Functional and Structural Studies on a Tryptic Fragment of Eucaryotic Elongation Factor Tu from Rabbit Reticulocytes†

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ABSTRACT: Treatment of eucaryotic elongation factor Tu (eEF-Tu; M_r 53 000) with trypsin results in cleavage of the factor at at least two sites, one and probably both of which are located near the amino-terminal end of the polypeptide chain. The products after exposure of eEF-Tu to trypsin for 2 h is a single polypeptide of 43 000 daltons (eEF-Tu^t) and as yet unidentified polypeptides of $M_r \leq 5000$. The presence of high glycerol conncentrations and GDP in the reaction mixture markedly retards the rate of tryptic cleavage, while GTP has little effect. When eEF-Tu is bound to eucaryotic elongation factor Ts in an eEF-T complex, it is much more resistant to the action of trypsin. The loss of factor activity during tryptic digestion (as measured by its ability to bind aminoacyl-tRNA to 80S ribosomes) is much slower than the rate of eEF-Tu^t formation, and 2-h digests containing only eEF-Tu^t are about 30% as active as the native enzyme. However, no ribosome-dependent activity is detectable after purification of eEF-Tu^t by ion-exchange chromatography, followed by gel filtration. Purified eEF-Tu^t binds guanine nucleotides, although with diminished activity compared with that of eEF-Tu. Amino-terminal sequence analyses of eEF-Tu^t reveal a striking sequence homology with the functionally related factor from *Escherichia coli* (EF-Tu). The first four residues of eEF-Tu^t, Gly-Ile-Thr-Ile, are identical with the first four residues of a 37 000-dalton tryptic fragment of *E. coli* EF-Tu, and other homologies are evident in the first twelve amino-terminal residues of the corresponding tryptic fragments.

Elongation factor Tu has been among the most well studied bacterial proteins. In addition to its role in protein synthesis, EF-Tu[1] along with EF-Ts forms part of bacteriophage Qβ replicase (Blumenthal el al., 1977), and the protein has been implicated in a variety of different cellular functions [for recent reviews, see Kaziro (1978), Blumenthal & Carmichael (1979), and Miller & Weissbach (1977)]. Recently the structure of this enzyme has received a great deal of attention. We now know the complete primary structure of EF-Tu (Arai et al., 1980; Jones et al., 1980), and details of the three-dimensional structure should be forthcoming (Morikawa et al., 1978; Jurnak et al., 1980; Kabsch et al., 1977).

By contrast, little is known about the structure of the corresponding factor in eucaryotic cells, designated here as eEF-Tu. What is known can be summarized briefly. eEF-Tu in all cells so far investigated is a basic single polypeptide chain of molecular weight 53 000 and with a p*I* of ca. 8.5 (Iwasaki et al., 1974; Slobin & Moller, 1976; Slobin, 1980). Like its procaryotic counterpart, eEF-Tu binds both aminoacyl-tRNA and GTP and is generally believed to transport aminoacylated tRNA to the A site of 80S ribosomes during protein synthesis. eEFTu also binds to an acidic polypeptide of 30 000 M_r, originally isolated by Iwasaki et al. (1976), which has all of the functional properties previously ascribed to EF-Ts and which is designated eEF-Ts (Slobin & Moller, 1977; Slobin, 1979). In addition, eEF-Tu can be complexed with a third polypeptide (β chain) of molecular weight 50 000 for which a function has not yet been found (Bollini et al., 1974; Slobin & Moller, 1976; Slobin, 1980; Hattori & Iwasaki, 1980). These three polypeptides have generally been isolated as stable high molecular weight aggregates and will be referred to here as eEF-T.[1]

The complex of eEF-Tu with eEF-Ts confers functional stability to the latter factor. Indeed, the one peculiar structural feature of the factor reported so far is its extreme lability in the absence of high concentrations of glycerol (Iwasaki et al., 1974; Nagata et al., 1976).

One of the simplest probes of protein structure has been to examine the susceptibility of enzymes to proteolytic enzymes. This approach has been rewarding in the case of EF-Tu where it has been shown that trypsin rapidly cleaves the factor at a limited number of sites to yield a core fragment which apparently retains some of the functional properties of the intact enzyme (Jacobson & Rosenbusch, 1976; Wittinghofer et al., 1980). The sensitivity of EF-Tu to trypsin has been used successfully as a probe to investigate structure–function relationships of the factor (Blumenthal et al., 1977; Douglass & Blumenthal, 1979).

In this report we present studies on the tryptic cleavage of eEF-Tu from rabbit reticulocytes. Many of the features of the reaction between eEF-Tu and trypsin were found to resemble those already reported for the bacterial factor. In particular, a trypsin-resistant fragment of molecular weight 43 000 could be isolated from digests of eEF-Tu. To our surprise, amino-terminal sequence analysis of this fragment revealed clear structural homology with the bacterial factor.

Experimental Procedures

Preparation of Elongation Factors, Ribosomes, and Aminoacyl-tRNA. Purified eEF-Tu and eEF-T were prepared from rabbit reticulocyte as described previously (Slobin, 1979, 1980). Both enzymes were approximately 90% pure as judged by electrophoresis in polyacrylamide gel slabs (see below). Ribosomes (80 S) were prepared from the brine shrimp *Artemia* as described elsewhere (Slobin & Moller, 1979). Pure

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[1] Abbreviations used: EF-Tu, procaryotic elongation factor Tu; EF-Ts, procaryotic elongation factor Ts; eEF-Tu, eucaryotic elongation factor Tu; eEF-Ts, eucaryotic elongation factor Ts; eEF-Tu^t, the 43 000-dalton fragment of eEF-Tu. The complex of eEF-Tu and eEF-Ts and a third polypeptide of molecular weight 50 000 (β chain), often referred to as EF-1, will be designated as eEF-T, in analogy with the designation of EF-T given to the corresponding complex of bacterial factors. eEF-Tu has been referred to by other investigators as EF-1_α or EF-1_L; eEF-Ts has been named EF-1_β. For a discussion of the nomenclature of these factors, see Slobin & Moller (1979).

