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Note

Chromatographic purification of cytoplasmic ribosomes from potato leaves

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In a previous paper[1] a method was described for isolating cytoplasmic ribosomes from young pea plants using a chromatographic procedure. Application of this technique to young potato leaves gives poor results. The ribosome yield, measured as the extinction value at 260 nm, is very small; an absorbance of about 1 per ml per g fresh weight has been obtained. The absorption spectrum of the preparation does not appear to be that of ribosomes, the sedimentation coefficient cannot be determined and no particles can be seen in the electron microscope.

Venekamp *et al.*[2] found that the irreversible adsorption of oxidised phenols to virus particles inpeded the release of these particles from a cellulose column. The above mentioned poor yields may be the result of the same phenomenon, as potato leaves contain more phenols than pea plants. For the purification of ribosomes from plant leaves, Venekamp *et al.*[3] used solvents containing cysteine and polyvinylpyrrolidone to counteract the oxidation of phenols and to combine phenolic compounds.

For research into the background of the resistance of potato leaves to *Phytophthora infestans* a quick, simple and quantitative ribosome estimation method is required, by which it is possible to purify ribosomes from a large number of leaf samples. The technique of Venekamp *et al.*[3] is not suitable for this. The method of Kliffen[1] involves less solvent changes and the composition of the solvents is more uniform.

In this article, cytoplasmic ribosomes are purified from potato leaves according to the method of Kliffen[1], with a major modification, *viz.*, that cysteine and polyvinylpyrrolidone are added to the solvents used in the first column.

MATERIALS AND METHODS

Plant material

Leaf parts of young shoots of potato plants are used.

Chromatographic columns and solvents

These are the same as in the previous paper[1]. The following are added to the solvents of the first column: 0.1% (w/v) K15 polyvinylpyrrolidone (Fluka, Buchs, Switzerland) of mol. wt. 10,000 (PVP) and 0.1% (w/v) L(+)-cysteine. The second column contained 15 g cellulose powder.

Purification

In comparison with the previous paper[1], the following changes have been introduced. Two similar portions of 50 g of fresh plant material are ground in 200 ml of a solvent, which apart from the substances already named also contains 0.1% (w/v) of PVP and 0.1% (w/v) of cysteine. In order to form a slurry, the homogenate is mixed with 400 g of sand and 24 g of cellulose powder. The mixture is added to the first column and washed through with 1000 ml of a solvent containing 0.004 *M* magnesium acetate, 0.4 *M* sucrose, 0.005 *M* Tris–acetic acid buffer (pH 7), 0.1% (w/v) PVP and 0.1% (w/v) cysteine. The first effluent is cloudy and has a brownish colour. Next, the solvent is changed by the omission of the magnesium acetate. After about 300 ml of this solution has passed through the column, about 400 ml of a solution of ultraviolet-absorbing material is eluted, this being opalescent and yellow-green in colour. This material is collected in fractions of about 40 ml. Each fraction is centrifuged for 10 min at 40,000×*g*, causing most of the green material to be spun from the solution. To the supernatant, PEG and magnesium acetate are added to final concentrations of 10% (w/v) and 0.004 *M*, respectively. The solutions thus produced from two such columns are combined and put through a second column, which is then washed through with 200 ml of a solution containing 10% (w/v) PEG, 0.004 *M* magnesium acetate, 0.4 *M* sucrose and 0.005 *M* Tris–acetic acid buffer (pH 7). Finally, 200 ml of 0.005 *M* Tris–acetic acid buffer (pH 7) are passed through, causing the cytoplasmic ribosomes to be eluted. The flow rate of the solutions through both columns is 5 ml/min; elution of the last fraction from the second column takes place at 2 ml/min.

