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ORIGINAL PAPER

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Archaeal elongation factor 1α from *Sulfolobus solfataricus* interacts with the eubacterial antibiotic GE2270A

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Abstract The thiazolyl-peptide antibiotic GE2270A, an inhibitor of the elongation factor Tu from *Escherichia coli* (*Ec*EF-Tu), was used to study the effects produced in the biochemical properties of the archaeal functional analogue elongation factor 1α from *Sulfolobus solfataricus* (*Ss*EF-1α). GE2270A did not substantially affect the poly(U)-directed-poly(Phe) incorporation catalyzed by *Ss*EF-1α and the formation of the ternary complex *Ss*EF-1α·GTP·Phe-tRNA^{Phe}. On the other hand, the antibiotic was able to increase the GDP/GTP exchange rate of *Ss*EF-1α; nevertheless, this improvement was not associated with an increase in the catalytic activity of the enzyme. In fact, GE2270A inhibited both the intrinsic GTPase of *Ss*EF-1α (GTPase^{Na}) and that stimulated by ribosomes. Interestingly, GTPase^{Na} of both intact and C-terminal-deleted *Ss*EF-1α resulted in a greater sensitivity to the antibiotic with respect to *Ss*EF-1α lacking both the M- and C-terminal domains. This result suggested that, similar to what is found for *Ec*EF-Tu, the M domain of *Ss*EF-1α is the region of the enzyme most responsible for the interaction with GE2270A. The different behavior observed in the inhibition of protein synthesis with respect to *Ec*EF-Tu can be ascribed to the different adaptive structural changes that have occurred in *Ss*EF-1α during evolution.

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Introduction

The archaeal elongation factor (EF)-1α from *Sulfolobus solfataricus* (*Ss*EF-1α), the functional homologue of the eubacterial elongation factor Tu (EF-Tu), belongs to the superfamily of GTPases (Dever et al. 1987) and is an essential component of the protein synthesis process (Masullo et al. 1991). Like elongation factor Tu from *Escherichia coli* (*Ec*EF-Tu), *Ss*EF-1α is also made of three structural domains: a GDP/GTP-binding domain (G domain), a middle (M)-, and a C-terminal domain (Vitagliano et al. 2001). The primary role of EF-Tu/*Ss*EF-1α is the delivery of the aminoacyl (aa)-tRNA to the A site of an mRNA-programmed ribosome during the elongation cycle (Abel and Journak 1996; Krab and Parmeggiani 1998). In its active form complexed with GTP, this EF carries the aa-tRNA on the ribosome (Bilgin et al. 1998; Raimo et al. 2000); following codon–anticodon recognition, GTP is hydrolyzed, and the resulting inactive form bound to GDP dissociates from the ribosome. The use of the antibiotic kirromycin—that, after GTP hydrolysis hinders the release of EF-Tu·GDP from the mRNA programmed ribosome (Krab and Parmeggiani 2002) and pulvomycin and GE2270A, both preventing the interaction between EF-Tu·GTP and aa-tRNA (Krab and Parmeggiani 2002)—has been of great help to elucidate the complex reaction mechanisms of the protein synthesis in eubacteria. These antibiotics do not seem to be specific for *Ec*EF-Tu only; in fact, kirromycin interacts with other EF-Tus purified from phychrophilic (Masullo et al. 2000), mesophilic (Smith and Paress 1978; Olsthoorn-Tieleman et al. 2002), and thermophilic eubacteria (Krasny et al. 2000) and with EF-1α from calf brain (Crechet and Parmeggiani 1986). Regarding the archaeal

protein synthesis instead, no inhibitory properties of kirromycin and pulvomycin were found (Cammarano et al. 1982), thus suggesting that the interaction with these antibiotics could be used as a phylogenetic marker for archaebacterial ancestry (Cammarano et al. 1985). However, in recent works, we were surprised to find that kirromycin was able to enhance the intrinsic GTPase activity of some *Ss*EF-1α mutants, but not that of the wild-type enzyme (Masullo et al. 2002a, b). In addition, fusidic acid, another eubacterial antibiotic acting on EF-G, was found to interact with its archaeal functional analogue *Ss*EF-2 (De Vendittis et al. 2002). These findings prompted us to investigate the effects of other eubacterial antibiotics on the biochemical properties of *Ss*EF-1α. The results reported in this paper showed that GE2270A was able to interact with *Ss*EF-1α by affecting its GTPase activity, though it did not have any relevant effect on protein synthesis.

