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# Dissociation of Guanosine Nucleotide·Elongation Factor G·Ribosome Complexes†

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ABSTRACT: The spontaneous dissociation of complexes containing elongation factor G (EF-G), the ribosome, and either GDP plus fusidic acid, guanyl-5′-yl imidodiphosphate, or guanyl-5′-yl methylene diphosphonate has been measured and it follows biphasic kinetics that can be resolved into two first-order decay rates. This suggests the existence of two classes of complexes with apparent dissociation rate constants ($k$) differing 5–20-fold. The values of $k$ and the distribution of complexes between the fast and the slowly decaying class depend on the conditions in which the dissociation occurs but not on the conditions in which the complexes are formed. Rapid transitions of complexes from one to the other class occur only when the chemical environment in which the dissociation takes place is modified. Thus, increasing the concentration of $NH_4Cl$ or adding the antibiotic thiostrepton accelerates the decay and converts slowly dissociating into fast dissociating complexes. In contrast, addition of misreading-inducing aminoglycoside antibiotics of the neomycin, kanamycin, streptomycin, and gentamicin (but not hygromycin) groups slows down the decay. For neomycin B at 10 $\mu$M, this effect is due to the conversion of fast into slowly decaying complexes. A model to explain the results involving conformational transitions of the complexes is proposed.

To study the function of elongation factor (EF) G,[1] an *Escherichia coli* protein involved in ribosomal translocation, the ability of the factor to interact with guanosine nucleotides and the ribosome in the absence of protein synthesis has been very valuable (for reviews, see Lucas-Lenard & Lipmann, 1971; Modolell & Vázquez, 1975). With GDP and with the nonhydrolyzable GTP analogues, guanyl-5′-yl methylene diphosphonate (Gpp($CH_2$)p) and guanyl-5′-yl imidodiphosphate (Gpp(NH)p), the interaction leads to formation of ternary complexes containing 1 mol of each of guanosine nucleotide, EF-G, and ribosomes (Bodley et al., 1970; Lin & Bodley, 1976; Eckstein et al., 1971; San-Millán et al., 1977). Recent observations are consistent with an ordered mechanism for the formation and dissociation of ternary complexes: EF-G binds guanosine nucleotide first, and then the ribosome; the ternary complex spontaneously dissociates into a guanosine nucleotide·EF-G complex, which then dissociates into its components (Rohrbach & Bodley, 1976; Girbés et al., 1977a). Moreover, the ternary complex containing GDP is stabilized by fusidic acid and it is usually studied in the form of a quaternary complex containing one molecule of this antibiotic (Bodley et al., 1970; Willie et al., 1975).

To gain further insight into the mechanism of breakdown and the properties of the ternary and quaternary complexes, we have now studied their spontaneous dissociation under conditions in which reactions leading to complex re-formation and interactions between complexes and free constituents have been halted by high dilution. The experimental decay curves obtained are consistent with the existence of at least two classes of interconvertible complexes with different dissociation rates. Moreover, the influence of ionic environment, temperature, and antibiotics on the rates of decay and on the interconversion between complexes has been examined.

## Experimental Procedure

*Materials.* Preparation of 1 M $NH_4Cl$ washed *E. coli* MRE600 ribosomes and EF-G has been described elsewhere (Modolell & Vázquez, 1973; Parmeggiani et al., 1971). One $A_{260}$ unit of ribosomes was assumed to be equivalent to 27.7 pmol (Koppel, 1974). Labeled guanosine nucleotides were from The Radiochemical Centre, Amersham. Their specific activities, determined by isotopic dilution (Modolell et al., 1973), were: [$^3$H]GDP, 7230 and 6820 cpm/pmol; [$^3$H]-Gpp(NH)p, 7860 and 4630 cpm/pmol; [$^3$H]Gpp($CH_2$)p, 1040 cpm/pmol; they were adjusted to convenient, lower values by addition of known amounts of unlabeled nucleotides. Counting efficiency was 30%. Antibiotics were gifts from the following sources: thiostrepton, Squibb Institute for Medical Research (Dr. B. Stearns); sisomicin, verdamicin, gentamicin $C_1$, and gentamicin $C_{1a}$, Schering Corp. (Dr. Waitz); kanamycins A and B, Bristol Laboratories (Dr. F. Leitues); tobramycin and hygromycin B, Lilly Laboratories; neamine (= neomycin A), neomycins B and C, and bluensomycin, Upjohn Co.; ribostamycin, Maiji Seika Kaisha, Ltd., Japan (Dr. I. Matsuda); paromomycin, Parke-Davis.

