Equilibrium Measurements of the Interactions of Guanine Nucleotides with *Escherichia coli* Elongation Factor G and the Ribosome†

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ABSTRACT: The interactions among *Escherichia coli* elongation factor G (EF-G), guanine nucleotides, ribosomes, and fusidic acid were investigated by a number of physical techniques. Equilibrium dialysis studies demonstrated the existence of a binary EF-G·GDP complex. This complex forms with a stoichiometry of ca 1:1 and an apparent K_a of 2.5×10^5 M^{-1}. While no evidence was obtained for the formation of a ribosome·GDP complex, in the presence of ribosomes, the apparent K_a for guanosine diphosphate (GDP) increased 40-fold over that for binding to EF-G alone. Although the apparent K_a increased, the stoichiometry remained ca. 1 mol of GDP/mol of EF-G. An upper limit of 1.3×10^7 M^{-1} was calculated for the K_a for binding of ribosomes to the EF-G·GDP complex. Fusidic acid had no effect on the apparent K_a's for either the EF-G·GDP or EF-G·β,γ-methyleneguanosine triphosphate (GMP-P(CH_2)P)·ribosome complexes, but markedly increased the K_a for GDP in the EF-G·GDP·ribosome complex without altering the stoichiometry. The apparent K_a for GDP was shown to be dependent upon the fusidic acid concentration. In addition, the rate of GDP exchange into the quaternary EF-G·GDP·ribosome·fusidic acid complex was inversely related to the fusidic acid concentration. All of the data obtained in these studies suggest that the formation and dissociation of complexes involving EF-G and guanine nucleotides is ordered. GDP is the first component to bind to EF-G, followed by the ribosome, and, finally, fusidic acid. This conclusion is consistent with the kinetic mechanism for the hydrolysis of GTP by EF-G and the ribosome proposed in the preceding paper of this issue (Rohrbach and Bodley (1976b). In addition to these binding studies, guanine nucleotides have also been shown to protect EF-G against both limited trypsinolysis and chemical modification by *N*-ethylmaleimide. These observations offer additional evidence for the existence of a guanine nucleotide binding site on EF-G.

In the preceding paper of this issue (Rohrbach and Bodley, 1976b), we reported a steady-state kinetic analysis of the uncoupled GTPase reaction catalyzed by elongation factor G (EF-G)[1] and the ribosome. The results of this analysis suggested that the reaction proceeds by an ordered mechanism in which GTP is the leading reactant and GDP is the last product released. In addition, these results indicated that a dead-end EF-G·ribosome complex also forms. This complex has been previously observed and the requirements for its formation have been well characterized (Lin and Bodley, 1976). In contrast, complexes of EF-G and guanine nucleotides have not been previously observed, and, in fact, preliminary experiments by us (Bodley, 1972) and others (Kaziro et al., 1969; Kuriki et al., 1970; Brot et al., 1971) in the past have failed to reveal such complexes.

In this communication, we report evidence for the existence of binary complexes of EF-G and guanine nucleotides based on the results of binding studies. In addition, the effects of ribosomes and fusidic acid on the dissociation of this complex are also examined. The results are consistent with the kinetic model proposed for the hydrolytic reaction (Rohrbach and Bodley, 1976b) and provide an explanation for the observed stabilization of the ternary EF-G·GDP·ribosome complex by fusidic acid.

Experimental Procedure

The nucleotides GTP and GDP were purchased from Sigma Chemical Co. β,γ-methyleneguanosine triphosphate (GMP-P(CH_2)P) was from Miles Laboratories. [^{3}H]GMP-P(CH_2)P was prepared by New England Nuclear Corp. and purified as previously described (Skar et al., 1975). Fusidic acid was a gift from Dr. W. O. Godtfredsen of Leo Pharmaceutical Products. *N*-ethylmaleimide (MalNEt) and bovine pancreatic trypsin were purchased from Sigma.

Ribosomes and elongation factor G (EF-G) were purified from *Escherichia coli* B by the methods of Bodley (1969) and Rohrbach et al. (1974), respectively.