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yeast phenylalanine transfer RNA (Boehringer) was charged with [^{14}C]phenylalanine (Schwarz/Mann, 460 Ci/mmol) by using crude *Escherichia coli* synthetase as described (Slobin & Moller, 1976).

Preparation and Purification of eEF-Tu^t. Solutions of eEF-Tu (0.5–1 mg/mL) in buffer A (50 mM Tris-HCl, pH 7.5, 5 mM magnesium acetate, 1 mM $CaCl_2$, 0.5 mM dithiothreitol, 0.5 mM ethylenediaminetetraacetic acid, and 25% (v/v) glycerol) were incubated at 25 °C with freshly dissolved *N*-tosyl-Lphenylalanine chloromethyl ketone treated trypsin (Worthington) at a protease concentration of 2% (w/w). The reaction was stopped by the addition of a 2-fold excess (w/w) of soybean trypsin inhibitor (Sigma Chemical Co.) over trypsin. For structural analysis eEF-Tu^t was purified by ion-exchange chromatography and gel filtration. Briefly, 10 mg of eEF-Tu which had been treated with trypsin for 2 h at 25 °C was chromatographed on a column (0.7 × 5 cm) of CM-Sepharose CL-6B (Pharmacia) previously equilibrated with Buffer B (20 mM Tris-HCl, pH 7.5, 100 mM KCl, and 1 mM 2-mercaptoethanol). The column was developed at 4 °C with a linear salt gradient (20 × 20 mL) between buffer B and the same buffer containing 300 mM KCl. Fractions (1 mL) were monitored by optical density and a protein peak containing eEF-Tu^t eluted at about 125 mM KCl. The fractions containing eEF-Tu were pooled, dialyzed against 0.1 M *N*-ethylmorpholine, pH 8.0, concentrated by negative pressure to about 1 mL, and chromatographed on a column (1 × 15 cm) of Sephacryl S-200 (Pharmacia) previously equilibrated with 0.1 M *N*-ethylmorpholine, pH 8.0. The fractions containing eEF-Tu^t (monitored by absorbency measurements at 280 nm) were pooled and taken to dryness on a Speed-Vac (Savant Instruments).

In an attempt to preserve activity, eEF-Tu^t was also purified on CM-Sepharose by using the buffers described above except that they contained 25 (v/v) glycerol. The factor eluted at a similar salt concentration (125 mM KCl).

Assays. The binding of [^{3}H]GTP (Amersham; 13 Ci/mmol) to eEF-Tu and eEF-Tu^t was measured by the nitrocellulose filter assay as described previously (Slobin & Moller, 1977). Under the assay conditions used, the binding of aminoacyl-tRNA is linear and stoichiometric with factor concentration up to approximately 10 pmol (0.53 μg) of factor (Iwasaki et al., 1974; Roobol & Moller, 1978; Slobin, 1980). The eEF-Tu used for most of the experiments reported here bound 0.7 pmol of aminoacyl-tRNA/pmol of factor. We have also tested factor with somewhat higher activity (0.9 pmol of aminoacyl-tRNA bound to 80S ribosomes per pmol of enzyme) and have obtained similar inactivation kinetics after treatment with trypsin. The binding of aminoacyl-tRNA to poly(U)-programmed 80S ribosomes from *Artemia* was assessed by the standard nitrocellulose filter technique (Slobin & Moller, 1979).

Amino Acid Sequence Analysis. Amino acid sequence analyses were performed on a Beckman 890-C sequenator equipped with a cold trap attachment and a Sequemat P-6 auto converter. A program similar to that of Brauer et al. (1975) employing 0.3 M Quadrol was used. The PTH-amino acids at each cycle of Edman degradation were identified by high-performance liquid chromatography on a Varian 5000 liquid chromatograph equipped with a Beckman-Altex dedicated PTH-amino acid reversed-phase Ultrasphere-ODS column. Separation was achieved by use of a linear gradient with an initial solvent of 4.2 mM sodium acetate (pH 5.55) containing 5% tetrahydrofuran up to 40% of the second solvent (which consisted of 10% tetrahydrofuran in acetonitrile (program supplied by Beckman Instruments). Confirmations of PTH-amino acids at some steps were made by isocratic runs using various proportions of the two solvents.

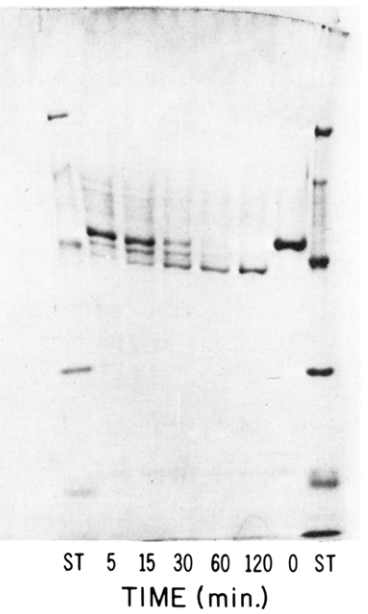

FIGURE 1: Kinetics of hydrolysis of eEF-Tu by trypsin. Native eEF-Tu was treated with trypsin under conditions described under Experimental Procedures. Aliquots containing 6 μg of factor were removed at the indicated times, and the tryptic hydrolysis was stopped by addition of soybean trypsin inhibitor. Samples were analyzed on 10% polyacrylamide slab gels in the presence of dodecyl sulfate according to the procedure of Laemmli (1970). Molecular weight standards at the left and right are as follows: (1) phosphorylase *b* (94 000); (2) bovine serum albumin (66 000); (3) ovalbumin (45 00); (4) carbonic anhydrase (30 000); (5) soybean trypsin inhibitor (21 000); (6) lysozyme (14 300).

