gel was cut out and rerun on an EDTA gel. Under these conditions the 1.1×10^6 -mol.wt. rRNA from the magnesium gel

was resolved into three components, the remaining core, mol.wt. 1.1×10^6 , 0.70×10^6 and 0.43×10^6 (Fig. 3c). The core 1.1×10^6 -mol.wt. rRNA was stable to a further EDTA electrophoresis. The 0.56×10^6 -mol.wt. rRNA from the magnesium

Fig. 5. Effect of Mg^{2+} , Ca^{2+} and EDTA on the fractionation of spinach chloroplast RNA. RNA was prepared from spinach leaf chloroplasts in the presence of Mg^{2+} and fractionated by (a) magnesium-, (b) calcium-, and (c) EDTA-electrophoresis. (d) Fractionation in a buffer containing neither EDTA nor Mg^{2+} (30 mM-tris, 36 mM- NaH_2PO_4 , 0.2% sodium dodecyl sulphate).

fractionation ran as a single component on electrophoresis in EDTA (Fig. 3d).

The position of the break in the 1.1×10^6 -mol.wt. rRNA varied with preparations from different plants. Magnesium fractionation of magnesium-prepared RNA from broad-bean chloroplasts resulted in the 1.1×10^6 - and 0.56×10^6 -mol.wt. rRNA species (Fig. 4a). When this preparation of RNA was run in the EDTA buffer, the amount of the 1.1×10^6 -mol.wt. rRNA decreased with the appearance of two main components with molecular weights of 0.67×10^6 and 0.53×10^6 , plus several

minor components with molecular weights of 0.92×10^6 , 0.63×10^6 , 0.50×10^6 and 0.34×10^6 (Fig. 4b). When the 1.1×10^6 -mol.wt. rRNA component from the magnesium gel was rerun in EDTA it was resolved to give the core 1.1×10^6 -mol.wt. rRNA plus the 0.67×10^6 - and 0.53×10^6 -mol.wt. components (Fig. 4c). Again the 0.56×10^6 -mol.wt. rRNA appeared completely stable when rerun in the EDTA buffer (Fig. 4d). Fractionation of a magnesium preparation of RNA from spinach chloroplasts by EDTA electrophoresis did not show two major breakdown products, but a range of components

Fig. 6. Effect of EDTA on the preparation of chloroplast RNA. RNA was prepared from radish cotyledon chloroplasts in the presence of EDTA and fractionated by (a) EDTA- and (b) magnesium-electrophoresis. RNA was prepared from spinach leaf chloroplasts in the presence of EDTA and fractionated by (c) EDTA- and (d) magnesium-electrophoresis.

with molecular weights of 0.67×10^6 , 0.50×10^6 , 0.45×10^6 , 0.39×10^6 and 0.34×10^6 (Fig. 5c). The stabilization of the 1.1×10^6 -mol.wt. rRNA was not specific for Mg^{2+} , since Ca^{2+} could be substituted in the electrophoresis buffer (Fig. 5b). Neither was the presence of the EDTA in the buffer necessary for the breakdown of the component, since fractionation in a buffer containing no EDTA gave a profile very similar to the normal EDTA electrophoresis (Fig. 5d).

Breakdown of the 1.1×10^6 -mol.wt. rRNA was greater when radish chloroplast RNA was prepared in the presence of EDTA rather than Mg^{2+} . On EDTA fractionation essentially no 1.1×10^6 -mol.wt. rRNA remained, and in addition the excess of 0.7×10^6 -mol.wt. RNA had also disappeared, with the appearance of 0.49×10^6 - and 0.24×10^6 -mol.wt. components. The sizes of these two latter components, and their relative amounts, suggested that they had arisen from breakdown of the 0.7×10^6 -mol.wt. component. Other components, with molecular weights of 0.87×10^6 , 0.34×10^6 and 0.20×10^6 were also present (compare Figs. 6a and 3b). Fractionation of this EDTA preparation of RNA by magnesium electrophoresis yielded a similar picture except that the 0.49×10^6 - and 0.43×10^6 -mol.wt. components were not completely resolved (Fig. 6b). Similarly with spinach chloroplast RNA, EDTA fractionation of an EDTA preparation differed from that of the magnesium preparation (compare Figs. 6c and 5c). More of the 0.49×10^6 -mol.wt. RNA was present, plus the additional components of 0.84×10^6 - and 0.24×10^6 -mol.wt.