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[1] Abbreviations used: EF-G, elongation factor G; Gpp($CH_2$)p, guanyl-5′-yl methylene diphosphonate; Gpp(NH)p, guanyl-5′-yl imidodiphosphate.

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*Ribosome Activation.* Prior to each experiment ribosomes were activated by incubating them at 30 °C for 30 min in mixtures (60–100 μL) containing: 50 mM $NH_4Cl$, 50 mM KCl, 20 mM magnesium acetate, 10 mM Tris-HCl, pH 7.8, 1 mM dithiothreitol, 0.1 mg/mL poly(U), 1.9 μM ribosomes, and 3–5% glycerol (carried over with the ribosomal preparation stored in 44% glycerol). The mixture was cooled to 0 °C and used immediately.

*Formation of Guanosine Nucleotide·EF-G·Ribosome Complexes.* Binding of guanosine nucleotides and EF-G to ribosomes was performed in mixtures (100–500 μL) containing, unless otherwise specified: 8 mM $NH_4Cl$, 8 mM KCl, 10 mM magnesium acetate, 10 mM Tris-HCl, pH 7.8, 1 mM dithiothreitol, 0.3 μM activated ribosomes, 30–90 μg/mL EF-G and either 1–2 μM [$^3$H]Gpp(NH)p (1040–2300 cmp/pmol), 1 μM [$^3$H]Gpp($CH_2$)p (410–500 cpm/pmol) or 0.5 μM [$^3$H]GDP (650–1840 cpm/pmol) plus 2.4 mM fusidic acid. After incubation at 30 °C for 30 min, portions of 15–20 μL were taken out, mixed with 2 mL of dilution buffer (10 mM $NH_4Cl$, 10 mM magnesium acetate, 10 mM Tris-HCl, pH 7.8, 0.1 mg/mL bovine serum albumin, and 2 mM fusidic acid when this antibiotic was present in the reaction mixture) and filtered through nitrocellulose membranes (Millipore, type HAWP). The filters were immediately washed twice with 2 mL of dilution buffer, dried, immersed in toluene containing 5 g/L of 2-(4-*tert*-butylphenyl)-5-(4-biphenylyl)-1,3,4-oxadiazole (butyl-PBD, Ciba), and counted. To account for nonspecific binding of [$^3$H]guanosine nucleotide to the filters, control mixtures without EF-G were run in parallel and their values (0.5–5% of the radioactivity retained in the presence of EF-G) were subtracted. The remaining reaction mixture was used immediately to study the dissociation of the guanosine nucleotide·EF-G·ribosome complexes.

*Dissociation of Guanosine Nucleotide·EF-G·Ribosome Complexes.* Portions (15–20 μL) or the whole reaction mixture (up to 450 μL) containing [$^3$H]guanosine nucleotide EF-G·ribosome complex were mixed with either 100 volumes of dilution buffer (complexes formed with GTP analogues) or 400 volumes of the same buffer containing 2 mM fusidic acid (complexes formed with GDP). When indicated, the dilution buffer contained compounds whose effect on complex decay was to be determined. After incubation at 0 or 30 °C for the indicated times, the mixtures (1.5–6 mL) or 2–6-mL portions of larger mixtures were filtered through nitrocellulose membranes. When samples were smaller than 6 mL, additional dilution buffer was added to the filters to keep the total volume of filtered fluid constant at 6 mL. Without further washing, the filters were immediately removed from the filtration apparatus and were dried and counted as described above. Controls without EF-G showed that the nonspecific trapping of radioactivity by filters was not significantly different following this procedure or the more conventional one above described. Their values were subtracted from those of the complex containing mixtures. Control experiments using ribosomes labeled in vivo with [$^3$H]leucine showed that, under the condition of 400-fold dilution (but not at 100-fold dilution), ribosomes were lost from the incubation mixture (probably by absorption to the glass walls of containers) at a linear rate of 6.5% per h. Results were not corrected for this loss since it was estimated that its effect on $k_1$, $X_1$, and $X_2$ (see Results) was negligible and it would increase $k_2$ by only 6–16%.

