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Interactions between Human Translocation Factor, Guanosine Triphosphate, and Ribosomes*

Engin Bermek and Heinrich Matthaei

ABSTRACT: Incubation of the human translocation factor TF II with [^{14}C]GTP and ribosomes results in the formation of a high molecular weight [^{14}C]GTP complex which is retained on nitrocellulose filters and can also be recovered in the exclusion peak after gel filtration through Sephadex G-25. The guanosine nucleotide present in this complex appears to be GDP. Despite extensive purification, the TF II protein fraction alone reveals a slight GTP binding activity. In addition to promoting the binding of [^{14}C]GTP to the ribosome, TF II itself is bound to the ribosome in the presence of a guanosine nucleotide. GDP can substitute for GTP in the reaction leading to the binding of TF II to ribosomes. The TF II–GDP–ribosome complex is stabilized by the presence of fusidic acid and its formation is inhibited by showdomycin. GTP increases the heat stability of TF II, but does not protect the factor against inactivation by showdomycin. The resistance of TF II to showdomycin is, however, increased when the factor is in the ribosome-bound state.

Studies in recent years have established many aspects of the mechanisms in polypeptide-chain elongation on the bacterial ribosome. Not all of these aspects have been explored to the same extent in mammalian polypeptide-chain elongation. The findings thus far suggest a basic analogy between the elongation mechanisms in bacterial and mammalian systems.

Regarding the translocation step, bacterial G factor as well as the corresponding mammalian factor TF II reveal some similar functional properties: both factors possess a ribosome-dependent GTPase activity (Nishizuka and Lipmann, 1966a; Felicetti and Lipmann, 1968) and transform bound AA-tRNA in the presence of GTP into a puromycin-reactive state (Haenni and Lucas-Lenard, 1968; Skogerson and Moldave, 1968a). Both systems are inhibited by fusidic acid, a specific translocation inhibitor (Tanaka *et al.*, 1968; Malkin and Lipmann, 1969). Furthermore, the bacterial G factor as well as the mammalian translocation factor possess SH groups, which are essential for their functional integrity (Nishizuka and Lipmann, 1966b; Kaziro *et al.*, 1969; Sutter and Moldave, 1966; Mosteller *et al.*, 1966).

Nonetheless, some differences seem to exist between the two systems. No interchangeability has thus far been observed between the bacterial and the mammalian translocation factors. Recently, however, it was demonstrated that the bacterial binding factor T can replace the mammalian factor in a cell-free system derived from rabbit reticulocytes (Krisko *et al.*, 1969). Furthermore, the mammalian translocation factor has been shown to be inactivated by diphtheria toxin in the presence of NAD (Collier, 1967), whereas bacterial pro-

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tein synthesis is not affected by diphtheria toxin at all. Such differences suggest that the known mechanism of bacterial translocation may not be unrestrictively applied to the corresponding process in mammalian systems.

A more detailed characterization of the mammalian translocation process and of the mammalian translocation factor appears, therefore, to be a prerequisite to gaining further insight into the mechanisms involved in mammalian elongation. Work has been carried out in our laboratory on the purification and characterization of the human translocation factor. This communication presents an account of the interactions occurring between the human translocation factor TF II, ribosomes, and GTP.

Materials and Methods

Ribosomes and elongation factors used were prepared from human tonsils as previously described (Bermek *et al.*, 1970). The ribosomes were purified by washing with 1 M KCl and 10 mM $MgCl_2$ in homogenization buffer, in addition to our standard purification procedure involving sucrose and 0.5 M NH_4Cl washes as well as treatment with 0.5% DOC[1] (Bermek *et al.*, 1970). After these steps the ribosome fraction contained only monomers and was free of detectable elongation factors. TF I used corresponded to fraction IV (Bermek and Matthaei, 1970). For [^{14}C]GTP-binding assays, TF II fraction V proteins from the DE 32 step (Bermek and Matthaei, 1970) were further purified by chromatography on a phosphocellulose column as described (Galasinski and Moldave, 1969). After this phosphocellulose step, human TF II will be designated fraction VI; it is ~50% pure. Polyacrylamide gel electrophoresis of TF II fraction VI proteins reveals three to four minor bands in addition to the main band containing TF II activity. A detailed report of the purification of the human translocation factor is in preparation.

tRNA (*E. coli*) was purchased from Schwarz BioResearch and 1.6% charged with L-[^{3}H]phenylalanine (The Radiochemical Centre, Amersham; specific activity 1000 Ci/mole) as described previously (Matthaei *et al.*, 1966). [^{14}C]GTP (specific activity 33 Ci/mole) and [^{14}C]UTP (specific activity 197 Ci/mole) were obtained from the Radiochemical Centre, Amersham, and [^{14}C]ATP (specific activity 21 Ci/mole) and [^{14}C]-CTP (specific activity 21 Ci/mole) from Schwarz BioResearch. Poly(U), GTP, GDP, and GMP were obtained from Boehringer (Mannheim). The purities of the guanosine nucleotides were controlled by paper or thin-layer chromatography. Showdomycin was a product of P-L Biochemicals, Milwaukee, Wis. Fusidic acid was kindly supplied by Dr. Godtfredsen, Leo Pharmaceutical Products Trading, Copenhagen. 2-Mercaptoethanol was a product of Fluka AG, Buchs, Switzerland.

