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Studies on ATPase(GTPase) Intrinsic to *E. coli* Ribosomes (1)

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(1) *Escherichia coli* 70S ribosomes showed intrinsic ATPase and GTPase activities, although they were much lower than those of rat liver ribosomes. The latter activity was higher than the former one. (2) The ATPase activity was inhibited by GTP and GMP-P(NH)P, and the GTPase activity was inhibited by ATP and AMP-P(NH)P, indicating a close relationship between the two enzymes. (3) Elongation components alone or in combination enhanced the ATPase activity, indicating the possible correlation of ribosomal ATPase with elongational components. (4) Vanadate at the concentrations that did not inhibit the GTPase activities of EF-Tu and EF-G, depressed the poly(U)-dependent polyphe synthesis, suggesting that ribosomal ATPase (GTPase) participates in peptide elongation by inducing positive conformational changes of ribosomes required for the attachment of elongational components.

Key words: ATPase, *E. coli*, GTPase, 70S ribosome.

Peptide elongation reactions are performed on ribosomes. A new amino acid is attached to the ribosomal A site as an aminoacyl-tRNA-EF-Tu-GTP complex by the codon-anticodon interaction in small subunits. The subsequent peptide bond formation is catalized by large subunits. The resulting peptidyl-tRNA and associated mRNA are translocated from the A site to the P site in a GTP-dependent manner by EF-G. Two elongation factors possess GTPase stringent for GTP, which is essential in peptide elongation.

In addition to extensive biochemical, genetic, and structural analyses of bacterial ribosomes and elongation factors, cryoelectron microscopy and X-ray crystalography have revealed high-resolution views of ribosomes with attached mRNA and protein factors. From these studies, the conformation of ribosomes is thought to be extensively changed during peptide elongation (*1–5*). However, components including such conformational changes have not been clarified completely, although dynamic changes in secondary structure of 16S rRNA due to unstable base pairing were visualized (*5*).

In this respect, it was reported that peptide elongation can be carried out by ribosomes themselves in the absence of factors and GTP. Therefore, the ability carrying the above-mentioned function is an inherent property of ribosomes (*6, 7*).

Our previous report (*8*) described some properties of ATPase intrinsic to rat liver ribosomes and its role in peptide elongation. The ribosomal ATPase activity was found to be enhanced by elongational components (poly U, phe-tRNA^{phe}, EF-1α, and EF-2); and two ATPase inhibitors, AMP-P (NH) P and vanadate, inhibited poly (U)-dependent polyphe synthesis of rat liver 80S ribosomes, although they did not inhibit the GTPase of EF-1α, and EF-2. From these results we assumed that ATPase intrinsic to 80S ribosomes may participate in peptide elongation by inducing positive conformational changes of ribosomes.

The structural and functional similarities between procaryotic and eucaryotic ribosomes, together with the factor-free elongation by *Escherichia coli* ribosomes (*6, 7*), prompted us to extend the study of ATPase of mammalian 80S ribosomes to that of *E. coli* 70S ribosomes. Since EF-Tu and EF-G possess no ATPase, it may be preferable to study ribosomal ATPase in order to elucidate the conformational changes of ribosomes during peptide elongation as described below.

MATERIALS AND METHODS

Materials—Labeled compounds and other chemicals were described previously (*8, 10*). Buffer A: 20 mM Tris-HCl, pH 7.6, 10 mM Mg-acetate, 100 mM NH_4Cl, 3 mM 2-mercaptoethanol.

The Preparation of E. coli 70S Ribosomes—Crude ribosomes were prepared from *E. coli* A19 or W3100 by a slight modification of the method of Kajiro *et al.* from S100 without DNase treatment (*9*). Crude ribosomes were washed twice with 1 M NH_4Cl containing Buffer A and finally suspended in Buffer A. After adjusting NH_4Cl concentration to 1 M, the suspension was left to stand at 2°C for 10 min, then layered on a 5 to 20% linear sucrose density-gradient in Buffer A. The gradient was centrifuged at 40,000 rpm for 90 min in an RPS 40T rotor (Hitachi), then the A_{254} was continously monitored with an ISCO automatic gradient fractionator. After adding the same amount of Buffer A, the mixture was concentrated with a Diaflow membrane. 70S ribosomes were stored in small portions at –85°C.

