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New antibiotic that acts specifically on the GTP-bound form of elongation factor Tu

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The new thiazolyl peptide antibiotic GE2270 A, isolated from *Planobispora rosea* strain ATCC 53773, is shown to inhibit bacterial protein biosynthesis *in vitro* by affecting specifically the GTP-bound form of elongation factor Tu (EF-Tu). The 'off' rate of EF-Tu·GTP is slowed down 400-fold, locking GTP on EF-Tu, whereas EF-Tu·GDP is unaffected. Therefore, on the EF-Tu·guanine nucleotide interaction, GE2270 A mimicks the effect of aa-tRNA. In line with this, the binding of aa-tRNA to EF-Tu·GTP is hindered by the antibiotic, as shown by the absence of a stable ternary complex and the inhibition of the enzymatic binding of aa-tRNA to the ribosome. This blocks the elongation cycle. GE2270 A does not essentially modify the intrinsic GTPase activity of EF-Tu, but impairs the stimulation by ribosomes of this reaction. The negative effect of GE2270 A on the EF-Tu·GTP interaction with aa-tRNA bears similarities with that of the structurally unrelated pulvomycin, whereas marked differences were found by comparing the effects of these two antibiotics on EF-Tu·GDP. This work emphasizes the varieties of the transitional conformations which tune the EF-Tu interaction with GTP and GDP.

Key words: aminoacyl-tRNA/antibiotic/GTPase/GTP-binding proteins/protein biosynthesis

Introduction

The new antibiotic GE2270 A, recently isolated from *Planobispora rosea* strain ATCC 53773 and found to be specifically active on gram-positive and anaerobic microorganisms, belongs structurally to the family of thiazoyl peptide antibiotics (Selva *et al.*, 1990) which includes a number of inhibitors of bacterial protein biosynthesis acting on the ribosome, such as thiostrepton, siomycin, nosiheptide and micrococcin (Vazquez, 1977). In this work we report that GE2270 A also strongly inhibits protein biosynthesis in the *in vitro* system of *Escherichia coli*, but its action is directed towards elongation factor Tu(EF-Tu), the carrier of aminoacyl-tRNA (aa-tRNA) to the ribosome and the most abundant protein of the bacterial cell (Miller and Weissbach, 1977; Kaziro 1978; Bosch *et al.*, 1983). EF-Tu, a model for the class of GTP-binding proteins (Barbacid, 1987; Gilman, 1987; Woolley and Clark, 1989), is known to be the target of two antibiotics, kirromycin and pulvomycin (Parmeggiani and Swart, 1985). Kirromycin, the better characterized of these two antibiotics, has played a crucial role in the understanding of the EF-Tu functions and genetic organization (reviewed in, Bosch *et al.*, 1983; Parmeggiani and Swart, 1985). This induced us to investigate the mechanism of action of this new antibiotic. GE2270 A has been found to alter the active form of EF-Tu, the EF-Tu·GTP complex, leading to the blockage of the elongation process. Our results put a new emphasis on the fine mechanisms tuning the conformational transitions of the GTP- and GDP-bound states of EF-Tu.

Results

The antibiotic GE2270 A and its action in protein biosynthesis

As shown in Figure 1A, the antibiotic GE2270 A consists of a chain originating from 14 modified amino acids resulting in 6 thiazole rings and one pyridine, arranged in a cyclic structure with a side chain (Selva *et al.*, 1990). Structurally, this compound reveals evident similarities with other thiazolyl peptides, especially in the chromophoric central part. Among the components of this family, micrococcin is the most similar (Figure 1B; Walker *et al.*, 1977; Bycroft and Gowland, 1978). No structural similarity is shared with pulvomycin (Figure 1C; Smith *et al.*, 1985) or kirromycin (Maehr *et al.*, 1980).

As shown in Figure 2, GE2270 A is a strong inhibitor of protein biosynthesis *in vitro* as determined in a minimal *E.coli* purified system for poly(U)-directed poly(Phe) synthesis. A 50% inhibition occurs at 0.4 μM GE2270 A.

