# CHARACTERIZATION OF PRODUCTS OF PHOSPHOROLYSIS OF *Streptomyces aureofaciens* RIBOSOMAL RIBONUCLEIC ACID

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Received April 6th, 1976

The results obtained in this study show that under optimum reaction conditions only one third of ribosomal ribonucleic acid, isolated from *Streptomyces aureofaciens* and showing sedimentation constants of $S^0_{20,w} = 15{\cdot}62$ S and 22·72 S is degraded by polynucleotide phosphorylase from the same microorganism. This reaction gives rise to adenine, guanine, cytosine and uracil nucleoside 5'-diphosphates and to smaller molecular fragments of ribosomal ribonucleic acid which show a sedimentation coefficient about 9 S. Bentonite is an effective stabilizer of ribosomal ribonucleic acid during its incubation in an alkaline medium (pH 8·9; 37°C). The activity of polynucleotide phosphorylase remained unaffected by the presence of bentonite.

Polynucleotide phosphorylase is an enzyme catalyzing polyribonucleotide polymerization or depolymerization proceeding *via* the following reversible reactions:

$$(\mathrm{NMP})_n + n\,\mathrm{P_i} \overset{Mg^{2+}}{\rightleftharpoons} n\,\mathrm{NDP}$$

The polyribonucleotide chains are degraded during the phosphorolytic reaction by a processive exonucleolytic mechanism[1]. The physiological function of polynucleotide phosphorylase as well as of ribosomal ribonucleic acid (rRNA) has not been explained in full eyt. It is assumed that polynucleotide phosphorylase is a part of a multienzyme system responsible for the elimination of messenger RNA after its translation as well as for the regeneration of substrates for additional RNA synthesis[2-4]. The degradation of rRNA is believed to result from a combined action of endo- and exonucieases and of polynucleotide phosphorylase[5,6]. It appears that polyribonucleotide phosphorylase alone does not participate on the degradation of *Streptomyces aureofaciens* rRNA since *in vivo* its activity in *Streptomyces*

*Abbreviations used:* Pi — inorganic phosphate; NMP — adenine, guanine, cytosine, and uracil nucleoside 5'-monophosphates; poly A-polyadenylic acid; NDP-adenine, guanine, cytosine, and uracil nucleoside 5'-diphosphates.

*aureofaciens* systems culminates at the stage of active proteosynthesis and decreases during the phase of intensive rRNA degradation and of intensive chlortetracycline biosynthesis[7]. Conversely, rRNA degradation by enzymes bound to *S. aureofaciens* ribosomes increases in the presence of chlorotetracycline[8]. The phosphorolysis of polyribonucleotides depends on their primary, secondary, and tertiary structure; the rate of ribonucleic acid phosphorolysis is relatively low[9–11]. The existence of a relationship between the secondary and tertiary structure of ribonucleic acids and their phosphorolysis has been evidenced by the results of studies on the phosphorolysis of specific transfer RNA's[12,13]. The phosphorolysis of rRNA *in vitro* has not been studied in detail. In this study we examined the phosphorolysis of *Streptomyces aureofaciens* rRNA *in vitro* by polynucleotide phosphorylase from the same organism and characterized the products of this reaction.

## EXPERIMENTAL

*Material.* Adenosine 5'-diphosphate, guanosine 5'-diphosphate, cytidine 5'-diphosphate, uridine 5'-diphosphate sodium salts were from Calbiochem, Los Angeles, USA. Sephadex G-75 was a product of Pharmacia, Uppsala, Sweden. Dowex 1-X8, 200–400 mesh, in $Cl^-$ form, was from Fluka, FRG, Bentonite and sodium dodecyl sulphate from Serva, Heidelberg, GFR. Phenol was a product of Lobachemie, Wien, Austria. [$^{32}$P]$K_2HPO_4$, specific activity 9·2 mCi/m. was supplied by the Institute for Research, Production and Use of Radioisotopes, Prague. The membrane filters were from Synthesia, Pardubice. The mycelium from the 27th hour of plant fermentation of *S. aureofaciens* (Biotika, Slovenská Ľupča) was used as a source for the isolation of ribosomes and polynucleotide phosphorylase. The mycelium was washed twice with 0·9% NaCl and stored at −20°C.