Equilibrium Dialysis and Gel Filtration. All dialyses were performed at 4 °C in a solution (buffer A) composed of 10 mM Tris-Cl, pH 7.4, 20 mM magnesium acetate, 10 mM NH_4Cl, and 5 mM β-mercaptoethanol. Buffer A (0.5 or 0.25 ml) containing ribosomes and/or EF-G was placed in dialysis tubing and dialyzed against 4–4.5 ml of various concentrations of tritiated nucleotides (in buffer A) until equilibration was attained (48 h). When fusidic acid was included, it was placed both inside and outside the dialysis bag. Aliquots (10 or 20 μl) were removed from inside and outside the dialysis bags for radioactivity determination. Chromatographic analysis indicated that negligible breakdown of nucleotide had occurred.

EF-G·GDP interactions were also examined by filtration through a Sephadex G-25 column (0.4 × 45 cm) according to the procedure of Hummel and Dreyer (1962). The column was equilibrated with buffer A containing 2.58×10^{-6} M [^{3}H]GDP. EF-G (10 μl, 10^{-4} M) was incubated at 25 °C for 5 min with 90 μl of equilibration solution. After incubation, 90 μl was applied to the column and fractions were collected at room temperature and their radioactivity was determined.

Protection of EF-G by Nucleotide from MalNEt Inactivation. Reaction mixtures (53 μl containing buffer A, EF-G

† From the Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received February 9, 1976.* This investigation was supported by grants from the National Institutes of Health (GM-17101 and GM-21359). This is XXII in the series: "Studies on Translocation". The preceding paper is M. S. Rohrbach and J. W. Bodley (1976b).

[1] Abbreviations used are: EF-G, elongation factor G; GMP-P(CH_2)P, β,γ-methyleneguanosine triphosphate; MalNEt, *N*-ethylmaleimide; FA, fusidic acid; R, Ribosome; GTPase, guanosine triphosphatase; GTP,GDP, guanosine tri- and diphosphates.

TABLE I: Summary of Association Constants for Reactions Involving Nucleotides, EF-G, Ribosomes, and Fusidic Acid.

Complex Formation	K_a (M^{-1})
EF-G + GTP ⇌ EF-G·GTP	2.7×10^4 [a]
EF-G + GDP ⇌ EF-G·GDP	2.5×10^5
	6.1×10^4 [a]
EF-G + R ⇌ EF-G·R	$>2 \times 10^4$ [b]
R + GDP ⇌ R·GDP	$<10^4$
EF-G·GDP + R ⇌ EF-G·GDP·R	$<1.3 \times 10^7$
	6.7×10^6 [a]
EF-G·GDP·R + FA ⇌ EF-G·GDP·R·FA	2.6×10^6 [c]

[a] Determined kinetically by Rohrbach and Bodley (1976b). [b] Estimated from nonequilibrium measurements by Lin and Bodley (1976). [c] Determined by equilibrium dialysis using 24,25-dihydrofusidic acid by Willie et al. (1975).

(312 pmol)) and various amounts of tritiated nucleotide (GDP or GTP) were incubated at 25 °C for 5 min. Aliquots (2 μl) were removed prior to the addition of MalNEt and assayed by quantitation of the EF-G·ribosome·[^{3}H]nucleotide·fusidic acid complex by Millipore filtration (Highland et al., 1971) to determine the value for 100% activity. After the addition of 3 μl of MalNEt (4.25×10^{-3} M, final) and incubation for 2.5 min, 2-μl aliquots were assayed to determine the amount of inhibition.

EF-G Protection by GDP from Trypsinolysis. EF-G was subjected to limited trypsin digestion and electrophoresed on sodium dodecyl sulfate–polyacrylamide slab gels according to the procedures of Skar et al. (1975). Three digestion tubes were prepared with each containing 750 μl of 100 mM Tris–Cl, pH 7.4, 530 μg of EF-G, and 7 μg of trypsin. One of the three tubes also contained 20 mM magnesium acetate and a second tube 20 mM magnesium acetate plus 10 mM GDP. The tubes were incubated at 37 °C. Aliquots were removed at timed intervals and proteolysis was terminated with sodium dodecyl sulfate (2% final concentration) and prepared for gel electrophoresis as previously described (Skar et al., 1975).

