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tRNA of the Fungus *Pleurotus ostreatus*. Preparation and Purification by the Method of Preparative Gel Electrophoresis

tRNA z grzyba *Pleurotus ostreatus*. Otrzymywanie i oczyszczanie metodą elektroforezy preparatywnej na żelu poliakryloamidowym

т-РНК из гриба *Yleurotus ostreatus*. Получение и очистка методом препаративного электрофореза на полиакриламидовом геле

INTRODUCTION

Preparative polyacrylamide gel electrophoresis plays an important role in the preparation of nucleic acids. Many authors (1—4) using this method have obtained highly purified preparations of RNA fractions, both on a large and small scale (from milligram to microgram amounts). The equipments used for this purpose differ greatly both in their construction and system of elution. We have used a maximally simplified apparatus for a single elution which gave pure tRNA on a semi-preparative scale. The short separation time, the simplicity of the apparatus and the acceptor activity of the preparations obtained have qualified this method for routine use in our laboratory.

MATERIAL AND METHODS

Mycelium of *Pleurotus ostreatus* Jacq., strain No 53 obtained from the Laboratory for Anatomy and Physiology of Plants J. E. Purkynje University, Brno, Czechoslovakia, was grown stationary on Lindeberg and Holm's (5) mineral medium. 100—120 g (at a time) of a fresh mass of six-week-old mycelium was used for the experiments.

Preparation of RNA: after filtration off the medium, mycelium (about 100 g) was washed twice with SSC (0.15 M sodium chloride in 0.015 M sodium citrate) and homogenized with phenol in the following proportions: 30 ml of SSC and 70 ml of phenol per 30 g of fresh mass of mycelium. Before homogenization, 15 g of alumina, 500 mg of SDS and 0,2 ml of diethylpyrocarbonate (DEP) was added. The homogenate was shaken for 1 hr, then centrifuged and RNA was precipitated with 2 volumes of ethanol from the aqueous phase. The precipitate was extracted according to Kirby (6) for the removal of polysaccharides, after being dissolved in a small volume of SSC and precipitated again with ethanol. After 1 hr at —20°C the precipitate was centrifuged and

dried over P_2O_5. From 100 g of fresh mycelium 150—200 mg of RNA was obtained.

For electrophoresis on polyacrylamide gel there was used the apparatus shown in Fig. 1. 50—100 mg of RNA were dissolved im 1 ml of 0.1 M trisglycine buffer pH 9.1 and incubated for 30 min. at 37°C for deacylation of tRNA. After incubation the solution of RNA was poured onto a 4% gel column (25 mm high and 20 mm in diameter). The gel was made in a tris-boric acidmagnesium chloride-ethylendiamine sodium tetraacetate (90 mM⁺90 mM⁺10 mM⁺2 mM) buffer according to P h i l o p s and T i m k o (7). The same buffer

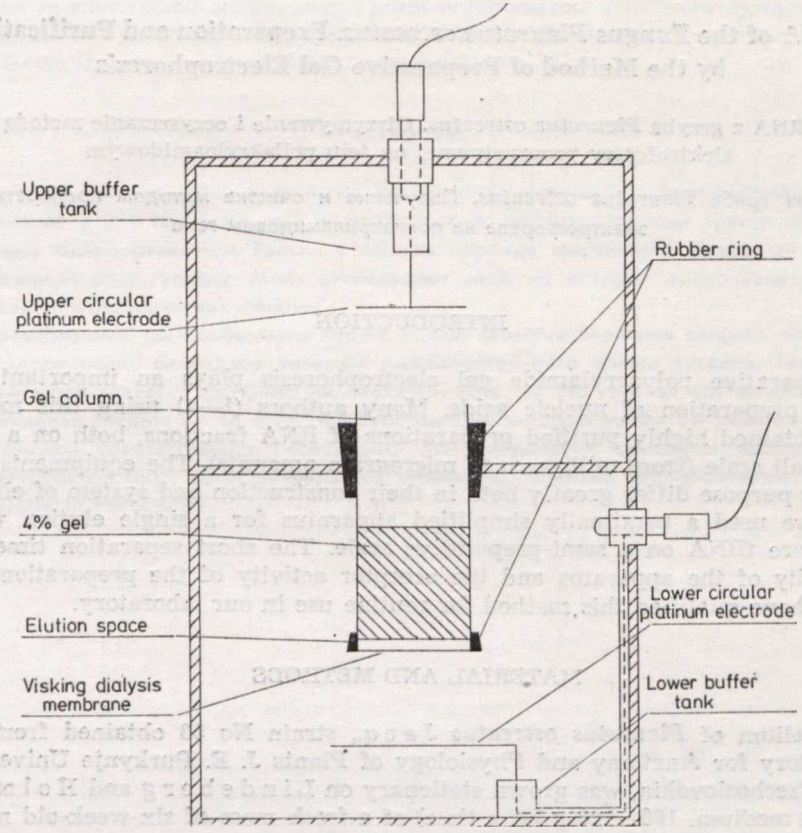

Fig. 1. Scheme of the apparatus for preparative polyacrylamide gel electrophoresis