*Isolation of ribosomes and ribosomal ribonucleic acid.* The ribosomes were isolated by the method of fractional centrifugation according to Spirin and coworkers[14]. rRNA was isolated by the method of Kirby[15] as modified by Dingman and Sporn[16].

*Activity of polynucleotide phosphorylase and phosphorolysis of ribosomal ribonucleic acid.* The enzyme (E.C. 2.7.7.8) was prepared according to Šimúth and coworkers[17]. Its specific activity was 200 μmol of [$^{14}$C]AMP incorporated into poly A in one hour by one milligram of the protein. The optimum conditions of phosphorolysis of *S. aureofaciens* RNA were determined by Šimúth and coworkers[18]. The standard incubation mixture (0·2 ml) contained in μmol: Tris-HCl (pH 8·9) 10; $NH_4Cl$ 0·50; EDTA 0·002; $K_2H^{32}PO_4$, specific activity about 0.05 μCi/μmol 3; or $K_2HPO_4$ 3; $MgCl_2$ 1. Bentonite 0·050 mg, rRNA $2A_{260nm}$ units; polyribonucleotide phosphorylase 50 μg. Reaction time 2 hours at 37°C. The [$^{32}$P] ribonucleoside diphosphates resulting from rRNA phosphorolysis in the presence of $K_2H^{32}PO_4$ were measured according to Singer[19] in a Vakutronic VA-G-20 apparatus using a GM tube. The ribonucleoside diphosphates were separated from rRNA by gel filtration on Sephadex G-75. The standard incubation mixture after rRNA phosphorolysis (volume 1 ml) was applied to a Sephadex G-75 column equilibrated with 0·05M Tris-HCl buffer, pH 7·0, containing 0·15M-NaCl and 0·001M EDTA. The fraction containing high polymers was used for the determination of the sedimentation coefficients of rRNA.

*Analytical ultracentrifugation.* The sedimentation coefficients of rRNA were determined by the sedimentation velocity method in Beckman Model E Ultracentrifuge at 44000 rev./min at 20°C

AnD rotor and a standard aluminum cell at a 70° angle of the schlieren diaphragm were used for the determination by the schlieren technique. The same rotor with a two-sector cell and UV-optics with photoelectric scanner were used for the measurement at 260 nm. The sedimentation coefficients were calculated according to Schachman[20]; the value of partial specific volume of rRNA 0·53 ml/g (Stanley[21]) was used in the calculation of the sedimentation constants. The kinematic viscosity of 0·05M Tris-HCl buffer, pH 7·0, containing 0·15M-NaCl and 0·001 M EDTA was measured in an Ubbelohde U2 viscosimeter at 20°C.

*Identification of products of phosphorolysis of ribosomal ribonucleic acid.* The low molecular weight products of rRNA phosphorolysis were analyzed by ion exchange chromatography on Dowex 1-X8 in $Cl^-$-form. The ribonucleoside diphosphates were also identified by paper chromatography and autoradiochromatographically in the Krebs–Hems solvent system[22], isobutyric acid–1M-$NH_4OH$–0·1M-EDTA (250 : 150 : 4, v/v). The identity of NDP was also determined from UV spectra recorded in a Beckman DB spectrophotometer.

## RESULTS AND DISCUSSION

*S. aureofaciens* rRNA with sedimentation constants $S^0_{20,w}$ = 15·62 S and 22·72 S was used as a substrate in the phosphorolytic reaction. The UV spectrophotometric characteristics of the rRNA isolated indicate a good deproteinization of the sample since the absorbance ratio $A_{260nm}/A_{280nm}$ was in the range 2·0—2·2. Since the incuba-

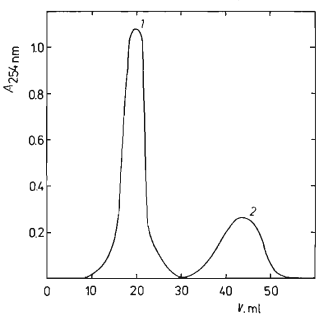



FIG. 1

Separation of Products of Phosphorolysis of rRNA

Column of Sephadex G-75 (1·4 × 19 cm); flow rate 0·5 ml/min; eluant 0·05M Tris-HCl (pH 7·0) containing 0·15M-NaCl and 0·001M-EDTA, *1* residual rRNA, *2* mixture of nucleoside diphosphates.