## Results

*Kinetics of Complex Dissociation: Effects of Temperature and Ionic Environment.* Guanosine nucleotide·EF-G·ribosome complexes spontaneously undergo the association–dissociation reaction (Rohrbach & Bodley, 1976; Girbés et al., 1977a):

$$\text{guanosine nucleotide·EF-G·ribosome} \rightleftharpoons \text{guanosine nucleotide·EF-G} + \text{ribosome} \quad (1)$$

In the context of this work, guanosine nucleotide stands for either GDP or the GTP analogues Gpp(NH)p or Gpp($CH_2$)p. With GDP, fusidic acid has always been present in the mixtures since it stabilizes the ternary complex and displaces equilibrium 1 toward association (Bodley et al., 1970; Baca et al., 1976). To measure the dissociation of these complexes in the absence of the association reaction of equilibrium 1, we have diluted standard, concentrated mixtures containing the complexes with either 400 volumes (complexes formed with GDP) or 100 volumes (complexes formed with GTP analogues) of dilution buffer (see Experimental Procedure) and determined, at time intervals, the amounts of labeled guanosine nucleotide remaining in complex with the aid of the nitrocellulose filter technique. (Binary guanosine nucleotide·EF-G complexes are unstable and are not retained by filters (Arai et al., 1975; Marsh et al., 1975; Rohrbach & Bodley, 1976).) The dilutions used, but not substantially lower ones, effectively stopped the association reaction since no ternary or quaternary complexes were detected in mixtures containing the components for complex formation that were diluted prior to the addition of labeled guanosine nucleotide and incubated at 30 °C for 30 min (not shown). Moreover, a study of the dependence of complex decay on the degree of dilution showed that the rates of dissociation increased only up to a 50–100-fold dilution, thereafter remaining constant up to the highest dilution examined (800-fold; not shown).

The time courses of the spontaneous decay, at 0 and 30 °C and at several $NH_4Cl$ concentrations, of complexes containing either [$^3$H]Gpp(NH)p or [$^3$H]GDP are shown in Figure 1. With [$^3$H]Gpp(NH)p and at either 10, 40, or 170 mM $NH_4Cl$ (panels A, C, and E, respectively), the decay was always faster at 30 °C than at 0 °C. The opposite was observed with [$^3$H]GDP at 10 mM $NH_4Cl$ (panel B), but at higher $NH_4Cl$ concentrations (40 and 100 mM, panels D and F, respectively) there was little difference between the decay at each temperature. Increasing concentrations of $NH_4Cl$ in the range from 10 to 200 mM progressively accelerated complex breakdown, the [$^3$H]GDP-containing complex being more sensitive than that containing [$^3$H]Gpp(NH)p (Figures 1 and 2A). Another difference between these two complexes was that, at the lowest concentration of $NH_4Cl$ tested (0.13 mM), the [$^3$H]GDP-containing complex was maximally stable, while that containing [$^3$H]Gpp(NH)p decayed more rapidly than at 10 mM $NH_4Cl$ (Figure 2A). Addition of 2 mM sodium fusidate to this last complex did not modify its increased lability at 0.13 mM $NH_4Cl$ (not shown). Figure 2B shows that both complexes were most stable at concentrations equal to or higher than 15 mM magnesium acetate, but at low concentrations the [$^3$H]Gpp(NH)p-containing complex was the most stable.