Materials and methods

Chemicals, buffers, and enzymes

Labeled compounds and chemicals were as already reported (Masullo et al. 1997). The following buffers were used: buffer A—20 mM Tris-HCl (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$; buffer B—20 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 1 mM DTT, 3.6 M NaCl; and buffer C—50 mM imidazole·acetate (pH 7.5), 10 mM $MgCl_2$, 400 mM NaCl, 1 mM DTT. GE2270A was prepared as 50 mM stock solution in 100% dimethyl formamide (DMF) and stored at −80°C (Selva et al. 1991; Landini et al. 1992; Möhrle et al. 1997).

Elongation factor 1α from *Sulfolobus solfataricus* and its modified forms were produced and purified as already reported (Masullo et al. 1997; Arcari et al. 1999).

*Ss*EF-1α assays

Isolation of total tRNA, ribosomes, and other macromolecular component for poly(U)-directed poly(Phe) synthesis assay from *S. solfataricus* were performed as already described (Masullo et al. 1997). The preparation of [^{3}H]Phe-*Ec*tRNA^{Phe} or Phe- *Sc*tRNA^{Phe}, the formation of the ternary complex *Ss*EF-1α·[γ−^{32} P]·GTP·Phe-*Sc*tRNA^{Phe}, and the protection against spontaneous deacylation of [^{3} H]Phe-*Ec*tRNA^{Phe} were carried out as already described (Arcari et al. 1999; Raimo et al. 2000).

The ability of *Ss*EF-1α to exchange [^{3}H]GDP for GTP was assayed as described (Masullo et al. 1997).

The GTPase activity was measured in the presence of either 3.6 M NaCl (Masullo et al. 1994) or 1.6 μM ribosomes (M. Masullo, unpublished results). The reaction was followed kinetically at 60°C, and the amount of [^{32}P]$_i$ released was determined in 50-μl aliquots as already reported (Masullo et al. 1994).

The effect of GE2270A on the heat inactivation of *Ss*EF-1α was evaluated by incubating 4 μM protein in buffer A for 10 min at selected temperatures in the 70–99°C interval. After the heat treatment, 40-μl aliquots were cooled on ice for 30 min and then analyzed for their [^{3}H]GDP-binding ability as above reported. Unless otherwise indicated, in all the assays, the experiments carried out in the absence of GE2270A were performed in the presence of the same amount of DMF as carried over by antibiotic solution.

Native polyacrylamide electrophoresis at pH 4.3 was carried out on 15% gel as already reported (Reisfeld et al. 1962). Samples (20 μl) were diluted 1:1 with loading buffer and applied to the polyacrylamide gel; the electrophoresis run was carried out at 4°C, using a buffer at pH 4.5 as electrode solution. Gels were stained with Coomassie Brilliant Blue R-250.

Results

Effects of GE2270A on the functional properties of *Ss*EF-1α

The ability of GE2270A to inhibit protein synthesis in *Sulfolobus solfataricus* was evaluated on a purified cell-free system reconstituted in vitro with the components of protein synthesis isolated from this archaeon. As reported in Fig. 1, the antibiotic exerted only a very

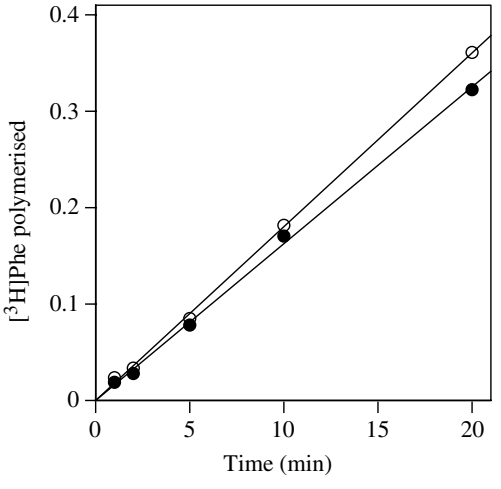

Fig. 1 Effect of GE2270A on the rate of poly(U)-directed poly(Phe) synthesis promoted by elongation factor 1α from *Sulfolobus solfataricus* (*Ss*EF-1α). A volume of 300 μl of the reaction mixture contained 25 mM Tris-HCl (pH 7.5), 19 mM magnesium acetate, 10 mM NH_4Cl, 10 mM dithiothreitol, 2.4 mM ATP, 1.6 mM GTP, 0.16 mg/ml poly(U), 3 mM spermine, 0.25 μM *Ss*ribosome, 80 μg/ml *Ss*tRNA, and 2.4 μM [^{3}H]Phe (specific activity, 5,432 cpm/pmol). The reaction was started by addition of 0.5 μM final concentration of *Ss*EF-1α in the absence (*open circles*) or presence (*filled circles*) of 50 μM GE2270A and carried out at 60°C. At the times indicated, 50-μl aliquots were withdrawn, chilled on ice, and then analyzed for the amount of [^{3}H]Phe incorporated, reported as mol [^{3}H]Phe polymerised·mol *Ss*EF-1α^{-1}·min^{-1}

weak inhibitory effect on the rate of poly[^{3}H]Phe synthesis catalyzed by *Ss*EF-1α.