Other Procedures. Conditions for slab polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate as well as for two-dimensional electrophoresis have been described elsewhere (Slobin, 1980). Acrylamide gels stained with Coomassie brilliant blue R250 were destained with 25% (v/v) ethanol–7% (v/v) acetic acid and scanned with a Transidyne Model 2510 densitometer equipped with Transidyne Model 2500 control module. Protein was determined by using the dye-binding method of Bradford (1976). We have found that this procedure overestimates eEF-Tu content by a factor of 1.7 using bovine γ-globulin as a standard.

Other Materials. Unlabeled nucleotides and poly(uridylic acid) were purchased from Sigma. Reagents used for amino acid sequence analysis were obtained from Beckman Instruments. All other chemicals used were reagent grade.

Results

Kinetics of Hydrolysis of eEF-Tu by Trypsin. The time course of the hydrolysis of eEF-Tu by trypsin was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The results, shown in Figure 1, indicate a progressive disappearance of the starting eEF-Tu and the appearance of two polypeptides of molecular weights 48 000 and 43 000, respectively. The 48 000-dalton polypeptide is likely to be an intermediate in the hydrolysis of eEF-Tu as its appearance precedes that of the 43 000-dalton polypeptide. It then declines in amount until by 2 h it has virtually disappeared from the digestion mixture (Figure 2). The 43 000-dalton polypeptide, designated eEF-Tu^t, accumulates during the digestion, accounting for slightly more than 90% of the stained material on the polyacrylamide gel at 2 h (Figure 2). No other stained bands were observed except for material which migrated with

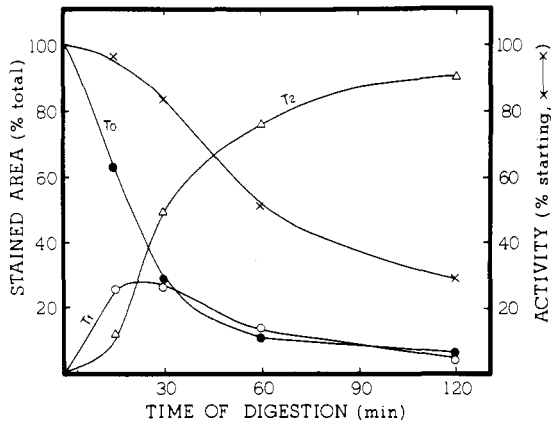

FIGURE 2: Densimetric analysis of the kinetics of hydrolysis of eEF-Tu by trypsin. The polyacrylamide gel shown in Figure 1 was scanned by using a Transidine densitometer (see Experimental Procedures), and the relative amounts (percent of total stained area) of starting eEF-Tu (T0), 48 000-dalton polypeptide hydrolysis intermediate (T1), and 43 000-dalton fragment (T2) are plotted as a function of digestion time. Elongation factor activity, as measured by eEF-Tu-dependent binding of aminoacyl-tRNA to 80S ribosomes, after various times of exposure to trypsin is also depicted. One-hundred percent activity corresponds to 3.8 pmol of aminoacyl-tRNA bound to 80S ribosomes from *Artemia* by 0.29 μg of factor (5.5 pmol) under standard assay conditions.

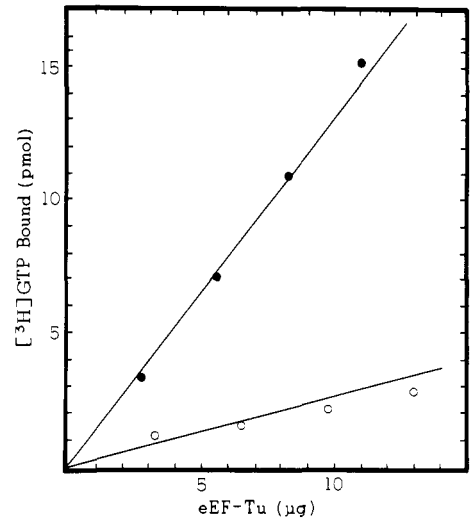

FIGURE 3: Binding of [^{3}H]GTP by eEF-Tu (●) and eEF-Tu^t (○). The binding of [^{3}H]GTP by eEF-Tu and eEF-Tu^t was determined after incubation of the factor with 5 μM nucleotide in a 40-μL reaction volume for 5 in at 37 °C as described elsewhere (Slobin & Moller, 1977).

the dye front. Attempts to visualize the 10 000 daltons of polypeptide which was cleaved by trypsin from eEF-Tu by raising the acrylamide concentration have so far proved unsuccessful.

Our experiments suggest that eEF-Tu is initially cleaved into a 48 000-dalton polypeptide and a 5000-dalton peptide; further cleavage produces eEF-Tu^t and another 5000-dalton peptide. Both 5000-dalton peptides are then probably degraded to smaller fragments. Further incubation of eEF-Tu with trypsin (up to 4 h at 25 °C) did not result in any further breakdown of eEF-Tu^t into smaller polypeptides (results not shown).