*Complex Heterogeneity.* The decay curves of Figure 1 do not fit simple, first-order kinetics, since the plots of ln ($C_t/C_0$) vs. time ($C_0$ representing the concentration of complex at the start of the decay and $C_t$ the concentration remaining after $t$ min of decay) did not yield straight lines (Figure 1, insets). Inspection of these plots suggested that the curves might result from the sum of two linear decay processes. Consequently, it was assumed that two classes of complexes were present, those in one class decaying much faster than those in the other. To resolve the decay curves it was further assumed that, after 45–75 min of breakdown (depending on the overall velocity

Table I: Effect of Temperature, $NH_4Cl$ Concentration, and Antibiotics on the Apparent Dissociation Rate Constants of Fast-Decaying ($k_1$) and Slowly Decaying ($k_2$) EF-G·Ribosome Complexes Containing Either [$^3$H]GDP plus Fusidic Acid or [$^3$H]Gpp(NH)p and on the Initial Molar Ratio ($X_1$) of Fast-Decaying Complexes[a]

| guanosine nucleotide | temp (°C) | $NH_4Cl$ (mM) | antibiotic[b] | $k_1 \times 10^3$ ($s^{-1}$) | $k_2 \times 10^4$ ($s^{-1}$) | $X_1$ |
|---|---|---|---|---|---|---|
| [$^3$H]GDP | 30 | 10 | | 0.8 ± 0.2 | 1.1 ± 0.2 | 0.27 ± 0.05 |
| | 30 | 40 | | 1.2 ± 0.1 | 2.3 ± 0.1 | 0.75 ± 0.03 |
| | 30 | 100 | | 2.6 ± 0.1 | 1.4 ± 0.7 | 0.93 ± 0.04 |
| | 0 | 10 | | 1.2 ± 0.3 | 1.0 ± 0.4 | 0.51 ± 0.06 |
| | 0 | 40 | | 1.4 ± 0.3 | 1.7 ± 0.1 | 0.69 ± 0.07 |
| | 0 | 100 | | 2.4 ± 0.2 | 1.4 ± 0.8 | 0.93 ± 0.04 |
| [$^3$H]Gpp(NH)p | 30 | 10 | | 1.5 ± 0.1 | 1.2 ± 0.2 | 0.81 ± 0.01 |
| | 30 | 40 | | 1.3 ± 0.2 | 2.3 ± 0.5 | 0.80 ± 0.03 |
| | 30 | 170 | | 3.1 ± 0.6 | | 0.98 |
| | 0 | 10 | | 1.3 ± 0.4 | 0.7 ± 0.2 | 0.45 ± 0.12 |
| | 0 | 40 | | 1.4 ± 0.2 | 0.8 ± 0.2 | 0.49 ± 0.06 |
| | 0 | 170 | | 3.0 ± 0.2 | 3.0 ± 0.3 | 0.69 ± 0.04 |
| [$^3$H]Gpp($CH_2$)p | 30 | 40 | | 1.1 ± 0.1 | 1.7 ± 0.3 | 0.75 ± 0.01 |
| | 0 | 40 | | 1.0 ± 0.2 | 0.6 ± 0.2 | 0.49 ± 0.08 |
| [$^3$H]GDP | 30 | 10 | Thios, 3 μM | 0.9 ± 0.1 | 1.1 ± 0.6 | 0.93 ± 0.01 |
| | 30 | 10 | Thios, 10 μM | 1.5 ± 0.2 | | 0.95 ± 0.01 |
| [$^3$H]Gpp(NH)p | 30 | 40 | Thios, 3 μM | 3.4 ± 0.9 | | 0.95 |
| | 30 | 40 | Thios, 10 μM | 4.4 ± 0.4 | | 0.99 |
| | 30 | 40 | Neo B, 10 μM | 1.0 ± 0.1 | 2.0 ± 0.2 | 0.36 ± 0.02 |

[a] Results, expressed as mean values ± standard error of the mean, are averages of two to six experiments performed with two different ribosomal and EF-G preparations. Method of calculation of $k_1$, $k_2$, and $X_1$ is described in the text. [b] Thios, thiostrepton; Neo B, neomycin B.

