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CHROMATOGRAPHIC PURIFICATION OF CYTOPLASMIC RIBOSOMES FROM PEA PLANTS

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SUMMARY

A chromatographic procedure was developed for the purification of ribosomes. Young pea plants were ground in a solvent containing polyethylene glycol, magnesium acetate, sucrose, and tris(hydroxymethyl)aminomethane–acetic acid buffer (pH 7). The slurry was poured onto a column of cellulose and sand and the impurities washed out using a solvent containing magnesium acetate, sucrose, and tris(hydroxymethyl)-aminomethane–acetic acid buffer (pH 7). The ribosomes were eluted by omitting magnesium acetate from the developing solvent. The effluent was centrifuged at 40000 × *g* for 10 min. To achieve a higher degree of purification and concentration of the ribosomes, the chromatographic procedure was repeated with the supernatant. The solvent used for the grinding medium was used to eliminate more undesirable components from the eluate containing the ribosomes. This method completely separates chloroplasts and most of the other unwanted impurities from the ribosomes. The final preparation contained highly purified ribosomes, as evidenced by ultraviolet spectra, sedimentation constants and electron micrographs.

INTRODUCTION

A chromatographic procedure was developed by VENEKAMP AND MOSCH[1] for the purification of a number of rod-shaped plant viruses. After addition of a solution containing polyethylene glycol (PEG) to homogenates of freshly infected leaves, the viruses and chloroplasts were retained by cellulose adsorbents. Impurities were washed out with a similar solution. The virus was eluted by decreasing the PEG content of the developing solvent.

VENEKAMP *et al.*[2] reported the purification of some spherical viruses by the use of a similar procedure. These viruses have a size comparable to that of ribosomes. An attempt was therefore made to isolate ribosomes from plant material by employing the same procedures used for virus isolation. This method has several advantages besides its simplicity, such as the high purity that can be attained[3] and the complete recovery of the isolated viruses[1]. According to VENEKAMP AND MOSCH[4] these results may be due to the stabilizing effect of polyethylene glycol. This phenomenon had already been mentioned by ALBERTSSON[5] and ALBERTSSON AND BALTSCHEFFSKY[6].

MATERIALS AND METHODS

Plant material

Pea plants, *Pisum sativum* L. var. Rondo, were grown to a height of 5 to 10 cm. The upper parts including the first unfolded leaves were harvested.

Chromatographic columns

For the first column, 200 g of white sand (50 mesh) and 12 g of cellulose (Whatman fibrous powder, CF11) were mixed to form a homogeneous slurry in 100 ml of an aqueous solution containing 10 % PEG 6000 (Carbowax 6000, Carbide and Carbon Chemicals Co., U.S.A.), 0.4 *M* sucrose, 0.004 *M* magnesium acetate, and 0.005 *M* tris(hydroxymethyl)aminomethane–acetic acid buffer (pH 7). This was poured into a chromatographic column 6 cm in diameter. The second column (diameter 3 cm) consisted of 10 g of cellulose powder, suspended in the above-mentioned solvent. The chromatographic columns used in these studies were cooled by attached mantles with alcohol circulated in them at 4°.

Purification

Fresh plant material (25 g) was homogenized for 30 sec in a Waring Blendor with 100 ml of 0.005 *M* tris(hydroxymethyl)aminomethane–acetic acid buffer (pH 7) containing 10 % PEG, 0.4 *M* sucrose and 0.004 *M* magnesium acetate. The homogenate was mixed with 200 g of white sand and 12 g of cellulose powder to form a slurry. This mixture was applied to the first column and washed with 900 ml of a solvent containing 0.4 *M* sucrose, 0.004 *M* magnesium acetate and 0.005 *M* tris(hydroxymethyl)-aminomethane–acetic acid buffer (pH 7). The initial effluent was turbid and had a yellowish-brown color. Next the solvent was modified by eliminating magnesium acetate. After 150 ml of the modified solution passed through the column, a subsequent quantity of 250 ml of this solution eluted UV-absorbing material. The effluent was centrifuged in a Sorvall refrigerated centrifuge RC-2 at 40000 × *g* for 10 min.

PEG and magnesium acetate were added to the supernatant to give final concentrations of 10 % and 0.004 *M*, respectively. This solution was then passed through the second column. This column was then washed with 100 ml of 10 % PEG, 0.4 *M* sucrose, 0.004 *M* magnesium acetate, and 0.005 *M* tris(hydroxymethyl)aminomethane–acetic acid buffer (pH 7). Finally, 100 ml of a similar solvent minus PEG was passed through the column. To concentrate the ribosome fraction the last effluent was made 10 % with respect to PEG and the mixture centrifuged at 40000 × *g* for 30 min. The precipitate was resuspended in 15 ml of water and the resulting suspension was stirred carefully for 1 h followed by centrifugation at 40000 × *g* for 10 min. The supernatant contained the cytoplasmic ribosomes.

