ON THE IDENTIFICATION OF THE TWO *E. coli* SUPERNATANT PROTEINS WHICH STIMULATE THE INITIATION OF POLYPEPTIDE SYNTHESIS

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SUMMARY

The two *E. coli* supernatant proteins which were previously designated as factors A and B, and which were shown to activate ribosomes for initiation, are very probably factors EF-Ts and EF-G, respectively. An EF-Ts preparation, homogeneous when analyzed by polyacrylamide gel electrophoresis, contained considerable A activity. The EF-Ts and A activities differed, however, in their heat sensitivity and in the amount of the preparation required. An electrophoretically-homogeneous preparation of EF-G retained strong B activity. The amount of the preparation required for EF-G and B activity and the heat sensitivity of the two activities were similar. GTP enhanced the activation of the ribosomes by factors A and B.

We reported recently on the isolation of two proteins from the *E. coli* supernatant which stimulate the initiation of polypeptide synthesis by activating the ribosomes (1). One protein, designated factor A, was clearly different from the elongation factor EF-G. This protein also appeared to differ from EF-Tu and EF-Ts in that it could not substitute for an EF-T preparation in polypeptide synthesis, and the EF-T preparation could not effectively substitute for it in ribosome activation. The latter observation, however, did not demonstrate unequivocally that factor A was different from EF-Ts, since EF-Ts could have been present in the EF-T preparation in limiting amounts and could have been complexed with EF-Tu. The second protein, designated factor B, had considerable EF-G activity; its identity with the elongation factor could not be ruled out.

Studies carried out with highly purified preparations of EF-Ts and EF-G

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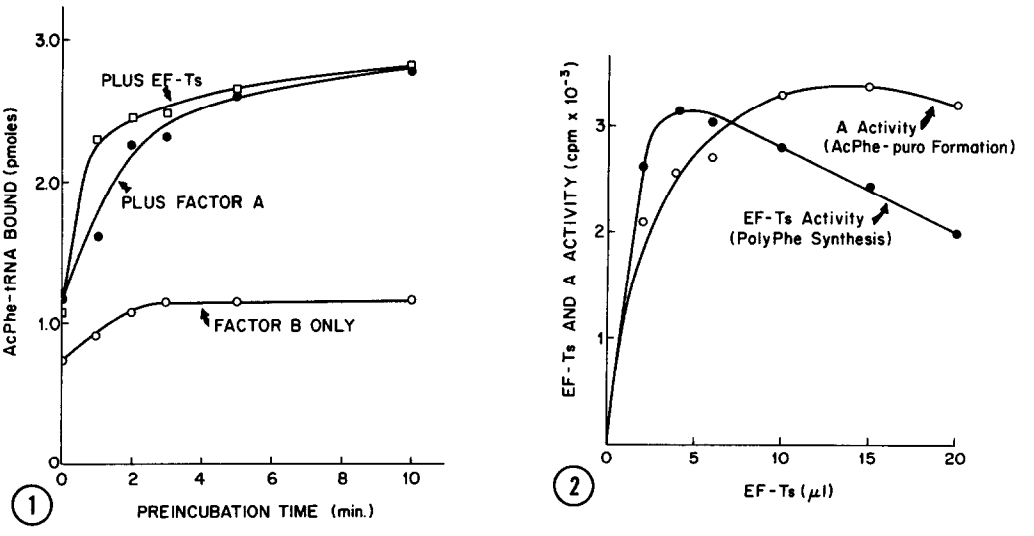


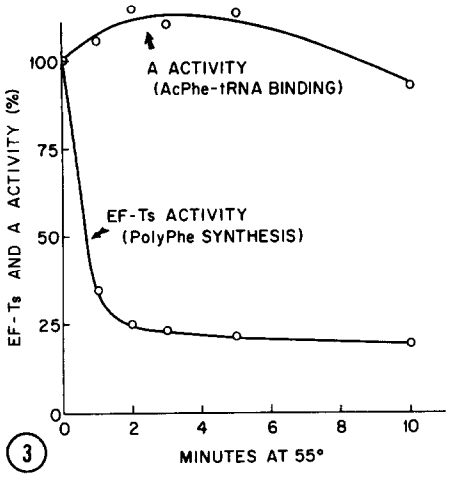
Fig. 1. A activity of EF-Ts preparation. Binding of AcPhe-tRNA was carried out at 15° for 30 sec with sucrose-washed ribosomes (see Methods). The ribosomes were preincubated at 37° with the components and for the times indicated before binding was initiated with poly(U) and AcPhe-tRNA.