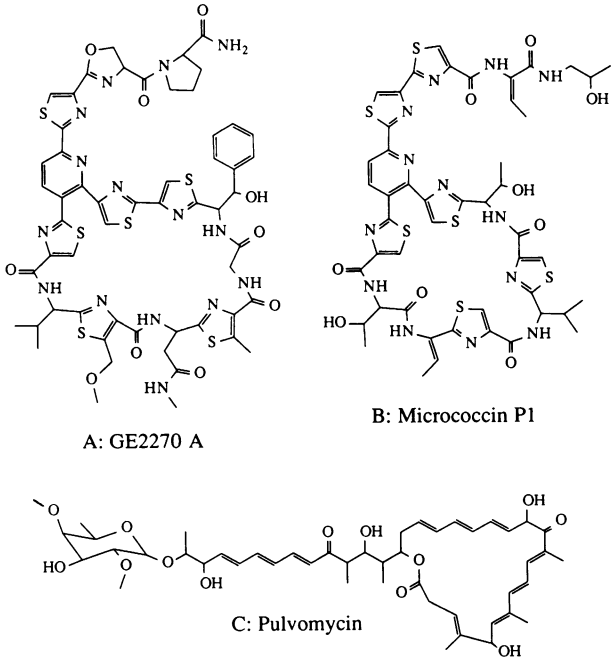

Fig. 1. Chemical structures of GE2270 A (**A**), micrococcin P1 (**B**) and pulvomycin (**C**).

The same effect is obtained with 0.4 μM kirromycin or 1 μM pulvomycin. In experiments not shown, EF−Tu pre-incubated with GE2270 A and passed through a Sephadex G25 column to eliminate the free antibiotic was inactive in poly(Phe) synthesis. In contrast, the activity of 70S ribosomes exposed to the same treatment was unaffected. These results suggested that GE2270 A interferes with EF−Tu.

The target of GE2270 A: elongation factor Tu

We first investigated the interactions with GTP and GDP, the two ligands controlling the switching on and off of the EF−Tu functions in the elongation of the polypeptide chain. As with all guanine nucleotide-binding proteins, GTP induces the active EF−Tu conformation capable of interacting with aa-tRNA and ribosomes. The EF−Tu complex with GDP is characterized by a much smaller dissociation constant ($K_{d(EF-Tu\cdot GDP)}$ = 1 nM) than the complex with GTP ($K_{d(EF-Tu\cdot GTP)}$ = 0.5 μM). This is a consequence of the slower dissociation and the faster association of the former complex (Arai *et al.*, 1974; Fasano *et al.*, 1978). The presence of aa-tRNA stabilizes EF−Tu·GTP which shows a half-life of 88 min (versus 2 min in the absence of aa-tRNA) and a K_d as EF−Tu·GDP (Fasano *et al.*, 1978).

The only remarkable effect induced by GE2270 A on the kinetics of the EF−Tu·guanine nucleotide interaction is a striking decrease of the dissociation rate of the EF−Tu·GTP complex by 400-fold (Figure 3), i.e. an effect even stronger than that of aa-tRNA. The half-life of the EF− Tu·GTP complex at 0°C becomes 830 min. The association rate of this complex is only slightly increased (10−20%) whereas neither the association nor the dissociation rate of the EF−Tu·GDP complex is modified (see Figure 6). As a consequence, the K_d of EF−Tu·GTP is decreased by two orders of magnitude (from 0.5 μM to 1.2 nM), approaching that of EF−Tu·GDP. The affinity of GE2270 A for EF−Tu·GTP, measured as the concentration inducing 50% of the effect on the dissociation rate obtained with saturating amounts of the antibiotic (50 μM), was found to be ~ 5 nM.

These results show a clear similarity between the effects of GE2270 A and aa-tRNA. Both stabilize EF−Tu·GTP without modifying the dynamics of EF−Tu·GDP.