was in the gel and in the tanks. The capacity of each tank was 500 ml. The elution space was sealed off the bottom by the Visking dialysis membrane type 27/32 (Serva). The average diameter of pores of this type of membrane allows particles of a mass up to 10,000 daltons to permeate. Electrophoresis was run for 30 min. at 40 mA (constant current). The time of electrophoresis was chosen upon examining the velocity of tRNA from *E. coli* (commerical, BDH) migration with the sedimentation coefficient 4S. This time was sufficient for tRNA from *Pleurotus ostreatus* with the sedimentation coefficient 3.95 S, taking into consideration a slower migration caused by high

molecular weight impurities. After 30 min., electrophoresis was stopped and tRNA was recovered from the elution space with ethanol.

Analytical gel electrophoresis was performed in the same gel at 2.5 mA per column (5×70 mm). Twenty micrograms of tRNA were applied to the top of the column. After electrophoresis, gels were stained with toluidine blue, and the optical density was measured at 644 nm in the densitometer TLD — 100 Vitatron.

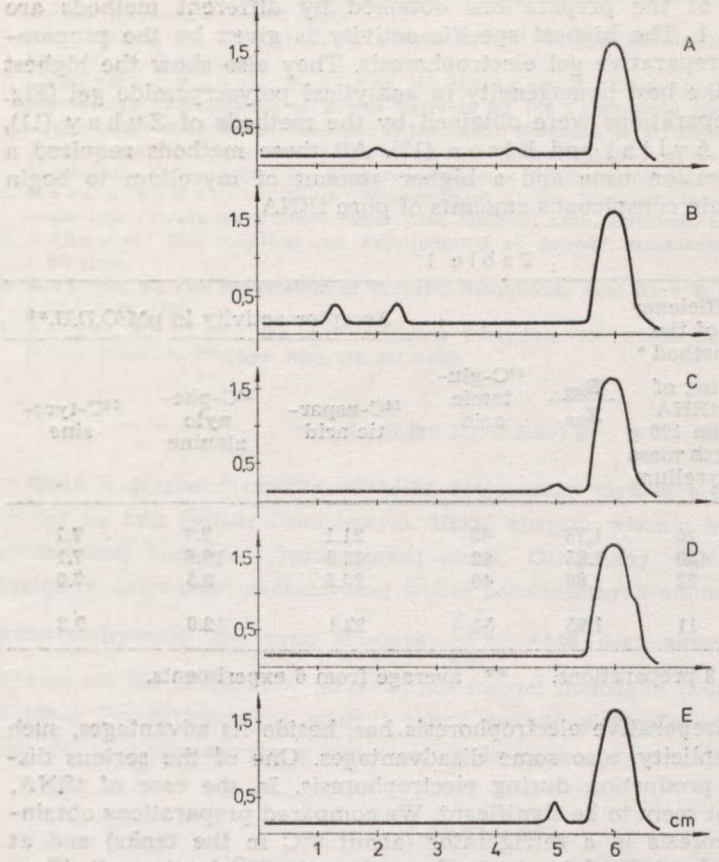

Fig. 2. Analytical gel electrophoresis of several preparations of tRNA: A — tRNA obtained according to Zubay, B — tRNA obtained according to Avital and Elson, C — tRNA obtained according to Gutcho, D — tRNA obtained by preparative gel electrophoresis, E — *E. coli* tRNA from BDH

Acceptor activity of tRNA was examined in homogenous system using aminoacyl-tRNA synthetases from the same fungus. The synthetases were obtained by column chromatography on DEAE-cellulose in similar manner as that of L e b e r m a n n et al. (8) and on aminohexyl Sepharose according to J a k u b o w s k i and P a w e ł k i e w i c z (9). The details will be described in another paper. The aminoacylating mixture contained 100 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (Hepes), *pH* 7.3, 10 mM $MgCl_2$, 10 mM KCl, 2.5 mM $ATPNa_2$, 1 mM dithiothretol (DTT), 150 μg of tRNA and 80—100 μg

of enzymatic protein. The volume of a sample was 150 µl and the time of incubation 30 min. at 37°C. Radioactivity was determined in the toluene scintillator according to Mans and Novelli (10) in the Packard scintillation counter 3320.

RESULTS AND DISCUSSION

The properties of the preparations obtained by different methods are presented in Table 1. The highest specific activity is given by the preparations obtained by preparative gel electrophoresis. They also show the highest optical purity and the best homogeneity in analytical polyacryamide gel (Fig. 2). Comparative preparations were obtained by the methods of Zubay (11), Gutcho (12) and Avital and Elson (13). All these methods required a much longer preparation time and a higher amount of mycelium to begin with in order to obtain conspicuous amounts of pure tRNA.