Elongation Factors—EF-G crystalline (*11*), EF-Tu GDP

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Abbreviation: AMP-P(NH)P, 5′-adenylyl imidodiphosphate; GMP-P(NH)P, 5′-guanylyl imidodiphosphate; DTT, dithiothreitol; TCA, trichloro-acetic acid; iP, inorganic phosphate.

(crystalline), and EF-Ts (*12*) highly purified from *E. coli* were the gift of Dr. Y. Kajiro. Nucleotide-free EF-Tu was prepared from EF-Ts-GDP by passing it through a column of Dowex 1 (*12*).

Preparation of [^{3}H]phe-tRNA—E. coli S100 fraction (1 mg of protein) (*7*) was dialyzed overnight against 20 mM Tris-HCl, pH 8.0, 8 mM Mg-acetate, 150 mM NH_4Cl, and 0.05 mM DTT, then incubated with [^{3}H]phenylalanine (12 nmol, 300 μCi) and 24 mg of *E. coli* tRNA (Boehringer) at 37°C for 30 min in a solution containing 0.1 mM Tris-HCl, 8 mM $MgCl_2$, and 2.5 mM ATP in a total volume of 2 ml. tRNA was purified by the SDS-phenol method followed by Sephadex G-50 column chromatography (0.75 × 7 cm) (*10*). A total of 8.5 mg of tRNA containing 2.3 × 10^3 DPN/μg of phe-tRNA was obtained.

Poly (U) -Dependent Polyphe Synthesis—A mixture of 1 pmol of 70S ribosomes with 480 pmol of [^{3}H]tRNA containing 3.2 × 10^4 DPN of [^{3}H]Phe-tRNA in 0.04 mM GTP, 12 pmol of EF-Tu GDP, 2 pmol of EF-Ts, 2.8 pmol of EF-G, and 2.5 μg of poly (U) in 50 mM Tris-HCl (pH 7.6), 8 mM Mg-acetate, 150 mM NH_4Cl, and 0.05 mM DTT (total volume, 20 μl) was incubated at 37°C for 10 min. The amount of polyphe synthesized was measured as hot TCA-insoluble ^{3}H radioactivity by the method described previously (*8*). The results were expressed as pmol [^{3}H]Phe-tRNA incorporated into the hot TCA-insoluble fraction.

Assay of ATPase and GTPase Activities—To compare the ATPase or GTPase activities of 70S ribosomes with those of 80S ribosomes, 1.7 pmol of 70S ribosomes was incubated with 0.125 mM [γ-^{32}P]ATP, or [γ-^{32}P]GTP (1 μCi) in 50 mM Tris-HCl (pH 7.6), 6 mM $MgCl_2$, 100 mM NH_4Cl, 1 mM DTT (total volume, 20 μl) at 37°C for 30 min. In some experiments, especially the assay of the GTPase of EF-Tu or polyphe synthesizing system, 10 μM [γ-^{32}P]GTP was used. The hydrolysis of labeled nucleotide was measured by the isobutanol–benzene method described previously (*10*). The activity was expressed as pmol [^{32}P]iP hydrolyzed per pmol 70S ribosomes or the elongation factor per min.

RESULTS AND DISCUSSION

It was important to prepare 70S ribosomes free from contamination by elongation factors. To preserve the conformation of ribosomes, the following mild procedures were used: ribosomes were washed twice with 1 M NH_4Cl, then subjected to sucrose density-gradient centrifugation after suspension in 1 M NH_4Cl containing Buffer A. Repeated centrifugation described above or treatment with octyl-glucoside to remove membranes (*13*) did not change the ATPase activity of 70S ribosomes, indicating that they were of sufficient purity to examine the ATPase activity intrinsic to *E. coli* ribosomes.

Basic Properties of ATPase of 70S Ribosomes—The ATPase and GTPase activities of 70S ribosomes in the presence of 0.125 mM [^{32}P]nucleotide were 0.3 and 0.6 pmol iP hydrolyzed/pmol ribosomes/min, respectively. These were much lower than the corresponding activities of rat liver ribosomes (6 pmol and 4.5 pmol). The GTPase activity of EF-G was notably low, about 40% of ribosomal GTPase at 0.125 mM [^{32}P]GTP; and that of EF-Tu was only 18% of ribosomal GTPase at 10 μM [^{32}P]GTP. I (K.O.) consider that the less compact structure of 70S ribosomes in comparison with mammalian 80S ribosomes requires less energy of ATP (GTP) for the conformational change during peptide elongation, and the low ATPase activity is sufficient to supply this energy. Kawakita and Iwasaki (*9*) reported that the GTPase activity of *E. coli* ribosomes washed 4 times with 1 M NH_4Cl was 0.2 pmol iP hydrolyzed/pmol ribosomes/min under similar conditions to ours. They also noted ribosomal ATPase activity, but attributed it to contamination by membranes.