Functional Properties of eEF-Tu^t. Measurement of eEF-Tu activity by the factor-dependent binding of aminoacyl-tRNA to ribosomes indicates that eEF-Tu is only partially inactivated during the course of conversion to eEF-Tu^t (Figure 2). The kinetics of inactivation show that at all times studied there is substantially more eEF-Tu activity in the digestion mixture than can be accounted for by the presence of either native eEF-Tu or the intermediate 48 000-dalton hydrolysis product. After treatment with trypsin for 2 h, the digest possesses about 30% of starting eEF-Tu activity while less than 5% of the protein behaves as native eEF-Tu on dodecyl sulfate–polyacrylamide gels. We have repeated this experiment several times and have always observed substantially more eEF-Tu activity than could be accounted for by unmodified factor in the digestion mixture. However, when eEF-Tu^t was purified from tryptic digests of the eEF-Tu by CM-Sepharose chromatography using buffers containing 25% (v/v) glycerol, little, if any, ribosome-dependent factor activity was observed. It is possible that a small peptide fragment which is necessary for activity remains associated with eEF-Tu^t in digests but is lost upon subsequent purification of eEF-Tu^t (see Discussion).

Less doubt can be raised about the capacity of eEF-Tu^t to bind guanine nucleotides. Purified eEF-Tu^t retains the ability to bind GTP (Figure 3) although this binding has been reduced to about 20% of that of the native enzyme. The native enzyme bound 0.073 pmol of GTP/pmol, a value in agreement with most (Legocki et al., 1974; Slobin & Moller, 1976; Grasmuk et al., 1978) but not all (Nagata et al., 1976) studies on eEF-Tu. It is possible that this rather inefficient binding reflects the relatively low binding constant of eEF-Tu for guanine nucleotides compared with that of EF-Tu (Roobol & Moller, 1978; Nolan et al., 1974; Nagata et al., 1976; Miller & Weissbach, 1977). Preliminary experiments using the ability of the factor to retard aminoacyl-tRNA hydrolysis indicate that eEF-Tu^t can interact with aminoacyl-tRNA, although again with significantly diminished effectiveness compared with that of the native enzyme.

Effect of Guanine Nucleotides and Glycerol on the Tryptic Hydrolysis of eEF-Tu. Substantial evidence has accumulated that the structure EF-Tu is altered when the factor binds guanine nucleotides (Kaziro, 1978; Miller & Weissbach, 1977). In particular, it has been noted that the rate of trypsin cleavage of EF-Tu is retarded significantly when GDP is bound to the enzyme (Douglass & Blumenthal, 1979). Similar structural changes in eEF-Tu upon binding guanine nucleotides may be inferred from the capacity of eEF-Tu·GTP, but not eEF-Tu·GDP, to bind aminoacyl-tRNA (Nagata et al., 1976). In an attempt to demonstrate directly the effect of guanine nucleotides on eEF-Tu structure, we investigated their ability to alter the course of tryptic cleavage of the enzyme. The results, given in Figure 4, show a dramatic reduction in the rate of tryptic cleavage of eEF-Tu in the presence of GDP. The presence of GTP, on the other hand, has much less effect on the hydrolytic reaction, although it also seems to retard tryptic cleavage. In addition, less of the 48 000-dalton intermediate cleavage product can be detected when GTP is present in the hydrolysis mixture (compare results in Figure 4 with those in Figure 1).

As mentioned in the introduction, eEF-Tu activity is remarkably unstable in the absence of high concentrations of glycerol. The buffers for the tryptic digestion of eEF-Tu all include 25% (v/v) glycerol. The reason for this inclusion is made apparent in Figure 4 where the time course for the hydrolysis of the factor in the absence of glycerol is presented. Essentially all of the eEF-Tu is completely degraded by trypsin to small peptides (M_r <10 000) in 15 min at 25 °C when glycerol is excluded from the reaction mixture. That the major effect of glycerol in the reaction mixture was on eEF-Tu conformation and not on trypsin activity was demonstrated in separate experiments, where it was shown that the presence of 25% (v/v) glycerol in reaction mixtures had little effect on

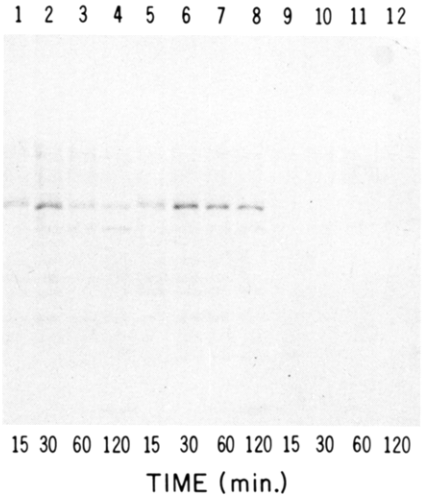

FIGURE 4: Effect of guanine nucleotides and glycerol on the kinetics of hydrolysis of eEF-Tu by trypsin. Native eEF-Tu was treated with trypsin under varying conditions for varying times, and the reaction mixtures were analyzed on polyacrylamide gels as described in the legend to Figure 1. Digestion conditions were the same as described in the legend to Figure 1 except for the following additions or omissions: lanes 1–4, 0.5 M GTP; lanes 5–8, 0.5 mM GDP; lanes 9–12, minus glycerol. The eEF-Tu used in this experiment was only about 80% pure and contained some minor components of lower molecular weight.

the kinetics of trypsin hydrolyses of bovine serum albumin.