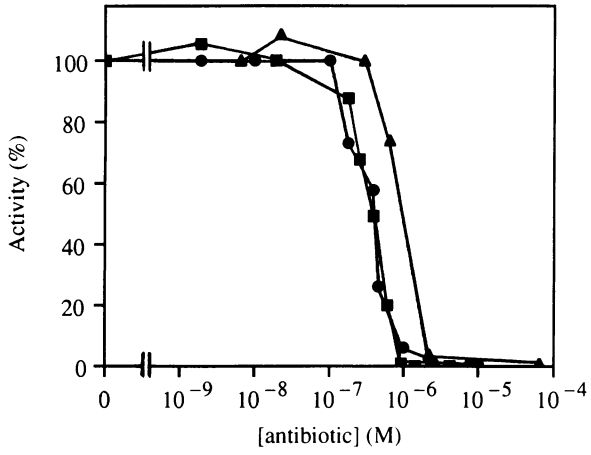

Fig. 2. Inhibition of poly(U)-directed poly(Phe) synthesis. The reaction mixture (75 μl) contained 20 mM Tris−HCl, pH 7.5, 90 mM NH_4Cl, 7 mM $MgCl_2$, 7 mM 2-mercaptoethanol, 1 mM ATP, 0.5 mM GTP, 1 mM phospho*enol*pyruvate, pyruvate kinase (40 μg/ml), 0.4 μM EF−Tu, 0.3 μM elongation factor Ts, 0.1 μM elongation factor G, 0.4 μM ribosomes, poly(U) (80 μg/ml), a saturating amount of phenylalanyl-tRNA synthetase, 120 μg bulk *E.coli* tRNA, 5 μM [^{14}C]Phe (200 c.p.m./pmol), GE2270 A (■), kirromycin (●) or pulvomycin (▲) at the indicated concentrations. After 10 min at 30°C, at which time the rate of the reaction is linear, 60 μl were spotted on Whatman 3MM filter discs and assayed for incorporation of [^{14}C]Phe into hot trichloroacetic acid insoluble material (Swart *et al.*, 1987). The results are expressed as a percentage of the activity obtained in the absence of the antibiotics.

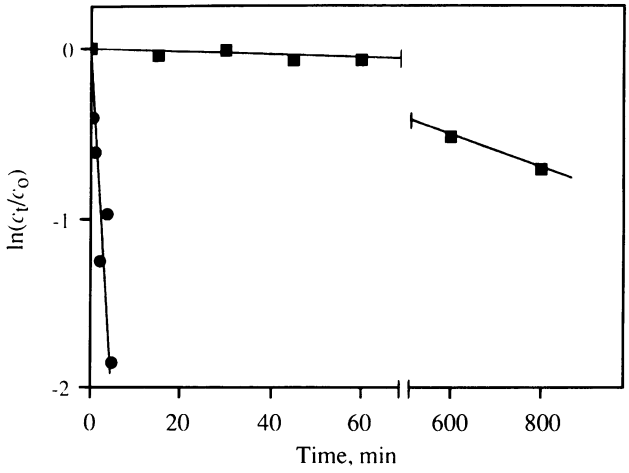

Fig. 3. Effect of GE2270 A on the dissociation rate of the EF−Tu·GTP complex. Nucleotide-free EF−Tu [20 pmol, prepared as described in Fasano *et al.* (1978)], was incubated in 1 ml standard buffer with 400 pmol of [γ-^{32}P]GTP (specific activity: 12 000 c.p.m./pmol) at 0°C for 20 min in the presence (■) or absence (●) of 50 μM GE2270 A. The dissociation reaction was started by the addition of a 1000-fold excess of unlabelled GTP. At the indicated times, 100 μl aliquots were withdrawn and EF−Tu-bound radioactivity determined by the nitrocellulose filter method. The c_0 is the initial concentration of the EF−Tu·GTP complex and c_t the concentration at the different times t (Fasano *et al.*, 1978).

