Cross-reactivity studies on the interaction between elongation factors Tu and Ts from *Streptomyces aureofaciens* and *Escherichia coli* in the GDP exchange reaction

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Received 17 March 1986; revised version received 22 April 1986

The method of purification of elongation factor Ts from *Streptomyces aureofaciens* is described. Purified elongation factors Ts from *S. aureofaciens* and *Escherichia coli* were tested in cross-reactivity studies with elongation factors Tu from both species in a GDP exchange reaction under equilibrium and non-equilibrium conditions. Experiments have revealed that slower spontaneous release of GDP from *S. aureofaciens* EF-Tu is compensated for by higher affinity of homologous EF-Ts towards EF-Tu and thus the initial rates of EF-Ts catalysed GDP exchange can be kept the same in both *E. coli* and *S. aurefaciens* in vitro systems.

Elongation factor Ts *Elongation factor Tu* *Purification* (E. coli, Streptomyces) *GDP exchange* *Cross-reactivity*

1. INTRODUCTION

Elongation factors Tu (EF-Tu) and Ts (EF-Ts) participate in the polypeptide chain elongation cycle during protein synthesis in procaryotes. EF-Tu catalyzes the binding of aminoacyl-tRNA to ribosomes and EF-Ts accelerates the dissociation of the EF-Tu·GDP complex [1,2]. The release of GDP from EF-Tu is a rate-limiting step in in vitro poly(U) translation in the absence of EF-Ts [3]. The presence of EF-Ts in the system stimulates the rate of EF-Tu·GTP generation by a factor of 2000 [3].

In our previous studies on EF-Tu from *Streptomyces aureofaciens* we have found the GDP dissociation rate constant of this factor to be 3-times lower than that of EF-Tu from *Escherichia coli* [4]. One question of interest is whether this difference in spontaneous GDP release also affects the rate of recycling of EF-Tu in vitro in the presence of EF-Ts or whether the slower spontaneous dissociation of GDP is compensated for by an enhanced 'catalytic' effectivity of EF-Ts from *S. aureofaciens*. We therefore purified EF-Ts from *S. aureofaciens* and compared its effect with that of *E. coli* EF-Ts on EF-Tu from *E. coli* as well as from *S. aureofaciens*. The effects of EF-Ts on these GDP exchange reactions were studied under equilibrium and non-equilibrium conditions.

Similar studies have been performed with elongation factors from *Caulobacter crescentus* [5] and two thermophilic bacteria [6,7]. In all these studies EF-Ts from *E. coli* did not catalyze GDP exchange on any of these heterologous EF-Tus.

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

Here we show that both EF-Ts factors can catalyze a nucleotide exchange even when combined with a heterologous EF-Tu. We could demonstrate that the slower spontaneous dissociation of GDP from *Streptomyces* EF-Tu is counterbalanced in the presence of the homologous EF-Ts. We interpret this to mean that the higher affinity for the EF-Tu–EF-Ts pair from *S. aureofaciens* counteracts the slow spontaneous release rate of GDP from *S. aureofaciens* EF-Tu. The result is that the EF-Ts-catalyzed exchange rate is the same in the two systems.

2. MATERIALS AND METHODS

2.1. *Materials*

EF-Tu·GDP in an aggregated state was purified from vegetative cells of *S. aureofaciens* strain 84/25 as described in [8]. The purification of EF-Tu·GDP from *E. coli* followed the method of Leberman et al. [9]. EF-Ts from *E. coli* was obtained through a procedure of Arai et al. [10] and [^{3}H]GDP was purchased from Amersham International (Amersham).

2.2. *Nucleotide exchange assay*

EF-Ts was quantitated by measurement of exchange between GDP bound to EF-Tu and free [^{3}H]GDP as described [10].

