INTERCONVERTIBILITY OF TWO CHROMATOGRAPHICALLY SEPARABLE FORMS OF *ESCHERICHIA COLI* ELONGATION FACTOR Tu

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1. Introduction

The bacterial protein synthesis elongation factor Tu (EF-Tu) has been suggested to be involved in many different cellular mechanisms. Beside its role in the translational machinery (reviewed [1]), it is a subunit of the RNA bacteriophage Qβ replicase [2], it has also been proposed as part of the regulation mechanism for the ribosomal RNA transcription [3,4]. Further, EF-Tu was suggested to have actin-like properties [5,6] and be membrane-associated [7,8]. We showed that the EF-Tu can be separated by column chromatography on DEAE Sephadex A-50 into two different subpopulations, one of which appeared to be selectively depleted from the supernatant during sedimentation of ribosomes [9]. Moreover, two widely separated loci were found for the protein on the *E. coli* genome [10] and the gene products appear to have a different affinity for the ribosome [11,12]. This gave a rationale for the heterogeneity of the EF-Tu population. When we set out to purify further and characterize the two forms of EF-Tu, we found that they were apparently interconvertible. This and the ribosomal involvement in this interconversion is the subject of this communication.

2. Materials and methods

Essentially, all methodology has been detailed [9]. DEAE Sephadex columns were 1.2 × 20 cm, run with gradients as in [9] and standardized with 110 mg protein loading. In addition, high salt extraction of ribosomes was following the procedure in [13], and ribosomal subunit preparation and analytical sucrose gradients [14]. The two forms of EF-Tu, EF-Tu1 and EF-Tu2, are as defined [9], being numbered in order of elution from DEAE Sephadex.

3. Results

In [9] we showed that the EF-Tu1 form of the bacterial elongation factor Tu was selectively depleted from the supernatants of an S100 fraction by high speed centrifugation, whereas the EF-Tu2 activity remained in the soluble fraction. We attempted to purify EF-Tu1 further by extraction of the protein from the ribosomal pellet with a high-salt washing procedure as used for the purification of the initiation factors [13]. The EF-Tu1 extract was run on a DEAE Sephadex A-50 column under exactly the conditions in [9]. The elution profile is shown in fig.1B. Much to our surprise, the EF-Tu1 activity eluted at the position of EF-Tu2 and not as expected at the EF-Tu1 position. Figure 1A shows the position at which EF-Tu2 derived from the S100 fraction from the same preparation eluted from a parallel column.

The discovery of the apparent conversion of the EF-Tu1 protein to a form eluting on a DEAE Sephadex column at the position of EF-Tu2 after the high salt treatment prompted us to treat the ribosomal pellet in a more careful way. We therefore gently resuspended it in the column low salt buffer and it was run on the DEAE Sephadex column without any

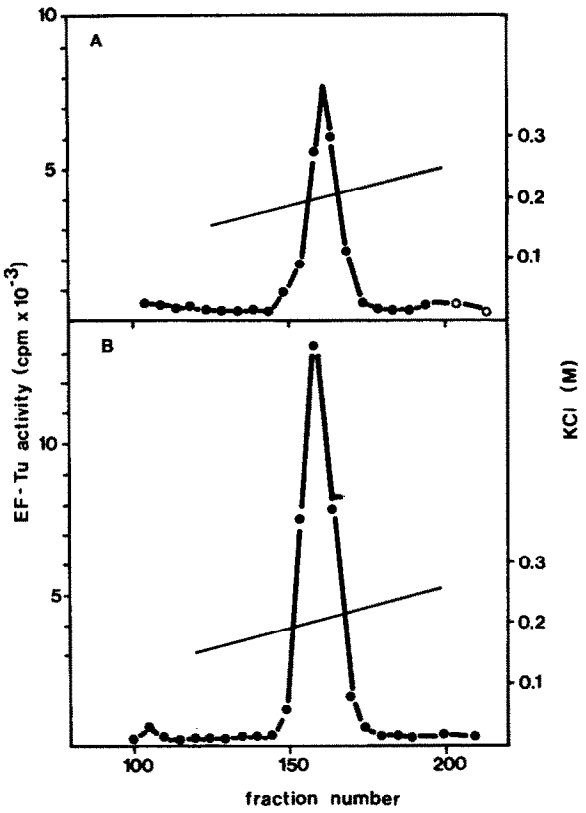

Fig.1. Chromatography of the EF-Tu extracted from the ribosomal pellet. The S30 fraction was centrifuged for 180 min at 50 000 rev./min in a Beckmann Ti 50 rotor, and the high-salt extract from the ribosomal pellet was run on DEAE Sephadex A-50 (B). As control, EF-Tu2 isolated from the S100 supernatant of the same preparation was run in parallel on an identical column (A).

