FATE OF SPECIFIC RIBONUCLEOSIDE TRIPHOSPHATASES ON PURIFICATION AND RECONSTITUTION OF THE RIBOSOMAL SYSTEM OF ESCHERICHIA COLI

By

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SUMMARY

A study was made of the relative changes in RNA, protein, and four specific triphosphatases ~~which occur when a crude extract~~ During separation of E. coli ~~is separated~~ into ribosomal and supernatant fractions, and when the purified ribosomes are subsequently recombined with "supernatant". The following results were obtained:

1. In the course of the centrifugal purification of the ribosomal system there is a net and irreversible loss of material from the "heavy" and "light" particle fractions, the loss of protein being relatively greater than that of RNA.

2. Some, but not all of the protein lost from the ribosomes in the course of their purification is readsorbed when washed ribosomes are mixed with supernatant.

3. Both the protein and the RNA lost in the washings are qualitatively different from that remaining in either the ribosomes or the supernatant. Hence, the reconstituted system is not equivalent to the crude extract.

[redacted]

4. The four triphosphatase activities are differentially removed from the ribosomes during the washing procedure. Specifically, washing caused an increase in specific activity of UTPase, a partial loss of ATPase, a complete, but reversible loss of GTPase, and a complete and irreversible loss of CTPase. The results prove that four different enzymes are responsible for the four triphosphatase activities.

INTRODUCTION

The existence of specific ribonucleoside triphosphatases in crude extracts of E. coli was demonstrated in the preceding paper (1). Since the bulk of these enzymes appeared to be particle-bound, it seemed of interest to investigate their fate in the course of routine purification of ribosomal and supernatant fractions, as commonly employed for studies of amino acid incorporation. This is the subject of the present communication.

METHODS AND MATERIALS

All methods of preparation and analysis were as described in the two preceding papers (1, 2), except that all samples, as well as the sucrose density gradients (SDG) were prepared and kept in 0.01 M Mg acetate, 0.005 M Tris, pH 7.1. This lower pH was chosen to facilitate the adsorption to the ribosomal fractions of total protein (2), though not necessarily of triphosphatases (1).

An aliquot of crude extract was centrifuged on a SDG, and analyzed while ribosomes and supernatant were being prepared from the same extract. (The ribosomes were taken through three cycles of centrifugation.) The next day ribosomes and supernatant fraction were mixed in a proportion approximating that of the crude extract; this reconstituted system was then compared with ribosomes and supernatant fraction alone.

RESULTS

The distributions of RNA and protein in the isolated fractions and reconstituted system, as compared to that in the crude extract, are given in Figs. 1 and 2. It is seen that protein and RNA were lost mainly from the heavy and light particle fractions, as expected from the centrifugation schedules employed in the washing of the ribosomes. The relative loss of protein, however, was greater than that of RNA, as shown by the increased RNA to protein ratio of the ribosomes, as compared to the crude extract (Fig. 3). The RNA to protein ratio profile of the reconstituted system was remarkably similar to that of the crude extract, as some, though not all, of the protein lost during purification was readsorbed when ribosomes were mixed with supernatant.

As seen in the preceding paper (1) the bulk of the specific triphosphatase activities is sedimentable in the crude extracts, and hence appears in the crude ribosomal pellets. In the process of washing the ribosomes, however, these activities are differentially lost. CTPase was the most labile, it being removed completely in the first wash. Next in lability was GTPase which was very low after the second wash. ATPase activity was also reduced by washing, but the ultimate level of activity in the purified ribosomes varied greatly with both the conditions of purification, as well as the nature of a given preparation. (The ribosomes in the present experiment had a rather higher activity than usual.) The UTPase activity was most refractory to removal by washing, and all ribosomal preparations examined to date contained this activity. Under the particular conditions of the present experiment, the specific UTPase activity was actually greater in the purified ribosomes than in the crude ones. Fig. 4 shows the distribution of UTPase activity in a crude ribosomal pellet (which, however, was low

in ATPase activity), and Fig. 5 shows the distribution of UTPase and ATPase in the ribosomes used in the present experiment. (This preparation was free of CTPase, and essentially free of GTPase activity.) It should be noted that the enzyme peaks in the purified ribosomes are as sharp or sharper than in the crude extract, showing that ribosomes remain heterogeneous even after purification (see also (2)). Also, the specific activity of the enzymes is highest in the heavy regions of the gradient, just as in crude extracts (1) and very low in the main peak. The same is true of ribosomes of pea seedlings (3) and of reticulocytes (4).