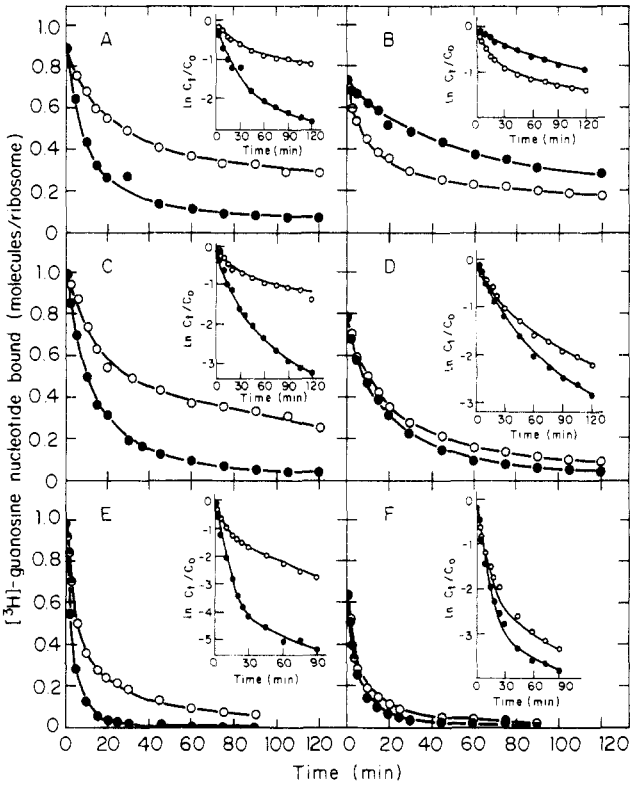


FIGURE 1: Time courses of the decay at 30 (●) or 0 °C (○) of EF-G·ribosome complexes containing either [$^3$H]Gpp(NH)p (A, C, E) or [$^3$H]GDP plus fusidic acid (B, D, F). Experiments were conducted as described in the text. $NH_4Cl$ concentration was: 10 mM (A, B), 40 mM (C, D), 100 mM (F), and 170 mM (E). Insets: The data of the corresponding panel were replotted, $C_0$ being the initial concentration of complex and $C_t$ that remaining after $t$ min of incubation. Lines in insets are theoretical curves derived as indicated in the text.

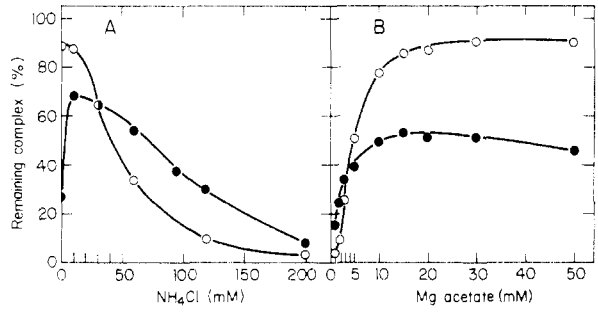


FIGURE 2: Effect of $NH_4Cl$ (A) and magnesium acetate (B) concentration on the decay of EF-G·ribosome complexes containing either [$^3$H]Gpp(NH)p (●) or [$^3$H]GDP plus fusidic acid (○). Decay was allowed to occur for 15 (A) or 30 min (B) at 30 °C. One hundred percent represents between 0.42 and 0.81 molecules of [$^3$H]guanosine nucleotide bound per ribosome.

of the process which was sensitive to the chemical environment, Figure 1), only slowly decaying complexes remained in the reaction mixtures. Then, a straight line was fitted to the data points beyond this time using the least-square method and the dissociation rate constant $k_2$ and initial molar ratio $X_2$ of the slowly decaying complexes were obtained. By subtracting from the total amount of complexes the amount of slowly decaying complexes present at each time (estimated from $k_2$ and $X_2$), the amounts of fast-dissociating complexes at early decaying times were calculated. The values showed a good fit to a straight line and the dissociation rate constant $k_1$ was obtained. Figure 1 (insets) shows that the theoretical decay curves obtained from the sum of the two linear rates fit nicely the experimental dissociation data.