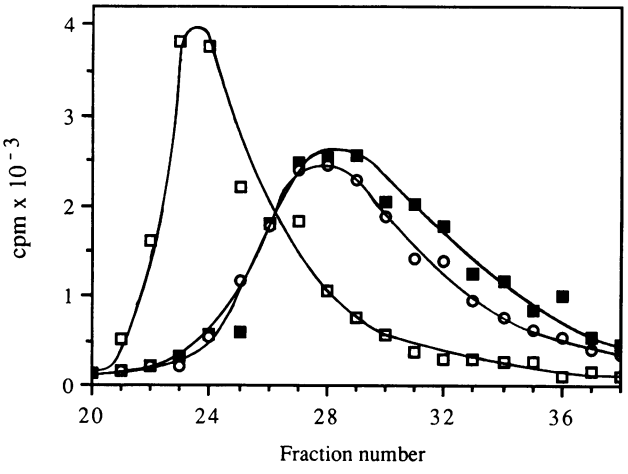

Fig. 4. Effect of GE2270 A on the formation of the ternary complex EF−Tu·GTP·Phe-tRNA^{Phe}. EF−Tu·GDP (540 pmol in 58 μl standard buffer) was converted to EF−Tu·GTP by incubation at 30°C for 15 min with 2 mM phospho*enol*pyruvate, pyruvate kinase (40 μg/ml) and 0.5 mM GTP. The mixture cooled on ice was added with 200 pmol [^{14}C]Phe-tRNA^{Phe} (specific activity: 300 c.p.m./pmol) in 40 μl of standard buffer (□) or standard buffer containing 50 μM GE2270 A (■). After 15 min at 0°C the mixture was applied to an Ultrogel AcA54 column (IBF) (0.6 × 65 cm) and eluted with standard buffer. Fractions of 150 μl were collected and analysed for the presence of radioactivity. As a reference the elution pattern of [^{14}C]Phe-tRNA^{Phe} in the absence of EF−Tu (○) is shown.

Effect on the EF-Tu interaction with aa-tRNA and the ribosome

The next question was whether the antibiotic affected the interaction of EF-Tu with aa-tRNA or the ribosome. In the presence of GE2270 A EF-Tu·GTP loses the ability to form a stable ternary complex with aa-tRNA, as tested on AcA54 gel filtration (Figure 4). This result suggests that the negative effect on the interaction with aa-tRNA caused by EF-Tu-bound GE2270 A represents a major reason for the interruption of the elongation cycle. In line with this possibility, GE2270 A also impairs the EF-Tu-dependent binding of Phe-tRNA^{Phe} to poly(U)-programmed ribosomes carrying N-Ac-Phe-tRNA in the P site, as illustrated in Figure 5. The amount of the antibiotic needed for a 50% inhibition was 0.7-0.8 μM.

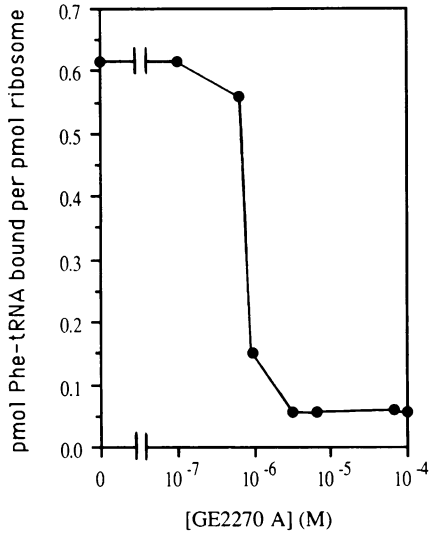

Fig. 5. EF-Tu-dependent binding of Phe-tRNA^{Phe} to the A site of poly(U)-programmed ribosomes as a function of GE2270 A concentrations. The final reaction mixture contained in 50 μl standard buffer 0.2 μM ribosomes, 40 μg/ml poly(U),, 0.3 μM N-Ac-Phe-tRNA^{Phe}, 0.1 mM GTP, 2 mM phospho*enol*pyruvate, 40 μg/ml pyruvate kinase, 0.35 μM EF-Tu, 0.2 μM [^{14}C]Phe-tRNA^{Phe} (specific activity: 300 c.p.m./nmol) and GE2270 A at the indicated concentrations. EF-Tu, GTP was prepared as described in Figure 4 and Ribosomes, poly(U) and N-AcPhe-tRNA^{Phe} were preincubated for 15 min at 30°C. The reaction was started by the addition of Phe-tRNA^{Phe} to the other components. After 6 min. at 30°C, 40 μl samples were filtered through nitrocellulose membranes which were washed twice with 2 ml standard buffer. In the absence of EF-Tu, 0.07 pmol [^{14}C]Phe-tRNA^{Phe} were bound per pmol ribosome.