The ATPase activity of *E. coli* ribosomes was increased slightly by heating at 50°C for 4 min (about 130% of the control), although heating at 70°C decreased it markedly (about 60% of the control). These results may indicate that the ribosomal ATPase of *E. coli* is more heat-stable than that of mammalian ribosomes (*8*).

Competitive Effects of GTP, AMP-P(NH)P, and GMP-P(NH)P on ATPase, and Effects of ATP, AMP-P(NH), P and GMP-P(NH)P on GTPase of E. coli Ribosomes—Since *E. coli* ribosomes showed both ATPase and GTPase activities, it appeared important to clarify the relationship between the two activities. For this purpose we used competitive methods. As shown in Fig. 1(1), the ATPase activity of *E. coli* ribosomes was inhibited concentration-dependently by GTP, GMP-P(NH)P, and AMP-P(NH)P. On the other hand, their GTPase activity was inhibited strongly and concentration-dependently by GMP-P(NH)P and ATP, and somewhat weakly by AMP-P(NH)P. These findings indicate an intimate relationship between the two enzymes in *E. coli* ribosomes. Similar results were obtained in mammalian ribosomes (*14*) and their small subunits (*10*).

Effects of Elongational Components on the ATPase Activity of E. coli Ribosomes—The results are shown in Fig. 2. Poly(U) enhanced the ATPase activity concentration-dependently up to 2 μg, although the extent of stimulation was rather small. Phe-tRNA increased the activity up to 20 μg, depending on the concentration. EF-Tu and EF-G en-

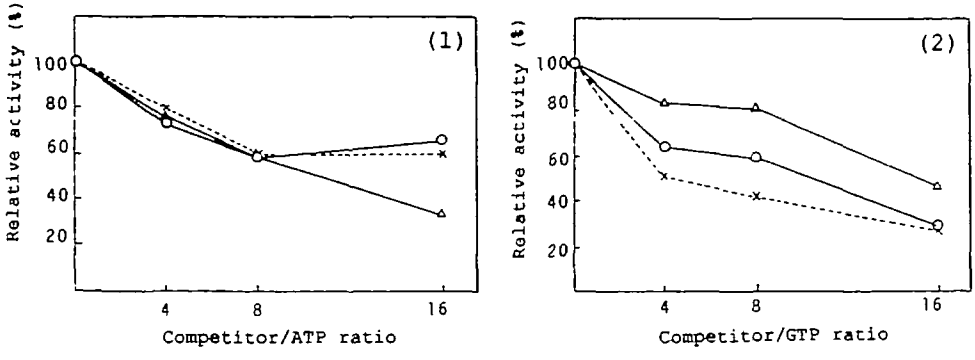

Fig. 1. **(1) Inhibition of the ATPase activity of *E. coli* ribosomes by GTP, AMP-P(NH)P, and GMP-P(NH)P.** 10 μM [^{32}P]-ATP was used. ○, GTP; △, GMP-P(NH)P; ×, AMP-P(NH)P. **(2) Inhibition of the GTPase activity of *E. coli* ribosomes by ATP, AMP-P(NH)P, and GMP-P-(NH)P.** 10 μM [^{32}P]GTP was used. ○, ATP; △, AMP-P(NH)P; ×, GMP-P(NH)P.

hanced the activity definitely and concentration-dependently.

