DETECTION OF SYNTHETIC ACTIVITY OF POLYNUCLEOTIDE PHOSPHORYLASE IN RIBOSOMES OF Escherichia coli ATCC No. 7020

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Ribosomes of Escherichia coli ATCC No. 7020 contain two enzymes which catalyze the synthesis of polyadenylic acid (poly-A): polynucleotide phosphorylase (PNPase) and poly-A polymerase (PAPase). It was shown that 70-80% of the synthetic activity of PNPase determined by the reaction of polymerization of UDP-C^{14} and ADP-C^{14} is bound with the E. coli ribosomes and only 20-30% is found in the S-100 fraction. Absence of phosphorylation of ADP to ATP and independence of the incroporation of ADP-C^{14} of the action of PAPase were found.

In a study of the activity of polynucleotide phosphorylase (nucleosidediphosphate-polynucleotide-nucleotidyl transferase; 2.7.7.8) (PNPase) in subcellular fractions of Escherichia coli ATCC No. 7020 the authors found that ribosomes isolated from this microogranism exhibit considerable activity in the synthesis of polyadenylic acid (poly-A) from ADP-C^{14}. At the same time the virtually complete absence of synthesis of poly-A was observed when the postribosomal (S-100) fraction was used as the enzyme [1].

The object of the present investigation was to make a more detailed study of the synthetic and phosphorolytic activity of PNPase in the various subcellular fractions of E. coli ATCC No. 7020.

EXPERIMENTAL METHOD

A culture of E. coli ATCC No. 7020 was grown at 37°C in nutrient medium. The cells were collected by centrifugation, broken up by grinding with Al_2O_3, suspended in standard buffer [5] with the addition of deoxyribonuclease (2 μg/ml), and centrifuged at 30,000 g for 20 min. The resulting supernatant (fraction S-30) was used to obtain ribosomes and to determine PNPase activity. Ribosomes were obtained by centrifuging the S-30 fraction at 144,000 g for 2.5 h in a Spinco L ultracentrifuge and the resulting residue of ribosomes was suspended in standard buffer. The supernatant (fraction S-100) and the ribosomal preparation were used to determine the activity of the enzyme.

The incubation mixture for the polymerization reaction contained in 0.55 ml (in μmoles): ADP-C^{14} or UDP-C^{14} 0.12-0.18 (47,000-60,000 counts/min), ADP or UDP 0.7, $MgCl_2$ 0.25, Tris-HCl buffer, pH 8.0, 100. The concentration of enzyme protein was 150-200 μg per sample. The samples were incubated and treated by the method described previously [2]. Radioactivity was measured with a liquid scintillation counter (Picker Nuclear).

The unit of synthetic activity of PNPase was taken to be the amount of enzyme catalyzing the incorporation of 1 μmole ADP-C^{14} or UDP-C^{14} into the acid-insoluble material in 1 h at 37°C.

The unit of phosphorolytic activity of PNPase was taken to be the quantity of enzyme catalyzing the liberation of 1 μmole ADP-p^{32} or UDP-p^{32} in 1 h at 37°C.

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TABLE 1. Synthesis of poly-A from ADP-C^{14} and of poly-U from UDP-C^{14} Catalyzed by Different Subcellular Fractions of E. coli

Substrate	Fractions	Activity of PNPase (in units)	Specific PNPase activity (in units per mg protein)
ADP-C^{14}	S-30	7,81	0,13
	S-100	1,24	0,036
	Ribosomes	6,5	0,2
UDP-C^{14}	S-30	22,3	1,75
	S-100	6,69	0,72
	Ribosomes	15,12	1,72

TABLE 2. Phosphorolysis of poly-A and poly-U Catalyzed by Different Subcellular Fractions of E. coli

Substrate	Fractions	Activity of PNPase (in units)	Specific PNPase activity (in units per mg protein)
Poly-A	S-30	1,47	0,148
	S-100	1,31	0,13
	Ribosomes	0,172	0,027
Poly-U	S-30	9,84	0,7
	S-100	9,1	0,92
	Ribosomes	0,848	0,08

TABLE 3. Specificity of PNPase Bound with Ribosomes Relative to ADP and ATP

Sample	Composition of samples incubated	Amount of poly-A synthesized (in counts/min/sample)	%incorporation
1	ADP-C^{14} (0.01 μmole) + ADP (1 μmole)	2072	5
2	ADP-C^{14} (0.01 μmole) + ATP (1 μmole)	66	0,16
3	ATP-C^{14} (0.01 μmole) + ATP (1 μmole)	458	2