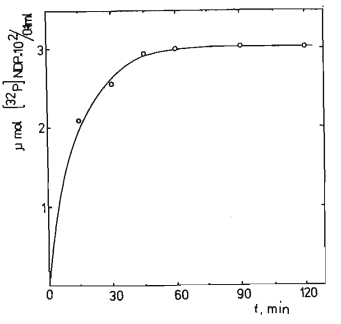



FIG. 2

Time Dependence of Phosphorolysis of rRNA

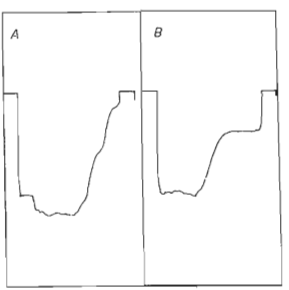
The phosphorolysis of rRNA was allowed to proceed at 37°C in the standard incubation mixture (see Experimental).

tion of rRNA in the standard phosphorolysis mixture in the absence of polynucleotide phosphorylase led to a decrease of the sedimentation coefficients of rRNA (the sedimentation coefficients decreased to about 16 and 13 S after 2 and 24 hours respectively of incubation under these conditions), experimental conditions were developed under which the sedimentation characteristics of rRNA remained unaltered. We used bentonite, a well-known inhibitor of ribonucleases, which does not affect the activity of polynucleotide phosphorylase in the phosphorolytic reaction. The addition of 0·025 mg of bentonite per one $A_{254nm}$ unit of rRNA in 0·1 ml of the reaction mixture does not change the sedimentation characteristics of rRNA even after 24-h incubation at 37°C. Chromatography on Sephadex G-75 (Fig. 1) of the standard incubation mixture after rRNA phosphorolysis showed that one third of rRNA only is phosphorylated under optimum reaction conditions. The optimum quantity of enzyme was that which brought about the highest yield of adenine, guanine, cytosine, and uracil nucleoside 5'-diphosphates; an additional quantity of the enzyme did not increase the yield. As shown in Fig. 2, rRNA degradation by this quantity of enzyme stops after 90 min of incubation. The first peak obtained by chromatography on Sephadex G-75 represent polyribonucleotide chains of sedimentation coefficient about 9 S (Fig. 3). The second peak is represented by a mixture of adenine, guanine, cytosine and uracil nucleoside 5'-diphosphates. The identity of the products of rRNA phosphorolysis was shown by ion exchange chromatography on Dowex 1 in $Cl^-$-form, by paper chromatography, autoradiochromatographically as well as by UV spectra measurement. The results obtained permit us to postulate that all 16 S and 23 S molecules of *S. aureofaciens* rRNA are attacked by polynucleotide

FIG. 3

Sedimentation Characteristics of rRNA after Phosphorolysis

*A* blank experiment: rRNA incubated in standard phosphorolysis mixture in the absence of polynucleotide phosphorylase, 2 h at 37°C. The values of the sedimentation coefficients are 14 S and 24 S. *B* rRNA in standard phosphorolysis mixture in the presence of polynucleotide phosphorylase, 2 h at 37°C. The value of the sedimentation coefficient is 9 S. The records were made by means of the UV optics of the analytical ultracentrifuge 16 min after the rotor had achieved its working speed.



phosphorylase and that the factor limiting this phosphorolytic degradation is the structure of the substrate itself, similarly to the phosphorolysis of specific transfer ribonucleic acids[12,13,23].

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Translated by V. Kostka.