Action of Trypsin on eEF-T. Experiments designed to investigate the stability of eEF-T to trypsin are illustrated in Figure 5, which depicts the kinetics of disappearance of eEF-Tu from the eEF-T complex under varying digestion conditions. So that these results could be obtained, tryptic digests of eEF-T were subjected to analysis on polyacrylamide gels in the presence of sodium dodecyl sulfate and the stained gel was analyzed by densitometry. Although the β chain of eEF-T is not well resolved from eEF-Tu on dodecyl sulfate–acrylamide gel (Slobin, 1979), we have found that reticulocyte eEF-T contains a variable amount of the β polypeptide and that the 53 000-dalton band of eEF-T represents predominantly (≥70%) eEF-Tu[2] (Slobin, 1980; R. V. Clark and L. I. Slobin, unpublished observations).

The results shown in Figure 5 indicate that, unlike eEF-Tu, the eEF-Tu in eEF-T is rather resistant to trypsin in the presence of glycerol. Guanine nucleotides appear to retard the rate of tryptic hydrolysis, which GDP having a stronger effect than GTP. However, even in the absence of glycerol, the major digestion product of eEF-T is the 43 000 polypeptide eEF-Tu^t. This result contrasts strongly with the rapid hydrolysis of eEF-Tu to small peptides when the factor is treated with trypsin in the absence of high glycerol concentrations. The eEF-Ts in eEF-T appears to be rapidly hydrolyzed by trypsin in the absence of glycerol, although the relatively faint staining of this polypeptide compared with eEF-Tu makes it difficult to assess the reaction quantitatively.

These conclusions are supported strongly by measurements of factor activity after exposure to trypsin for varying times (Figure 6). The eEF-Tu dependent binding of aminoacyl-tRNA to 80S ribosomes is unaffected by trypsin in the presence of 25% (v/v) glycerol, whereas, in the absence of glycerol, there is a progressive increase in the rate of loss of factor activity when digestion is done in the presence of GDP and GTP and in the absence of a guanine nucleotide. Under

[2] That the stained band below eEF-Tu in Figure 5, lanes 1–4, is eEF-Tu^t and not a product of β-chain hydrolysis was confirmed by analysis of two-dimensional gels of the tryptic digest (data not shown; however, see Figure 7).

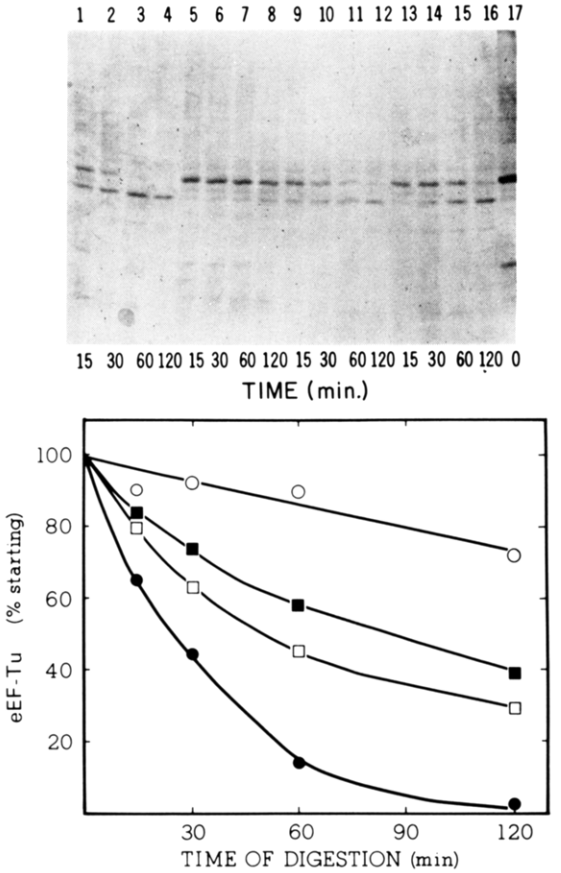

FIGURE 5: Kinetics of the hydrolysis of eucaryotic elongation factor T by trypsin. Native eEF-T from rabbit reticulocytes (Slobin, 1979) was treated with trypsin as described for eEF-Tu under Experimental Procedures except that glycerol was absent from digestion mixtures unless otherwise indicated. Aliquots of the hydrolysis mixtures containing 9 μg of protein were removed at the time indicated and treated with a 2-fold excess (w/w) of soybean trypsin inhibitor, and the reaction products were analyzed by polyacrylaimde gel electrophoresis as described in the legend to Figure 1. All digestion mixtures had identical compositions except for the following additions: lanes 1–4, no additions; lanes 5–8, 25% (v/v) glycerol; lanes 9–12, 0.5 mM GTP; lanes 13–16, 0.5 mM GDP. Lanes 17 contained the eEF-T prior to trypsin treatment; the bands are eEF-Tu (M_r 53 000) and eEF-Ts (M_r 30 000). Below the reproduction of the dodecyl sulfate–polyacrylamide gel is a densitometric analysis of the kinetics of hydrolysis of the 53 000 M_r components of eEF-T (eEF-Tu) by trypsin under varying digestion conditions. The gel was scanned as described in the legend to Figure 1, and the percentage of the starting eEF-Tu is plotted as a function of digestion time. All digestion mixtures had identical compositions except for the following additions: (●) no additions; (□) 0.5 mM GTP; (■) 0.5 mM GDP; (○) 25% (v/v) glycerol.

all conditions investigated eEF-T activity is significantly less affected by trypsin than eEF-Tu.