Poly(U)-dependent Phe_n synthesis was assayed as previously described (Bermek *et al.*, 1970). Amounts of elongation factors used are indicated in the legends.

[^{14}C]GTP binding was assayed after incubation for 5 min at 37°. Reaction mixtures (100 μl) contained, if not otherwise indicated, 8 mM $MgCl_2$, 60 mM KCl, 50 mM Tris-HCl (pH 7.4), 8.5–9.5 mM 2-mercaptoethanol, 15 μM [^{14}C]GTP, and 3–5 A_{260} units of ribosomes. Amounts of TF II fraction VI proteins used were as indicated in the legends. Reactions were started by the addition of [^{14}C]GTP. After incubation, the reaction mixtures were diluted with 3–4 ml of cold wash buffer containing 10 mM $MgCl_2$, 20 mM Tris-HCl (pH 7.4), and 20 mM KCl; they were passed through Selectron filters (Schleicher & Schüll, 0.45-μm pore size, 25-mm diameter). The filters were then dried under an infrared lamp and counted in 2 ml of toluene containing 0.4% 2,5-diphenyloxazole in a Packard liquid scintillation counter (TriCarb). Blanks were subtracted routinely from all values. Blank values (0.5–0.8 pmole) were determined by filtering 1.5 nmoles of [^{14}C]GTP in 100-μl reaction mixtures without TF II and ribosomes through Selectron filters.

Heat Inactivation of TF II. TF II fraction V proteins (20 μg) were heated for 5 min at the indicated temperatures in 20 μl of standard enzyme buffer (dialysis buffer), containing 50 mM Tris-HCl (pH 7.4), 0.1 mM K-EDTA, 7 mM 2-mercaptoethanol, and 250 mM sucrose. GTP, if present, was 2 mM. After heating, tubes were chilled in ice and the assay volume increased to 100 μl by addition of the remaining components required for standard Phe_n synthesis. Radioactivity incorporated by assays lacking TF II (approximately 1.6 pmoles/1-ml reaction mixture) were subtracted.

Complex Formation between TF II and Ribosomes. TF II fraction V or VI proteins and ribosomes were incubated in the amounts indicated for 5 min at 37°. Reaction mixtures of 1- or 3-ml final volume contained 8 mM $MgCl_2$, 60 mM KCl, 50 mM Tris-HCl (pH 7.4), 1 mM GTP, and 8–9 mM 2-mercaptoethanol. After incubation, 1-ml reaction mixtures were layered over 1 ml of homogenization buffer containing 5 mM $MgCl_2$, 25 mM KCl, 50 mM Tris-HCl (pH 7.4), 7 mM 2-mercaptoethanol, and 250 mM sucrose; samples were centrifuged in an MSE Type 10 × 10 rotor for 4 hr at 150,000*g*. Alternatively, 3-ml reaction volumes were layered after incubation over 8.5 ml of homogenization buffer and centrifuged for 3–4 hr at 150,000*g* in a Spinco Type 50 Ti rotor. Pelleted ribosomes, complexed with TF II, were resuspended in homogenization buffer. The final ribosome concentration was adjusted to 200 A_{260} units/ml.

Preincubation of TF II or TF II–Ribosome Complexes with Showdomycin. TF II fraction VI proteins (72 μg) or 6 A_{260} units of ribosomes (preincubated with 12 μg of TF II fraction VI proteins per A_{260} unit and isolated as described above) were incubated at 37° in 150-μl reaction mixtures (either with or without 9.1 mM showdomycin). Incubation mixtures with free TF II contained 40 mM Tris-HCl (pH 7.4), 5.6 mM 2-mercaptoethanol, and 200 mM sucrose, those with TF II-complexed ribosomes 4 mM $MgCl_2$, 20 mM KCl, 40 mM Tris-HCl (pH 7.4), 5.6 mM 2-mercaptoethanol, and 200 mM sucrose. At the times indicated 25-μl aliquots were withdrawn and added to reaction tubes in ice containing, in 75 μl, the remaining components required for the standard Phe_n synthesis assay. The reaction mixtures were then immediately incubated at 37° for 30 min. The final concentration of showdomycin in the polymerization assay was 2.3 mM in the presence of 9 mM 2-mercaptoethanol; showdomycin caused no inhibition of the reaction at this concentration. Radioactivity incorporated by reaction mixtures lacking TF II (approximately 1.1 pmoles/ml) were subtracted.

Resolution of a High Molecular Weight [^{14}C]GTP Complex by Gel Filtration on Sephadex G-25. The G-25 column (0.6 × 12 cm) was equilibrated and eluted (at 4°) with 8 mM $MgCl_2$, 60 mM KCl, 50 mM Tris-HCl (pH 7.4), 7 mM 2-mercaptoethanol, and 250 mM sucrose. For samples with fusidic acid, the elution buffer contained, in addition, 0.1 mM fusidic acid. Incubation conditions were as in the filter assays. The incuba-

[1] Abbreviations used are: DOC, sodium deoxycholate (sodium salt); Phe_n, polyphenylalanine; A_{260} unit, the quantity of material in 1 ml of a solution giving an optical density of 1.0 at 260 mμ in a light path of 1 cm; [^{3}H]Phe-tRNA, unfractionated tRNA acylated with [^{3}H]phenylalanine; ADP–ribose, adenosine diphosphoribose.