The low triphosphatase activity in the supernatant has been previously noted (1). It seems likely, moreover, that most of this activity is still due to small amounts of incompletely sedimented particles, since when an aliquot of "supernatant" (previously centrifuged for 4 hours at 145,000 g in a Spinco rotor No. 50, and upper two thirds taken) was analyzed on a SDG, each of the triphosphatase activities sedimented in a series of well-defined peaks (Fig. 6). While the positions of the peaks were the same for all activities, the activity of each enzyme relative to the others was greatly different from peak to peak. It should also be pointed out that since the bulk of the protein stayed essentially on top of the gradient (Fig. 2), the specific activity (per mg protein) of the sedimenting enzyme peaks was very high. Another noteworthy fact is that, whereas neither the RNA nor the protein profiles alone (Figs. 1 and 2) showed any signs of resolution, a number of components were discernible in the RNA to protein ratio profile of the supernatant (Fig. 3). The valleys (protein-rich components) in the latter correspond to the peaks of enzyme activity.

Fig. 7 shows the distribution of each of the four triphosphatases in the reconstituted system compared with that of the individual components

on the one hand, and with that of the crude extract, on the other. Each of the four enzymes behaves differently. Most remarkable is the almost total loss of the CTPase activity of the crude extract upon purification of the ribosomal system. A large portion of the total ATPase activity is also lost, but some remains; note, however, that the supernatant seems to inhibit the ATPase activity in the ribosomes, since the activity of the reconstituted system is lower than that of the ribosomes. The behaviour of the GTPase is interesting in that, although it can be readily washed off the ribosomes, it can also be readsorbed from the supernatant. Finally, very little UTPase is lost, so that the enzymatic profile of the reconstituted system is remarkably similar to that of the crude extract in terms of total activity, although the reconstituted system is more active in terms of activity per mg protein.

The loss or enrichment of the enzymes relative to that of total protein is brought out more clearly in Fig. 8, which shows a comparison of specific activities. Besides the relative enrichment in UTPase activity, it is particularly noteworthy that the GTPase activity in the purified system is almost exactly equivalent to that of the unfractionated extract in terms of specific activity.

DISCUSSION

The importance of the present results, besides strongly substantiating the claim of the previous paper (1) that the four triphosphatase activities reside in physically distinct entities, lies in showing that the protein lost from the ribosomes in the course of their purification is qualitatively different from either the supernatant or the remaining ribosomal proteins. This finding is of relevance to studies on amino acid incorporation in vitro, quite regardless of any functional importance of these particular

triphosphatases, because it shows up the possibility that other enzymes of potential importance might be lost in the wash fluids and cannot be replaced by the supernatant.

Moreover, the fact that GTPase lost on washing is readsorbed to its original level of specific activity when washed ribosomes are mixed with supernatant, whereas CTPase, although present in the supernatant in comparable amounts to GTPase (cf. Fig. 6), is not readsorbed, shows that washing destroyed the binding site for one, but not the other enzyme. Since the solubilization of the enzymes by ribonuclease (1) or pre-incubation of the crude extract (5) suggests that the binding of the enzymes is mediated by RNA, it further follows that the RNA, as well as the protein, lost in the washes is qualitatively different from the remaining ribosomal RNA and, similarly, cannot be replenished from the supernatant.

With these results, together with those in the two preceding papers (1, 2), the present study succeeded in its original objective of showing that many possible and previously unrecognized differences exist between ribosomal preparations which look identical by conventional sucrose density gradient analysis. The next step will be to determine the relevance, if any, of some of these changes to the mechanism of protein synthesis.

ACKNOWLEDGEMENTS

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LEGENDS FOR FIGURES

Fig. 1. Distribution of RNA.

The broken lines represent purified ribosomes and supernatant, respectively; o.... o.... crude extract; ●—●, reconstituted system.

Fig. 2. Distribution of protein. Legends as in Fig. 1.

Fig. 3. RNA to protein ratio profiles. Legends as in Fig. 1.

Fig. 4. Distribution of UTPase activity in a crude ribosomal pellet.

The ribosomes were prepared, recycled once, and analyzed in 0.005 M Tris, pH 7.4, 0.005 M $Mg(Cl)_2$. RNA to protein ratio was 0.7. The broken line represents the distribution of RNA.

Fig. 5. Enzymatic activities of ribosomes washed three times in 0.01 M Mg acetate, 0.005 M Tris, pH 7.1 (RNA/protein 1.41).

