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PURIFICATION OF CYTOPLASMIC RIBOSOMES BY COLUMN CHROMATOGRAPHY

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SUMMARY

In experiments with potato plants cellulose columns adsorbed cytoplasmic ribosomes from a solvent containing polyethylene glycol and magnesium ions. The subsequent omission of magnesium ions from the solvent resulted in liberation of ribosomes from the column. This is independent of the polyethylene glycol content (not more than 5%) of the eluting solvent.

Ribosomes from bean, pea and tobacco plants and from *Nicotiana glutinosa*, *Nicotiana rustica* and *Lolium multiflorum* could be purified by means of the chromatographic procedure developed for potato ribosomes.

INTRODUCTION

Kliffen[1] modified the chromatographic procedure of Venekamp and Mosch[2] to purify ribosomes from young pea plants. A column of a mixture of sand and cellulose with a solvent containing 10% polyethylene glycol, 0.004 *M* magnesium acetate, 0.4 *M* sucrose and 0.005 *M* Tris–acetic acid buffer (pH 7), was used to adsorb high-molecular-weight substances from crude plant material. Omission of magnesium ions and polyethylene glycol from the solvent induced elution of ribosomes.

Venekamp *et al*[3]. described the adsorption of some rod-shaped plant viruses and ribosomes on cellulose by application of a solvent containing 5% polyethylene glycol, 1% ammonium acetate and 0.004 *M* magnesium acetate. After omission of ammonium acetate and magnesium acetate from this solvent tobacco mosaic virus could be collected in the effluent. This preparation might contain a certain amount of ribosomes. An investigation of the liberation of ribosomes from the column by use of a solvent containing 5% polyethylene glycol, 1% ammonium acetate and no magnesium acetate is yet to be carried out.

Potato virus X and ribosomes were adsorbed on the column from a solvent containing only 5% polyethylene glycol and 0.004 *M* magnesium acetate. Omission of the magnesium acetate resulted in elution of ribosomes. However, a part of the amount of potato virus X in the column was eluted simultaneously. When the elution schedule used for tobacco mosaic virus was used, then potato virus X was eluted only with 3% polyethylene glycol.

White clover mosaic virus and ribosomes could also be adsorbed on the column from a 5% polyethylene glycol- and 0.004 *M* magnesium acetate-containing solvent. In this case omission of magnesium acetate yielded only ribosomes. The virus was liberated after reducing the polyethylene glycol content to 1%. It may be of advantage to reduce the polyethylene glycol content, *e.g.*, to 3%, before the omission of magnesium acetate. This may result in the collection of a larger quantity of ribosomes.

An adaptation of the elution of ribosomes to that of viruses will be considered. This included the question whether the ribosome elution induced by absence of magnesium ions will be independent of the polyethylene glycol and ammonium acetate contents of the the eluting solvent. Some solvent series useful for virus purification will be used to answer this question.

Criteria such as sedimentation coefficient, characteristics of absorption spectra and diameter of the particles in electron micrographs were used to identify the nucleoproteins.

The possibility of general applicability of this purification procedure is another question. Ribosomes from six plant species were studied to approach this question.

MATERIALS AND METHODS

Potato plants (var. Bintje)

Potato plants (var. Bintje) were grown in the greenhouse at a temperature of 20–22°. Leaf samples of 100 g fresh weight were analysed for ribosome content by chromatographic procedure 7 of Venekamp *et al.*[3]. Instead of 0.1% sodium diethyldithiocarbamate, 0.1% K15 polyvinylpyrrolidone (Fluka, Buchs, Switzerland; MW 10,000) and 1% cysteine were used.

In six experiments the following series of solvents for the second series of col-

TABLE I

COMPOSITION OF SOLVENTS FOR THE SECOND SERIES OF COLUMNS IN THE TWO-STEP CHROMATOGRAPHIC RIBOSOME PURIFICATION PROCEDURE

For the two-step chromatographic procedure see Venekamp *et al.*[3]. The second step is the final ribosome purification.
PEG = polyethylene glycol 6000; NH_4OAc = ammonium acetate; PVP = polyvinylpyrrolidone; Cys = cysteine; $Mg(OAc)_2$ = magnesium acetate; Tris = Tris-acetic acid buffer, pH 7.

