RIBOSOMAL RIBONUCLEIC ACIDS FROM *STREPTOMYCES GRISEUS*

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Nucleic acids from *Streptomyces griseus* 178 were isolated during cultivation. After their fractionation on a column of methylated serum albumin adsorbed on Kieselguhr, the 16 S and 23 S RNA were isolated. To characterize RNAs their sedimentation coefficients, T_m and nucleotide composition were determined. During cultivation of *S. griseus* 178 rRNA level reaches two maximum peaks and the production of streptomycin influences nucleic acids of the producer organism.

In our previous paper[1] we have shown that the level of nucleic acids in *S. griseus* 178 cultivation has two peaks: the first in the logarithmic phase of growth and the second during antibiotic biosynthesis. The decrease in RNA level during fermentation and the period during which the second increase of RNA level is observed depends on the intensity of streptomycin (STM) biosynthesis. Proportionally with STM production the second RNA peak was formed. More detailed study of RNA from this strain is the subject of this paper.

Materials and Methods

Producing strain:

Streptomyces griseus 178, a strain producing approximately 2,000 μg of STM per ml, was cultivated in the following medium: 3% soy-meal extract, 4% glucose, 0.25% NaCl, 0.6% $(NH_4)_2SO_4$, 0.05% KH_2PO_4, pH adjusted to 7.2~7.3; sterilisation for 45 minutes at 1.2 atm. 120°C. The cultivation (rotary shaker, 220 r.p.m., 100~120 hours) was carried out in 500-ml flasks, containing 60 ml of medium at 28°C.

Streptomycin determination:

Determination of streptomycin was carried out by the maltol method according to SCHENCK and SPIELMAN[2] combined with the method of BOXER *et al*[3]. The accuracy of the chemical determination of STM was checked by the microbiological agar plate assay according to HESS[4] using *Bacillus subtilis* 6633 as a test organism.

Isolation of total nucleic acids:

The washed mycelium was suspended in 0.005 M Tris buffer with 0.01 M Mg^{2+}, pH 7.4 and then homogenized in a blendor ETA MIRA (5×3 min) in an ice bath. Nucleic acids were isolated by phenol method according to KIRBY[5] combined with the method of ISHIHAMA *et al*[6]. Then they were dissolved in 0.05 M phosphate buffer, pH 6.7, containing 0.3 M NaCl.

Fractionation of nucleic acids:

Total nucleic acids were fractionized according to MANDELL and HERSHEY[7], and SUEOKA and CHENG[8] on a column of methylated serum albumin adsorbed on Kieselguhr (MAK). Hyflo Super Cel Kieselguhr and human serum albumin (Calbiochem) were used. The column (2×10 cm), was eluted with NaCl gradient (0.3~1.1 M) in 0.05 M phosphate buffer pH 6.7. Approx. 4 mg of RNA were applied on the column. (concentration 1 OD_{260} in 1 ml of the same buffer). RNA fractions obtained were desalted on a Sephadex G-100 column, and concentrated on a membrane filter "Synpor 10" (Synthesia, Pardubice, Czechoslovakia).

Sedimentation measurements:

An analytical ultracentrifuge Beckman Spinco, Model E, was used for the measurements of sedimentation coefficients. The centrifugation was carried out in double-sector Al cell at 44,000 rev/min. at 20°C. The sample concentration was 1.5 mg per ml in 0.05 M Tris buffer, pH 7.0, containing 0.1 M NaCl. Calculation was done according to SCHACHMAN[9].

Base composition of RNA:

RNAs were hydrolysed in 0.3 N KOH at 37°C for 18 hours according to OSAWA *et al*[10]. The hydrolysate was chromatographed on DEAE Sephadex A–25 column (1.2×60 cm). Elution was carried out with a linear NaCl gradient (from 0.0 to 0.5 M) in 0.05 M sodium carbonate - bicarbonate buffer, pH 9.2. The flow rate was 24 ml per hour. The absorbancy at 254 nm was registered on a UV-analyzer. The fractions of nucleotides after concentration in vacuum at 30°C, were chromatographed on Whatman No. 1 paper in a system isobutyric acid - NH_4OH - H_2O (66: 1: 3 by vol.). The spots of nucleotides were eluted in 0.1 N HCl at laboratory temperature for 24 hours, and their spectra were registered on a Beckman spectrophotometer DB in 0.1 N HCl and in 0.1 N KOH.

The thermal stability of RNA:

The measurements were carried out on a Unicam SP 800 spectrophotometer with heated cells and automatic registration of temperature. Samples (concentration 1.0 OD_{260} per ml) dissolved in 0.05 M phosphate buffer containing 0.15 M NaCl, pH 6.7, were used. From the absorbancy differences measured at 260 nm while gradually increasing the temperature (2°C per minute) the percentage of hyperchromicity was calculated.

