Ribosomes in Dormant and Germinating Conidia of *Aspergillus oryzae*

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Ribosomes were isolated from dormant and germinating conidia of *Asp. oryzae* No. 13. The ribosomes which consisted of 80 S were easily dissociated into 40 S and 60 S in low Mg^{++} buffer. Polyribosomes were not found in dormant conidia, but were found in germinating conidia. Ribosomes in *Aspergillus* fungi consisted of almost equal amount of RNA and protein, and the base compositions of RNA were alike, as compared as ribosomal RNA between dormant and germinating conidia.

INTRODUCTION

Ribosomes are considered to represent the protein synthesizing machinery in living cells, and their physico-chemical properties have been studied with various kinds of organisms.^{1~4)} In fungi, Minagawa et al.⁵⁾ have studied the base composition of ribosomal ribonucleic acid (RNA) in conidia and hyphae of *Neurospora crassa*, and Henney and Storck⁶⁾ have reported the base composition of total RNA, ribosomal RNA, and soluble RNA in conidia and hyphae of *Asp. oryzae*. Their studies have revealed that the base composition are almost similar between the two phases of life cycle. The present work was undertaken to investigate the state of ribosomes in dormant and germinating conidia of *Asp. oryzae*. The ribosomes in other *Aspergillus* fungi also have been studied.

MATERIALS AND METHODS

Conidia

Conidia of *Asp. oryzae*, No. 13 was obtained from

1) J. E. M. Midgley, *Biochim. Biophys. Acta*, **61**, 513 (1962).
2) M. L. Petermann and M. G. Hamilton, "Protein Biosynthesis", Academic Press, p. 233 (1961).

3) F. Chao and H. K. Schachman, *Arch. Biochem. Biophys.*, **61**, 220 (1956).

4) M. M. Tayler and R. Storck, *Proc. N. A. S.*, **52**, 958 (1964).

5) T. Minagawa, B. Wagner and B. Strauss, *Arch. Biochem. Biophys.*, **80**, 442 (1959).

6) H. R. Henney and R. Storck, *Proc. N. A. S.*, **51**, 1050 (1964).

Yamasa Shoyu Co., Ltd., conidia of *Asp. candidus*, *Asp. sojae*, *Asp. oryzae* E, and *Asp. oryzae* U were gifted from Kojiya Sanzaemon Co., conidia of *Asp. oryzae* A1 and *Asp. oryzae* Y were from Kon-no Koji Co.

Buffer systems

Two types of buffer were used: TM 1 buffer (0.01M Tris-HCl, and 0.005M $Mg(CH_3COO)_2$, pH 7.4), and TM 2 buffer, (0.01M Tris-HCl and 0.0001M $Mg(CH_3COO)_2$, pH 7.4).

Preparation of ribosomes from conidia

Conidia peste (500 mg of conidia) to which 1 g of alumina (500 mesh) had been added was ruptured by hand grinding for 5 min with chilling mortar and pestle. Ribosomal fraction was prepared by extraction with 20 ml of TM 1 buffer. The subsequent fractionation is shown in Fig. 1. These procedures were carried out in a cold room. The ribosomes thus obtained was suspended in 2 ml of TM 1 buffer to subject to analysis.

Chemical analysis

The amount of RNA was determined by the method of Kerr⁷⁾ and protein was by the method of Lowery et al.⁸⁾ Yeast ribonucleic acid (Sigma Chemical Co.) and bovine serum albumin (California Biochemical Corp.) were used as the standards for RNA and protein determination.

7) R. Kerr, *J. Biol. Chem.*, **159**, 211 (1945).

8) O. H. Lowery, N. J. Farr, and A. L. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

FIG. 1. Procedure for Fractionation of Ribosomes from the Conidia of *Asp. oryzae*

Nucleotide composition

Ribosomes were directly hydrolyzed with 1N HCl for 1 hr at 100°C,⁹⁾ and chromatographed on Tōyō Roshi No. 51A filter papers with *tert*-butanol-HCl-water (700:132:168 v/v) solvent system. After elution from papers with 0.1N HCl, each base was determined by the method of Marham and Smith.¹⁰⁾

Sedimentation analysis

Sedimentation coefficients were determined with a Hitachi analytical ultracentrifuge equipped with Schlieren optical system.

