# Polypeptide elongation factor Tu: observations on the interaction with DNase I and the aggregation at pH 6

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No evidence for the interaction of EF-Tu with DNase I could be found by measuring the activities of each of the two proteins in the presence of excess of the other. Gel filtration of a mixture of the two proteins also provided no evidence for interaction. Aggregation at pH 6, observed with various samples of EF-Tu, is apparently due to the aggregation of material which is not functional in the GDP exchange assay. These results indicate that there is no structural/evolutionary relationship between EF-Tu and eukaryotic actin.

*EF-Tu* *Actin* *DNase I* *GDP binding* *Aggregation* *Cytoskeleton*

## 1. INTRODUCTION

Polypeptide elongation factor EF-Tu has a well-defined role in bacterial protein biosynthesis [1,2]. Other properties have been attributed to this protein which might indicate a structural/evolutionary relationship to actin. These properties of polymerisation [3,4] and binding to DNase I [4] together with other actin-like properties [5], have led from the suggestion of an evolutionary relationship between the two proteins [4] to the possibility of EF-Tu being a component of a bacterial internal cytoskeleton [3].

In contrast to this, during the isolation of EF-Tu [6] no inhibition is observed by the large excess of EF-Tu released on cell lysis, of the small amount of DNase I used to digest bacterial DNA. Furthermore, during this isolation procedure, the behaviour of the protein on gel filtration would argue against extensive polymerisation. Direct evidence against EF-Tu constituting a bacterial cytoskeleton comes from the immunocytochemical study of the localization of EF-Tu within *Escherichia coli* [7].

At least one review of contractile proteins accepts the possibility of an evolutionary relationship between actin and EF-Tu [8], but subsequent primary structure determination of EF-Tu [9] and the results presented below show that any structural/evolutionary relationship is unlikely.

*Abbreviations:* EF-Tu, bacterial polypeptide elongation factor Tu; DNase I (EC 3.1.4.5), bovine pancreatic ribonuclease I; DTE, dithioerythritol

## 2. MATERIALS AND METHODS

EF-Tu was isolated from *E. coli* (MRE 600) by ion-exchange chromatography and gel filtration [6]. DNase I, with a specific activity of 2813 units/mg, was from Worthington. Salmon sperm DNA (sodium salt) and RNase A were from Sigma.

The GDP binding assay for EF-Tu was as previously described [10]. DNase I activity was monitored by following the initial rate of hydrolysis of DNA as measured by the increase in optical density at 260 nm. Polymerisation of EF-Tu was monitored by measuring the apparent increase of optical density at 340 nm due to light scattering. Gel filtration was performed with a column (1 × 90 cm) of AcA 54 (LKB).

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The buffers used were: buffer A, 2 mM Tris–HCl, 0.3 mM $MgCl_2$, 0.135 mM DTE, 0.025 mM GDP, 0.01 mM EDTA, pH 7.5 [3]; buffer B, 100 mM Tris–HCl, 1 mM $CaCl_2$, 0.1 mM DTE, 10 mM $MgCl_2$, pH 7.9; 200 mM cacodylate, pH 6. Buffer B is similar to the buffer used in [4] except that the Tris concentration is 100 and not 10 mM. The pH values of the buffers were adjusted after the addition of all components.

## 3. RESULTS

The interaction of skeletal muscle actin with DNase I leads to the formation of a tight stoicheiometric 1:1 complex (binding constant $10^8$ $M^{-1}$) with the mutual inhibition of the component activities [11,12]. The effect of EF-Tu on the enzymatic activity of DNase I in catalysing the hydrolysis of DNA is shown in fig.1, where it can be seen that no inhibition is observed even with a 100-fold molar excess of elongation factor.

To study the possible effect of DNase I on one property of EF-Tu, the ability of EF-Tu to exchange [$^3$H]GDP in the presence of excess DNase I was measured. Triplicate samples of EF-Tu·GDP (20 μl, 0.45 mg/ml) with or without the addition of DNase I (20 μl, 3.1 mg/ml; 10-fold molar excess) were incubated at room temperature for 1 h in 200 μl [$^3$H]GDP assay mixture. The amount of [$^3$H]GDP bound to the EF-Tu was measured by the standard cellulose nitrate filter procedure [10]. No significant differences were observed between the samples with or without DNase I.