Results

We have described in our previous paper[1] that the level of RNA in *S. griseus* 178 cells has two peaks, the first in the 24th hour and the second between the 70~80th hour. For RNA isolations samples were taken at such time intervals as to detect the known peaks and decrease in the RNA level. We have studied 10 fermentations in this way and our results were reproducible. One of these experiments is represented in Fig. 1 and Fig. 2. The absorbancy ratio of the isolated total nucleic acids at 260 nm and 280 nm was determined, with the coefficient value from 1.9 to 2.0. Fig. 1 shows fractionation of nucleic acids on MAK column into 4 fractions. 4 S RNA, DNA, 16 S RNA, and 23 S RNA are eluted at salt concentration 0.4 M, 0.74 M, and 0.87 M respectively. The data obtained show a small difference from the data determined for nucleic acid fractions isolated from *Escherichia coli* and fractionated on MAK[7,11,12]. Fig. 1 shows the fractionation of nucleic acids on MAK column in the 22nd, the 53rd, the 70th and the 94th hour of cultivation, respectively. The decrease of 16 S and 23 S RNA levels and the increase of the fraction eluted by 0.4 M NaCl gradient in the 53rd hour of fermentation was found (Fig. 1B). Evidently the degradation of 16 S and 23 S RNA takes place and probably degradation products are eluted already at this salt concentration. Fig. 1C showing the fractionation of nucleic acids from the 70th hour of fermentation is similar to Fig. 1A. Compared to the 53rd hour of fermentation 16 S and 23 S RNA levels are increased. In the 94th hour of fermentation 16 S and 23 S RNA levels decrease again and the absorbancy of the peak eluted by the gradient 0.4 M NaCl is increased (Fig. 1D). The changes in 16 S and 23 S RNA levels during cultivation which were estimated after fractionation of nucleic acids on a MAK column are shown in Fig. 2 in which also STM biosynthesis is presented. 16 S RNA and 23 S RNA fractions were assayed on an analytical ultracentrifuge and sedimentation coefficients were calculated. For 16 S and 23 S RNA samples the sedimentation coefficients 15.4 S (Fig. 3) and 23.8 S (Fig. 4) respectively, were determined. For the fraction eluted on MAK with gradient 0.4 M NaCl the sedimentation coefficient 4.2 S was determined.

Fig. 1. The fractionation of nucleic acids on MAK column.

A: The nucleic acids isolation from 22nd hour of fermentation

B: The nucleic acids isolation from 53rd hour of fermentation

C: The nucleic acids isolation from 70th hour of fermentation

D: The nucleic acids isolation from 94th hour of fermentation

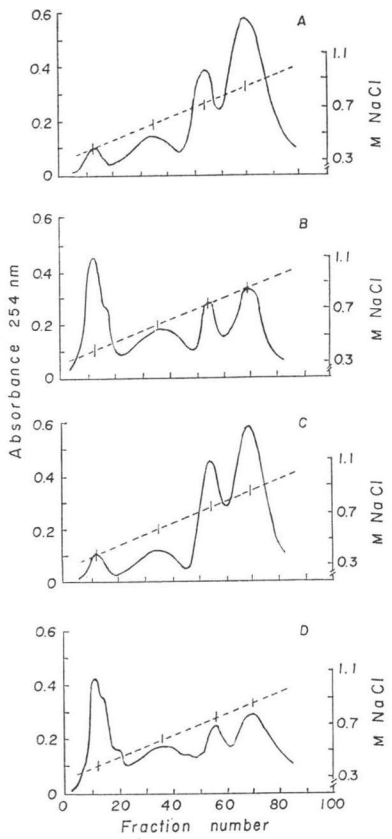


Fig. 2. The change of the rRNA levels and streptomycin production during cultivation.

The values of 16 S and 23 S RNA were estimated after the fractionation of nucleic acids on MAK column.

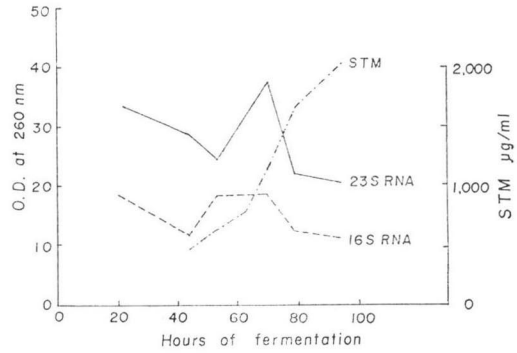


Fig. 3. The sedimentation velocity patterns of sample corresponding to 16 S RNA.