RESULTS

Ribosomes in dormant conidia of *Asp. oryzae* No. 13

Fig. 2 shows the Schlieren diagrams obtained from the ribosomal fraction of dormant conidia. Characteristics are; majority of the particles consist of 80 S particles and the fast moving faint boundary is 120 S which is probably a dimer of the 80 S particles. The average content of RNA and protein were analyzed in triplicate. The results are shown in Table I together with the nucleotide composition of ribosomal RNA. The ribosomes

FIG. 2. Schlieren Diagram of Purified Ribosomes from Dormant Conidia of *Asp. oryzae* No. 13.

Centrifugation was performed at 20°C and concentration of the ribosomes was about 7 mg per milliliter of TM 1 buffer. The picture was taken after 15 min. at 34,450 r.p.m.

described above were, after being dialyzed against TM 2 buffer for 18 hr at 4°C, subjected to ultracentrifugal analysis. Fig. 3 shows the Schlieren diagram of the dialyzed ribosomes. Two boundaries with the sedimentation coefficient of 60 S and 40 S are observed. There is no sign of 80 S particles. The 40 S and 60 S particles may represent the component of 80 S ribosomes.

9) J.D. Smith and R. Markham, *Biochem. J.* (London), **46**, 509 (1950).

10) R. Markham and J.D. Smith, *Biochem. J.* (London), **49**, 401 (1951).

FIG. 3. Schlieren Diagram of Ribosomes Containing about 2 mg per Milliliter Which had been Dialyzed against Low Mg^{++} Buffer.

Centrifugation conditions are the same as described in Fig. 2.

Ribosomes in germinating conidia of *Asp. oryzae* No. 13

Conidia (1 g) were suspended in 1 liter of Terui's medium having the following composition: glucose, 3%; KH_2PO_4 , 0.1%; $MgSO_4 \cdot 7H_2O$, 0.05%; KCl, 0.05%; NH_4NO_3 , 0.3%;

$FeSO_4 \cdot 7H_2O$, 0.001%; and polypeptone, 0.1%, delivered into a 5 liter Erlenmeyer flask and incubated at 26°C on a rotary shaker. Two hundred and fifty ml of the broth was withdrawn from the culture after 0, 60, 180, and 240 min respectively. Microscopic observation revealed that more than 90% of the conidia sprouted germ tubes after 240 min incubation. The culture fluid was centrifuged at $3,000 \times g$ for 10 min to collect the germinating conidia. The germinating conidia thus prepared were disrupted by the method described above, and the supernatant fluid after centrifugation at $15,000 \times g$ for 15 min was subjected to sedimentation analysis. The Schlieren patterns of the extracts are shown in Fig. 4. Only 80 S peak was observed in dormant conidia, but the germinating conidia contained considerable amount of 125 S, 160 S, and 185 S particles besides 80 S particles. These large ribosomes may be polyribosomes. The extract from the 180 min conidia was centrifuged at $105,000 \times g$ for 90 min, and ribosomes (mixture of 80 S to 185 S) were collected by the method described in Fig. 1.

a

b

c

FIG. 4. Schlieren Profiles of Germinating Conidia of *Asp. oryzae* No. 13.

a) Only 80 S ribosome unit is present in dormant conidia. Centrifugation was performed at 20°C and concentration of the ribosomes was about 15 mg per milliliter of TM 1 buffer.

b) Polyribosomes can be detected in 60 min. conidia. Centrifugation conditions are the same as described in the above except concentration, which was about 5 mg/ml.

c) 80 S ribosomes, and polyribosomes are found in 240 min. conidia. Centrifugation conditions are the same as in b. Pictures were taken after 10 min at 34,450 r.p.m.