Table I summarizes the values for $k_1$, $k_2$, and $X_1$ ($X_2$ being equal to $1 - X_1$) under a variety of conditions. With the [$^3$H]GDP-containing complex dissociating at 30 °C, changing the $NH_4Cl$ concentration from 10 to 40 mM dramatically increased $X_1$ from 0.27 ± 0.05 to 0.75 ± 0.03, while $k_1$ was much less affected. At 100 mM $NH_4Cl$, almost all complexes ($X_1$ = 0.93 ± 0.04) decayed rapidly and $k_1$ was approximately three times larger than at 10 mM $NH_4Cl$. At 0 °C, increasing $NH_4Cl$ concentration from 10 to 100 mM also increased $k_1$ by a similar factor, but the change in $X_1$ was less marked, since at 10 mM $NH_4Cl$ already half of the complexes decayed with fast kinetics. With the [$^3$H]Gpp(NH)p-containing complex decaying at 30 °C, similar variations of $k_1$ with alterations

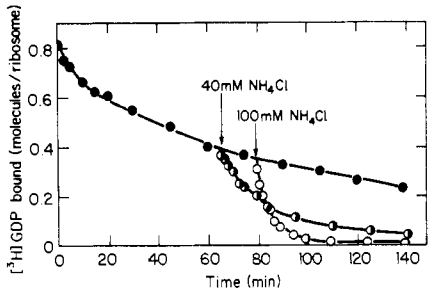


FIGURE 3: Effect of increasing $NH_4Cl$ concentration from 10 to either 40 or 100 mM in the dissociation of [$^3$H]GDP·EF-G·ribosome·fusidic acid complex. At the times indicated by arrows, portions of the mixture containing the complex decaying at 30 °C were taken out and mixed with sufficient $NH_4Cl$ to raise its concentration to the indicated values. Incubation at 30 °C was continued and analysis of complexed [$^3$H]GDP performed as described in the text.

in $NH_4Cl$ concentration were observed, but $X_1$ showed lower variability since at 10 mM $NH_4Cl$ it was already 0.81 ± 0.01. At 0 °C the pattern of changes was similar to that of the complex containing [$^3$H]GDP. Complexes containing [$^3$H]Gpp($CH_2$)p decayed with slightly slower rates than those containing [$^3$H]Gpp(NH)p.

*Interconversion between Complexes.* Complexes containing either [$^3$H]GDP or [$^3$H]Gpp(NH)p were formed at 0 and at 30 °C and in the presence of either 14 or 40 mM $NH_4Cl$. In all cases, the conditions of formation had no influence on $k_1$, $k_2$, $X_1$, and $X_2$ (not shown); these parameters were solely determined by the chemical environment in which the decay took place. These results suggested a rapid interconversion between the two types of complexes when ionic conditions or temperature were changed. This was verified in the experiment of Figure 3, in which raising the $NH_4Cl$ concentration of a mixture containing complexes undergoing decay from 10 to either 40 or 100 mM immediately increased the rate of dissociation. Moreover, the kinetic parameters of the new rates ($k_1 = 1.0 \times 10^{-3}$ s$^{-1}$, $k_2 = 1.4 \times 10^{-4}$ s$^{-1}$, $X_1 = 0.77$ at 40 mM $NH_4Cl$; $k_1 = 2.6 \times 10^{-3}$ s$^{-1}$, $X_1 = 0.96$ at 100 mM $NH_4Cl$) were equal, within experimental error, to those of a population of complexes decaying from zero time in 40 or 100 mM $NH_4Cl$, respectively (Table I).

*Effect of Antibiotics in Complex Decay.* Many aminoglycoside antibiotics capable of inducing misreading stabilized the [$^3$H]Gpp(NH)p·EF-G·ribosome complex (Figure 4). In the range from 3 to 30 μM, neomycins B and C were the most effective, while the related antibiotic paromomycin was much less potent and ribostamycin and neamine were almost without effect. Antibiotics of the kanamycin group (kanamycins A and B and tobramycin) and of the gentamicin group (gentamicins $C_1$ and $C_{1a}$, sisomicin and verdamicin) had smaller effects than the neomycins, but some were fairly effective at concentrations between 30 and 100 μM. Antibiotics of the streptomycin group (streptomycin, dihidrostreptomycin, and bluensomycin) were active at 0.3 μM (50% inhibition of decay), but higher concentrations did not further inhibit complex dissociation. In contrast, hygromycin B was inactive, even at concentrations as high as 100 μM. Moreover, Table I shows that the inhibition of dissociation by 10 μM neomycin B was due mainly to conversion of fast into slowly dissociating complexes and not to a substantial decrease in the value of the dissociation rate constants. It remains to be clarified whether this mechanism of stabilization is applicable to the remaining effective antibiotics.