Stability of Ternary Complex and Fusidic Acid Concentration. The stabilization of the complex EF-G·ribosome·[^{3}H]GDP by fusidic acid was examined by Millipore filtration (Highland et al., 1971). One-milliliter solutions of buffer A, 280 μM [^{3}H]GDP (5 Ci/mol), 0.16 μM EF-G, and varying concentrations of fusidic acid, were incubated at 37 °C for 5 min. Fifty microliters was removed and added to 5 ml of cold buffer A containing the same fusidic acid concentration and immediately passed through a Millipore filter without washing (0 time). GDP (10 μl, 92 mM) was added to the remaining incubation mixture (kept at 37 °C) and aliquots (50 μl) were removed at timed intervals onto Millipore filters. The radioactivity retained on the filters was determined.

Results

Binary and Ternary Complex Formation. The previously unobserved binary complex, EF-G·GDP, was demonstrated by several established methods. The use of equilibrium dialysis clearly showed the formation of the complex. A Scatchard plot of the data derived from equilibrium dialysis experiments had a slope of -2.5×10^5 M^{-1} and an ordinate intercept of 0.87. Duplicate experiments resulted in similar values for both intercept and slope. From these data, it is apparent that approximately 1 mol of GDP is bound per mol of EF-G with an apparent association constant of 2.5×10^5 M^{-1}. When [^{3}H]GDP and EF-G were subjected to gel filtration by the method of Hummel and Dreyer (1962) through Sephadex G-25 equilibrated with the nucleotide, the complex was also observed. The binding constant obtained in this way ($K_a = 3 \times 10^5$ M^{-1}; average of two experiments) is in close agreement with that derived by equilibrium dialysis. Attempts to detect the EF-G·[^{3}H]GDP complex by Millipore filtration were unsuccessful, despite the fact that EF-G is itself retained (Lin and Bodley, 1976). This may have resulted from rapid dissociation of the complex during washing of the filters.

Examination of the binding of [^{3}H]GDP to EF-G in the presence of ribosomes was also performed by equilibrium dialysis. A Scatchard plot of the data derived from equilibrium dialysis experiments had a slope of -10^7 M^{-1} and an ordinate intercept of 0.95. From these data, it is apparent that in the presence of ribosomes, approximately 1 mol of GDP is bound per mole of EF-G with an apparent association constant of 10^7 M^{-1}. The 40-fold increase in the apparent association constant for GDP in the presence of ribosomes suggested that the dissociation of the EF-G·GDP·ribosome complex is ordered with ribosomes leaving first. An upper limit for the association constant for the binding of ribosomes to the EF-G·GDP complex can be calculated from the equation: $K_a = K_1 + K_1K_2[R]$ (see Appendix). In this equation, K_1 is the association constant for the EF-G·GDP complex and the concentration of free ribosomes is equal to the difference between the total ribosomal concentration and the concentration of EF-G. The binding constant (K_2) calculated in this way is 1.3×10^7 M^{-1}, and represents an upper limit for K_2.

While the incusion of fusidic acid had no effect on the binding of GDP to EF-G, it significantly affected the association of the nucleotide with EF-G and ribosomes by increasing the K_a without affecting the stoichiometry of binding. These results will be presented below in conjunction with experiments performed to probe the basis for fusidic acid stabilization of the ternary complex.

While we were able to observe the binary EF-G·GDP complex, technical problems prevented the detection of comparable interactions with GTP and GMP-P(CH_2)P either by equilibrium dialysis or gel filtration. Such complexes are predicted to occur by kinetic analysis (Rohrbach and Bodley, 1976b). In the case of GTP, trace amounts of nucleotidase converted radiolabeled GTP to GDP during equilibrium dialysis, while high background levels of radioactivity obscured possible EF-G·GTP interactions during filtration through Sephadex G-25. The low specific activity of [^{3}H]GMP-P(CH_2)P prevented its being used in gel filtration and, for unexplained reason(s), we did not observe the complex by equilibrium dialysis.