Fig. 2. Saturation curves for A and EF-Ts activities of EF-Ts preparation. A and EF-Ts activities were measured by AcPhe-puromycin formation and polyphenylalanine synthesis, respectively, as described in the text. Salt-washed ribosomes were used without preincubation, and incubation was carried out at 37°, for 5 min for the puromycin reaction and 1 min for polyphenylalanine synthesis. The EF-Ts preparation contained 1.55 mg of protein per ml.

now indicate that factor A is very probably identical with EF-Ts in spite of a sharp difference in the heat stability of the two activities, and that factor B is identical with EF-G. A stimulatory effect of GTP on the activation of the ribosomes by the purified factors A and B has also been observed.

METHODS

Ac[^{14}C]Phe-tRNA, salt-washed ribosomes, initiation factors, and factor A were prepared from *E. coli* B as reported previously (1-3). The preparation of factor B (1) was modified slightly by an increase in the time for the heating step at 55° from 10 to 15 min. EF-Ts and EF-Tu were prepared according to the procedure of Arai *et al.* (4), and sucrose-washed ribosomes, by that of Nishizuka *et al.* (5). The EF-Ts preparation exhibited a major band and a faint trace of a second band when analyzed by polyacrylamide gel electrophoresis. The major band co-migrated with authentic EF-Ts (experiment generously carried out by Dr. T. Blumenthal). EF-G was prepared by the method of Rohrbach *et al.* (6), except that binding of the factor to the ribosomes was omitted, and additional chroma-



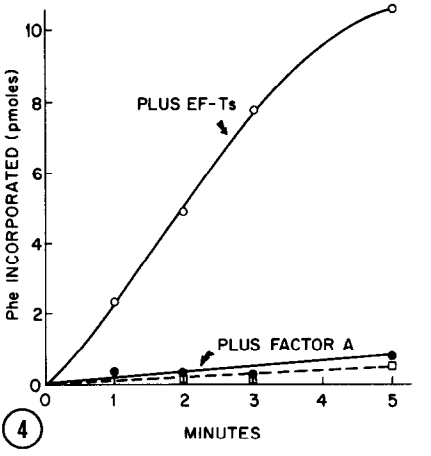


Fig. 3. Heat sensitivity of A and EF-Ts activities. The EF-Ts preparation was heated at 55° in a solution containing 20 mM imidazole-HCl, pH 7.4, 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, and 12% glycerol. At the indicated times, samples were taken, cooled in ice, and assayed for A and EF-Ts activities by AcPhe-tRNA binding and polyphenylalanine synthesis, respectively, without preincubation (see Figs. 1 and 2).

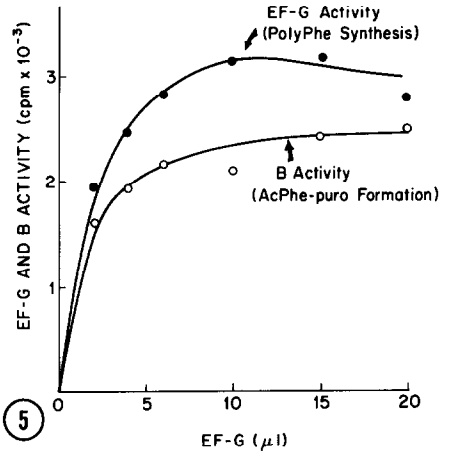
Fig. 4. Absence of EF-Ts activity in factor A preparation. Polyphenylalanine synthesis was carried out as described for Fig. 2, except that sucrose-washed ribosomes were used.

tography on Sephadex G-150 was performed as a final step. The EF-G preparation revealed a single band in polyacrylamide gel electrophoresis.

The assay conditions for A and B activities by Phe-tRNA binding and by the puromycin reaction, and for EF-T and EF-G activities by polyphenylalanine synthesis, were as described previously (1). Unless indicated all factors were added in saturating amounts, about 2-3 µg for EF-Tu, 2-4 µg for EF-Ts, 8-15 µg for factor A and 3-4 µg for EF-G or factor B.

RESULTS

To settle unambiguously the question whether factors A and EF-Ts are distinct proteins, we purified EF-Ts and tested it for A activity. The results, presented in Fig. 1, show that the EF-Ts preparation had considerable A activity; it was as effective as factor A in activating the ribosomes for the binding of AcPhe-tRNA. Our most highly purified preparation of EF-Ts, which contained high A activity, was essentially homogeneous according to analysis by polyacrylamide gel electrophoresis, and the principal protein co-migrated with authentic EF-Ts (see Methods).