Additional effects of GE2270-A: a comparison with other antibiotics

Inhibition of the interaction between EF-Tu·GTP and aa-tRNA has also been ascribed to pulvomycin (Wolf *et al.*, 1978; Pingoud *et al.*, 1982). Although it is not clear whether these authors used the intact antibiotic or a fragment of it (cf. Wolf *et al.*, 1978 and Smith *et al.*, 1985), we could observe a similar inhibitory effect, using pulvomycin preparations showing the same UV spectrum as described for the intact molecule (results not illustrated). In order to compare the action of the two antibiotics on the GTP- and GDP-bound forms of EF-Tu, we investigated the dynamics of the EF-Tu·GTP and EF-Tu·GDP complex formation in the presence of pulvomycin, an unexplored aspect of the action of this antibiotic. Pulvomycin (50 μM) also inhibits the dissociation of EF-Tu·GTP (half-life at 0°C: 400 min, not shown). However, differently from GE2270 A, it enhances most strongly the dissociation rate of the EF-Tu·GDP complex (Figure 6A) and to a lesser extent the association rate of EF-Tu·GDP (twice, see Figure 6B) and EF-Tu·GTP (five times, not shown). If GE2270 A and pulvomycin are added simultaneously, the strong stimulation of the latter antibiotic on the dissociation rate of ET-Tu·GDP disappears (Figure 6A), indicating that GE2270 A is dominant over pulvomycin. Moreover, this result shows that GE2270 A can bind to EF-Tu·GDP without affecting the properties of this complex.

Concerning the intrinsic GTPase of EF-Tu at 100 mM NH_4Cl, GE2270 A does not essentially affect this activity, in contrast to pulvomycin and especially to kirromycin which was taken as reference due to its well-known ability to enhance markedly this activity (Table I). At a higher NH_4Cl

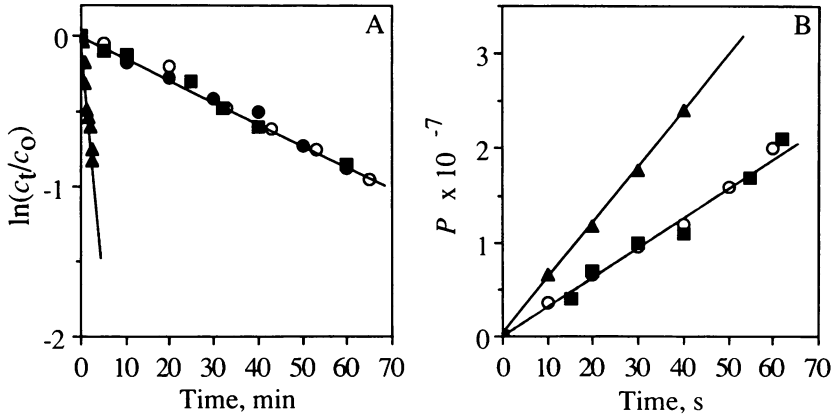

Fig. 6. Influence of pulvomycin on the dissociation and association rates of EF-T·GDP as compared to GE2270 A. **Panel A**: Dissociation rate of the [^{3}H]GDP·EF-Tu complex. The experimental conditions were as in Figure 3, except that [^{3}H]GDP (20 pmol, specific activity 6300 c.p.m./pmol) replaced [γ^{32}P]GTP, in the absence (○) or presence of 50 μM GE2270 A (■), 50 μM pulvomycin (▲) or 50 μM GE2270 A + 50 μM pulvomycin (●). **Panel B**: Association rate of the [^{3}H]GDP·EF-Tu complex. The reaction mixture (1 ml of standard buffer) contained nucleotide-free EF-Tu (10 pmol) in the absence (○) or presence of 50 μM pulvomycin (▲) or 50 μM GE2270 A. (■). The association reaction was started by the addition of 20 pmol [^{3}H]GDP. At the indicated times, 100 μl aliquots were withdrawn and the remaining [^{3}H]GDP·EF-Tu was determined by the nitrocellulose filter method. P = [1/(b) − a]ln[a(b − x)/b(a − x)] = association rate constant·t, where a is the initial concentration of [^{3}H]GDP, b is the initial concentration of EF-Tu and x is the concentration of the EF-Tu·[^{3}H]GDP complex formed at the different times t.