DISCUSSION

Instability of the 1.1×10^6 -mol.wt. rRNA has been observed in essentially all preparations of chloroplast RNA (see Loening & Ingle, 1967), and has caused difficulty in the interpretation of such studies (e.g. Spencer & Whitfield, 1966). Of the range of higher and lower plants studied (Loening & Ingle, 1967; Ingle & Burns, 1968) only *Pinus* and *Euglena* (Scott, Munns, Graham & Smillie, 1970) contained chloroplast RNA that was stable on EDTA electrophoresis. From quantitative studies of the RNA components from radish cotyledons it was concluded that the 1.1×10^6 -mol.wt. rRNA was cleaved into two pieces, of 0.70×10^6 - and 0.43×10^6 -mol.wt. This cleavage could, however, be prevented by the presence of Mg^{2+} during both the preparation and fractionation of the RNA. The specific cation requirements for the 1.1×10^6 -mol.wt. rRNA stabilization have not yet been studied, other than to show that Ca^{2+} acts in a similar way to Mg^{2+} . The magnesium effects described in this paper are not therefore specific to Mg^{2+} but are probably a general bivalent cationic

effect. Once it was possible to keep the 1.1×10^6 -mol.wt. rRNA intact, it could be shown that on removal of Mg^{2+} the 1.3×10^6 -, 0.70×10^6 - and 0.56×10^6 -mol.wt. rRNA species were all stable, whereas much of the 1.1×10^6 -mol.wt. rRNA dissociated into 0.70×10^6 - and 0.43×10^6 -mol.wt. components, in agreement with the earlier indirect conclusions (Ingle, 1968).

The amount of the 1.1×10^6 -mol.wt. rRNA remaining after EDTA fractionation varied not only from plant to plant (Fig. 1, Loening & Ingle, 1967), but also with the physiological state of the tissue. The percentage of 1.1×10^6 -mol.wt. rRNA that remained as core 1.1×10^6 -mol.wt. rRNA varied from 35 to 15% in experiments with young or old radish cotyledons respectively. The core 1.1×10^6 -mol.wt. rRNA could be re-electrophoresed in EDTA buffer with no further cleavage. Since it has been shown that newly synthesized 1.1×10^6 -mol.wt. rRNA, as measured by the incorporation of a radioactive precursor, is stable in the absence of Mg^{2+} (Ingle, 1968), the core 1.1×10^6 -mol.wt. rRNA is probably a measure of more recently synthesized molecules.

The stage in the RNA preparation and fractionation procedure at which the EDTA is added affects the end result. Whereas a magnesium preparation of radish chloroplast RNA contained 15 to 35% of core 1.1×10^6 -mol.wt. rRNA, an EDTA preparation contained essentially no core 1.1×10^6 -mol.wt. rRNA, and the 0.70×10^6 -mol.wt. breakdown component was further cleaved (compare Figs. 3b and 6a). This presumably indicates that different sites within the RNA molecule have different affinities for Mg^{2+} . Exposure of the chloroplasts to EDTA before magnesium preparation and fractionation did result in subsequent dissociation of the 1.1×10^6 -mol.wt. rRNA. The sensitivity of the 1.1×10^6 -mol.wt. rRNA towards EDTA also depended on whether RNA was prepared from total tissue or from isolated chloroplasts (compare Figs. 2a and 6a). Further, whereas magnesium fractionation of an EDTA preparation from total tissue usually improved the yield of 1.1×10^6 -mol.wt. rRNA, compared with EDTA fractionation (Fig. 2), it had no such stabilizing effect on the 1.1×10^6 -mol.wt. rRNA in EDTA preparations from isolated chloroplasts. These differences presumably reflect the ionic content of the tissue, much of the EDTA being effectively removed by chelation with other ions in preparations from total tissue, whereas more of the EDTA may be available for chelation of ions involved in RNA stability in chloroplast preparations.

Although the number and size of 1.1×10^6 -mol.wt. rRNA cleavage products vary in detail from plant to plant (e.g. Figs. 3, 4 and 5), the collection of values in Fig. 7 indicates that cleavage occurs

Fig. 7. Cleavage products of the chloroplast 1.1×10^6 -mol.wt. rRNA. The results obtained from fractionations, such as Figs. 3, 4, 5 and 6 are summarized to show the range of cleavage products from a variety of plant species. ■, Major fragments; □, minor components.

