Phytochemistry, Vol. 21, No. 2, pp. 273–275, 1982.
Printed in Great Britain.

0031-9422/82/020273-03$03.00/0

EFFECT OF RYE EMBRYO RIBOSOME NUCLEASE ON DOUBLE-STRANDED RNA

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(*Received* 30 *May* 1981)

Key Word Index—*Secale cereale*; Gramineae; rye; nuclease; double-stranded RNA; ribosomes.

Abstract—In extracts obtained by treating rye embryo ribosomes with 0.5 M NH_4Cl, nuclease activity was noted towards double-stranded RNA from virus of *Penicillium chrysogenum* and towards synthetic poly (A)–poly (U) and poly (I)–poly (C) complexes.

INTRODUCTION

Nucleolytic activity towards single-stranded RNA has been observed in the homogenate, nuclei, plastids, mitochondria, cytosol and ribosomes of rye embryos [1]. Two ribonucleases which degraded exclusively single-stranded RNA [2, 3] were isolated and purified from rye germ cytosol. Nuclease isolated from the nucleoplasm of rye germ nuclei did not show activity towards poly (A)–poly (U) [4]. Nuclease of type I purified by salt washing from the ribosome fraction of rye germs has no hydrolysing properties towards double-stranded RNA (Siwecka, unpublished work). Reports are so far lacking on enzymes degrading double-stranded RNA in higher plants.

The presence of double-stranded RNA was revealed in the chloroplasts of tobacco leaves [5]. In a cell-free system from wheat germs no nucleolytic activity towards double-stranded RNA was observed [6].

This paper presents data concerning the activity of an NH_4Cl extract of cytoplasmic ribosomes of rye germs towards synthetic and viral double-stranded RNA structures and considers the possibility of washing off the proteins with this activity from the ribosomes.

RESULTS AND DISCUSSION

The data presented in Table 1 show that in the rye germ clusters, obtained by treating ribosomes with 0.5 M NH_4Cl, activity towards double-stranded RNA of virus from *Penicillium chrysogenum* has *ca* 50% of the activity directed toward single-stranded RNA. Degradation of rRNA and tRNA from *E. coli*, tRNA from yeast, synthetic double-stranded complexes poly (A)–poly (U) and poly (I)–poly (C) as well as of the hybrid poly (dT)–poly (rA) was also observed. The high activity of the wash towards poly (C) and poly (U), twice as high as that towards single-stranded RNA, suggests that the enzyme present in the wash probably preferentially degrades diester bonds close to the pyrimidine bases.

The activity towards the double-stranded complex poly (A)–poly (U) is exhibited both by the ribosome and the cytosol fraction of rye germs (Table 2). The highest activity level towards poly (A)–poly (U) as compared to that towards single-stranded RNA is observed in the ribosome fraction washed twice with 0.5 M NH_4Cl. Further treatment of ribosomes with this salt releases from them a greater part of activity towards poly (A)–poly (U) and only 27% of activity towards this complex remains bound to this fraction. On the other hand, *ca* 40% of activity towards single-stranded RNA remains bound to the ribosome fraction washed 3× with NH_4Cl. This allows the conclusion that such washing releases the major part of the enzyme-hydrolysing double-stranded RNA structures. The higher total activity level towards poly (A)–poly (U) in ribosomes washed once or twice with NH_4Cl as compared with the activity of unwashed ribosomes is evidence that the enzyme-degrading double-stranded structures associated with ribosomes occur partly in latent form.

The data obtained by us differ from those of Grill *et al.* [8] who did not observe nucleolytic activity

Table 1. Nucleolytic activities of ammonium chloride wash from rye germ ribosomes

Substrate	Sp. act. (units/mg)	Rel. act.
Single-stranded RNA from wheat germ	325	1.0
Double-stranded RNA from virus of *Penicillium chrysogenum*	152	0.46
rRNA from *E. coli*	235	0.72
tRNA from *E. coli*	210	0.64
tRNA from yeast	155	0.47
Poly (dT)–poly (rA)	210	0.64
Poly (A)–poly (U)	255	0.78
Poly (I)–poly (C)	115	0.35
Poly (A)	250	0.76
Poly (C)	745	2.29
Poly (U)	660	2.03