However, using the same GTP analogue, we did determine its association constant for its binding to EF-G in the presence of ribosomes. By equilibrium dialysis in the presence of excess ribosomes it bound to the complex with a stoichiometry of 1:1 (nucleotide:EF-G) and an apparent K_a of 1.7×10^7 M^{-1}. Fusidic acid had no observable effect on either the K_a or stoichiometry.

Experiments were performed by equilibrium dialysis to detect a ribosome·GDP complex. If such an interaction occurs, the association constant must be $<10^4$ M^{-1}, the lower limit of sensitivity of our system.

A summary of the association constants determined in our laboratory of interactions involving guanine nucleotides, EF-G, ribosomes, and fusidic acid is presented in Table I.

Protection of EF-G by Nucleotides from MalNEt Inactivation and Proteolysis. Rohrbach and Bodley (1976a) demonstrated that MalNEt inhibits EF-G by modifying a cysteine

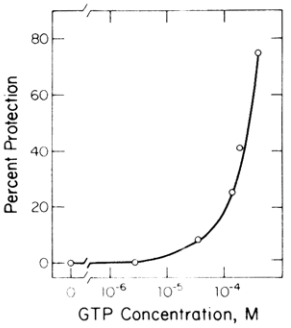

FIGURE 1: Protection of EF-G by GTP from MalNEt inactivation. The experimental conditions are described under Experimental Procedure.

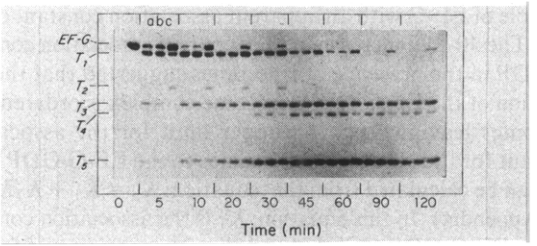

FIGURE 2: Protection of EF-G by GDP from trypsinolysis. Conditions for proteolysis and polyacrylamide gel electrophoresis are described in the text. The three samples bracketed at the indicated time of proteolysis are from digests containing buffer and trypsin plus (a) EF-G, (b) EF-G and magnesium acetate, (c) EF-G + magnesium acetate + GDP. The gel was stained with Coomassie blue.

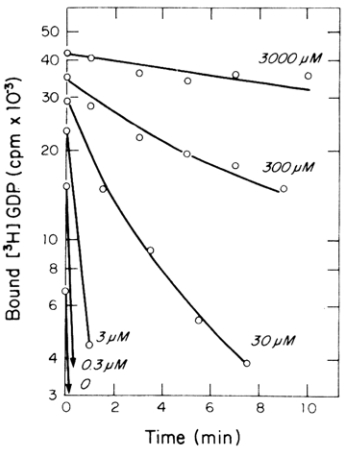

FIGURE 3: Influence of fusidic acid on the rate of exchange of [^{3}H]GDP from EF-G·GDP·R·FA. The effect of various concentrations of fusidic acid (0–3000 μM) on the rate of GDP exchange was determined as described under Experimental Procedure. The complex was formed in the presence of 0.4 μM [^{3}H]GDP and "chased" with 92 μM unlabeled GDP.

residue. While the modified enzyme could bind to ribosomes, it could not form any complexes involving nucleotides. It was therefore concluded that the modified cysteine residue was at or near the nucleotide binding site. In light of the existence of the binary complex, EF-G·GDP, experiments were performed to determine if the inclusion of GDP or GTP would prevent inactivation by MalNEt, providing further proof of the involvement of the modified cysteine residue in nucleotide binding and additional proof of the existence of the binary complex.

Figure 1 illustrates that GTP indeed prevents inactivation of EF-G by MalNEt. The enzyme remained about 75% active after exposure to MalNEt in the presence of 5.7×10^{-4} M GTP. The same amount of MalNEt yields maximum inhibition of the unprotected enzyme. The relatively large amount of nucleotide required for protection is probably due to the fast reaction of MalNEt with the cysteine sulfhydryl group as soon as the protecting nucleotide dissociates. Similar protection from MalNEt inactivation was obtained with GDP (data not shown).