TABLE I. RNA AND PROTEIN CONTENTS, AND THE NUCLEOTIDE COMPOSITIONS OF THE RIBOSOMAL FRACTION OF DORMANT AND GERMINATING CONIDIA

	Protein	RNA	A	G	C	U	G+C	Purine/ Pyrimidine	6 amino 6 keto	AU GC
Dormant conidia of <i>Asp. oryzae</i>	45%	55%	25.8	31.4	21.8	21.0	53.2	1.34	0.91	0.88
Germinating conidia of <i>Asp. oryzae</i>	47%	53%	25.2	32.1	22.5	21.6	54.6	1.27	0.91	0.88

A: Adenylate, G: Guanylate, C: Cytidylate, U: Uridylate.
 6 amino=6 amino nucleotides (adenylic and cytidylic acids)
 6 keto =6 keto nucleotides (guanylic acid and uridylic acid)

TABLE II. RNA AND PROTEIN CONTENTS, AND THE NUCLEOTIDE COMPOSITIONS OF THE RIBOSOMAL FRACTIONS OF *Aspergillus* FUNGI

	RNA	Protein	U	C	A	G	G+C	Purine/ Pyrimidine	6 amino 6 keto	AU GC
<i>Asp. oryzae</i> Y	55.8%	44.2%	20.0	22.2	23.7	34.1	56.7	1.37	0.91	0.78
<i>Asp. oryzae</i> A1	52.0	48.0	22.1	21.7	23.3	32.9	54.6	1.30	0.82	0.83
<i>Asp. oryzae</i> U	53.0	47.0	21.3	21.3	24.8	32.8	54.1	1.36	0.86	0.91
<i>Asp. candidus</i>	55.5	44.5	23.2	20.3	22.0	34.5	54.8	1.30	0.74	0.83
<i>Asp. sojae</i>	58.1	41.9	23.8	21.8	23.5	30.9	52.7	1.19	0.83	0.89

U: Uridylate, C: Cytidylate, A: Adenylate, G: Guanylate.
 6 amino=6 amino nucleotides (adenylic and cytidylic acids)
 6 keto =6 keto nucleotides (guanylic and uridylic acids)

The RNA and protein contents as well as the nucleotide composition of the ribosomal fraction are shown in Table I.

Ribosomes in conidia of other *Aspergillus* fungi

Ribosomes fractions prepared from conidia of *Asp. oryzae* Y, *Asp. oryzae* A1, *Asp. oryzae* U, *Asp. sojae* and *Asp. candidus* by the method described in a previous section were subjected to sedimentation analysis. They consisted of 80 S particles. The RNA and protein contents are shown in Table II together with the nucleotide composition.

DISCUSSION

On the bases described above, it may be concluded that the principal form of ribosomes in dormant conidia of *Aspergillus* fungi is of 80 S type. This observation is consistent with the results reported in *Neurospora crassa* and *Asp. niger*.^{5,6)} The 120 S particles in the purified ribosomal fraction were probably artifact, because, as shown in Fig. 4, no 120 S particles were observed in the extract directly

prepared from dormant conidia. It was reported in the case of *Asp. niger* that the 80 S ribosomes could dissociate into 40 S and 60 S only by treatment of EDTA. In *Asp. oryzae*, however, the 80 S ribosomes easily dissociated into 40 S and 60 S in low Mg⁺⁺ buffer. This is the only difference observed. The direct evidence that 125 S, 160 S, and 185 S particles found in the germinating conidia of *Asp. oryzae* were polyribosomes which were reported in other organisms⁶⁾ is still poor. Nevertheless, these large particles were found in only germinating conidia, and the incorporation of C¹⁴-uracil into cold TCA insoluble fraction began at 60 min after incubation,¹¹⁾ therefore, the large particles may be polysomes. The ribosomes in *Aspergillus* fungi consist of almost equal amount of RNA and protein, and base compositions of RNA are alike, as compared as ribosomal RNA, among the different species and between the dormant and germinating conidia.

11) K. Horikoshi, in preparation.