The broken line represents RNA; x... x., ATPase; ▲—▲, UTPase. The upper part represents specific activities, the lower one, total activities.

Fig. 6. Distribution of triphosphatase activities in the supernatant fraction.

x --- x -, ATPase; o .... o .., GTPase; ▲—▲— UTPase; and ● — ●, CTPase. The fastest peak corresponds to about 60s.

Fig. 7. Comparison of total triphosphatase activities of crude extract (●— ●—), purified ribosomes (▲--▲—), supernatant fraction (x -- x --), and the reconstituted system (o— o—).

Fig. 8. Comparison of specific triphosphatase activities of crude extract. Legends as in Fig. 7.

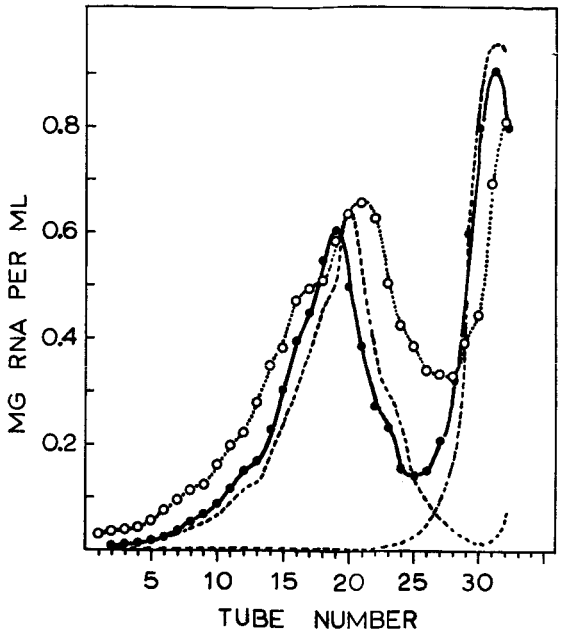


Figure 1

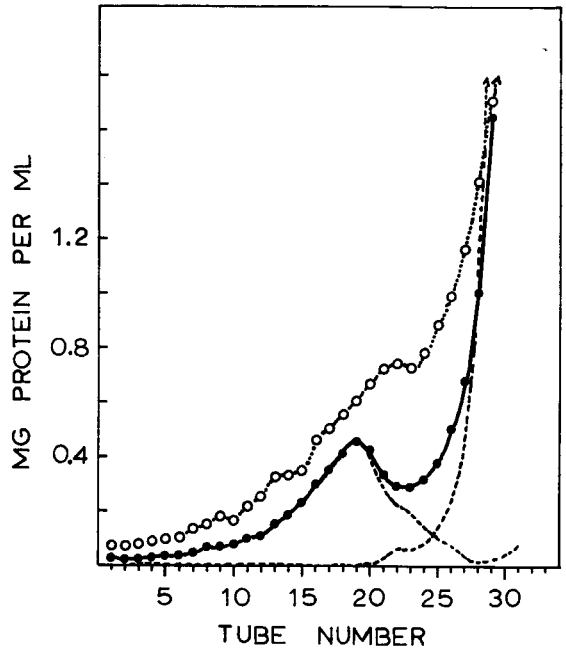
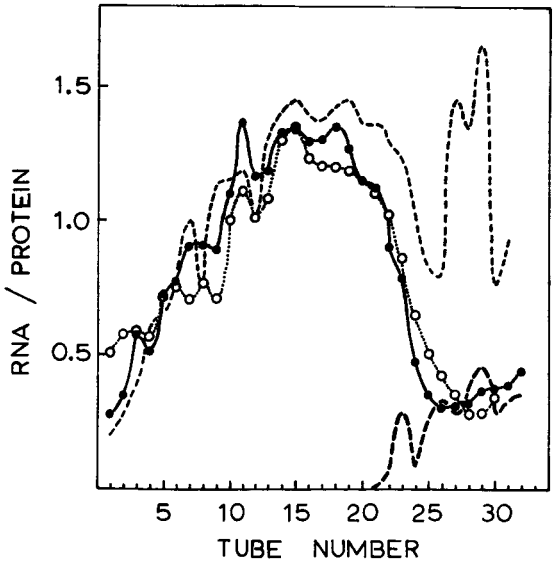