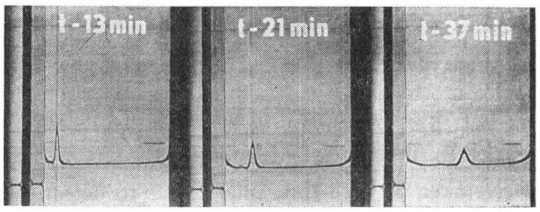
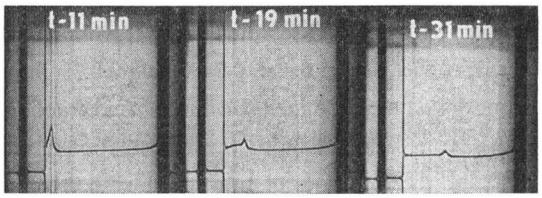


Fig. 4. The sedimentation velocity patterns of sample corresponding to 23 S RNA.



Chromatography mononucleotides of the RNA hydrolysate from the 22nd hour of fermentation are shown in Fig. 5. Table 1. shows the composition of mononucleotides in RNAs. The analysed RNAs are characterised by the increased amount of GMP in comparison with the other mononucleotides. The hyperchromicity dependence of different RNA on the temperature is shown in Fig. 6. For the total RNA $T_m = 55°C$ was determined. For 23 S RNA, 16 S RNA, and 4 S RNA temperature values 57°, 59° and 60°C, respectively, were found.

Discussion

It is evident from the results given in Fig. 1 and Fig. 2 that after intense synthesis of 16 S and 23 S rRNAs their decrease and later again their increase takes place.

RNA synthesis in the second peak is accompanied by antibiotic production. In the logarithmic phase of growth during which the antibiotic is not synthetized the first increase in the RNA level can be

Fig. 5. Products of RNA hydrolysis on DEAE Sephadex A–25 chromatography.

The RNA isolated at 22nd hour of fermentation.

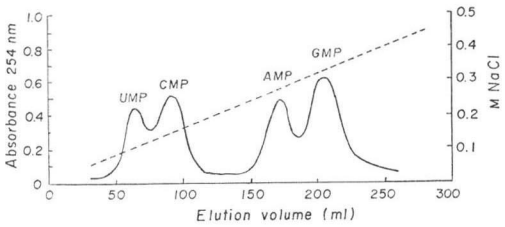
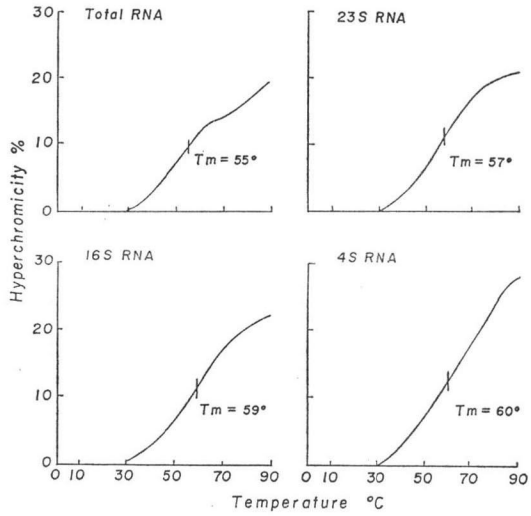


Table 1. Nucleotide composition of RNAs from *S. griseus*.

RNA	Amount of nucleotides (mole %)				
	G	A	C	U	$\frac{G+C}{A+U}$
Total	29.1	25.6	22.7	23.6	1.05
23 S	30.4	24.1	24.8	20.6	1.23
16 S	29.5	23.9	24.7	21.8	1.18
4 S	32.2	21.7	26.6	19.4	1.43

Fig. 6. Melting curves of ribonucleic acids of *S. griseus*.

Total RNA, 23 S RNA, 16 S RNA and 4 S RNA in 0.05 M phosphate buffer with 0.15 M NaCl, pH 6.7.



observed followed by an RNA decrease during which antibiotic production is initiated. Further streptomycin synthesis goes hand in hand with further RNA level increase[1]. Fractionation of the isolated RNAs has shown that mostly rRNAs are responsible for changes in the total RNA level in this streptomycete. These results are in accordance with our findings[1].

It is interesting that the polynucleotide phosphorylase activity (which was measured by ADP-phosphate exchange as well as by ADP-polymerization) during *S. griseus* 178 cultivations has also two maxima which correlate with maxima of RNA[13].

Sedimentation coefficients 16 S and 23 S of the isolated rRNAs of *S. griseus* are in agreements with the results of LOENING[14] which determined 16 S and 23 S sedimentation coefficients of rRNAs isolated from bacteria and actinomycetes.

We consider the onset of STM synthesis to be the cause of 16 S and 23 S RNA decrease because also the addition of exogenous STM in the 24th hour of fermentation causes rRNA decrease as compared with the control. This decrease is very pronounced in the 72nd hour of fermentation and in the 96th hour complete degradation of 16 S and 23 S RNA can be observed[15]. For the time being, there is no explanation for the phenomenon that in the producing phase a further increase of the RNA level takes place in the mycelium.

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