Table 1

Preparation obtained according to:	Efficiency of the method *		Acceptor activity in pM/O.D.U.**		
	mg of tRNA from 100 g fresh mass mycelium	$\frac{E_{280}}{E_{260}}$	^{14}C-glutamic acid	^{14}C-aspartic acid	^{14}C-phenylo alanine	^{14}C-tyrosine
Zubay	26	1.78	43	21.1	9.7	7.1
Gutcho	30	1.85	42	16.0	10.8	7.1
Avital and Elson	22	1.80	40	20.5	9.5	7.0
Preparative gel electrophoresis	11	1.95	51	22.1	12.0	9.3

* Average from 3 preparations, ** average from 6 experiments.

The method of preparative electrophoresis has, beside its advantages, such as rapidity and simplicity, also some disadvantages. One of the serious disadvantages is heat production during electrophoresis. In the case of tRNA, however, this did not seem to be significant. We compared preparations obtained during electrophoresis in a refrigerator (about 8°C in the tanks) and at room temperature (about 20°C in the tanks and up to 30° in the gel). They did not show any differences in acceptor activities. As it is possible to separate both degradation products and high molecular RNA fractions, we have considered this method suitable and used it in our laboratory.

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REFERENCES

1. Malaciński G. M.: Preparative Gel Electrophoresis of Ribonucleic Acids. Anal. Biochem. 38, 288 (1970).

2. McCarthy D., Hawkes S. P., Lander D. E.: Small Scale Preparative Gel Electrophoresis of Ribonucleic Acids. Biochim. Biophys. Acta 277, 301-9 (1972).
3. Shimada K., Sekikawa K., Fujinaga K., Ito Y.: A New Device of Preparative Polyacrylamide Gel Electrophoresis and Its Application to Analysis of Cellular RNA. Anal. Biochem. 51, 456 (1973).
4. Jacobson A., Lodish H.: A Simple and Inexpensive Procedure for Preparative Gel Electrophoresis of RNA. Anal. Biochem. 54, 513 (1973).
5 Lindeberg G., Holm H.: Occurrence of Tyrosinase and Laccase in Fruit Bodies and Mycelia of some *Hymenomycetes*. Physiol. Plant. 5, 100 (1952).
6. Kirby K. S.: A New Method for the Isolation of Ribonucleic Acids from Mammalian Tissues. Biochem. J. 64, 405 (1956).
7. Philops G. R., Timko J. L.: Simple Method for Characterization of 5 S RNA. Anal. Biochem. 45, 319 (1972).
8. Leberman R., Bon Schuetz H., Dittgen R., Ulmar G.: A Scheme for the Preliminary Fractionation of *E. coli* Extracts for the Large-Scale Isolation of Tryptophanyl — and Lysyl-tRNA Synthetases and tRNA. Anal. Biochem. 51, 111 (1973).
9. Jakubowski H., Pawełkiewicz J.: Chromatography of Plant Amino-Acyl-tRNA Synthetases on w-Aminoalkyl Sepharose Columns. FEBS Letters 34, 150 (1973).
10. Mans R. J., Novelli G. G.: Measurement of the Incorporation of Radioactive Amino Acids into Protein by a Filter-Paper Disk Method. Arch. Biochem. Biophys. 94, 48 (1961).
11. Zubay G.: The Isolation and Fractionation of Soluble Ribonucleic Acid. J. Mol. Biol. 4, 347 (1962).
12. Gutcho S.: The Preparation of Transfer Ribonucleic Acid from *E. coli*. Biochim. Biophys. Acta 157, 76 (1968).
13. Avital S., Elson D.: A Convenient Procedure for Preparing Transfer RNA from *E. coli*. Biochim. Biophys. Acta 179, 297 (1969).

STRESZCZENIE

tRNA z grzyba *Pleurotus ostreatus* oczyszczono metodą elektroforezy preparatywnej na żelu poliakryloamidowym. Użyto aparatu własnej konstrukcji o bardzo uproszczonej budowie i jednorazowej elucji. Otrzymany tRNA przebadano pod względem aktywności akceptorowej wobec poszczególnych aminokwasów oraz spektrofotometrycznie. Uzyskano stosunek $\frac{E_{260}}{E_{280}} = 1{,}95$ oraz aktywności akceptorowe wyższe niż dla preparatów otrzymanych innymi metodami (Zubay, Avital i Elson, Gutcho). Przedyskutowano wady i zalety metody elektroforezy preparatywnej na żelu poliakryloamidowym w zastosowaniu do kwasów rybonukleinowych.

РЕЗЮМЕ

Для очистки т-РНК из гриба *Pleurotus ostreatus* методом препаративного электрофореза был применен упрощенный аппарат с однократной элюцией (аппарат собственной конструкции). Исследовалась акцепторная активность т-РНК в отношении некоторых аминокислот. Кроме того, были проведены спектрофотометрические исследования. В результате акцепторная активность оказалась большей, чем у препаратов, полученных другими методами (Zubay, Avital и Elson, Gutcho), а отношение $\frac{E_{260}}{E_{280}}$ выносило 1,95. Рассмотрена возможность применения этого метода для рибонуклеиновых кислот.