Structure of eEF-Tu^t. eEF-Tu from 2-h tryptic digests of eEF-Tu was purified by CM-Sepharose chromatography and gel filtration. The weaker binding of eEF-Tu^t to CM-Sepharose, compared with that of starting eEF-Tu, suggested that eEF-Tu^t is somewhat less basic than the starting factor. This suggestion was confirmed by electrophoresis of a mixture of eEF-Tu and eEF-Tu^t using a two-dimensional acrylamide gel system. The result, shown in Figure 7, indicates that eEF-Tu^t has an isoelectric point about 0.2 pH unit lower than that of eEF-Tu. [The p*I* of reticulocyte eEF-Tu is approximately 8.5 (Slobin, 1980).]

Purified eEF-Tu^t was taken for amino acid sequence analysis by using a Beckman sequenator. One predominant PTH-amino acid was released at each cycle (Table I), except at step 2 where approximately equal quantities of isoleucine and as-

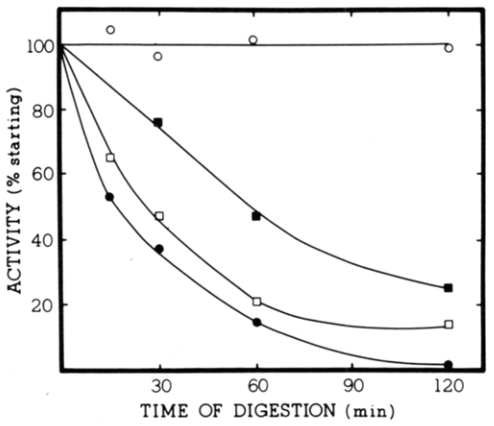

FIGURE 6: Kinetics of inactivation of eEF-T upon treatment with trypsin. Factor activity was measured as described in the legend to Figure 2. One-hundred percent activity corresponded to 3.5 pmol of aminoacyl-tRNA bound to 80S ribosomes by 0.68 μg of eEF-T. All digestion mixtures had identical compositions except for the following additions: (●) no additions; (□) 0.5 mM GTP; (■) 0.5 mM GDP; (○) 25% (v/v) glycerol. It should be noted that the eEF-Tu activity of eEF-T is completely inactivated by treatment with trypsin for 2 h at 25 °C [see (○) in the figure], whereas considerable factor activity remains after tryptic digestion of eEF-Tu for the same time (see Figure 2). The explanation for this apparent discrepancy is that eEF-Tu^t, like eEF-Tu, is rapidly inactivated in the absence of glycerol.

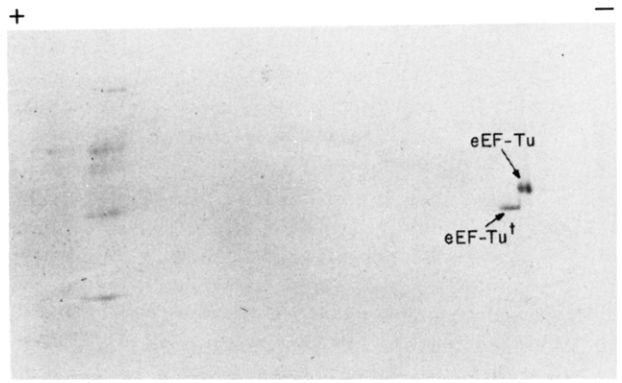

FIGURE 7: Two-dimensional gel of eEF-Tu and eEF-Tu^t. Approximately equal amounts (15 μg) of eEF-Tu and eEF-Tu^t were subjected to two-dimensional gel electrophoresis as described elsewhere (Slobin, 1980). The molecular weight standards shown at the left are, in order of decreasing molecular weight, phosphorylase *b* (94000), bovine serum albumin (66000), ovalbumin (45000), and carbonic anhydrase (30000). The difference in p*I* between eEF-Tu and eEF-Tu^t (by using the middle of each band as a reference) was found to be 0.2 pH unit. The pH gradient of the first dimension isoelectric focusing gel was determined by pH measurements at 5-mm intervals using a Pro-philer (Bio-Rad Laboratories) and a combination microelectrode (M1-410 microcombination pH probe, Microelectrodes, Inc.).

partic acid were found. The sequence of the first 12 amino-terminal residues of eEF-Tu^t is given in Figure 8, together with the comparable sequence for EF-Tu^t (Jacobson & Rosenbusch, 1976; Nakamura et al., 1977; Jones et al., 1980).

Table I: Automated Sequenator Analysis of eEF-Tu^t

cycle	residue identified by HPLC	yield (nmol)
1	Gly	3.9
2[a]	Ile	1.1
3	Thr	1.0
4	Ile	1.8
5	Asp	1.9
6	Ile	1.1
7	Ser	*b*
8	Leu	1.3
9	Trp	0.9
10	Val	0.3
11	Phe	0.4
12	Glu	0.6

[a] Cycle 2 contained approximately equal quantities of PTH-isoleucine and PTH-aspartic acid in one of our sequence determinations. [b] PTH-serine could not be quantitated by HPLC.

Discussion

The work presented in this report establishes for the first time a structural homology between procaryotic EF-Tu and the functionally analogous factor in eucaryotic cells. Both EF-Tu and eEF-Tu are cleaved by trypsin at a limited number of sites at the amino-terminal end of the molecule. In the case of the bacterial protein, it has been shown that cleavage occurs at three adjacent sites on the primary structure: arginine-44, arginine-58, and lysine-56 (Wittinghofer et al., 1980). The rates of hydrolysis at the two arginine residures are about the same, but that at the lysine residue is much slower. Eucaryotic elongation factor Tu is also cleaved at at least two sites. Cleavage at the first site appears to be relatively rapid and results in the appearance of a 48000-dalton polypeptide. Subsequently, the 48000-dalton fragment is trimmed to a 43000 molecular weight polypeptide which we designate eEF-Tu^t. Since we have not yet detected low molecular weight polypeptides as hydrolysis products, it remains possible that there are multiple cleavage sites in eEF-Tu. (Alternatively, of course, the 5000-dalton polypeptides sequentially released from eEF-Tu are further rapidly hydrolyzed to very small oligopeptides.)