* Molecular weights determined relative to the cytoplasmic rRNA species of mol.wt. 1.3×10^6 and 0.70×10^6 .

Fig. 8. Effect of Mg^{2+} on the fractionation of total RNA prepared from *Euglena*. Total RNA was prepared from a bleached mutant of *Euglena* in the presence of Mg^{2+} , and fractionated by (a) magnesium- and (b) EDTA-electrophoresis.

frequently in three particular regions of the 1.1×10^6 -mol.wt. molecule to yield 0.7×10^6 - plus 0.4×10^6 -mol.wt. components, 0.9×10^6 - plus 0.2×10^6 -mol.wt. components or a single 0.5×10^6 - 0.6×10^6 -mol.wt. product from a median cleavage. With radish RNA the 0.70×10^6 - plus 0.43×10^6 -mol.wt. components account for better than 90% of the loss of 1.1×10^6 -mol.wt. rRNA. In other plants cleavage occurs at two (French bean) or three (pea) positions within the 1.1×10^6 -mol.wt. rRNA. A minor 0.3×10^6 -mol.wt. component, which is not accounted for on the basis of the three cleavage

regions described, is present in many of the species studied.

Cleavage of the 1.1×10^6 -mol.wt. rRNA could result simply from nuclease action during the extraction process. However, the nuclease involved would have to be highly specific, both for the 1.1×10^6 -mol.wt. rRNA, since the other three rRNA species are not hydrolysed, and also for one or two positions within the 1.1×10^6 -mol.wt. rRNA. It would also be necessary for the Mg^{2+} to unite the two or more fragments into the intact configuration. Since newly synthesized 1.1×10^6 -mol.wt. rRNA is

stable under these conditions, it seems more likely that the cleavage occurs *in vivo* in the ribosome. The newly assembled ribosome presumably contains the intact 1.1×10^6 -mol.wt. rRNA, and with time the RNA is cleaved at one or two particular points, the specificity for which could be conferred by the configuration of the RNA within the 50S ribosomal subunit. The cleavage products would be essentially fixed within the ribosome structure, allowing Mg^{2+} to maintain the intact configuration. The Mg^{2+} may act as a direct linkage between two fragments or, more likely, it could maintain the integrity of the 1.1×10^6 -mol.wt. molecule by means of its effect on the secondary and tertiary structure of the molecule. Removal of the Mg^{2+} by chelation with EDTA would result in separation of the fragments, that could not be reassembled in solution by the addition of Mg^{2+} .

There are several more specific examples of unstable rRNA species in the literature. The large RNA component of the cytoplasmic ribosome of *Euglena* is unstable (Brawerman & Eisenstadt, 1964; Zeldin & Schiff, 1967). Breakdown of this 1.3×10^6 -mol.wt. rRNA is largely prevented by magnesium electrophoresis (Fig. 8), suggesting that this particular instability is similar to that of the chloroplast 1.1×10^6 -mol.wt. rRNA. A noteworthy situation exists with *Amoeba*, where under normal conditions of extraction and fractionation two rRNA species were obtained, but when the ionic strength of the electrophoresis buffer was decreased the heavy component disappeared (Loening, 1968). Although most bacteria contain a stable 1.1×10^6 -mol.wt. rRNA it has been reported that certain conditions of preparation of RNA from *E. coli*, resulted in the conversion of 1.1×10^6 -mol.wt. rRNA into the 0.56×10^6 -mol.wt. component. This breakdown was prevented by the presence of Mg^{2+} (Midgley, 1965). The magnesium stabilization of *E. coli* is completely stable to EDTA electrophoresis (Loening & Ingle, 1967; Loening, 1968). The

1.1×10^6 -mol.wt. rRNA is very unstable in *Rhodospseudomonas spheroides*, which was initially reported to contain only the light (0.56×10^6 -mol.wt.) rRNA (Lessie, 1965). Recently 0.53×10^6 -mol.wt. and 0.42×10^6 -mol.wt. rRNA components have been isolated from the large ribosomal subunit, and pulse-labelling experiments also showed the presence of 1.1×10^6 -mol.wt. rRNA, which was presumed to give rise to the smaller components (Marrs & Kaplan, 1970).

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