The antibiotic thiostrepton binds to the ribosomal 50S subunit and prevents all subsequent interactions of EF-G with the ribosome (Modolell & Vázquez, 1975, review). This

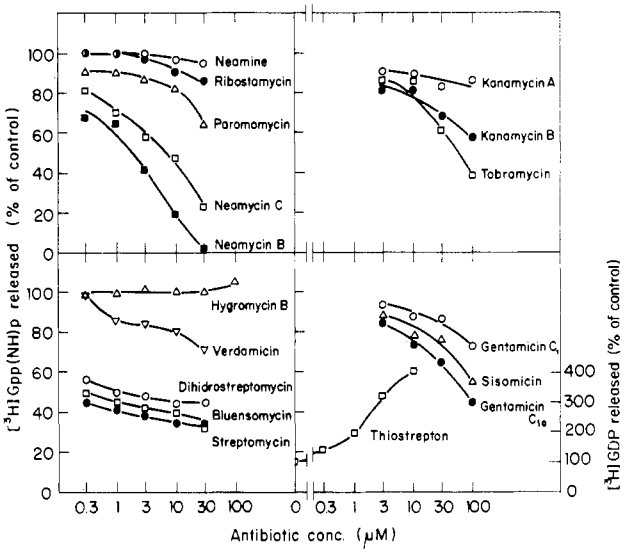


FIGURE 4: Effect of antibiotics on the decay of EF-G·ribosome complexes containing either [$^3$H]Gpp(NH)p (aminoglycoside antibiotics) or [$^3$H]GDP plus fusidic acid (thiostrepton). Decay took place at 30 °C for 15 min (with [$^3$H]Gpp(NH)p) or 20 min (with [$^3$H]GDP). One hundred percent represents the decay in the absence of antibiotic, 0.35 and 0.42 ([$^3$H]Gpp(NH)p) and 0.11 ([$^3$H]GDP) molecules of guanosine nucleotide released per ribosome.

antibiotic interacts with guanosine nucleotide·EF-G·ribosome complexes since, at concentrations between 0.3 and 10 μM, it stimulated their dissociation (Figure 4). Moreover, Table I shows that this stimulation was due to an almost quantitative conversion of slowly into fast decaying complexes and also to an increase in the dissociation rate constant.

## Discussion

The spontaneous dissociation of labeled guanosine nucleotide·EF-G·ribosome complexes follows biphasic kinetics and the experimental data obtained can be closely fitted by theoretical curves derived from the sum of two first-order decay processes (Figure 1). The simplest explanation for these results is to assume that the population of complexes is heterogeneous and contains two types of complexes with different rates of decay. The results also indicate that during the dissociation reaction the two classes of complexes do not rapidly interconvert, for such an interconversion would promote a mixed first-order decay rate rather than a biphasic decay resolvable into two distinct linear components. On the other hand, the two classes of complexes do interconvert when the chemical environment in which dissociation occurs is modified (Table I and Figure 3). Thus, increasing temperature and $NH_4Cl$ concentrations generally favor a conversion of the slower into the faster decaying complexes (see, however, Table I), while the antibiotic neomycin B favors the reverse conversion. To reconcile these apparently paradoxical findings (two noninterconvertible populations of complexes that become rapidly interconvertible on modification of the environment), a simplest explanation would assume that each complex can exist in two metastable conformations. A change in the environment might trigger structural rearrangements during which an increased structural flexibility would facilitate the transitions between the metastable states through common, short-lived intermediate conformation(s). The possible relationship of these two states to conformational transitions that ribosomes undergo during polypeptide synthesis (for example, the pre- and posttranslocated conformations (reviewed in Modolell & Vázquez, 1975)) is obscure. However, the following correlation is specially suggestive. Belitsina & Spirin (personal com-