Table 2. Release of nucleolytic activities from rye germ ribosomes

Fraction	Total vol. (ml)	Total protein (mg)	Activity towards Single-stranded RNA		Activity towards Poly (A)–poly (U)		Activity towards Poly (A)–poly (U)
			Total activity (units)	Sp. act. (units/mg)	Total activity (units)	Sp. act. (units/mg)	Activity towards single-stranded RNA
Cytosol	300.0	660.00	43800.0	66.4	36900	55.9	0.84
Unwashed ribosomes	2.0	21.00	323.4	15.4	248	11.8	0.77
NH_4Cl wash I	9.0	8.20	747.0	91.0	486	59.3	0.65
Ribosomes washed ×1	2.3	11.30	96.6	8.6	324	28.7	3.35
NH_4Cl wash II	9.0	1.35	104.4	77.3	139	102.7	1.33
Ribosomes washed ×2	2.7	9.85	54.0	5.5	319	32.3	5.90
NH_4Cl wash III	9.0	0.18	81.0	450.0	18	100.0	0.22
Ribosomes washed ×3	1.9	7.60	132.8	17.5	67	8.9	0.51

towards double-stranded RNA in a cell-free system (containing ribosomes) from wheat germs.

Earlier, Robertson and Mathews [9] observed activity towards double-stranded RNA in the cytosol and ribosomal fractions of Krebs II ascites cells. Similarly, Shanmungam [10] found in cultures of embryonal mouse cells that activity towards double-stranded RNA is present in the cytoplasmic fraction and is also associated with ribosomes and microsomes. It has lately been reported that ribonuclease specific for double-stranded RNA was isolated by salt washing of the ribosomes of Ehrlich ascites cells [11].

The results of our investigation supplement these observations by reporting the occurrence in higher plants of enzymes degrading double-stranded RNA, associated with ribosomes.

EXPERIMENTAL

Plant material. Commercial rye (*Secale cereale* L.) germs were cleaned by sieving from starch contamination. Meshes of 1.2 and 0.6 mm were used. The germs remaining on the 0.6-mm sieve were taken for expts.

Reagents. The following synthetic substrates were used: poly (A)–poly (U); poly (I)–poly (C); poly (dT)–poly (rA); poly (U), poly (C) and poly (A) supplied by Miles. rRNA and tRNA from *E. coli* and rRNA from yeast were also products of Miles. Highly polymerized wheat germ RNA (grade A) from Calbiochem was used and double-stranded virus RNA of *Penicillium chrysogenum* was kindly supplied by Dr. H. Sierakowska.

Isolation of ribosomes. The germs were hand-homogenized in a mortar cooled with ice in 13 μM KPi buffer containing 0.25 M sucrose and 10 μM $MgCl_2$, pH 7.3. The homogenate was filtered through three layers of nylon and then centrifuged at 12000 *g* for 15 min. Triton X-100 was added to the supernatant to give a 0.5 concn and the supernatant was centrifuged at 40000 *g* for 30 min. The sediment was discarded, and to the supernatant 2-mercaptoethanol was added to give a concn of 10 mM. The ribosomes were sedimented at 105000 *g* for 2 hr. The remaining supernatant constituted the cytosol fraction.

Removal of proteins with nucleolytic activity from ribosomes. The ribosomes were suspended in 10 μM Tris–HCl buffer, containing 0.5 M NH_4Cl and 10 μM Mg acetate, pH 7.8, and left to stand for 18 hr with constant stirring. The ribosome sediment thus treated was then sedimented by centrifugation at 150000 *g* for 2 hr. Nucleolytic activity was determined in the ribosomal salt wash eluate thus obtained.

Activity determination. Nucleolytic activity was determined by the method described in ref. [7], but with application of a different activity unit. One unit of activity was the amount of enzyme which under the experimental conditions caused an increase in $\Delta A_{260}^{1\,cm} = 0.1$. Sp. act. is expressed in units/1 mg protein.

Protein determination. Protein was determined by the method of ref. [8] using the procedure for insoluble proteins. Purified trypsin was used as standard.

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