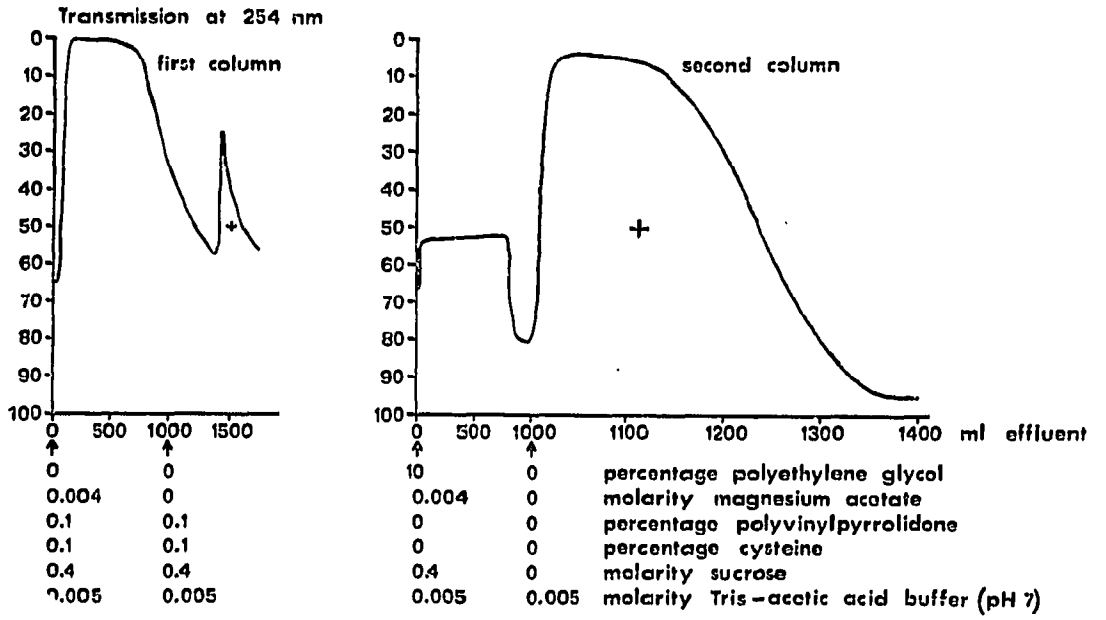

Fig. 1. Purification of ribosomes from potato leaves on cellulose columns. Column 1 (6 × 6 cm) consists of a mixture of 200 g sand (50 mesh) and 12 g cellulose. Column 2 (3 × 9 cm) contains 15 g cellulose. The composition of the solvents is indicated beneath the arrows. The absorption of the effluents is recorded by an LKB Uvicord absorption meter at 254 nm. The presence of ribosomes is indicated by a plus sign.

RESULTS

As shown by Fig. 1 all effluents exhibit a strong absorption at 254 nm. However, part of the absorption is caused by the PVP present in the solvents. The presence of cytoplasmic ribosomes in the second effluent of the second column can be demonstrated in the following ways. The absorption spectrum exhibits an absorption maximum and minimum at 260 and 240 nm, respectively; the ratio of these absorption values is 1.4. Large quantities of free particles with a diameter of 30 nm can be seen in the electron microscope. 80S particles can be demonstrated according to the method of Markham[4], especially in the top fractions. The yield of ribosomes measured as the absorbance per ml, per g fresh weight at 260 nm is about 12.

DISCUSSION

The high yield of ribosomes suggests that cysteine and PVP prevent oxidation of the phenols during purification. This is a confirmation of the results of Venekamp *et al.*[3] PVP and cysteine are no longer used in the second column as PVP strongly absorbs ultraviolet light.

Concentration of the ribosomes by means of precipitation with PEG seems poorer than in the experiments with pea leaves[1], as too many ribosomes are irreversibly precipitated. In order to obtain a high final concentration, the ribosome fractions of two first columns are added to one second column and for the same reason the second column is run slowly during elution of the ribosomes. With this modification the top fractions are practically free of PEG and sucrose. This and the high concentration of the ribosomes gives very good conditions for estimation of the S value. Separate portions of the second fraction of the first column should be centrifuged to remove the green material and to prevent, as much as possible, the precipitation of ribosomes under the influence of the PEG remaining from the first fraction. During the elution of the second fraction, the PEG concentration decreases and that of the ribosomes relatively increases.

As potato leaves are one of the most difficult objects from which to isolate nucleoproteins, one can assume that the modification described here makes the method suitable for general use in the purification of cytoplasmic ribosomes from plant leaves.

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