In order to identify the partial reaction catalyzed by *Ss*EF-1α possibly affected by GE2270A, we tested the ability of *Ss*EF-1α to interact with aa-tRNA in the absence or in the presence of the antibiotic. As shown in Fig. 2, the amount of ternary complex formed between heterologous Phe-*Sc*tRNA^{Phe}, [$\gamma-^{32}$P]GTP, and *Ss*EF-1α was almost identical either in the absence or in the presence of GE2270A. This behavior was confirmed by determining the ability of *Ss*EF-1α to protect [^{3}H]Phe-*Ec*tRNA^{Phe} against spontaneous deacylation (Fig. 3).

Effect of GE2270A on the interaction between *Ss*EF-1α and guanine nucleotides

As shown in Fig. 4, the presence of 50 μM GE2270A caused a 1.6-fold faster [^{3}H]GDP/GTP exchange rate on *Ss*EF-1α; whereas in the case of [^{3}H]GDP/GDP exchange, both rates were almost identical.

The selective action of GE2270A on the *Ss*EF-1α·GTP was also evident by analyzing the relative mobility of *Ss*EF-1α on native PAGE (Fig. 5). In fact, the migration of *Ss*EF-1α·GDP was not changed after incubation with the antibiotic and was also identical to that of the *Ss*EF-1α·GTP complex. If the latter was incubated with GE2270A, a small retardation was observed instead.

To better evaluate this behavior, we tested the effect of the antibiotic on the intrinsic GTPase of *Ss*EF-1α triggered by high concentrations of NaCl (Masullo et al. 1994) or stimulated by *S. solfataricus* ribosomes (M. Masullo, unpublished results). The results shown in Fig. 6 clearly indicate that GE2270A affected the kinetics of both GTPases of *Ss*EF-1α, and the inhibition level depended on the antibiotic concentration (Fig. 7). An almost-complete inhibition was observed at 50 μM GE2270A for both GTPase^{Na} and GTPase^{r}. The concentrations of GE2270A for half-inhibition of the

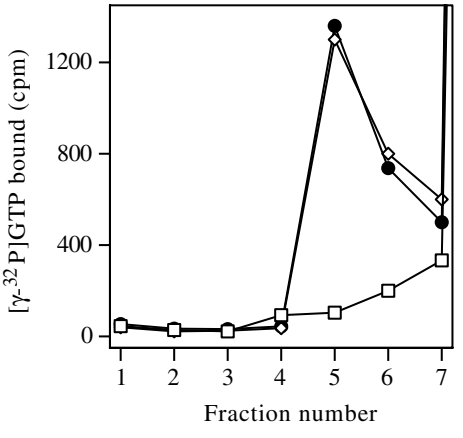

Fig. 2 Effect of GE2270A on the formation of the ternary complex between [$\gamma-^{32}$P]GTP, Phe-*Sc*tRNA^{Phe}, and *Ss*EF-1α. A volume of 35 μl of buffer A, in which 50 mM KCl was substituted for 60 mM NH_4Cl, contained 1 μM [$\gamma-^{32}$ P]GTP (specific activity, 8,300 cpm/pmol), 1 mM phosphoenolpyruvate, 40 μg/ml pyruvate kinase, 0.2 μM *Ss*EF-1α (*open squares*), and 1 μM Phe- *Sc*tRNA^{Phe} in the absence (*open circles*) or presence (*filled circles*) of 50 μM GE2270A. The reaction mixture was incubated for 10 min at 0°C, and then 30 μl was loaded onto a Sephadex G-25 column (0.4×15 cm), equilibrated, and eluted at 4°C with the reaction buffer. A volume of 100-μl fractions were collected and counted for radioactivity. Blanks run in the absence of *Ss*EF-1α were subtracted