Table I. Effect of GE2270 A, pulvomycin and kirromycin on the EF−Tu-dependent GTPase activity in the presence and absence of ribosomes[a]

	GTP hydrolyzed (mmol·mol EF−Tu^{-1}·min^{-1})	
Additions	EF−Tu	EF−Tu+ribosomes
none	4 (10)	76 (20)
GE2270 A	5 (7)	34 (12)
pulvomycin	16 (28)	36 (28)
kirromycin	110 (200)	1020 (290)

[a]The reaction mixture (50 μl) contained in 50 mM imidazole acetate, pH 7.5, 10 mM $MgCl_2$, 100 or 400 (results within brackets) mM NH_4Cl, 1 mM dithiothreitol, 15 pmol of EF−Tu·GDP, 600 pmol of [γ-^{32}P]GTP (specific activity 2000 c.p.m. pmol^{-1}), pyruvate kinase (40 μg/ml) and phospho*enol*pyruvate (2 mM). EF−Tu·GDP was converted into EF−Tu·GTP as reported in the legend to Figure 4. The reaction was started by adding NH_4Cl to the appropriate concentration and GE2270 A, pulvomycin or kirromycin to a final concentration of 50 μM. The temperature of incubation was 37°C. When indicated 40 pmol of ribosomes were also present. GTP hydrolysis was measured kinetically by withdrawing 10 μl samples after 4, 8, 12 and 16 min. During this time the reaction was linear.

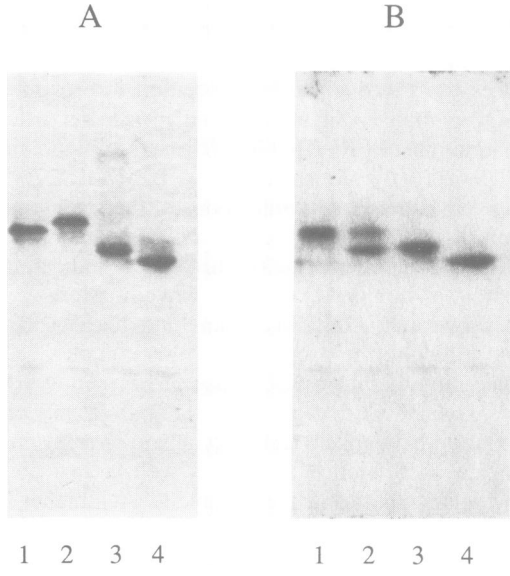

Fig. 7. Effect of GE2270 A, pulvomycin and kirromycin on the migration velocity of native EF−Tu on PAGE. **A**: EF−Tu·GDP (46 pmol) was incubated in 10 μl of 50 mM imidazole acetate (pH 7.6), 10 mM NH_4Cl, 10 mM $MgCl_2$, 1 mM dithiothreitol in the absence of antibiotics (lane 1) or in the presence of 50 μM GE2270 A (lane 2), 50 μM pulvomycin (lane 3) or 50 μM kirromycin (lane 4) for 15 min at 0°C. **B**: EF−Tu·GTP (46 pmol prepared as described in the legend to Figure 4) was incubated in a total volume of 10 μl buffer plus or minus antibiotics as in **A**. The mixtures were applied to non-denaturing, discontinuous 12% PAGE at 8°C in a mini gel apparatus (SE250, Hoefer Scientific Instruments) using an electrophoresis buffer containing 5 mM Tris−glycine, pH 8.3, supplemented in the upper chamber with 20 μM GDP in **A** and with 20 μM GTP in **B**.

concentration (400 mM, values in brackets), GE2270 A somewhat inhibits the intrinsic GTPase of EF−Tu, in contrast to pulvomycin and kirromycin. GE2270 A reduces the ribosome-dependent stimulation on the EF−Tu GTPase at both concentrations of salt, an effect evoked by pulvomycin to a greater extent. Kirromycin always reinforces the stimulatory action of ribosomes (see also, Parmeggiani and Swart, 1985).