Skar et al. (1975) demonstrated that trypsinolysis of native EF-G produced discrete cleavage products which arise from sequential scissions in the protein. The first cleavage of EF-G (74 000 daltons) results in a fully active (ability to complex with guanine nucleotides and ribosome) 71 000-dalton fragment (T_1). The next cleavage produces inactive peptides T_3 + T_5. Our observation that EF-G binds GDP suggested the possibility that bound nucleotide might protect active fragment T_1 from proteolysis if its scission and inactivation occurs at or near the nucleotide-binding site.

Figure 2 is a time course of tryptic digestion of EF-G in the presence of GDP analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The five cleavage products described by Skar et al. (1975) are reproduced in this experiment both in the presence and absence of GDP. However, there is a clear demonstration of retardation of proteolysis by the nucleotide. After 20 min of incubation with trypsin in the presence of nucleotide, a substantial amount of native EF-G is present, while unprotected digests have no detectable amount of the intact protein. After 120 min of incubation, fragment T_1 is still present in the digest containing GDP and absent from the unprotected digests.

Stabilization of EF-G·GDP·R by Fusidic Acid. Fusidic acid and its equally active analogue, 24,25-dihydrofusidic acid, have been previously shown to greatly increase the stability of the EF-G·GDP·R complex by forming the quaternary EF-G·GDP·R·FA complex (Okura et al., 1971; Willie et al., 1975). While this effect has been demonstrated, its physical basis has not been examined. In light of the observations indicating that the dissociation of the ternary complex is ordered with GDP as the last component to dissociate, it seems likely that the basis for fusidic acid stabilization is that the dissociation of the quaternary complex is also ordered. GDP cannot leave until first fusidic acid followed by the ribosome have dissociated. If this is the case, then both the rate of GDP exchange into the quaternary complex and the apparent association constant for GDP should be dependent upon the fusidic acid concentration.

The dependence of the rate of GDP exchange on the fusidic acid concentration is shown in Figure 3. Complexes were formed with radiolabeled GDP at various fusidic acid concentrations, and the rate of exchange of unlabeled GDP into the complex was quantitated by Millipore filtration. As can be seen, the rate of GDP exchange decreases as the fusidic acid concentration is raised up to 3000 μM fusidic acid. This would suggest that the dissociation of GDP from the quaternary complex is negligible.

The fusidic acid concentration dependence of the apparent association constant for GDP is described by the following equation (see Appendix):

$$K_a = K_1 + K_1K_2[R] + K_1K_2K_3[R]\,[FA]$$

The value for K_1, the association constant for the binding of GDP to EF-G, was determined by equilibrium dialysis as described above. The association constant (K_2) for the binding of ribosomes to EF-G·GDP has also been determined, and K_3 was determined to be 2.6×10^6 M^{-1} by Willie et al. (1975) using 24,25-[^{3}H]dihydrofusidic acid. When varying concen-

TABLE II: Effect of Fusidic Acid on Binding of [^{3}H]GDP to EF-G·Ribosome Complex.[a]

Fusidic Acid Concn ($M \times 10^{-7}$)	Theor K_a ($M^{-1} \times 10^7$)	Obsd K_a ($M^{-1} \times 10^7$)
None	1.0	1.0
1	1.3	2.8
3	1.8	3.1
10	3.6	5.9
30	8.6	9.4
100	25.4	15.0

[a] The theoretical and experimental K_a's were determined as described in the text.

trations of fusidic acid were included in the equilibrium dialysis experiments, the apparent K_a for GDP increased in a concentration dependent manner, and there was reasonable correlation between the theoretical and experimentally determined K_a's (Table II).

Discussion

In the preceding paper of this issue, (Rohrbach and Bodley, 1976b) a steady-state kinetic analysis of the mechanism of GTP hydrolysis catalyzed by EF-G and the ribosome was presented. This analysis indicated that the reaction occurs via an ordered mechanism with GTP as the first reactant bound to EF-G and GDP as the last product released. A number of predictions which are readily testable by binding studies result from this mechanism. The first prediction is the formation of binary complexes involving EF-G and guanine nucleotides. Secondly, since the mechanism is ordered, the addition of ribosomes to EF-G and guanine nucleotides should cause a pronounced increase in the apparent association constant for the guanine nucleotide. Finally, if fusidic acid can bind only to the ternary EF-G·GDP·ribosome complex, the apparent association constant should be dependent upon the fusidic acid concentration.