We have also not established whether the polypeptide(s) removed from eEF-Tu in the conversion to the 48000-dalton cleavage product originates from the amino-terminal or carboxyl-terminal portion of eEF-Tu. However, as is the case with the procaryotic factor, the amino-terminal residue of native eEF-Tu is blocked (unpublished observation). This observation permits the conclusion that at least one of the sites of tryptic hydrolysis on the native enzyme is at an amino-terminal locus. There are reasons to believe that all the trypsin-accessible sites on the native eEF-Tu are at the amino-terminal end of the enzyme (see below).

It has been well documented that eEF-Tu activity is extremely unstable in the absence of high concentrations of glycerol (Nagata et al., 1976; Slobin & Moller, 1976). The

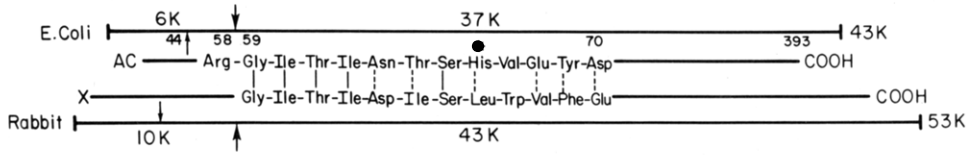

FIGURE 8: Amino-terminal sequence of eEF-Tu^t. For comparison, the amino-terminal sequence of the 37000-dalton polypeptide produced by the action of trypsin on EF-Tu from *E. coli* is shown. The solid arrows indicate the sites of trypsin cleavage on the native factors. In the case of eEF-Tu, these sites are proposed and have not yet been definitely established (see the text for a discussion). The X at the amino-terminal end of eEF-Tu indicates a blocked α-amino group. The amino-terminus of EF-Tu is *N*-acetyl-Ser. The asterisk above the His residue in the bacterial factor indicates it is affinity labeled by N^{ϵ}-bromoacetyl-Lys-tRNA (see the text). The dashed lines connecting the two sequences indicate that amino acid residue in one sequence can be converted to the residue shown in the other sequence by a single base substitution in the genetic code.

rather rapid conversion of native eEF-Tu to low molecular weight polypeptides by trypsin in the absence of glycerol suggests major structural alterations in the enzyme occur when glycerol is absent. These structural changes are apparently significantly modified when eEF-Tu is present in the eEF-T complex, since tryptic digestion of the latter in the absence of glycerol produces a product electrophoretically indistinguishable from eEF-Tu^t. This finding is in accord with our knowledge that eEF-Tu activity is stabilized by the presence of eEF-Ts (Slobin, 1979) and that active eEF-T can readily by isolated from cells by using buffers lacking high glycerol concentrations. The stability of eEF-Tu in the eEF-T complex is further substantiated by the essentially complete resistance of the factor to tryptic hydrolysis in the presence of 25% (v/v) glycerol.

Alterations of the structure of eEF-Tu by guanine nucleotides have been observed by using the rate of cleavage of the factor by trypsin as a conformational probe (Douglass & Blumenthal, 1979). When GDP was bound to EF-Tu, trypsin cleavage was slow, whereas the binding of GTP to the factor accelerated the rate of tryptic hydrolysis. GDP was found to exert a similar effect on the course of tryptic hydrolysis of eEF-Tu; i.e., the hydrolytic reaction was retarded by the nucleotide. However, the presence of GTP in the hydrolysis mixture seemed to have little effect on the rate of trypsin cleavage. Functional studies on both eEF-Tu and eEF-T after exposure to trypsin for varying times (Figures 2 and 5) generally support our conclusion based on dodecyl sulfate–acrylamide gel analysis.

Purified eEF-Tu^t binds guanine nucleotides, albeit with reduced affinity, indicating that the nucleotide binding site of the factor is only slightly altered by removal of 10000 daltons of polypeptide from the native enzyme by trypsin. The ability of eEF-Tu^t to bind aminoacyl-tRNA to ribosomes is less certain. The rate of loss of enzymatic activity during tryptic digestion of eEF-Tu is significantly slower than the rate of appearance of eEF-Tu^t. Furthermore, after 2 h of tryptic digestion 25–30% of eEF-Tu activity remains while only about 5% of the starging eEF-Tu is present in the digest. Nonetheless, purified eEF-Tu^t lacked activity in ribosome-dependent assays. A possibility is that eEF-Tu^t is active only under conditions where the low molecular weight fragments produced by tryptic digestion remained attached to it. Removal of these low molecular weight fragments from eEF-Tu^t by purification of the latter polypeptide may abolish enzyme activity. In this regard it is well established that the tryptic fragments of EF-Tu under native conditions form a strong complex which apparently crystallizes in various space groups (Kabsch et al., 1977; Morikawa et al., 1978; Jurnak et al., 1977). Although controversy has surrounded the activity (or lack thereof) of trypsin-cleaved eEF-Tu (Jurnak et al., 1977); Arai et al., 1976; Jacobson & Rosenbusch, 1976), a recent report indicates that the complex of EF-Tu tryptic fragments is active in aminoacyl-tRNA binding and polyphenylalanine synthesis (Wittinghofer et al., 1980).