Solvent number	*Component level (%)*					*Component level (M)*	
	PEG	*NH_4OAc*	*Glucose*	*PVP*	*Cys*	*$Mg(OAc)_2$*	*Tris*
1	5	1	4.5	0.1	0.1	0.004	0.01
2+	5	1	4.5			0.004	0.01
2−	5	1	4.5				0.01
3+++	5		4.5	0.1	0.1	0.004	0.01
3+	5		4.5			0.004	0.01
3−	5		4.5				0.01
4+	3		4.5			0.004	0.01
4−	3		4.5				0.01
5+	1		4.5			0.004	0.01
5−	1		4.5				0.01
6+						0.004	0.01
6−							0.01

umns, given in Table I, were used. Experiment 1: 1, 2+, 2−; experiment 2: 1, 2+, 6+, 6−; experiment 3: 3+++, 3+, 4+, 5+, 6+, 6−; experiment 4: 3+++, 3+, 4+, 5+, 5−; experiment 5: 3+++, 3+, 4+, 4−; experiment 6: 3+++, 3+, 3−.

The effluents of the last solvents, indicated by a minus sign, were centrifuged at 40,000 × *g* for 10 min. The pellets were resuspended in 5 ml of 0.01 *M* Tris–acetic acid buffer of pH 7.

The wavelengths of maximum and minimum absorbances and the ratio of maximum and minimum absorbances of each suspension were measured by means of a DB-G spectrophotometer (Beckman, Fullerton, Calif., U.S.A.) with a Sargent-Welch (Skokie, Ill., U.S.A.) recorder. From the maximum absorbances the absorbances/ml·g fresh weight were calculated.

Sedimentation was investigated with a Beckman–Spinco Model E analytical ultracentrifuge (An-D rotor) at a speed of 35,600 rpm and the sedimentation coefficients were calculated according to the method of Markham[4].

Ten- and hundred-fold dilutions of the suspensions were used for electron microscopic studies. The magnification of the palladium shadowcast preparations to determine the diameters of the particles was × 22,140 or × 66,600.

Other plants

Six-weeks-old plants of *Phaseolus vulgaris* (Bataaf), *Pisum sativum* (Rondo), and *Lolium multiflorum* (Tetila), eight-weeks-old plants of *Nicotiana tabacum* (White Burley) and nine-weeks-old plants of *Nicotiana glutinosa* and *Nicotiana rustica* were used for ribosome purification according to experiment 1, mentioned above. These plants were grown under the same conditions as the potato plants. Ultraviolet (UV) absorption spectra, sedimentation and electron micrographs were studied as described above.

RESULTS AND DISCUSSION

Potato plants

To avoid the chelating effect of sodium diethyldithiocarbamate, cysteine and polyvinylpyrrolidone were used. Cysteine reduces phenolic compounds combined with polyvinylpyrrolidone, as shown by Loomis and Battaile[5].

Omission of magnesium ions from one of the solvents induced the elution of nucleoproteins. According to the wavelengths of maximum and minimum absorbances, ratios of maximum and minimum absorbances, sedimentation coefficients and particle diameters, given in Table II, these nucleoproteins appeared to be 80S ribosomes. This is in agreement with the observations of Kliffen[1]. The liberation of ribosomes is qualitatively and quantitatively independent of the polyethylene glycol and ammonium acetate contents of the eluting solvent. An exception to this is the liberation of ribosomes with solvent 6+ (Fig. 1). This solvent contains magnesium ions and eluted a large amount of nucleoproteins from the column. The characteristics of the UV absorption spectrum, the sedimentation constant and the particle diameter are the same as those of ribosomes. According to the calculated absorbance/ml·g fresh weight, solvent 6+ eluted about half of the amount of ribosomes liberated by the polyethylene glycol-containing solvents (Fig. 1).

TABLE II

CHARACTERISTICS OF UV ABSORPTION SPECTRA, SEDIMENTATION CONSTANTS, AND DIAMETERS OF RIBOSOMES, PURIFIED BY TWO-STEP CHROMATOGRAPHIC PROCEDURES ON CELLULOSE COLUMNS

For the chromatographic procedure see Venekamp *et al.*[3]. Series of solvents (numbers given in Table I) in the second step as given in Materials and methods. Data are averages of 20 measurements.