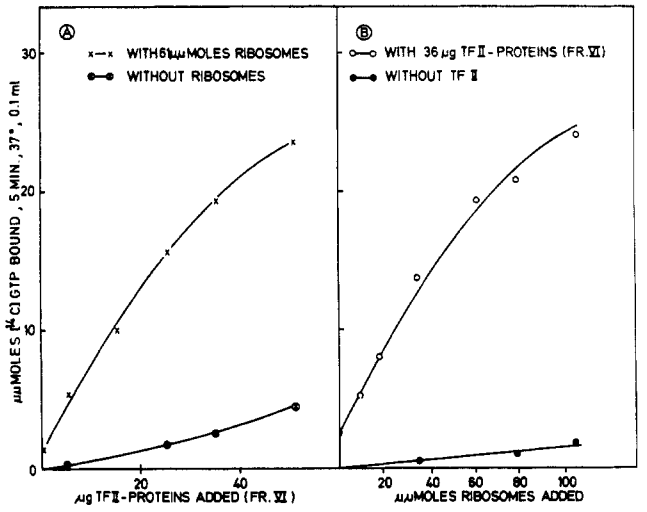

FIGURE 1: Effect of TF II and ribosome concentration on [^{14}C]GTP binding. Reaction conditions were as described under Methods. TF II (A) or ribosome (B) concentrations were varied as indicated. Counting efficiency for ^{14}C was 79.5%.

tion time, however, was reduced to 3 min. The concentration of fusidic acid in the reaction mixtures, if present, was 2 mM. After incubation, 75-μl aliquots removed from 100-μl reaction mixtures were applied to the column and eluted at a flow rate of 0.25 ml/min. After discarding the first 0.5 ml of eluate, ~185-μl fractions were collected. Aliquots (150 μl) from each fraction were counted in 5 ml of Bray's solution (Bray, 1960) in a Packard liquid scintillation counter. The counting efficiency for ^{14}C in 5 ml of Bray's solution was 74%. Background values (0.23 pmole), determined by counting 5 ml of Bray's solution, were subtracted from all values.

Results

TF II-Mediated [^{14}C]GTP Binding to Ribosomes. If, after incubation of [^{14}C]GTP with TF II and ribosomes, the reaction mixtures were passed through nitrocellulose filters, some radioactivity was retained on the filters. This retention of radioactivity was largely dependent upon the presence of both TF II and ribosomes and increased with increasing amounts of both these components (Figure 1). A slight [^{14}C]GTP binding activity was detectable also with increasing amounts of TF II proteins alone. In the presence of a sufficient amount of TF II, approximately 1 pmole of [^{14}C]GTP/3–4 pmoles of ribosomes[2] could be retained on the filters. TF II and ribosome dependent binding activity was apparently specific for GTP, as can be seen in Table I. The extent of the retention of CTP and UTP was negligible. Some ATP binding could, however, be observed in the presence of TF II proteins alone as well as in the presence of TF II and ribosomes. A chromatographic analysis of the product of [^{14}C]GTP binding on poly(ethyleneimine)-cellulose thin layers (Randerath and Randerath, 1964) showed that the ribosome-bound nucleotide was to at least 90% or greater in the form of GDP. The formation of a high molecular weight [^{14}C]GTP complex could be demonstrated also by gel filtration on a Sephadex G-25 column (Figure 2). In this case, the presence of both TF II and ribosomes was required for the recovery of the radioactivity in the exclusion peak fractions (A). TF II and ribosomes alone showed only