It is possible that a complex between DNase I and EF-Tu could be formed without the inhibition of their respective enzymatic and other properties. Such a complex would have a molecular mass and probably a Stokes' radius greater than the component proteins, although the latter might be largely determined by the asymmetric EF-Tu [13]. It would therefore be expected that such a complex would elute before or together with EF-Tu on gel filtration. Fig.2 shows the results of fractionating a sample containing 1.28 mg EF-Tu·GDP and 0.087 mg DNase I (molar ratio 10:1) on a column of AcA 54. It is clear that the DNase I activity is not eluted before or with EF-Tu activity, indicating that no complex is formed between the two proteins.

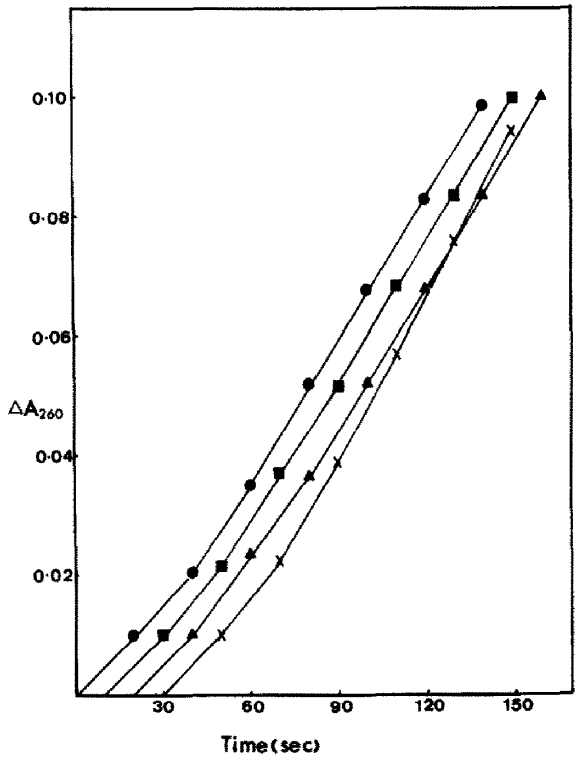


Fig.1. Depolymerisation of DNA by DNase I. Salmon sperm DNA (3 ml, 0.06 mg/ml) was incubated with DNase I (10 μl, 0.031 mg/ml) and various amounts of EF-Tu·GDP at 20°C, and the change in absorbance at 260 nm was measured. (a) (●—●) without EF-Tu·GDP; (b) (■—■) with 10 μl EF-Tu·GDP (0.045 mg/ml), curve displaced by 10 s for clarity; (c) (▲—▲) with 10 μl EF-Tu·GDP (0.45 mg/ml), curve displaced by 20 s; (×—×) with addition of 10 μl EF-Tu·GDP (4.5 mg/ml), curve displaced by 30 s.

Using the conditions described in [3], the aggregation of EF-Tu·GDP at pH 6 and 21°C was followed by monitoring the apparent increase of absorbance at 340 nm due to the scattering of light by aggregates. With two samples of the protein, which had GDP binding activities of 14000 and 17000 pmol/mg respectively, an increase in absorbance was observed on incubation at pH 6 (see, e.g., fig.3).

A third sample, which was freshly prepared and had a GDP binding activity of 20000 pmol/mg, showed no change in absorbance even after 30 min incubation at pH 6. Portions of the aggregated samples were removed from the spectrophotometer cuvette, centrifuged for 15 min at

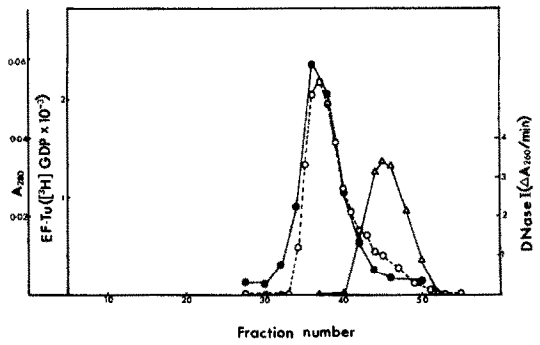


Fig.2. Gel filtration of EF-Tu·GDP with DNase I. A 120-$\mu$l sample containing 1.28 mg EF-Tu·GDP and 0.087 mg DNase I (relative molar concentrations 10:1) was applied to a column of AcA 54 equilibrated in buffer B. Fractions of 1.2 ml were collected and assessed for absorbance at 280 nm (○---○), [$^3$H]GDP exchange (●——●) and DNA depolymerisation (△——△).