Figure 2



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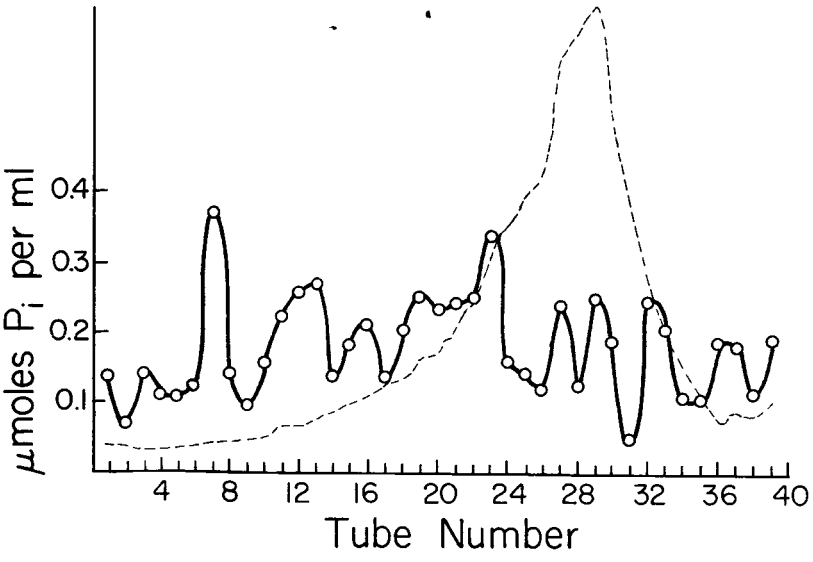