Interaction of EF-Ts with EF-Tu in GDP exchange was tested in EF-Ts titrations either under non-equilibrium conditions at 0°C, with an incubation time of 5 min with a maximum Ts:Tu ratio of 1:5 or under equilibrium conditions at 25°C for 30 min with a maximum Ts:Tu ratio of 6:1. The amount of [^{3}H]GDP bound to EF-Tu was determined by a standard nitrocellulose filter assay [8]. In the case of the non-equilibrium reaction the amount of [^{3}H]GDP bound in the absence of EF-Ts was subtracted from obtained values as blank and in both cases the amount of bound nucleotide was expressed in per cent of maximal binding activity at the most efficient combination of the factors.

2.3. *Purification of EF-Ts from* S. aureofaciens

For the purification of EF-Ts from *S. aureofaciens* we used the active fraction of the factor obtained from column chromatography on DEAE-Sephadex following the procedure in [8]. The fraction eluted from the column at 0.22–0.24 M KCl was precipitated with solid ammonium sulfate to 70% saturation. The sediment was resuspended in a small amount (5 ml) of buffer A (20 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate, 50 mM KCl, 10 mM 2-mercaptoethanol, 20 μM GDP) and dialysed against the same buffer overnight. The dialysed fraction was supplemented with GDP to a concentration of 60 μM and incubated for 10 min at 30°C to separate EF-Ts from the complex with EF-Tu. The fraction was then applied to a DEAE-Sepharose CL-6B column (2.6 × 40 cm) developed with 1000 ml of a linear gradient from 0.05 to 0.2 M KCl in buffer A. Active fractions were combined and concentrated to 1 mg/ml of protein. The purified EF-Ts was dialysed against buffer containing 20 mM Tris-HCl, pH 7.5, 10 mM magnesium acetate, 10 mM 2-mercaptoethanol and 250 mM sucrose and stored at −60°C.

3. RESULTS AND DISCUSSION

3.1. *Analysis and* M_r *determination of EF-Ts from* S. aureofaciens

Purified EF-Ts was analysed on SDS gel slabs according to Laemmli [11] which revealed that the protein is electrophoretically homogeneous (fig.1A, lane 2) with no EF-Tu contamination. In the same system we determined the M_r of EF-Ts from *S. aureofaciens* as 31000 (fig.1B). The value is identical with that published for *E. coli* EF-Ts [12]. Agreement in the size was confirmed by a single protein band obtained on running EF-Ts from *S. aureofaciens* and *E. coli* in one lane of the gel (fig.1A, lane 4).

3.2. *Interaction of EF-Ts with EF-Tu in GDP exchange reaction*

We first studied the effect of low amounts of EF-Ts from *S. aureofaciens* or *E. coli* on the GDP exchange reaction in all possible combinations with both EF-Tus under non-equilibrium conditions. The results of these experiments are shown in fig.2. They reveal that both EF-Ts factors catalyze the nucleotide exchange reaction with EF-Tu from *S. aureofaciens* or *E. coli*. The extent of the reaction is linearly dependent on the EF-Ts concentration in a small concentration range up to 1 pmol/assay for all combinations of factors except for *S. aureofaciens* EF-Tu combined with *E.*