The selectivity of the action of GE2270 A on EF−Tu·GTP is evident by examining the mobility of EF−Tu on native PAGE (Figure 7); the migration velocity of EF−Tu·GDP is slightly retarded whereas that of EF−Tu·GTP is accelerated considerably, approximately as with pulvomycin, but less than with kirromycin. In contrast to GE2270 A, the mobility of EF−Tu·GDP in the presence of pulvomycin and kirromycin is even more accelerated than that of EF−Tu·GTP. Thus the differential effect on the GTP- and GDP-bound forms of EF−Tu is strongest in the presence of GE2270 A. In the case of GE2270 A, the presence of a second band migrating as the control of EF−Tu·GTP suggests a partial dissociation of the antibiotic from the EF−Tu·GTP complex or two different conformations. Under the chosen conditions of salt and temperature, the intrinsic GTPase activity of EF−Tu in the presence and in the absence of kirromycin or pulvomycin is negligible.

In view of the structural similitude of GE2270 A with that of other thiazolyl antibiotics known to act on the ribosome, especially micrococcin, we have carefully investigated whether GE2270 A could affect the ribosomal activities. GE2270 A neither influences the translocation of Ac-Phe-Phe-tRNA from A to P site, nor the ribosome-dependent GTPase activity of elongation factor G, two reactions which are respectively inhibited and stimulated by micrococcin (Otaka and Kaji, 1974; Cundliffe and Dixon, 1975; Cundliffe and Thompson, 1981; Spedding and Cundliffe, 1984). Peptide bond formation is not affected by GE2270 A either.

Discussion

The most remarkable feature of GE2270 A is the exclusive specificity of its action directed towards the GTP bound form of EF−Tu. As a major effect, GE2270 A blocks the dissociation of the EF−Tu·GTP complex, the only kinetic parameter of the EF−Tu−nucleotide interaction significantly modified. The specificity of GE2270 A underlines the conformational differences between the GTP- and GDP-bound form of EF−Tu, controlling the ability of the factor to interact with aa-tRNA and the ribosome. The effect of GE2270 A on EF−Tu·GTP and EF−Tu·GDP resembles that of aa-tRNA, which also strongly increases the affinity for GTP, inhibiting the off-rate of the EF−Tu·GTP complex, without influencing the dynamics and properties of EF−Tu·GDP. The analogy of the actions of GE2270 A and aa-tRNA on EF−Tu·GTP(GDP) is further comforted by the observation that EF−Tu·GTP·GE2270 A can neither form a stable complex with aa-tRNA nor position aa-tRNA on the A site of poly(U)-programmed ribosomes; consequently the elongation cycle of polypeptide synthesis is blocked. Whether the inhibition of the interaction between EF−Tu·GTP and aa-tRNA by GE2270 A depends on the overlapping of the EF−Tu sites for aa-tRNA and the antibiotic, being thus related to steric hindrance effects, or whether it purely follows a conformational inactivation of the GTP-bound state, remains to be established. In both cases the localization of the EF−Tu region interacting with GE2270 A will elucidate a crucial regulatory region for the activities of the factor.

As aa-tRNA, GE2270 A affects little the catalytic activity of EF−Tu, showing that in EF−Tu the increase of the affinity for the substrate is not necessarily associated with a stimulation of the GTPase activity. With kirromycin and

to a minor extent with pulvomycin the increased EF-Tu affinity for GTP is associated with a stimulation of GTP hydrolysis.

All three antibiotics so far found to act on EF-Tu display the common property to bring about tighter, anomalous EF-Tu·GTP complexes characterized by a slow dissociation. With GE2270 A, this effect is strongest, followed by pulvomycin and kirromycin. However, the last two antibiotics also affect the GDP-bound conformation of EF-Tu. Moreover, in the presence of kirromycin, not only EF-Tu·GTP but also EF-Tu·GDP can bind to aa-tRNA and the ribosome. Consequently, EF-Tu·GDP-kirromycin remains on the ribosome-mRNA complex after GTP hydrolysis and thereby inhibits peptide bond formation. The action of GE2270 A and pulvomycin shows basic similarities, in spite of the unrelated structures; they both inhibit the elongation cycle by blocking the formation of the ternary complex with aa-tRNA. As a major difference, pulvomycin strongly influences the dynamics of the EF-Tu·GDP complex, with effects resembling those of elongation factor Ts. Moreover, the EF-Tu·GE2270 A GTPase is susceptible to be enhanced by the ribosome to a higher degree than that of EF-Tu·pulvomycin. Concerning the functional relationships of their effects, i.e. whether they are dominant or recessive, GE2270 A abolishes the stimulatory effect of pulvomycin on the dissociation of the EF-Tu·GDP complex (Figure 6A). It is likely that this effect is the consequence of overlapping sites, involving common long-range effects.