The flow rate of the first column was 4 ml/min and that of the second column was 3 ml/min. The presence of UV-absorbing substances in the effluents was detected using an automatic recording LKB-Uvicord absorption meter at 254 nm.

RESULTS

The chromatographic effluents showed strong absorbance at 254 nm as indicated in Fig. 1. The first solvent containing 0.4 *M* sucrose, 0.004 *M* magnesium acetate, and

0.005 *M* tris(hydroxymethyl)aminomethane–acetic acid buffer (pH 7) eluted a large amount of yellowish-brown colored and UV-absorbing material. The effluent by the second solution containing 0.4 *M* sucrose and 0.005 *M* tris(hydroxymethyl)aminomethane–acetic acid buffer (pH 7) was pale green and turbid.

The initial effluent from the second column had a slight UV-absorption. The solution containing 0.4 *M* sucrose, 0.004 *M* magnesium acetate, and 0.005 *M* tris(hydroxy-

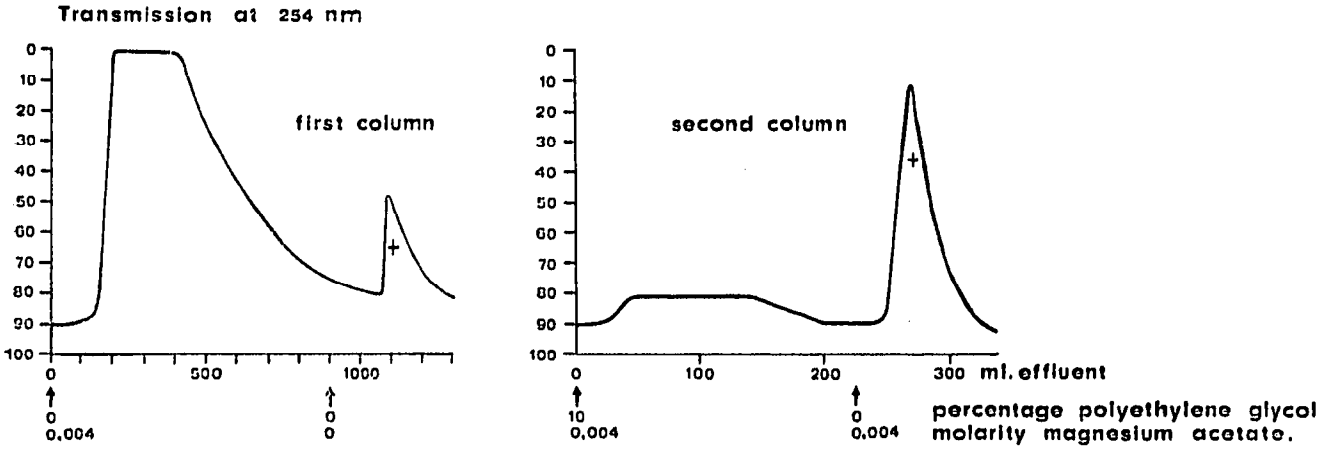

Fig. 1. Fractionation of ribosomes and other UV-absorbing substances from pea plant homogenates on cellulose columns. Column 1 (6 × 6 cm) is a mixture of 200 g of white sand (50 mesh) and 12 g of cellulose. Column 2 (3 × 6 cm) consists of 10 g of cellulose. Composition of the solvents: 0.4 *M* sucrose, 0.005 *M* tris(hydroxymethyl)aminomethane–acetic acid buffer (pH 7) and percentages of PEG and magnesium acetate as indicated by the arrows. Absorption of the effluents was recorded by the LKB Uvicord absorption meter at 254 nm. Presence of ribosomes is indicated by plus sign (+).