Experiment No.	*Wavelength of maximum absorption (nm)*	*Wavelength of minimum absorption (nm)*	*Ratio of maximum and minimum absorbances*	*Sedimentation coefficient (S)*	*Total ribosomes absorbance/ml·g fresh weight*	*Particle diameter (nm)*
1	258	238	1.56	80	0.73	29.8
2	258	238	1.64	82	0.69	30.4
3	258	239	1.46	79	0.75	30.1
4	257	238	1.65	83	0.71	29.5
5	258	237	1.62	80	0.72	29.7
6	258	237	1.52	80	0.74	30.2

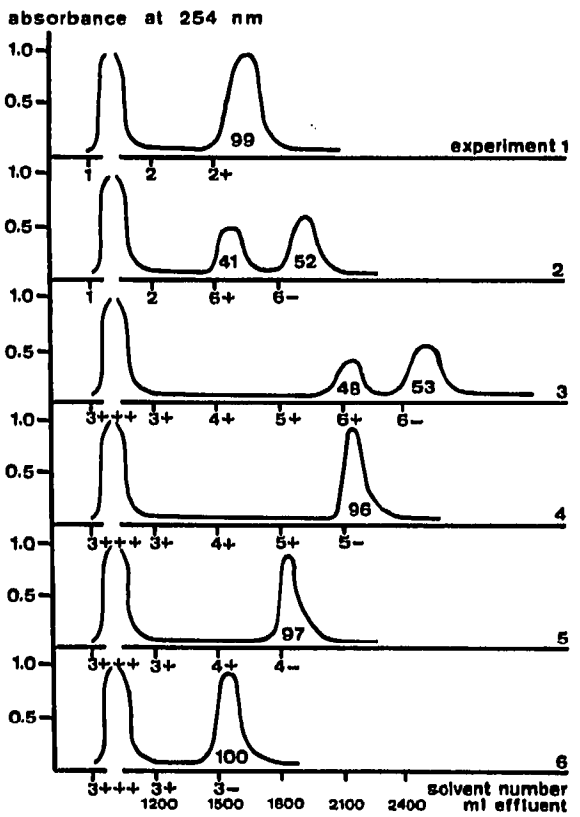

Fig. 1. Fractionation of ribosomes from "Bintje" potato plants by the second step of the two-step chromatographic procedure 7 of Venekamp *et al.*[3]. Compositions of the solvents numbered along the abscissa are indicated in Table I. The yield of ribosomes in the effluents is indicated as a percentage of the total amount of ribosomes in experiment 6. A ribosome content of 100% is equivalent to an absorbance/ml·g fresh weight of 0.74.

The wavelengths of maximum and minimum absorbances and the ratios of maximum and minimum absorbances of the ribosomes eluted with the polyethylene glycol-containing solvents are very similar. These data agree with those given by Marei and Romani[6] and Lynn *et al.*[7].

The estimation of the sedimentation constants resulted in 80S values. This means that the chromatographic procedures described in this paper yielded cytoplasmic ribosomes. The quantities are very similar and therefore independent of the eluting solvent. The electron microscopic preparations did not show any impurity.

From homogenates of healthy plant material no nucleoproteins could be eluted with solvents which usually liberate viruses. Ribosomes from this plant material eluted with the same solvent as did the ribosomes from diseased plants.

Other plants

The purified ribosomes from the plant species belonging to different families showed the same characteristics as those of the potato plants. The data are given in Table III. A simple elution schedule was used because questions concerning dependence of ribosome elution on solvent composition were not considered.

TABLE III

CHARACTERISTICS OF UV ABSORPTION SPECTRA AND SEDIMENTATION CONSTANTS OF RIBOSOMES PURIFIED BY TWO-STEP CHROMATOGRAPHIC PROCEDURES ON CELLULOSE COLUMNS

The chromatographic procedure is given by Table II, experiment 1.

Plant	*Wavelength of maximum absorption (nm)*	*Wavelength of minimum absorption (nm)*	*Ratio of maximum and minimum absorbances*	*Sedimentation coefficient (S)*
Phaseolus vulgaris (Bataaf)	258	239	1.50	80
Pisum sativum (Rondo)	257	241	1.31	80
Nicotiana tabacum (White Burley)	258	240	1.37	79
Nicotiana glutinosa	259	239	1.46	81
Nicotiana rustica	257	242	1.40	82
Lolium multiflorum	258	238	1.44	79

The technique described may be applied more generally. According to the results of Venekamp and Taborsky[8] the quantities of ribosomes extracted from 100 g of fresh material of different plant species are not comparable and are therefore not estimated.

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