120000 × $g$ in a Beckman Airfuge, and the supernatant and the resuspended pellet assayed for GDP binding activity. About 70% of the original activity was found in the supernatant fraction, and further activity could be recovered by washing the apparently insoluble precipitate. These results would tend to indicate that the material aggregating at pH 6 is predominantly inactive material, consistent with the observation in [3] that EF-Tu·GDP prepared by affinity chromatography polymerises less efficiently.

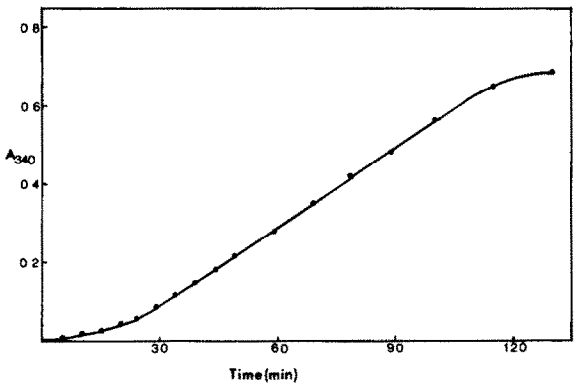


Fig.3. Aggregation of EF-Tu·GDP at pH 6. A sample of EF-Tu·GDP (2.7 ml, 1.25 mg/ml in buffer A, [$^3$H]GDP binding activity 17000 pmol/mg) was placed in a spectrophotometer cuvette thermostatted at 21°C. At zero time 0.3 ml 200 mM cacodylate buffer (pH 6) was added and the absorbance at 340 nm followed.

It should be stressed at this point that the GDP binding assay is dependent on some undefined property of EF-Tu, providing an affinity for cellulose nitrate. The loss of this property may be due to a particular type of denaturation, e.g., mild tryptic digestion resulting in cleavage of the polypeptide chain does not affect this property significantly [14], whereas occasionally samples of EF-Tu are isolated with specific activities as low as 10000 pmol/mg which exhibit only one band by SDS–polyacrylamide gel electrophoresis (PAGE) with a mobility corresponding to EF-Tu.

## 4. DISCUSSION

All attempts to demonstrate inhibition of DNase I activity by excess EF-Tu·GDP or conversely EF-Tu GDP binding activity by DNase I proved negative. The formation of a 'tight' complex [3], which might not affect some of the activities of the two proteins, is not observed by gel filtration (fig.2). It should be noted that the buffer used in the gel filtration experiment is similar to that used to demonstrate EF-Tu binding to immobilised DNase I [4] except that the concentration of the buffering reagent (Tris) is 100 mM and not 10 mM.

It is not easy to reconcile these results with those in [4] on the binding of EF-Tu to Sepharose-bound DNase I. It is possible that the buffering capacity of 10 mM Tris is not sufficient to maintain the correct pH on the addition of 10 mM $MgCl_2$ and acid denaturation might occur. Unfortunately, insufficient data are available on the activity of the EF-Tu used in that study to offer a reasonable explanation for the differing results.

Of the aggregates of EF-Tu·GDP produced under various conditions and examined by electron microscopy [3,4,15,16], only the crystalline filaments produced by 70% saturated ammonium sulphate [15,16], have been suitable for structural analyses, whereas the electron micrographs of aggregates of EF-Tu·GDP reproduced in [3,4] show little sub-structure. The results described above would indicate that the aggregation of EF-Tu·GDP observed at pH 6 and low ionic strength is probably due to the non-specific aggregation of material which is not active in at least one of the assay systems for EF-Tu activity, i.e., GDP and GTP binding. This is borne out by the properties

of the EF-Tu sample used in [3] which was only 70% active in binding GDP and 45% active in binding GTP. Thus, in terms of light scattering, the two sets of data are consistent but the interpretation is different since here the aggregated and non-aggregated material have been separated, whereas the author in [3] assumed that incubation at pH 6 led to complete aggregation of the sample and attempted no separation. It would therefore appear that there is no convincing evidence that native EF-Tu polymerises under low-salt conditions in vitro. This supports the direct observation of the absence of fibrous or filamentous assemblies of EF-Tu in *E. coli* in vivo [7].

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