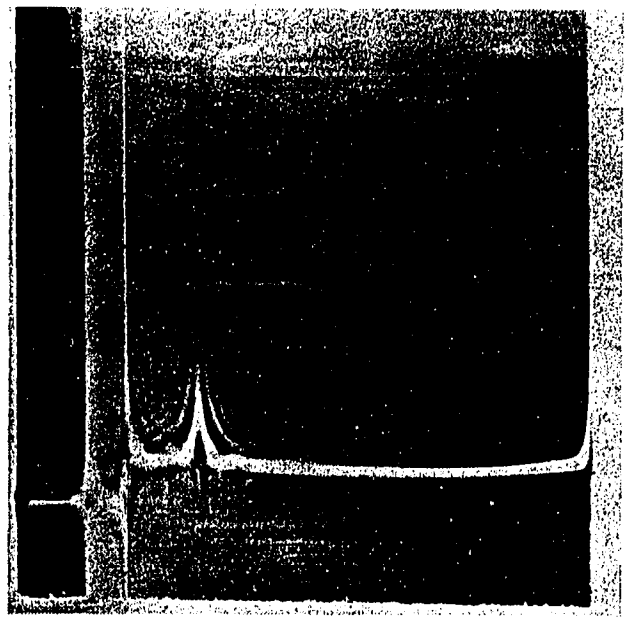

Fig. 2. Ultracentrifugal pattern of ribosomes isolated from homogenates of pea plants by means of chromatography on cellulose. Solvent constituents removed by centrifugation. Ribosomes dissolved in water.

methyl)aminomethane–acetic acid buffer (pH 7) eluted a large quantity of UV-absorbing substances from the second column. According to the studies described below, this effluent contained the ribosomes.

The absorption spectrum of the final preparation recorded by a combination of a Beckman UV spectrophotometer DB-G and a Sargent recorder Model SRLG, was characteristic of ribosomes. The maximum absorption was at 258 nm and the minimum absorption was at 238 nm. The ratio of these absorptions was 1.5.

The sedimenting behavior was studied with the aid of the Spinco analytical ultracentrifuge Model E. The pattern is given in Fig. 2. A sedimentation constant of 80 S was obtained based on the method of MARKHAM[7].

Electron microscopic studies of the final preparation failed to show the presence of impurities. The electron micrograph in Fig. 3 represents a portion of the pattern on the grid and is considered to be a representative field. The ribosomes were well separated from each other. An average diameter of 25 nm was found.