A binary complex between EF-G and GDP has been directly demonstrated in this report both by equilibrium dialysis and the gel filtration method of Hummel and Dreyer (1962). The apparent association constant calculated for this complex was 2.5×10^5 M^{-1}. This is in good agreement with the value of 6.1×10^4 M^{-1} determined kinetically (Rohrbach and Bodley, 1976b). The difference in these two values can, in part, be attributed to the buffers employed. In the kinetic studies, the buffers contained a higher concentration of ammonium ions in order to maximize hydrolysis. However, these higher ammonium ion concentrations tend to destabilize the binary complexes, resulting in a lower apparent association constant.

Previous attempts by other workers (Kaziro et al., 1969; Kuriki et al., 1970; Brot et al., 1971) to detect binary complexes of EF-G and guanine nucleotides have been unsuccessful. This can probably be attributed to the methods used for the analysis of these complexes which have involved the use of Millipore filtration or the use of gel filtration without the inclusion of nucleotide in the equilibration buffer. We, too, have been unable to detect this binary complex on nitrocellulose filters, probably due to its rapid dissociation upon washing.

In addition to the direct observations of the binary EF-G·GDP complex described above, evidence for the existence of this complex was also obtained from the observation that GDP protected EF-G from both limited trypsinolysis and chemical modification. As previously reported by Skar et al. (1975), native EF-G is cleaved by trypsin in a limited number of discrete sequential steps. While the presence of GDP does not alter either the number or order of tryptic scissions, the rate at which at least the first cleavage occurs is greatly reduced. From this it would appear that GDP, when bound, induces a conformational change in EF-G which makes the first trypsin-sensitive site less accessible to trypsin.

The observed protection of EF-G by GTP and GDP against inactivation by MalNEt supports the conclusion, reported by Rohrbach and Bodley (1976a), that the modification occurs at the nucleotide binding site. This conclusion was based on the observation that MalNEt-modified EF-G could form a complex with the ribosome but could not form any complexes involving nucleotides. At that time, it was not known that EF-G alone could bind guanine nucleotides, and experiments designed to demonstrate protection against inhibition by GDP or GTP alone were, therefore, not performed. During the preparation of this manuscript, Marsh et al. (1975) reported a similar protection of EF-G by GTP against inactivation by sulfhydryl-modifying reagents.

While we have been able to demonstrate the occurrence of a binary EF-G·GDP complex, we have not detected a binary complex involving ribosomes and guanine nucleotides. If such a complex occurs, its association must be very weak ($K_a < 10^4$ M^{-1}).

The inclusion of ribosomes along with EF-G and GDP in equilibrium dialysis experiments resulted in a 40-fold increase in the apparent association constant for GDP. This result is consistent with the dissociation of the ternary EF-G·GDP·ribosome complex being ordered with the ribosome as the first component to leave. If the dissociation were random, one would expect ribosomes to have only minimal, if any, effect on the apparent association constant for GDP.

The increased apparent association constant for GDP in the presence of fusidic acid can also be attributed to the ordered dissociation of complexes containing GDP. In agreement with the results reported by Willie et al. (1975) using the fusidic acid analogue 24,25-dihydrofusidic acid, we found that the only guanine nucleotide containing complex affected by fusidic acid was the EF-G·GDP·ribosome complex. This indicated that the only fusidic acid containing complex is the quaternary EF-G·GDP·ribosome·fusidic acid complex, and suggested that the dissociation of the quaternary complex is ordered with fusidic acid as the first component to dissociate. This conclusion is supported by two observations. First, the rate of GDP exchange into the quaternary complex is proportional to the concentration of fusidic acid. Secondly, an equation for the apparent association constant for GDP in the presence of fusidic acid was derived assuming that the dissociation was ordered with GDP as the last component to dissociate. The apparent association constants at various fusidic acid concentrations agreed with the theoretical values derived from the equation.