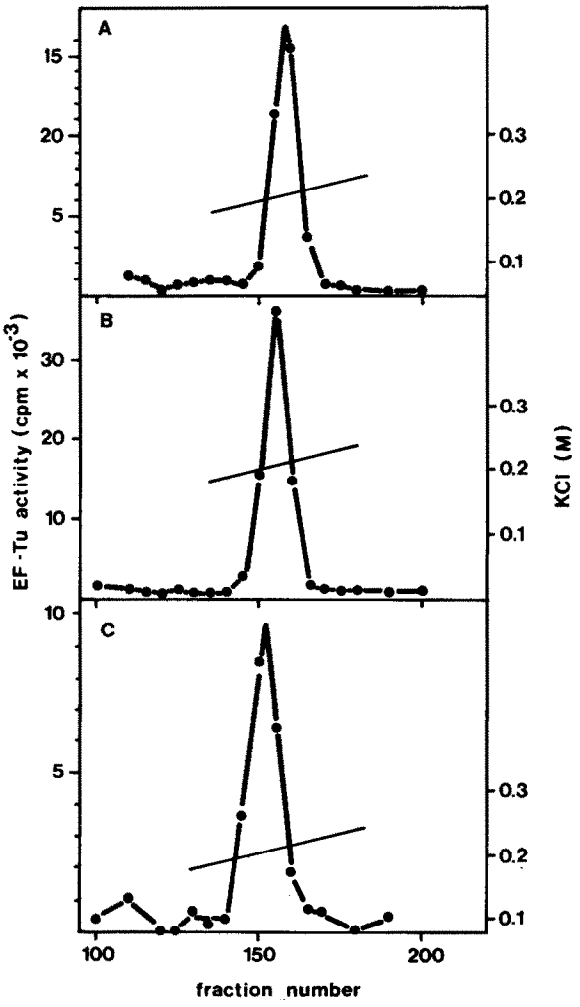

Fig.2. Rechromatography of the two forms of EF-Tu after Sephadex G-100 gel filtration and storage (several months, −20°C, 50% glycerol). The EF-Tu1 and EF-Tu2 obtained after the G-100 gel filtration step were rechromatographed individually (A and B, respectively) or as mixture (C) on DEAE Sephadex A-50.

further treatment. Under these conditions, the ribosomes appear in the break-through fraction of the column. Here again we failed to recover quantitatively EF-Tu activity as EF-Tu1, but we obtained a mixture of EF-Tu1 and EF-Tu2 (data not shown). From these results EF-Tu1 seems to be unstable. We therefore tested whether our purified preparations of EF-Tu1 and EF-Tu2 still eluted at their original position on a DEAE Sephadex column. The two forms of the EF-Tu protein which were purified through the Sephadex G-100 gel filtration column [9] were rerun on a DEAE Sephadex column. The results of the experiment are shown in fig.2. The EF-Tu2 emerged at the position expected (fig.2B). EF-Tu1 appeared at a higher KCl concentration than expected. This elution position was indistinguishable from that of EF-Tu2 (fig.2A). In addition, as shown in fig.2C, a single peak is obtained at the position of EF-Tu2 when a mixture of the two purified EF-Tu fractions was analysed. This is in dramatic contrast to earlier experiments, when it was shown that the fractions rechromatographed in the original positions if there was no intermediate handling. Thus, we conclude that

during purification EF-Tu1 was apparently converted into EF-Tu2.

A closer analysis of our findings seems to suggest a more intimate involvement of ribosomes in the phenomenon than originally supposed. The complete removal of ribosomes from the EF-Tu1 fraction, by a high-salt washing procedure, as well as a further purification step, apparently brought about a quantitative conversion of the EF-Tu1 form into EF-Tu2 (fig.1,2).

Our results imply an interaction of EF-Tu with ribosomes. Such an interaction has not been reported before for prokaryotic ribosomes, except under conditions where the GTP hydrolytic step of protein synthesis is blocked [15]. This was confirmed by sucrose gradient analysis (fig.3). When an S30 fraction was applied directly to a sucrose gradient, no EF-Tu activity was found at a position corresponding to ribosomes or ribosomal subunits, under ionic conditions corresponding approximately to the starting material applied to the DEAE Sephadex columns. This confirms the finding in [15]. On the other hand, a labile interaction of the corresponding eukaryotic factor, EF-1, with ribosomes, has been claimed [16].