The ATPase activity was increased by the combination of these elongational components. As shown in Table I, the ATPase activity in the presence of all components of the poly(U)-dependent polyphe-synthesizing system was about 5-fold higher than that in the presence of ribosomes alone. The omission of any one component clearly decreased the ATPase activity. Omitting EF-G and EF-Tu resulted in a marked decrease in the ATPase activity, suggesting their importance in this system. It was of interest that although poly(U) alone enhanced only slightly the ATPase activity, as mentioned above, the omission of poly(U) from the complete mixture decreased the activity to the larger extent, indicating that poly(U) contributes significantly to enhancement of ribosomal ATPase in the presence of the other components. These results are in good agreement with the effects of elongational components on the ATPase activity of mammalian ribosomes [Fig. 2 and Table III in the Ref. *8*]. As described in our previous reports (*8*, *10*), elongational components interact with different regions of 80S ribosomes (*15–19*). Ribosomal ATPase (GTPase) may be responsible for inducing conformational changes of ribosomes that allow the attachment of elongational components to ribosomes (*15–19*). The additive or synergistic changes of the ATPase activity of ribosomes by combinations of elongational components may be manifested by the combined attachment of elongational components to ribosomes.

In the case of bacterial ribosomes, Dabrowski *et al.* (*20*) recently showed that ribosomal contact sites of two tRNAs at the A site and P site hardly changed during the translocation reaction to P and E sites, suggesting that a movable ribosomal domain exists that tightly binds to tRNAs and carries them together in the translocation. Using three-dimensional cryoelectron microscopy, Gabashvili *et al.* (*5*) indicated the independent movements of the three main domains of the bacterial 30S subunits, namely, the head, the platform, and the main body. More recent analysis of bacterial ribosomes by X-ray crystalography has shown that the conformation of ribosomes and 30S subunits are extensively changed during peptide elongation (*3*, *21*).

We thought that ribosomal ATPase (GTPase) may be responsible for these conformational changes of ribosomes.

It must be added that when GTPase activity was measured in the system described above, the effect of EF-G was too strong due to uncoupled GTPase. As shown in Table I(2), in the presence of the full system, the GTPase activity was 17-fold higher than in the presence of the ribosomes alone, and omission of EF-G resulted in 10% of the activity of the complete system. Therefore, the effects of omitting

TABLE I. **Effects of components of the poly(U)-dependent polyphe-synthesizing system on ATPase and GTPase activities.**

	Additions							
Ribosomes (3 pmol)	+	+	+	+	+	+	+	−
Poly (U) (5 μg)	+	−	−	+	+	+	+	+
Phe-tRNA (10 μg)	+	−	+	−	+	+	+	+
Tu-GDP (2 pmol)	+	−	+	+	−	+	+	+
Ts (2.5 pmol)	+	−	+	+	+	−	+	+
G (1.5 pmol)	+	−	+	+	+	+	−	+
(1) ATPase activity	100	20	65	65	46	65	34	4
(2) GTPase activity	100	6	90	89	94	95	10	7

Incubation was carried out in 100 mM NH_4Cl, 10 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.6, and 1 mM DTT in the presence of 10 μM [γ-^{32}P]ATP or GTP (total volume, 20 μl).