EXPERIMENTAL RESULTS

Investigation of the activities of the S-30 (crude extract of E. coli), S-100, and ribosomal fractions in the reactions of synthesis of poly-A from ADP-C^{14} and of poly-U from UDP-C^{14} showed that most of the synthetic activity of the PNPase was connected with the ribosomes (Table 1). In the case of synthesis of poly-A from ADP-C^{14} about 80% of the enzyme activity was connected with the E. coli ribosomes and only 20% of the activity was detected in the S-100 fraction. In the reaction of polymerization of UDP-C^{14} most of the activity (70%) also was found in the ribosomal fraction and only 30% of it in the S-100 fraction.

It is also clear from Table 1 that the specific activity of PNPase bound with ribosomes in the reactions of polymerization of ADP-C^{14} and UDP-C^{14} was much higher than in the S-100 fraction and was practically the same as in the S-30 fraction. However, determination of the PNPase activity in the reaction of phosphorolysis of poly-A and poly-U in the various subcellular fractions of E. coli showed that about 99% of the phosphorolytic activity of the enzyme was present in the S-100 fraction and only 10-12% of the activity was connected with the ribosomes (Table 2).

It is important to note that in the reaction of phosphorolysis of poly-A and poly-U the highest specific activity, comparable with the S-30 fraction, was found in the S-100 fraction.

The results of these experiments thus demonstrate the structural dissociation of the two principal activities of PNPase in E. coli cells: synthetic, connected mainly with the ribosomes, and phosphorolytic, found in the S-100 fraction.

Meanwhile other workers have shown that 75% of the activity of polyriboadenylate polymerase (poly-A polymerase) (PAPase), which catalyzes the synthesis of polyriboadenylic acid from ATP in the presence of Mg^{++} and RNA, also is connected with the ribosomes [3, 4]. In the present series of experiments it was therefore necessary to rule out the presence of active phosphotransferases, capable of phosphorylating the ADP added to the incubation mixture into ATP, and to show that the synthesis of poly-A from ADP-C^{14} found in these experiments on the E. coli ribosomes is catalyzed by PNPase and not by PAPase. The results of these experiments are given in Table 3.

It will be seen in Table 3 that on addition of equimolar amounts (1 μmole) of nonradioactive ADP and ATP to the preparation of ADP-C^{14} incorporation of the label into poly-A, catalyzed by ribosomes, took place

only in the presence of ADP (samples Nos. 1 and 2). On the other hand, the appreciable incorporation of ATP-C^{14} into poly-A in the presence of nonradioactive ATP (sample No. 3) confirms the presence of PAPase in the ribosomes of E. coli as described by other workers.

The incubation sample in the reaction of poly-A synthesis from ADP-C^{14} (sample No. 1) also was analyzed by thin-layer chromatography on DEAE-cellulose. The results showed that after incubation of ADP-C^{14} with ribosomes no appreciable amounts of ATP-C^{14} were formed, and in conjunction with the absence of incorporation of ADP-C^{14} with nonradioactive ATP (sample No. 2) they confirm the hypothesis that phosphorylation of ADP to ATP does not take place under the experimental conditions used and that the incorporation of ADP-C^{14} is independent of the action of PAPase. The results also show that synthesis of poly-A and poly-U from ADP-C^{14} and UDP-C^{14} respectively is catalyzed by PNPase bound with the ribosomes of E. coli.

It is difficult at present to identify the role of the polyribonucleotides synthesized by PNPase and PAPase. It can, however, be postulated that the functions of these two enzymes in intact E. coli cells are different. This is shown indirectly by the following facts [1, 3]:

1. Poly-A-polymerase is bound with 70S-ribosomes, whereas, as the present experiments show, PNPase is found in the 30S-ribosomal subparticles.

2. On dissociation of 70S-ribosomes poly-A-polymerase was found in the 50S-subparticle, while PNPase in the present experiments remained bound with the 30S-subparticle.

3. PAPase activity was not found in particles heavier than 70S, whereas in the logarithmic phase of growth of E. coli it was found that up to 40% of the synthetic activity of PNPase was bound with the polyribosomes.

A further study of the relations between these two enzymes may shed light on their physiological role in the bacterial cell.

LITERATURE CITED

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