Figure 4

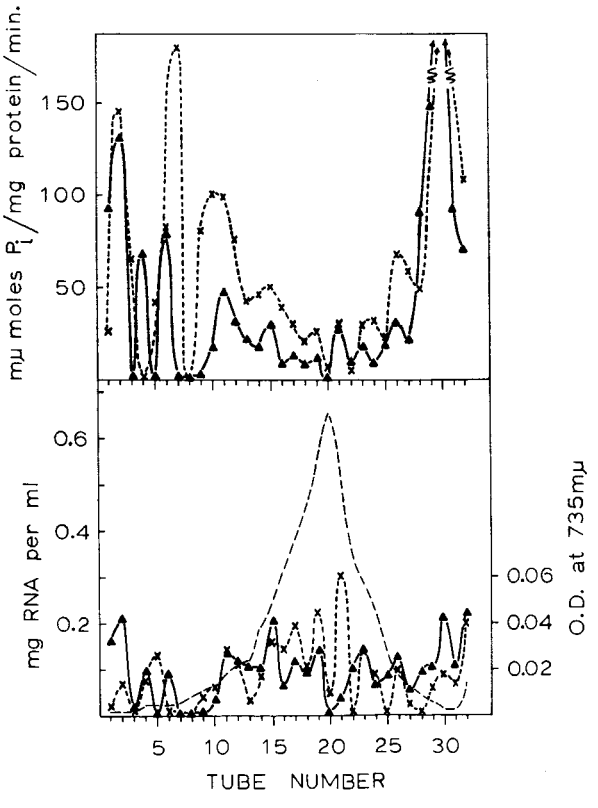

Figure 5

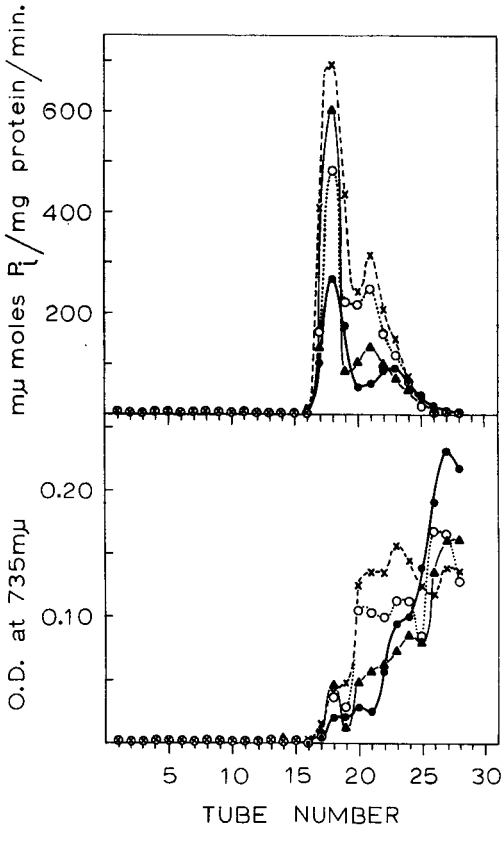

Figure 6

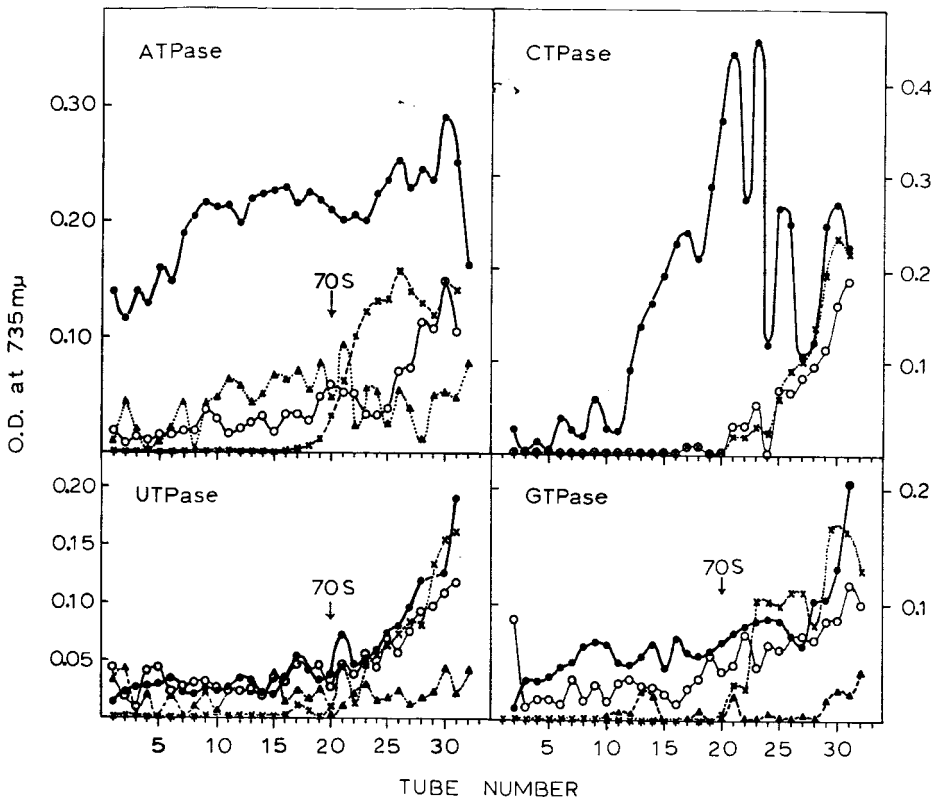

Figure 7

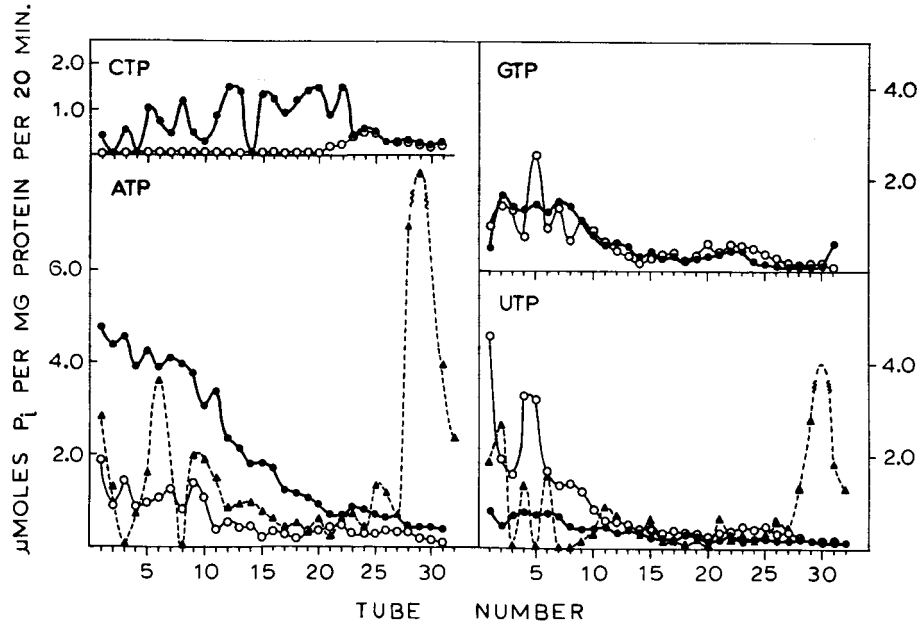

Figure 8