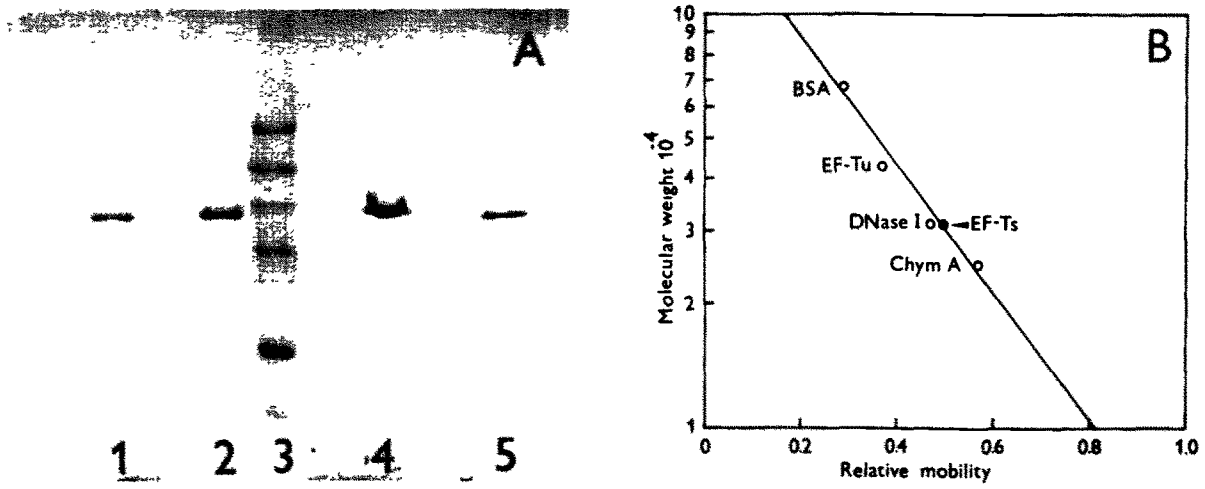

Fig.1. SDS gel electrophoresis and M_r determination of *S. aureofaciens* EF-Ts. (A) Electrophoresis was carried out according to Laemmli [11] in a 15% polyacrylamide gel. (1) EF-Ts from *E. coli* (4 μg); (2,5) EF-Ts from *S. aureofaciens* (4 μg); (3) reference proteins (from the top) – bovine serum albumin (M_r 67000), *E. coli* EF-Tu (M_r 43225), deoxyribonuclease I (M_r 31000), chymotrypsinogen A (M_r 25000) and myoglobin (M_r 17000); (4) mixture of EF-Ts from *E. coli* and *S. aureofaciens* (4 μg each). (B) The relative mobilities of reference proteins are plotted vs the logarithms of their M_r values and from the plot the M_r of *S. aureofaciens* EF-Ts was determined as 31000.

coli EF-Ts (fig.2A). This heterologous pair required 5-times higher concentrations of EF-Ts than the other three pairs to reach the same maximum amount of [^{3}H]GDP bound. This may be caused by a lower spontaneous rate of GDP dissociation from *S. aureofaciens* EF-Tu. It is particularly interesting that both homologous pairs of EF-Tu and EF-Ts, from *S. aureofaciens* and *E. coli*, exhibit practically identical initial rates of GDP exchange (fig.2A,B) under our conditions. It seems that EF-Ts from *S. aureofaciens* has been designed so that it can fully suppress the 'disadvantage' of the slower GDP dissociation rate connected with EF-Tu from *S. aureofaciens*.

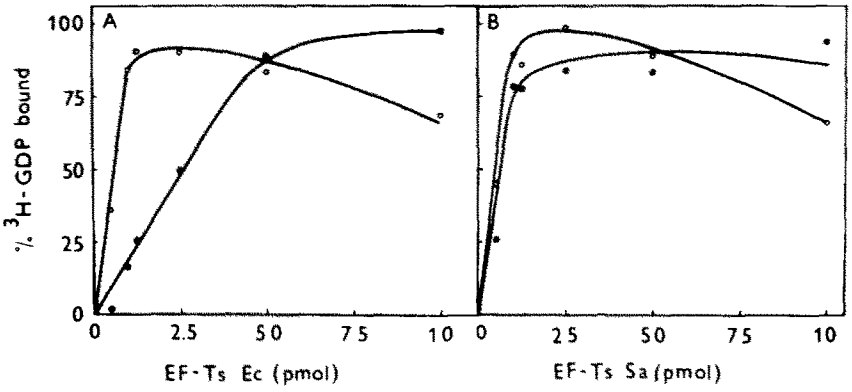

Fig.2. GDP exchange reaction on EF-Tu performed as an EF-Ts titration under non-equilibrium conditions with 50 pmol of respective EF-Tu and 154 pmol [^{3}H]GDP. Reaction mixtures were incubated for 5 min at 0°C. (A) Titration with EF-Ts from *E. coli* (Ec). (B) Titration with EF-Ts from *S. aureofaciens* (Sa). In both cases the effect on EF-Tu from *E. coli* (○—○) was compared to that on EF-Tu from *S. aureofaciens* (●—●).