munication) have observed that at 100 mM $NH_4Cl$ most ribosomes can effect the EF-G plus Gpp($CH_2$)p-promoted translocation of peptidyl-tRNA, and we find them forming almost exclusively fast dissociating complexes; furthermore, at 10 mM $NH_4Cl$ only about half of them translocate (even though the remaining inactive ribosomes can bind EF-G and Gpp($CH_2$)p) and we find them behaving heterogeneously in complex dissociation. Our preliminary results indicate that the 30S subunit may be responsible for the heterogeneous behavior since complexes formed on isolated 50S subunits undergo first-order dissociations. We would stress that functional heterogeneity has been described for other ribosomal activites like aminoacyl-tRNA binding (Glukhova et al., 1975), peptide bond formation (Maden et al., 1968), and antibiotic action (Pestka, 1972).

For any given condition of decay, the apparent dissociation rate constants of fast ($k_1$) and slowly ($k_2$) decaying complexes differed from 5- to 20-fold (Table I). $k_1$ was not significantly affected by temperature (values determined at 0 and 30 °C) but it increased by a factor of 2 to 3 by increasing the $NH_4Cl$ concentration from 10 to 100–170 mM. $k_2$ was larger at 30 °C than at 0 °C only with the GTP analogue containing complexes and was less affected than $k_1$ by increased $NH_4Cl$ concentrations. Marsh & Parmeggiani (1977) reported a $k$ of $3 \times 10^{-4}$ $s^{-1}$ for the slow decay phase of a GDP-containing complex dissociating at 30 °C in 10 mM $NH_4Cl$, 14 mM $MgCl_2$. This value is larger than that found now under very similar conditions ($k_2 = 1 \times 10^{-4}$ $s^{-1}$) probably because these authors studied complex breakdown by measuring the exchange of [$^3$H]GDP in the complex with a large excess of unlabeled GDP. We have observed (unpublished) that the exchange procedure consistently yields higher decay rates than the dilution method probably due to active displacement of labeled guanosine nucleotide (and EF-G) from ternary (and quaternary) complexes by binary unlabeled guanosine nucleotide·EF-G complexes.

It has been reported that EF-G protects the ribosome from thiostrepton inactivation (Highland et al., 1971, 1975). Our results indicate that the protection is not complete since thiostrepton interacts with guanosine nucleotide·EF-G·ribosome complexes and stimulates their dissociation (Figure 4 and Table I). A strong stimulation of decay, however, requires relatively high concentrations of the antibiotic (3–10 $\mu$M), while complete blockade (over 90%) of EF-G plus guanosine nucleotide interaction with the ribosome has been observed at 0.2–1 $\mu$M thiostrepton (Pestka, 1970; Highland et al., 1971; Modolell et al., 1971). Thus, thiostrepton seems to interact more easily with free ribosomes than with EF-G-complexed ribosomes. Moreover, the present findings indicate that the procedure of measuring complex dissociation in the presence of this antibiotic (Highland et al., 1971), while useful for obtaining qualitative results, should be used with great caution in quantitative studies.

Many misreading-inducing aminoglycoside antibiotics stabilize the ternary Gpp(NH)p·EF-G·ribosome complex, an observation that agrees with our previously reported inhibition by some of these antibiotics of the turnover of the GDP·EF-G·ribosome fusidic acid complex (Girbés et al., 1977b). Although we have recently shown that antibiotics of the neomycin, kanamycin, gentamicin, and hygromycin groups also interfere with ribosomal translocation (Cabanas et al., 1978a,b), there is no good quantitative correlation between the inhibition of this function and of the decay of ternary complex: hygromycin B inhibits translocation well but it does not impair ternary complex dissociation and streptomycin interferes well with the second reaction but only poorly with the first; in contrast, neomycins B and C strongly inhibit both reactions. Thus, the distortion of ribosomal structure provoked by each misreading-inducing aminoglycoside is probably more specific than it could be assumed from their common interference with aminoacyl-tRNA recognition.

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