All of the results reported here lead to the conclusion that the interactions among EF-G, guanine nucleotides, ribosomes, and fusidic acid occur through an ordered pathway. The first complex to form is the binary EF-G·guanine nucleotide complex. Once formed, the ribosome can bind to this complex to form the ternary EF-G·guanine nucleotide·ribosome complex. Finally, fusidic acid can bind to the ternary complex, but only when the guanine nucleotide is GDP. This ordered model obtained through binding studies is identical to that obtained by kinetic studies (Rohrbach and Bodley, 1976b) and therefore presents independent verification of the proposed mechanism for uncoupled GTP hydrolysis. In addition, it points out the

similarity between EF-G and the other protein synthesis factors, initiation factor 2 and elongation factor Tu, which catalyze GTP-dependent steps in protein synthesis. It is known that both of these factors bind GTP and that this binding is a prerequisite for their functional binding to the ribosome. It appears that EF-G functions in the same way.

Acknowledgment

We gratefully acknowledge the technical assistance of Ms. L. Lin, and the valuable suggestions of Dr. John Gander on the preparation of this manuscript.

Appendix

Derivation of Binding Constant. In deriving the equation for the apparent K_a for GDP to EF-G and the ribosome in the absence or presence of fusidic acid, the following assumptions were made: (a) the dissociation of all GDP-containing complexes is ordered with GDP as the last component to leave, and (b) on equilibrium dialysis, bound [^{3}H]GDP is found in EF-G·[^{3}H]GDP, EF-G·[^{3}H]GDP·R, and, when fusidic acid is present, EF-G·[^{3}H]GDP·R·FA.

$$K_1 = \frac{[\text{EF-G·GDP}]}{[\text{EF-G}]\,[\text{GDP}]}$$

$$= K_2 = \frac{[\text{EF-G·GDP·R}]}{[\text{EF-G·GDP}]\,[\text{R}]}$$

$$K_3 = \frac{[\text{EF-G·GDP·R·FA}]}{[\text{EF-G·GDP·R}]\,[\text{FA}]}$$

In the absence of fusidic acid

$$\bar{v} = \frac{\text{mol of GDP bound}}{\text{mol of EF-G}}$$

$$= \frac{[\text{EF-G·GDP}] + [\text{EF-G·GDP·R}]}{[\text{EF-G}] + [\text{EF-G·GDP}] + [\text{EF-G·GDP·R}]} \quad (1a)$$

From the equilibrium constants

$$[\text{EF-G·GDP}] = K_1\,[\text{EF-G}]\,[\text{GDP}]$$

$$[\text{EF-G·GDP·R}] = K_1K_2\,[\text{EF-G}]\,[\text{GDP}]\,[\text{R}]$$

and substituting into eq 1a and dividing through by [EF-G]

$$\bar{v} = \frac{K_1\,[\text{GDP}] + K_1K_2\,[\text{GDP}]\,[\text{R}]}{1 + K_1\,[\text{GDP}] + K_1K_2\,[\text{GDP}]\,[\text{R}]} \quad (2a)$$

Rearranging eq 2a

$$\bar{v} = \frac{(K_1 + K_1K_2\,[\text{R}])\,[\text{GDP}]}{1 + (K_1 + K_1K_2\,[\text{R}])\,[\text{GDP}]} \quad (3a)$$

Dividing through eq 3a by $(K_1 + K_1K_2\,[\text{R}])\,[\text{GDP}]$

$$\bar{v} = \frac{1}{\dfrac{1}{(K_1 + K_1K_2\,[\text{R}])\,[\text{GDP}]} + 1} \quad (4a)$$

and

$$K_a = K_1 + K_1K_2\,[\text{R}] \quad (5a)$$

By an analogous treatment, the apparent K_a in the presence of fusidic acid is:

$$K_a = K_1 + K_1K_2\,[\text{R}] + K_1K_2K_3\,[\text{R}]\,[\text{FA}] \quad (6a)$$

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