The selectivity of GE2270 A is particularly evident on native PAGE. EF-Tu·GDP and EF-Tu·GTP are affected in a markedly different manner, whereas kirromycin and pulvomycin modify the mobility of these complexes in a more similar way. The electrophoretic behaviour of EF-Tu·GTP·GE2270 A is similar to that of EF-Tu·GTP in the presence of pulvomycin. The isolation of EF-Tu species resistant to GE2270 A and to pulvomycin, a major project of our present research program, may yield additional information about the mechanism of action of these two antibiotics. Kirromycin-resistant EF-Tu species had a large impact not only on the functional and structural characterization of EF-Tu, but also on the mechanisms controlling the expression of the encoding *tuf* genes (Bosch *et al.*, 1983).

It is interesting that related basic structures as in GE2270 A and other thiazolyl antibiotics especially micrococcin, which share the same biosynthetic pathway, have evolved towards different targets as EF-Tu and ribosomes that are associated in their functions. Modifications of GE2270 A and micrococcin may be useful for identifying the structural elements responsible for their action. Important aspects that remain to be clarified are whether the isolated GTP-binding domain of EF-Tu (Parmeggiani *et al.*, 1987) may interact with GE2270 A and whether other GTP-binding proteins are affected, considering the structural and functional similarities of the members of this class of proteins.

The finding of a third antibiotic inhibiting protein biosynthesis via EF-Tu, emphasizes the importance of this factor as a crucial target for inhibition of bacterial cell growth. This GTP-binding protein displays a particularly complex regulation. It is not surprising that different inhibitors of its activity exploit the conformational transitions characterizing its basic switch mechanisms, revealing new aspects of the function-structural relationships of this enzyme.

Materials and methods

Biological components

Purified EF-Tu, elongation factor Ts and elongation factor G, Phe-tRNA synthetase, $tRNA^{Phe}$ (20-70% pure) and ribosomes were prepared from *E.coli* as reported (Parmeggiani and Sander, 1981). Purified GE2270 A (M_r: 1289) was obtained from Drs E.Selva and M.Denaro (Lepetit Research Centre) and stored at -30°C. Solution of 10 mM dimethylformamide at -30°C were stable for at least 4 weeks, thereafter showing a slow loss of activity. Kirromycin, obtained as mocimycin from Dr R.Beukers (Gist-Brocades), was used as a stable 3 mM solution in 100% methanol kept at -30°C (Wolf *et al.*, 1974). Pulvomycin, more than 98% pure, was isolated according to Smith *et al.* (1985). Its UV spectrum corresponds to that described for the intact molecule. A 10 mM solution of pulvomycin in methanol at -30°C was stable for at least several months. Micrococcin P (a mixture of micrococcin P1 and its ketone derivative P2 in a 7 to 1 ratio, Walker *et al.*, 1977) was kindly supplied by Dr J.Walker and stored as a stable 5 mM solution in dimethylformamide at -30°C. In the final reaction mixtures, organic solvents carried over with the antibiotics and glycerol from EF-Tu and ribosomes, never exceeded a 2% concentration and were also added to the controls.

Methods

Poly(U)-directed Poly(Phe) synthesis, dissociation constants, association and dissociation rate constants of the EF-Tu complexes with GDP or GTP, Phe-tRNA charging reaction, peptide bond formation, peptidyl-transferase activity, and binding of Phe-tRNA to the ribosomal A-site were measured as described (Chinali and Parmeggiani, 1973; Wolf *et al.*, 1974; Fasano *et al.*, 1977; Swart *et al.*, 1987). The GTPase activity of EF-Tu was determined as the liberation of $^{32}P_i$ from [γ^{32}P]GTP, using the charcoal method (Parlato *et al.*, 1981). The standard assay buffer contained 50 mM Tris-HCl, pH 7.6, 60 mM NH_4Cl, 7 mM $MgCl_2$ and 1 mM dithiothreitol. For other technical details, see legends to Figures.

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