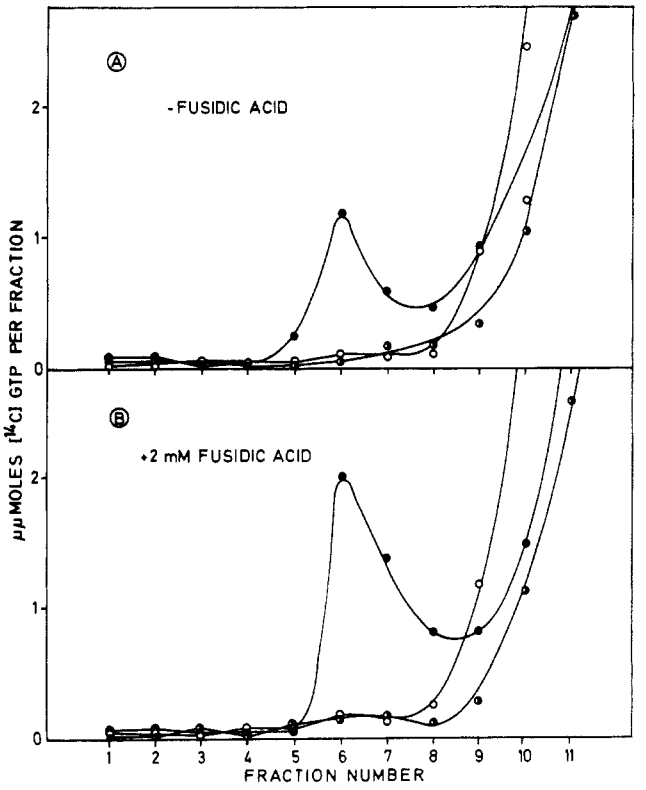

FIGURE 2: Demonstration of the formation of a high molecular weight [^{14}C]GTP complex by gel filtration on Sephadex G-25. Details of the experimental conditions are described in Methods. (A) (●) Complete system containing 5 A_{260} units (95.5 pmoles) of ribosomes and 25 μg of TF II fraction VI proteins; (○) TF II omitted; (◑) ribosomes omitted. (B) As in part A, but in the presence of fusidic acid. As a control 6 samples were incubated in parallel under the same experimental conditions as above. Without gel filtration, the bound radioactivity was directly determined by adsorption to Selectron filters. Samples corresponding to those from part A gave the following results: complete system = 24.3 pmoles; ribosomes omitted = 3.8 pmoles; TF II omitted = 1.1 pmoles. Samples corresponding to those from part B: complete system with 2 mM fusidic acid = 31.2 pmoles; ribosomes omitted = 3.7 pmoles; TF II omitted = 0.7 pmole. Counting efficiency for ^{14}C in 5 ml of Bray's solution was 74%, counting efficiency for ^{14}C on Selectron filters 58%.

TABLE I: Binding of Different Trinucleotides to TF II Proteins or Ribosomes.[a]

Nucleotides	[^{14}C]Nucleotides Bound (pmoles)		
	TF II[b]	Ribosomes[c]	TF II + Ribosomes
[^{14}C]GTP	0.9	0.6	12.7
[^{14}C]ATP	1.7	0.8	3.1
[^{14}C]UTP	0	0.1	0.2
[^{14}C]CTP	0	0.2	0.3

[a] Nucleotide binding reactions were carried out as described under Methods. [b] 16 μg of TF II fraction VI proteins and [c] 3 A_{260} units of ribosomes were used. Radioactive nucleotide concentration was 15 μM.

[2] The calculation of pmoles of ribosomes from A_{260} units was based upon the following approximation: 1 nmole of human ribosomes = 4 mg = 52.5 A_{260} units.

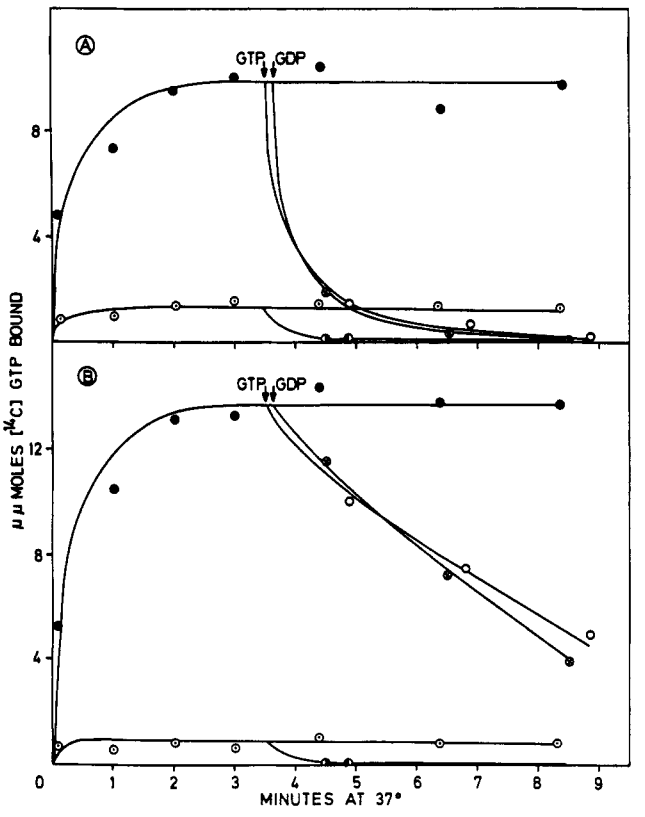

FIGURE 3: Kinetics of [^{14}C]GTP binding and of the exchange of the bound product with nonlabeled nucleotides. (A) 1.2-ml reaction mixtures contained 48 A_{260} units (917 pmoles) or no ribosomes, 160 μg of TF II fraction VI proteins, and 3.0 nmoles of [^{14}C]GTP. At the indicated times, 75-μl aliquots were taken and filtered. At 3 min 30 sec and 3 min 40 sec, 300-μl aliquots were withdrawn and added to other reaction mixtures containing, in 1 μl, 20 nmoles of unlabeled GTP or GDP. The remainder of the original reaction mixture served as a control. (●) Complete system (no unlabeled nucleotides added); (⊗) unlabeled GDP added; (○) unlabeled GTP added. Minus TF II blanks (0.8 pmole) were not subtracted from complete system samples. (⊙) Ribosomes were omitted (no unlabeled nucleotides added); (◑) unlabeled GDP added; (◐) unlabeled GTP added. Counting efficiency for ^{14}C was 79.5%. (B) Conditions were described in part A, but in the presence of 2 mM fusidic acid.