A few remarks about the purity of eEF-Tu^t are in order here. Although we have not established directly that purified eEF-Tu^t lacks low molecular weight peptide material, there is some reason to believe that most if not all small peptides present in the initial tryptic digest have been removed by gel filtration and subsequent CM-Sepharose chromatography. The purified eEF-Tu^t was found to possess an unambiguous amino-terminal sequence, a finding inconsistent with significant combination of the large tryptic fragment with small peptides. Furthermore, the markedly lower affinity of eEF-Tu^t for CM-Sepharose compared with that of the native factor suggests that basic peptides have been separated from eEF-Tu. Nonetheless, it remains possible that some peptide material, particularly the amino-terminal peptide of eEF-Tu which contains a blocked α-amino group, remains associated with eEF-Tu^t throughout the two-step purification procedure.

The remarkable similarity in the mode of action of trypsin on EF-Tu from *E. coli* and eEF-Tu from rabbit reticulocytes suggests that these two proteins possess common structural features. To our surprise, we found that the similarity between procaryotic and eucaryotic Tu extends to common amino acid sequences. Limited tryptic digestion of both factors gives a single high molecular weight polypeptide product (37000 for EF-Tu and 4300 for eEF-Tu). The amino-terminal sequence of the polypeptides is remarkably similar: The first four residues, Gly-Ile-Thr-Ile (positions 59–62 in the sequence of EF-Tu), are identical as is a Ser seven residues from the amino-terminal end of the sequence of both polypeptides (Figure 8). Of the remaining seven residues, six are related by single base substitutions in the genetic code (the exception being that valine at position 9 from the amino-terminal end of the bacterial factor fragment is replaced by tryptophan in the eucaryotic sequence). Other similarities are apparent. For example, a sequence Tyr-Asp in the bacterial factor is replaced by Phe-Glu in eEF-Tu^t. Preliminary results indicate that a threonine residue occupies a position 15 amino acids from the amino-terminal end of both tryptic fragments. Of course, the similarity in sequence must extend to the residue preceding the amino-terminal glycine. In EF-Tu that residue is arginine; the corresponding residue in eEF-Tu has not yet been identified.

The sequence homology between eEF-Tu^t and a corresponding tryptic fragment of EF-Tu is compelling evidence that the two proteins are evolutionarily related. Of course, only a complete sequence analysis of the eucaryotic protein can delineate the extent of that relationship. It may well be that the sequence at or near the trypsin-sensitive bonds of the two factors plays a particularly important role in factor function leading to its conservation and that other parts of the structure are less similar. In this regard it has been established that histidine residue 66 in EF-Tu (see Figure 8) is affinity labeled with N^{ϵ}-bromoacetyl-Lys-tRNA, suggesting that the amino-terminal sequence of the bacterial factor is in the vicinity of the tRNA binding site (Guerrier-Takada et al., 1981). The eucaryotic factor can be labeled with the same reagent, although with much lower efficiency (Johnson & Slobin, 1980). The lack of a histidine residue in eEF-Tu (Figure 8) at or near the site where EF-Tu is affinity labeled may be responsible for the reduction in labeling efficiency. For a further discussion implicating the amino-terminal portion of EF-Tu in aminoacyl-tRNA binding, see Jones et al. (1980).

If it is granted that EF-Tu and eEF-Tu share a related structure, then a number of consequences related to the evolution of the factor become evident. eEF-Tu contains approximately 10000 daltons more mass than the procaryotic factor. Tryptic cleavage trims eEF-Tu and EF-Tu of about 10000 and 6000 daltons, respectively. Since the amino-terminal sequences of the large tryptic fragments of EF-Tu and eEF-Tu are homologous, we can conclude that all of the extra mass in the eucaryotic factor cannot reside amino terminal to the site of tryptic cleavage. If, by analogy with EF-Tu, the tryptic cleavage of eEF-Tu occurs exclusively at the amino-terminal end of the protein, then about 4000 daltons of polypeptide (approximately 40 amino acid residues) has been inserted in the sequence of the factor at the amino terminus

and 6000 daltons somewhere else in the eEF-Tu[t] sequence. If the assumption concerning the sites of tryptic cleavage in eEF-Tu is correct, then there has been a noteworthy (67%) increase in the size of the eucaryotic factor at the amino-terminal end compared with that in EF-Tu while only a slight (16%) relative increase in mass throughout the remainder of the molecule.

Another suggestive piece of structural evidence which supports our view that tryptic cleavage of eEF-Tu, in analogy with the corresponding hydrolysis of EF-Tu, is confined to the amino-terminal portion of the molecule is the observation that in both enzymes the low molecular polypeptides removed by trypsin are significantly more basic than the intact molecule [Figure 7 and Jones et al. (1980)].

Despite the above-mentioned similarities between procaryotic and eucaryotic factors, there remain notable structural and functional differences. Unlike the *E. coli* factor, neither rabbit reticulocyte nor *Artemia* eEF-Tu is affected by the antibiotic kirromycin (unpublished observations). EF-Tu is an acidic protein (p*I* of 5.5; Arai et al., 1980), whereas eEF-Tu is basic. The eucaryotic factor binds to most types of nucleic acids (Kolb et al., 1978), whereas EF-Tu from *E. coli* is apparently restricted in its binding to aminoacyl-tRNA (Shulman et al., 1974; Domogatsky et al., 1978). Despite reports to the contrary (Krisko et al., 1969; Grasmuk et al., 1977), one of us has found that EF-Tu is unable to function in eucaryotic protein synthesis (Slobin, 1981). The significance of these changes in EF-Tu structure and function during the evolution from procaryotes to eucaryotes is unclear at present.

Finally, we believe the strong evidence for homology between EF-Tu and eEF-Tu reported in this paper is a fortiori reason for the abandonment of names for this factor (i.e., EF-1_L and EF-1_α; see footnote 1) which do not reflect a common evolutionary origin.

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