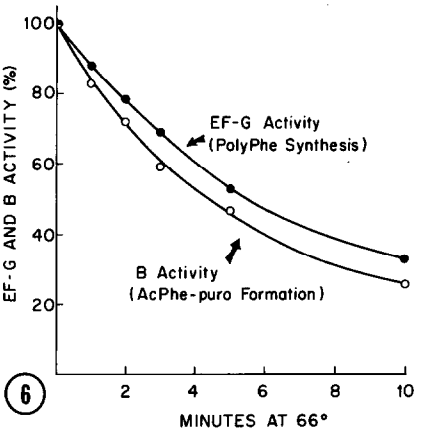


Fig. 5. Saturation curves for B and EF-G activities of EF-G preparation. Assay conditions were similar to those for Fig. 2, except that EF-Ts was present in all tubes and the amount of EF-G was varied. The protein concentration of the EF-G preparation was 0.31 mg/ml.

Fig. 6. Heat sensitivity of B and EF-G activities. Conditions for heating and assaying of the EF-G preparation were similar to those for the EF-Ts preparation, except that B and EF-G activities were monitored and heating was conducted at 66°.

Although it is probable that A and EF-Ts activities are due to the same protein, the amount of protein required for maximal activity and the heat sensitivity of the two activities differ. About three times as much of EF-Ts is needed to saturate the system for A activity as for EF-Ts activity (Fig. 2). The EF-Ts activity of an EF-Ts preparation is more sensitive to heat than its A activity (Fig. 3). About 75% of the EF-Ts activity is abolished by heating the protein at 55° for 2 min, whereas most of the A activity is retained even after 10 min. This difference undoubtedly accounts for the absence of EF-Ts activity in the factor A preparation (Fig. 4), which was purified in the usual way from crude supernatant heated for 10 min at 55° (1). Our preparation of EF-Ts was for some reason more heat-labile than those of others (4).

Identity of B factor and EF-G is indicated by the observation that a preparation of EF-G, homogeneous according to analysis by polyacrylamide gel

TABLE I

Effect of Various Nucleotides on Activation of Ribosomes by Factors A and B

Additions	AcPhe-tRNA Bound (pmoles)	
	with factors	without factors
None	2.5	1.0
GTP	3.7	1.2
GDPCP	1.9	1.0
GDP	2.8	1.2

Salt-washed ribosomes were preincubated for 10 min at 37°C with 2×10^{-4} M of the indicated nucleotides in the presence or absence of factors A and B. The binding assays were then carried out as described for Fig. 1.

electrophoresis (see Methods), still maintained good B activity (Fig. 5). The B and EF-G activities, unlike the A and EF-Ts activities, required about the same amount of protein for maximal activity and showed about the same sensitivity to heat (Fig. 6).

The activation of ribosomes by factors A and B is stimulated by GTP (Table I). When the ribosomes are preincubated with factors A and B in the presence of GTP, the effect of the factors on the binding of AcPhe-tRNA is enhanced. Neither GDP nor GDPCP (guanylylmethylene diphosphonate) substitutes for GTP. The GTP analogue, GDPCP, actually inhibits the action of the factors.

DISCUSSION

The high A activity in a homogeneous preparation of EF-Ts suggests that the A and EF-Ts activities are probably due to the same protein. The striking difference in heat sensitivity of the two activities, on the other hand, indicates that different catalytic sites of the protein are involved. Similarly, the B activity of highly purified EF-G suggests that B and EF-G activities are due to the same protein. In this case, the requirement of the same amount of EF-G for both activities, the similar heat sensitivity of the activities, and

the participation of GTP in both reactions, suggest the involvement of one or more common catalytic sites.

Although we suggested that the factors may induce a structural rearrangement of the ribosomes (1), we still have no clear indication how the two factors function to activate the ribosomes. Neither the known functions of EF-Ts and EF-G in polypeptide synthesis (7,8), nor the participation of EF-Ts as a constituent of QB replicase (9), provide any clues. The requirement for the 50S ribosomal subunit in the activation of the ribosomes (1) and the enhancement of the reaction by GTP are at least consistent with the known requirements for EF-G function in the translocation reaction. Interestingly, a possible, second function for EF-Ts in polypeptide synthesis was considered when direct phosphorylation of EF-Tu-GDP failed to fully eliminate the need for EF-Ts in polyphenylalanine synthesis (10).

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