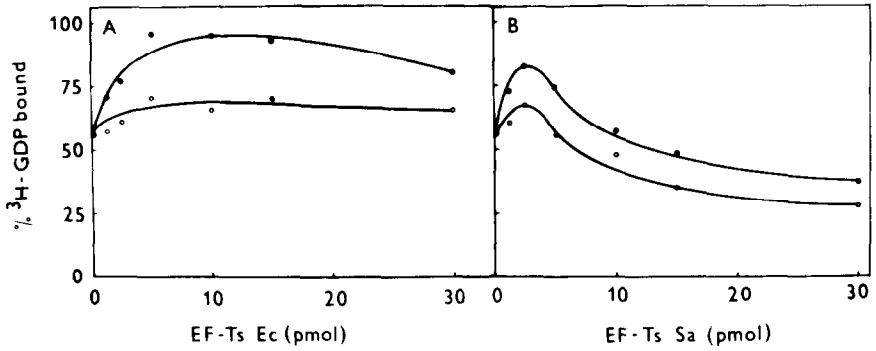

Fig.3. GDP exchange reaction on EF-Tu performed as an EF-Ts titration under equilibrium conditions with 5 pmol of the respective EF-Tu and 77 pmol [^{3}H]GDP. Reaction mixtures were incubated for 30 min at 25°C. (A) Titration with EF-Ts from *E. coli* (Ec). (B) Titration with EF-Ts from *S. aureofaciens* (Sa). In both cases the effect on EF-Tu from *E. coli* (○—○) was compared to that on EF-Tu from *S. aureofaciens* (●—●).

More information about the interaction of *S. aureofaciens* EF-Ts with both EF-Tu factors was obtained by studying nucleotide exchange reaction under equilibrium conditions. Since EF-Ts and GDP probably bind at or near the same site on EF-Tu [13] it is possible to inhibit the binding of [^{3}H]GDP to EF-Tu by increasing the concentration of EF-Ts. The extent of inhibition can subsequently be used to measure the relative binding affinity between EF-Tu and EF-Ts. The results of such experiments are shown in fig.3. With EF-Ts from *E. coli* very low inhibition of [^{3}H]GDP binding was observed over the total range of Ts concentrations used (fig.3A). However, for those pairs which contained EF-Ts from *S. aureofaciens* GDP binding on both EF-Tus was reduced to almost 50% of the maximum found with *E. coli* EF-Ts. This 50% reduction was observed already at a 3-fold excess of *S. aureofaciens* EF-Ts (fig.3B). This indicates that the binding affinity of EF-Ts from *S. aureofaciens* toward homologous as well as heterologous EF-Tu is higher than that of *E. coli* EF-Ts.

4. CONCLUSION

It has been shown previously that GDP and EF-Ts interact negatively on EF-Tu in *E. coli*. Thus the binding of GDP to EF-Tu lowers the affinity of EF-Tu for EF-Ts. The binding of EF-Ts reduces in a similar way the affinity of EF-Tu toward GDP [14]. In order for the rate of GDP exchange in *S. aureofaciens* in the presence of EF-Ts to be equal to that observed for *E. coli*, a compensation for the slow spontaneous exchange rate of *S. aureofaciens* EF-Tu must occur. We suggest that it is the high affinity of *Streptomyces* EF-Ts towards *Streptomyces* EF-Tu that leads to such a compensation. The presence of such a counterbalancing mechanism in itself gives at least indirect evidence for the existence of a transient ternary EF-Tu · GDP · EF-Ts complex in *S. aureofaciens*. It indicates further that the elongation cycle in *Streptomyces* may proceed via a similar reaction mechanism as recently proposed for *E. coli* [14,15].

ACKNOWLEDGEMENTS

We are indebted to Drs K. Janáček and J. Jonák for helpful advice and discussion. Dr M. Ehrenberg is gratefully acknowledged for his critical reading of the manuscript. This work was in part supported by the Swedish Cancer Society and Natural Science Research Council of Sweden.

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