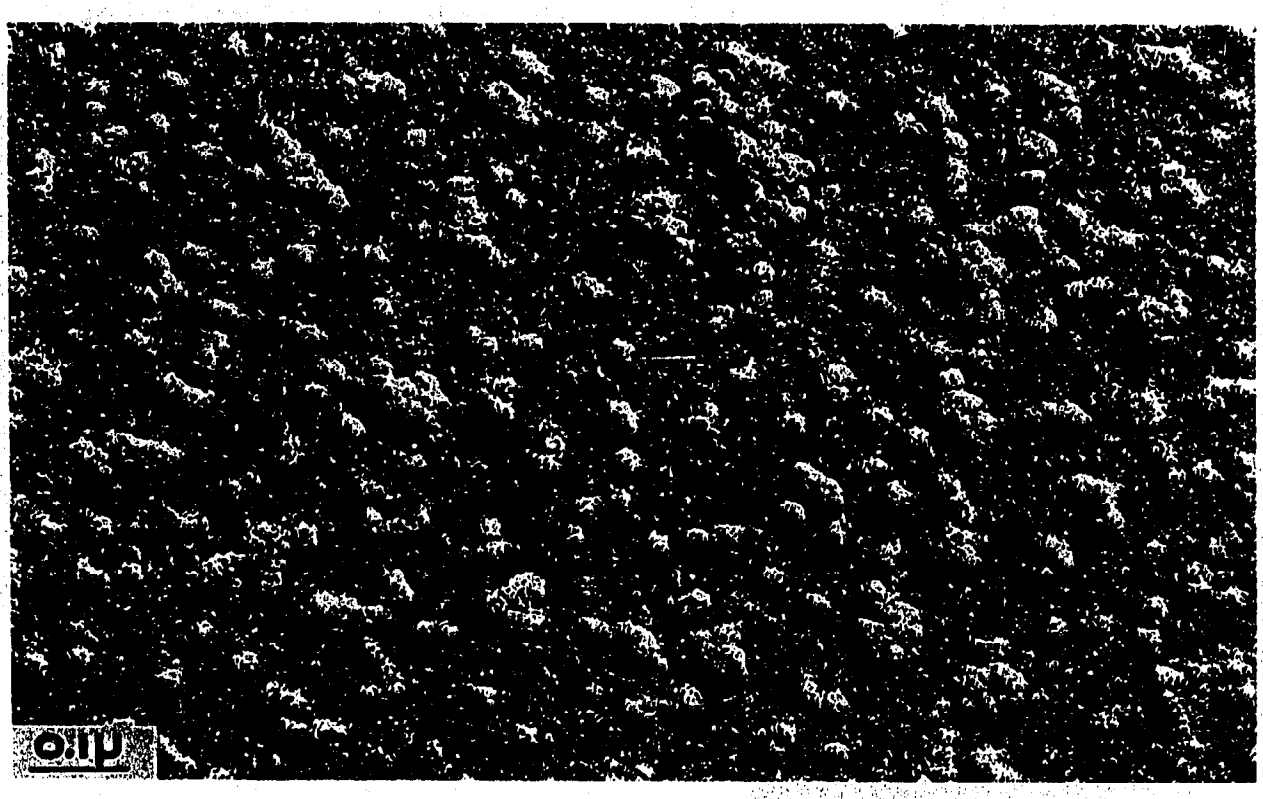

Fig. 3. Electron micrograph of ribosomes purified by chromatography on cellulose. Solvent constituents removed by centrifugation. Ribosomes dissolved in water. (Photograph: Technical and Physical Engineering Research Service, T.F.D.L., Wageningen).

DISCUSSION

This paper presents a procedure for the purification of ribosomes from young pea plants.

The packing material used for the columns is essentially cellulose because of its high capacity for adsorbing cell components. The column is especially efficient in ad-

sorbing chloroplasts and their fragments. Direct application of the plant homogenate on a cellulose column resulted in an immediate clogging of the system and hence the solvent flow was completely inhibited. The plant homogenate was mixed with white sand which reduced clogging. When the homogenate was mixed with white sand and cellulose, and at the same time the column cellulose was mixed with white sand, the solvent flow rate could be adjusted to 4 ml or more per min. The diameter of the column determined the flow rate also.

The stabilization of ribosomes by magnesium ions has been reported by many workers. BARKER AND RIEBER[8] described this effect on ribosomes from peas. ARGLEBE AND HALL[9] mentioned the same effect on ribosomes from bean plants. Consequently magnesium ions were added to the homogenate and to the solvents used in the procedures reported in this paper.

TS'O[10] and HSIAO[11] reported that low concentrations of ions and a pH of 7 reduced the dissociation of the ribosomes. A buffer system of 0.005 *M* tris(hydroxymethyl)aminomethane–acetic acid (pH 7) seemed to be suitable.

Sucrose at a concentration of 0.4 *M* produced an osmotic protection of the chloroplasts and mitochondria (see for example LYTTLETON[12]). This may be of importance in reducing the interactions between the breakdown products of these organelles and the ribosomes.

VENEKAMP AND MOSCH[13] reported that a solvent containing large amounts of PEG (8 percent or more) was necessary for adsorption of spherical virus particles similar in size to ribosomes. Here these particles are considered to behave like small virus particles. The presence of PEG is only necessary during the application of the homogenate on the column. Perhaps due to the effect of magnesium ions on the ribosomes and the presence of plant material in the column, elution with a solvent containing magnesium ions and no PEG removed undesirable components from the column while a solvent without magnesium ions eluted the ribosomes.

Removal of enzymes may have eliminated degradation of ribosomes during the purification procedure. This may partly account for a high recovery of ribosomes in the final preparation.

Using the first column the ribosomes could be purified in a short time, but UV-absorbing materials were tailing throughout the elution schedule. The purity of the ribosomes depended on the amount of the first solvent percolated through the column. Tailing did not occur during percolation through the second column.

The difference in the behavior of ribosomes in the first and in the second columns with respect to magnesium ions is likely due to the occurrence of various substances in the homogenate applied onto the first column. VENEKAMP AND MOSCH[1] described a similar effect in chromatographic virus purification. According to VENEKAMP *et al.*[3] protoplasmic membranes may be responsible for this phenomenon.

Cooling of the columns may have reduced enzymatic degradation of ribosomes.

The ribosome-carrying effluent from the second column is opaque but the color is pale-green. The final treatment of this effluent yielded a ratio of the absorption maximum and minimum similar to values found previously (see for example LYTTLETON[12]).

Three pieces of evidence suggest that this procedure yields pure cytoplasmic ribosomes. First, the UV absorption spectrum of the final preparation showed a maximum at 258 nm and a minimum at 238 nm; the ratio of these absorptions was 1.5.

Second, the sedimentation constant of the particles in this preparation was 80 S, consistent with the value reported among others by BARKER AND RIEBER[8]. Third, the electron micrograph represented a portion of the pattern on the grids and therefore was considered to be a representative field with particles, well separated from each other. The value for the diameter (25 nm) of the particles agrees with that reported for example by BONNER[14]. The absorption and sedimentation values of the preparations reported here suggest the absence of polysomes.

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