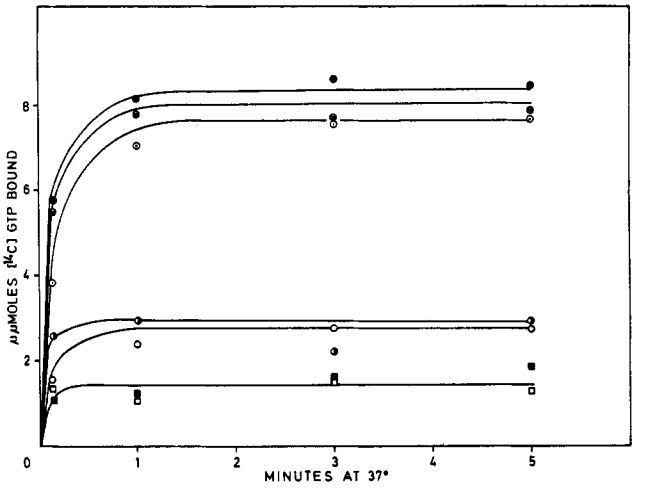

FIGURE 4: Effect of showdomycin on [^{14}C]GTP binding. (A) 350-μl reaction mixtures contained 14.4 A_{260} units (266 pmoles) of ribosomes, 46 μg of TF II fraction VI proteins, 8.6 mM 2-mercaptoethanol, and 1.5 nmoles of [^{14}C]GTP. Salt concentrations as described under Methods. At indicated times, 75-μl aliquots were taken and filtered. Minus TF II blanks (0.7 pmole) were not subtracted from complete system samples. (●) Control (complete system without showdomycin); (○) TF II fraction VI proteins were preincubated for 1 min at 37° with 18 mM showdomycin and 5.6 mM 2-mercaptoethanol. Remaining components required were then added to reach standard concentrations. Final concentration of showdomycin was 6.5 mM. (⊗) Ribosomes were preincubated with 18 mM showdomycin and 5.6 mM 2-mercaptoethanol for 1 min at 37°. Remaining components were added thereafter. (⊙) No preincubation, 6.5 mM showdomycin was added directly to the reaction mixture. (◑) No preincubation, 18 mM showdomycin was directly added to the reaction mixture; (■) TF II fraction VI proteins without ribosomes; (□) TF II fraction VI proteins without ribosomes, plus 18 mM showdomycin directly added to the reaction mixture containing 8.6 mM 2-mercaptoethanol. Counting efficiency for ^{14}C was 79.5%.

negligible quantities of radioactivity. Fusidic acid appeared to stabilize the bound radioactivity (B). Nitrocellulose filter assays carried out in parallel indicated, however, that only approximately 10–15% of the radioactivity otherwise retained on the filter could be recovered by the gel filtration method.

Exchange of the Bound Radioactive Nucleotide with External Unlabeled Guanosine Nucleotides. Kinetics of the binding of [^{14}C]GTP to ribosomes are shown in Figure 3. The binding to ribosomes was almost complete after 1 min. Upon addition of a great excess of cold guanosine nucleotides (GTP or GDP), a fast and complete exchange of the bound radioactive nucleotide with unlabeled GTP or GDP was seen. The extent of exchange was 80% complete 1 min after the addition of cold nucleotides. The presence of 2 mM fusidic acid in the reaction mixture resulted in a stimulation of [^{14}C]GTP binding to ribosomes (by approximately 40%) and a significant decrease in the rate of the subsequent exchange of the bound radioactive product with cold guanosine nucleotides. In this case, 1 min after the addition of unlabeled nucleotides only 15% of the bound radioactivity was exchanged.

Inhibition of [^{14}C]GTP Binding to Ribosomes by Showdomycin. The effect of showdomycin, a maleimide derivative antibiotic, upon GTP binding can be seen in Figure 4. Addition of a sufficient concentration of showdomycin to the reaction mixtures, or a 1-min preincubation of TF II with the same concentration of showdomycin, resulted in a significant inhibition of GTP binding to ribosomes. Preincubation of the ribosomes with the same concentration of showdomycin prior to the binding assay had no effect upon the binding of GTP to the ribosomes. Essentially the same results have been obtained with *N*-ethylmaleimide.

Binding of TF II to Ribosomes. In addition to catalyzing the binding of [^{14}C]GTP to ribosomes, TF II binds to ribosomes in the presence of a guanosine nucleotide (Figure 5). Ribosomes, preincubated with TF II in the presence of the same concentration of different guanosine nucleotides and isolated by centrifugation, were capable of Phe_n synthesis upon addition of only the binding enzyme. Other guanosine nucleotides could substitute for GTP to varying degrees in the promotion of TF II binding to ribosomes. GDP seemed to be at least as effective as GTP. Some TF II binding to ribosomes apparently occurred even in the absence of guanosine nucleotides. In this case, however, Phe_n synthesis displayed a prolonged (7–8 min) lag period.