The effect must then be ascribed to a weak interaction with the ribosomes. If such a weak interaction takes place, to be significant it must be on the EF-Tu part of the A site, which is known to be on the 50 S ribosomal subunit [17–19].

We therefore examined whether we could regenerate the modification by treatment of EF-Tu2 with ribosomes and if so, whether it was subunit-specific. The results in fig.4 show that (A) an alteration in the profile results from treatment of EF-Tu2 with the 50 S ribosomal subunit or (B) an equimolar mixture of 30 S and 50 S subunits, but not (C) with the 30 S subunit alone.

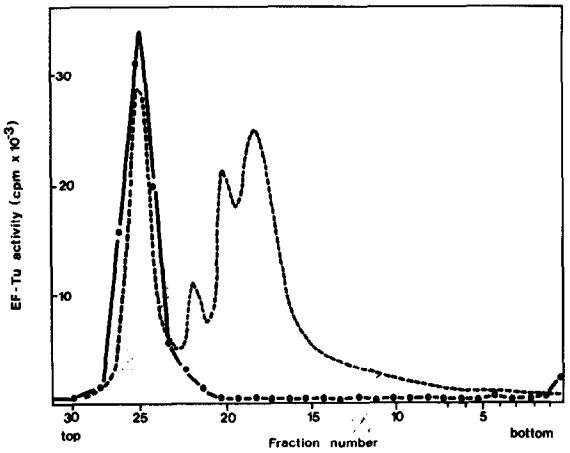

Fig.3. An S30 fraction (10 A_{260}· cm³) was applied to a 4 ml exponential sucrose gradient with 17.1% (w/v) sucrose at the top and 41% (w/v) sucrose in a constant volume mixing chamber. The gradient contained 20 mM Tris–HCl (pH 7.4), 10 mM $MgOAc_2$, 20 mM NH_4Cl and 5 mM β-mercaptoethanol. Centrifugation was at 1°C for 55 min at 56 000 rev./min in a Beckman SW56 rotor. The A_{260} peaks (– – –) correspond from the right to the left to 70 S ribosomes, 50 S and 30 S subunits, respectively, and to ribosome-free material. (•——•) EF-Tu activity.

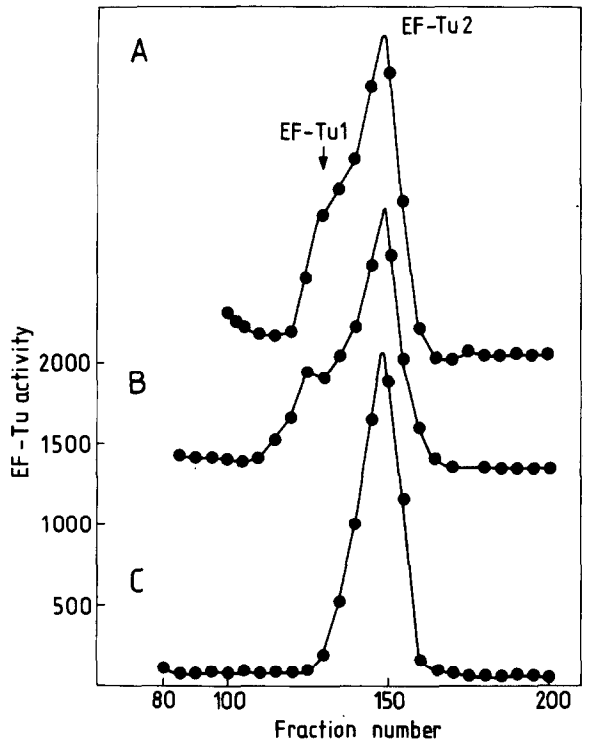

Fig.4. Effect of treatment of EF-Tu2 with ribosomal subunits. Ribosomal 50 S subunits (230 A_{260}·cm³; A), 30 S subunits (118 A_{260}·cm³; C) or both (B), prepared as in section 2 were added to an EF-Tu2 preparation as in fig.1(A) and fractionated on DEAE Sephadex. Gradient conditions and fraction volumes are also exactly as in fig.1. In this experiment, [^{14}C]GDP (spec. act. 492 mCi/mmol) was the substrate in the Tu-binding assay. For graphic reasons, the base lines of curves (A) and (B) have been arbitrarily displaced: the backgrounds are identical to that of curve (C).