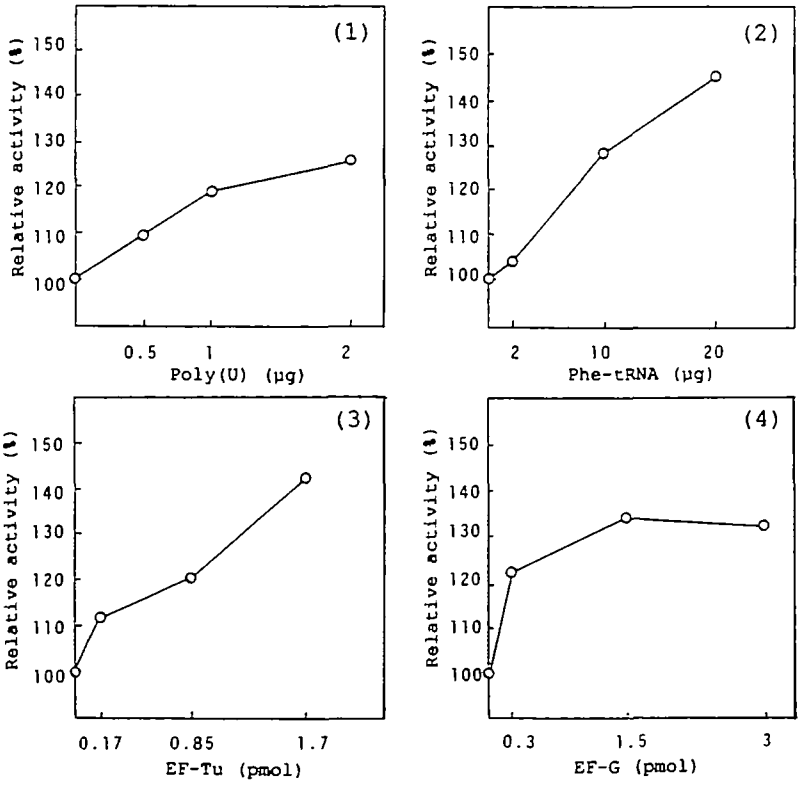

Fig. 2. **Effects of components of peptide elongation on the ATPase activity of *E. coli* ribosomes.** (1) effects of poly (U), (2) effects of phe-tRNA, (3) effects of EF-Tu, and (4) effects of EF-G. The ATPase activity of 70S ribosomes alone is set as 100%. 0.125 mM [γ-^{32}P]ATP was used.

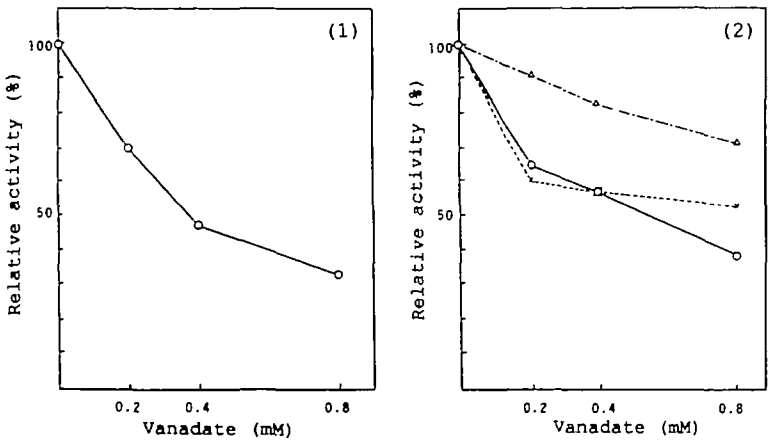

Fig. 3. **Effects of vanadate on poly (U)-dependent polyphe synthesis and on ATPase and GTPase activities of *E. coli* ribosomes and GTPase activity of polyphe-synthesizing system.** (1) Effects on polyphe synthesis. (2) Effects on ATPase and GTPase activities of *E. coli* ribosomes and GTPase activity of polyphe synthesizing system. 10 μM [^{32}P]GTP was used. ○, ribosomal GTPase; ×, ribosomal ATPase; △, GTPase of polyphe-synthesizing system.

the other components were very small, as also shown in Table I(2). Therefore, to examine the conformational changes of ribosomes, it is important to measure the ATPase activity of the system.

Effects of Vanadate on the Poly(U)-Dependent Polyphe Synthesis and ATPase and GTPase of 70S Ribosomes—In preliminary experiments, we examined the effects of vanadate on the GTPase activities of EF-G and EF-Tu. We found that, at concentrations up to 0.8 mM, it did not affect the GTPase activity of EF-G measured as uncoupled GTPase in the presence of 70S ribosomes, or that of EF-Tu, as was expected from the fact that the GTPase of the two elongation factors is stringent for GTP. In contrast, vanadate inhibited not only ATPase but also GTPase of 70S ribosomes, as described below.

As shown in Fig. 3(1), vanadate at concentrations up to 0.8 mM inhibited significantly and concentration-dependently the poly(U)-dependent polyphe synthesis. The result suggests the participation of ATPase (GTPase) of 70S ribosomes in peptide elongation, like the ATPase of mammalian 80S ribosomes (*8*). Vanadate inhibited not only ATPase but also GTPase of 70S ribosomes, and GTPase of the polyphe-synthesizing system [Fig. 3(2)]. In the case of rat liver 80S ribosomes, vanadate did not affect the GTPase of the polyphe-synthesizing system. The results suggest a more intimate relationship between the ATPase and GTPase of *E. coli* ribosomes than between those of mammalian ribosomes. Further study must be done to elucidate these situations.

The results of the present experiments indicate that ATPase (GTPase) intrinsic to *E. coli* 70S ribosomes participate in peptide elongation by inducing the positive conformational changes of ribosomes required for the attachment of elongational components to ribosomes, as in the case of mammalian 80S ribosomes.

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