Inactivation of TF II by Showdomycin or by Heat Treatment. The kinetics of inactivation by showdomycin of free or ribosome-bound TF II at 37° are illustrated in Figure 6. It can be seen that ribosomes provide a significant protection for TF II against inactivation by showdomycin: after preincubation with showdomycin for 1 min, approximately 90% of the ribosome-bound TF II activity was retained, compared

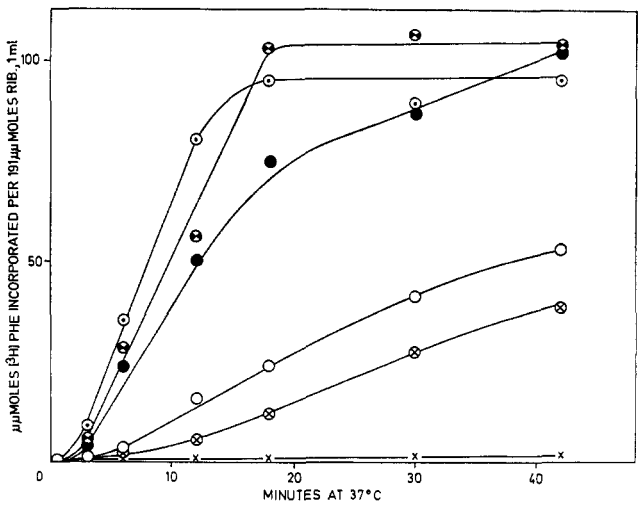

FIGURE 5: Effect of guanosine nucleotides upon the binding of TF II to ribosomes. Experimental conditions were as described under Methods. TF II fraction V proteins (265 μg) and 6.5 A_{260} units of ribosomes were incubated in 1 ml for 5 min at 37° in the presence or absence of 1 mM of the guanosine nucleotide indicated. The complexes formed were collected by centrifugation of the ribosomes. After resuspension in homogenization buffer, 3 A_{260} units (57 pmoles) ribosomes were assayed for TF II activity in Phe_n synthesis in a final volume of 300 μl. TF I fraction IV proteins (73 μg) and, if added directly to the assay, 122 μg of TF II fraction V proteins were present. (×) ribosomes preincubated without TF II; no TF II added afterwards; (⊗) ribosomes preincubated with TF II in absence of a guanosine nucleotide; (○) ribosomes preincubated with TF II and GMP; (●) ribosomes preincubated with TF II and GTP; (⊖) ribosomes preincubated with TF II and GDP; (⊙) ribosomes preincubated without TF II, TF II added directly to the reaction mixture. Counting efficiency for ^3H was 23.4%.

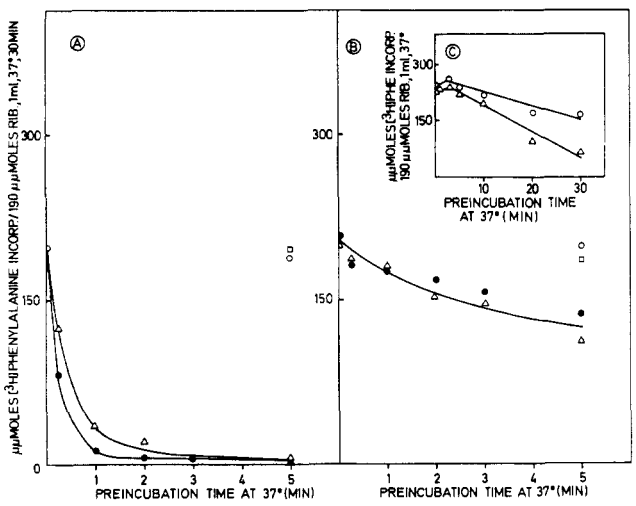

FIGURE 6: Kinetics of the inactivation of free *vs.* ribosome bound TF II and of ribosomes by showdomycin at 37° (as assayed in Phe_n synthesis). Preincubation conditions were as described under Methods. (A) (△) Free TF II fraction VI proteins were preincubated with showdomycin; (●) free TF II fraction VI proteins preincubated with showdomycin and 2 mM GTP; (○) TF II preincubated alone; (□) TF II preincubated with 2 mM GTP. (B) (△) TF II–ribosome complexes preincubated with showdomycin; (●) TF II–ribosome complexes preincubated with showdomycin and 2 mM GTP; (○) TF II–ribosome complexes preincubated alone; (□) TF II–ribosome complexes preincubated with 2 mM GTP. (C) 6 A_{260} units (115 pmoles) of ribosomes were preincubated for 30 min with or without 9.1 mM showdomycin and in the presence of 5.6 mM 2-mercaptoethanol. At indicated times, 25 μl-aliquots were withdrawn and assayed in Phe_n synthesis. (○) Ribosomes preincubated alone; (△) ribosomes preincubated with showdomycin. Counting efficiency for ^3H was 21%.