4. Discussion

Originally, our results suggested that EF-Tu1 and EF-Tu2 corresponded to the Tuf A and Tuf B gene products, respectively, since some evidence suggested a higher affinity of Tuf A gene product in ribosome binding reactions [12]. Our present finding of interconvertibility does not lend support to the idea. However, the presence of two subpopulations, one of which undergoes the EF-Tu1 ⇌ EF-Tu2 transition and one which does not, is not ruled out. The question of possible non-identity of the two gene products is still open. A slight difference of one spot in tryptic fingerprints of the Tuf A and Tuf B gene products was seen [20] but so far there has been no report of significant non-identity.

Our experimental data do not stringently require the existence of two genetically different proteins. It is also possible that in vivo only one part of the total EF-Tu population is converted to the EF-Tu1 form by its interactions with the ribosomes during protein biosynthesis, since it is now well established [8,21,22] that the bacterial cell overproduces EF-Tu relative to the level of the ribosomes and the other elongation factors.

Our results therefore strongly suggest the existence of two subpopulations of EF-Tu which are interconvertible. The nature of the difference is difficult to establish, since it seems impossible to obtain a pure species in the EF-Tu1 form. Some means of trapping the EF-Tu1 state would permit a definitive study of the two forms, as well as to give some insight into their possible role in protein synthesis.

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References

[1] Miller, D. L. and Weissbach, H. (1977) in: Molecular Mechanism of Protein Biosynthesis (Weissbach, H. and Peska, S. eds) pp. 323–373, Academic Press, New York.
[2] Blumenthal, T., Landers, T. A. and Weber, K. (1972) Proc. Natl. Acad. Sci. USA 69, 1313–1317.
[3] Travers, A., Kamen, R. I. and Schleif, R. F. (1970) Nature 228, 748–751.
[4] Travers, A. (1973) Nature 244, 15–18.
[5] Rosenbusch, J. B., Jacobson, G. R. and Jaton, J. C. (1976) J. Supramol. Struct. 5, 391–396.
[6] Beck, D. B., Arscott, P. G. and Jacobson, A. (1978) Proc. Natl. Acad. Sci. USA 75, 1250–1254.
[7] Jacobson, G. R., Tabacs, B. J. and Rosenbusch, J. P. (1976) Biochemistry 15, 2297–2303.
[8] Jacobson, G. R. and Rosenbusch, J. P. (1976) Nature 261, 23–26.
[9] Geiser, M. and Gordon, J. (1978) Proc. Natl. Acad. Sci. USA 75, 1140–1144.
[10] Jaskunas, S. R., Lindahl, L., Nomura, M. and Burgess, R. R. (1975) Nature 257, 458–462.
[11] Lemaux, P. G. and Miller, D. L. (1978) Mol. Gen. Genet. 159, 47–55.
[12] Miller, L. D., Nagarkatti, S., Laursen, R. A., Parker, J. and Friesen, J. D. (1978) Mol. Gen. Genet. 159, 57–62.
[13] Hershey, J. W. B., Renold-O'Donnell, E., Kolakofski, D., Dewey, K. F. and Thach, R. E. (1971) in: Methods in Enzymology (Grossman, L. and Moldave, K. eds) vol. 20, pp. 235–261, Academic Press, New York.
[14] Ramjoué, H.-P. R. and Gordon, J. (1977) Anal. Biochem. 83, 763–766.
[15] Lucas-Lenard, J., Tao, P. and Haenni, A.-L. (1969) Cold Spring Harbor Symp. Quant. Biol. 34, 455–462.
[16] Grasmuck, H., Nolan, R. D. and Drews, J. (1976) Eur. J. Biochem. 71, 271–279.
[17] Highland, J. H., Ochsner, E., Gordon, J., Hasenbank, R. and Stöffler, G. (1974) J. Mol. Biol. 86, 175–178.
[18] San José, C. Kurland, C. G. and Stöffler, G. (1976) FEBS Lett. 71, 133–137.
[19] Fabian, U. (1976) FEBS Lett. 71, 256–260.
[20] Furano, A. V. (1977) J. Biol. Chem. 252, 2154–2157.
[21] Furano, A. V. (1975) Proc. Natl. Acad. Sci. USA 72, 4780–4784.
[22] Mijajima, A. and Kaziro, Y. (1978) J. Biochem. (Tokyo) 83, 453–462.