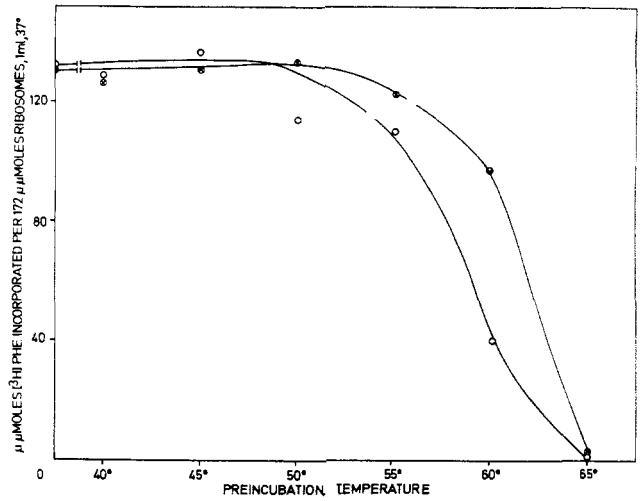

FIGURE 7: Effect of GTP on heat inactivation of TF II as assayed in Phe_n synthesis. The experimental conditions were as described in Methods. (○) TF II preincubated alone; (⊗) TF II preincubated with 2 mM GTP. Counting efficiency for ^3H was 23.4%.

with only 15% of the free TF II activity. As Figure 6C suggests, the inhibition of Phe_n synthesis observed after preincubation of TF II–ribosome complexes with showdomycin could be due solely to the inactivation of the factor. The ribosomes were apparently not affected by the antibiotic during the first 5-min preincubation. Longer incubation of the ribosomes at 37°, however, resulted in their gradual inactivation. This effect was amplified in the presence of the antibiotic. GTP appeared to have no protective effect on the inactivation of either the free or ribosome-bound TF II by showdomycin. As assayed in Phe_n synthesis, however, GTP does appear to protect TF II against heat inactivation (Figure 7). After 5 min at 60°, approximately 70% of the original factor activity remained in the presence of GTP, compared with only 30% without GTP.

Discussion

Results reported here suggest the formation of a ternary complex involving the human translocation factor, ribosomes, and GDP analogous to that observed in bacterial systems (Brot *et al.*, 1969; Parmeggiani and Gottschalk, 1969; Bodley *et al.*, 1970a). Formation of a TF II–GTP–ribosome intermediate and GTP hydrolysis probably precedes the formation of this TF II–ribosome–GDP complex. Whereas purified ribosomes alone do not significantly bind GTP, TF II fraction VI proteins alone display a slight but reproducible GTP binding activity. GTP binding activity of the partially purified fractions of the mammalian translocation factor have been observed also in rat liver (Raeburn *et al.*, 1968) and rabbit reticulocyte cell-free systems (Bodley *et al.*, 1970b). The high GTP binding activity of TF II fraction IV proteins is lost to a great extent in the subsequent steps of our purification procedure. This fact makes the specificity of the observed binding activity ambiguous. GTP shows no protective effect against showdomycin inactivation of TF II. On the other hand, GTP has been reported to protect partially rat liver translocation factor against diphtheria toxin promoted ADP ribosylation (Raeburn *et al.*, 1968). Furthermore, GTP increases the heat stability of human TF II rather significantly. The latter two observations might imply a direct interaction between

GTP and TF II before complex formation with the ribosome.

The low recovery of labeled guanosine nucleotide complexes after gel filtration indicates a definite instability of the GDP–ribosome complex from the human system. Fusidic acid only partially prevents the breakdown of the complex during gel filtration. As assayed by the nitrocellulose filter technique, fusidic acid does not appear to stimulate TF II- and ribosome-dependent [^{14}C]GTP binding to the same extent demonstrated in cell-free systems from *E. coli* (Bodley *et al.*, 1970a). However, as the kinetics of exchange of bound and unbound nucleotides indicate, fusidic acid significantly prevents release of bound guanosine nucleotide from human ribosomes. This is in agreement with the analogous mechanism of fusidic acid action in mammalian and bacterial translocation. The action of fusidic acid is apparently due to an interaction with the translocation factor and not with the ribosomes (Kinoshita *et al.*, 1968; Okura *et al.*, 1970). GDP seems to be as effective as GTP in the promotion of the binding of TF II to ribosomes. This observation suggests that GTP hydrolysis is not required for the binding of TF II to ribosomes. Stimulation of the binding of aminoacyl transferase II to rat liver ribosomes has, however, been reported to be greatest in the presence of GTP (Skogerson and Moldave, 1968b). Remarkably, some binding of TF II to ribosomes seems to occur even in the absence of guanosine nucleotides. The prolonged lag phase before the onset of Phe_n synthesis observed in this case might be due to a requirement for a conformational change of TF II when bound to ribosomes in the absence of a guanosine nucleotide. Binding of the translocation factor to ribosomes has been observed in the absence of a guanosine nucleotide: as assayed by diphtheria toxin dependent ADP ribosylation of the rabbit reticulocyte factor, as many as six translocation factor molecules could be bound to ribosomes of different origin in the absence of a guanosine nucleotide (Gill *et al.*, 1969). The extent of TF II binding to ribosomes occurring in the absence of a guanosine nucleotide is apparently reduced by a subsequent addition of guanosine nucleotides, EDTA, and moderate salt concentrations. These observations suggest that the mammalian translocation factor, when bound to ribosomes, might exist in two different conformational states or sites. Only TF II bound in the presence of a guanosine nucleotide appears to be immediately active in polypeptide-chain elongation.

The kinetics of inactivation of TF II by showdomycin show that TF II bound to ribosomes in the presence of GTP is more resistant than free TF II against this antibiotic. The slow rate of inactivation of the bound TF II might merely reflect the rate of release of the factor from the ribosomes. In addition, it might also be due to reduced accessibility of SH groups of TF II in the ribosome-bound state. Other interpretations are plausible. In experiments not shown, both ribosomes and ribosome-bound TF II appeared to be unaffected by the antibiotic at 0°; however, inactivation of free TF II by showdomycin occurred equally fast at 0 and 37°. Ribosomes are inactivated by showdomycin only at relatively high temperatures (37°) and in the course of prolonged incubation (>10 min). This suggests that prolonged incubation may result in conformational changes of the ribosome with possible exposure of their functional SH groups which are known to be involved in the binding of AA-tRNA (Heintz *et al.*, 1966; Bermek *et al.*, 1970). The showdomycin-promoted inactivation is apparently due to the alkylation of SH groups (Bermek *et al.*, 1970). The requirement of the mammalian translocation factor for SH compounds and its inactivation by SH group reactive agents have been previously reported (Sutter and Moldave, 1966; Mosteller *et al.*, 1966). The results reported here suggest the direct or indirect involvement of the SH groups of TF II in the formation of a ternary complex.

The observed stability of the TF II–ribosome complex might possibly be due to binding of TF II to ribosomes without a subsequent translocation. TF II is presumed to possess a high affinity for monomeric ribosomes. As recently reported, amino acid deprivation or puromycin treatment results in an increased binding of TF II to monomeric ribosomes *in vivo* (Smulson and Rideau, 1970). These authors found only small quantities of TF II associated with polyribosomal fractions actively involved in translocation. Results obtained from rat liver system indicate that the translocation factor is released from the ribosomes after completion of each translocation step (Siler and Moldave, 1969). These findings suggest a high affinity of TF II for ribosomes with peptidyl-tRNA bound in the acceptor site. Translocation of peptidyl-tRNA from the acceptor to the donor site might be a prerequisite for the release of TF II from the ribosome.

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Quantitative Procedures for Use with the Edman–Begg Sequenator. Partial Sequences of Two Unusual Immunoglobulin Light Chains, Rzf and Sac*

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ABSTRACT: Two methods of hydrolysis, with HI and with NaOH + $Na_2S_2O_4$, are described which permit amino acids to be regenerated from the thiazolinones produced by the Edman–Begg sequenator. Neither requires any additional extractions or prior conversion of the thiazolinones to other derivatives. Use of both methods enables all residues normally encountered in the automatic degradation of proteins to be unambiguously identified and quantitated with an amino acid analyzer, except that cysteine is not distinguishable from serine without some additional manipulation. Equations are developed to correct the resulting data for the systematic errors of automatic sequencing and to reduce them to an easily inspectable form in which all the quantitative information relevant to a sequence determination can be displayed in a single graph. An outline of computer programs to process the data is given. The procedures are illustrated by partial sequence determinations for 67 and 50 positions, respectively, of two smaller than usual immunoglobulin light chains. The two chains are Rzf [Deutsch, H. F. (1965), *Immunochemistry 2*, 207] and Sac [Lewis, A. F., Bergsagel, D. E., Bruce-Robertson, A., Schachter, R. K., and Connell, G. E. (1968), *Blood 32*, 189]. Rzf shows no evidence of a deletion in the region sequenced, but the light chain Sac has a major deletion of about 68 residues in its variable region.

The sequenator of Edman and Begg (1967) has already proved of considerable value in sequencing purified proteins (see, for example, Niall and Edman, 1967; Niall *et al.*, 1970). When mixtures of proteins are unavoidable, as with the light chains of antibodies from normal individuals, the usefulness of the sequenator can be considerably enhanced by making the procedure quantitative. In this paper we describe methods permitting the recognition and quantitation by regular amino acid analysis of all amino acids normally encountered in the sequential degradation of proteins. Equations are developed to correct the resulting data for systematic errors of automatic sequencing and to reduce them to an easily inspectable form. In an Appendix programs are described for the reduction of these data by a computer, but it can be done manually. The application of the quantitative procedures is illustrated by the partial sequencing of two unusual immunoglobulin light chains, Rzf (Deutsch, 1965) and Sac (Lewis *et al.*, 1968; Smithies *et al.*, 1971; Parr *et al.*, 1971). Quantitative data obtained by application of the same procedures to a study of mixtures of immunoglobulin light chains from normal humans and rabbits will be described elsewhere.

Materials and Methods

Proteins. Much of the work in developing the methods was carried out with a typical κ_{II} Bence-Jones protein, Dil. In preparing this protein, the patient's urine was deionized with 0.05–0.1 its volume of 20–50 mesh mixed bed resin, filtered, and then treated with approximately 6 g of water-washed DEAE-cellulose in its acetate form per liter of urine. These procedures were repeated, if necessary, until essentially all protein was removed from the urine. The batches of DEAE-cellulose on which the protein was absorbed were combined and packed in a column on top of approximately twice the

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