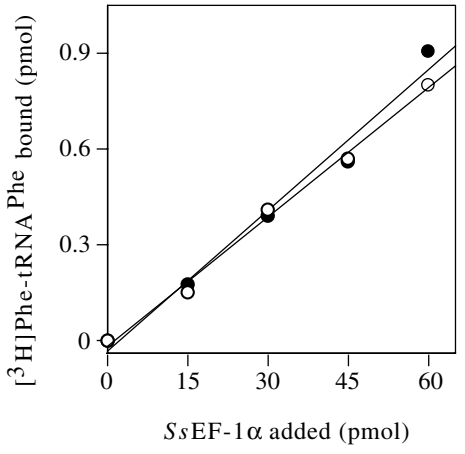

Fig. 3 Effect of GE2270A on the protection against spontaneous deacylation of [^{3}H]Phe-*Ec*tRNA^{Phe} by *Ss*EF-1α. A volume of 30 μl reaction mixture containing 25 mM Tris-HCl buffer (pH 7.8), 10 mM NH_4Cl, 10 mM DTT, 20 mM magnesium acetate, and 5.3 pmol of [^{3}H]Phe- *Ec*tRNA^{Phe} (specific activity 2,065 cpm/pmol) were preincubated for 1 h at 0°C to allow ternary complex formation in the presence of the indicated amount of *Ss*EF-1α·GppNHp complex, in the absence (*open circle*) or in the presence (*filled circle*) of 50 μM GE2270A. The deacylation reaction was carried out for 1 h at 50°C and the residual [^{3}H]Phe- *Ec*tRNA^{Phe} was determined as reported in Materials and methods

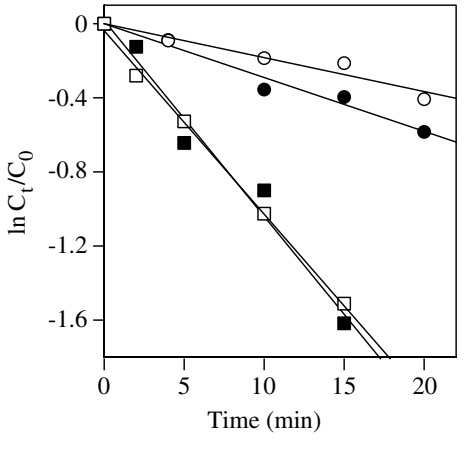

Fig. 4 Effect of GE2270A on the nucleotide exchange rate of *Ss*EF-1α. A volume of 200 μl reaction mixture contained 0.5 μM *Ss*EF-1α·[^{3}H]GDP (specific activity, 580 cpm/pmol) in buffer A. The exchange reaction was started by adding 1 mM final concentration of GDP (*squares*) or GTP (*circles*) in the absence (*open symbols*) or in the presence (*filled symbols*) of 50 μM GE2270A. At the times indicated, 40-μl aliquots were withdrawn, and the amount of residual radioactive binary complex was determined. Data were analyzed according to first-order kinetics

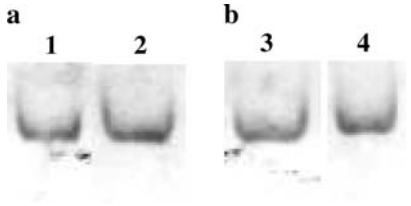

Fig. 5a, b Effect of GE2270A on the migration velocity of *Ss*EF-1α on nondenaturing PAGE. A sample of 20 μM of *Ss*EF-1α·GDP (**a**) and *Ss*EF-1α·GppNHp (**b**) was incubated for 20 min at 60°C in the absence (*lanes 1* and *3*, respectively) or in the presence of 100 μM GE2270A (*lanes 2* and *4*, respectively). Gels were run using electrophoresis buffers containing 10 μM GDP (**a**) or GppNHp (**b**)

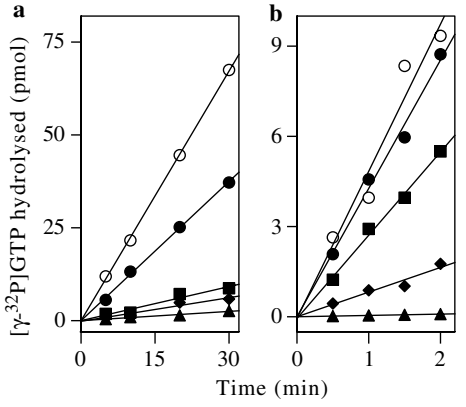

Fig. 6a, b Effect of GE2270A on the kinetics of GTPase of *Ss*EF-1α. **a** NaCl-dependent GTPase. A volume of 250 μl reaction mixture contained 0.3 μM *Ss*EF-1α and 50 μM [$\gamma-^{32}$P]GTP (specific activity, 230 cpm/pmol) in the absence (*open circles*) or in the presence of 5 μM (*filled circles*), 10 μM (*filled squares*), 20 μM (*filled diamonds*) or 50 μM (*filled triangles*) GE2270A in buffer B. **b** Ribosome-dependent GTPase. A volume of 250 μl reaction mixture contained 1.0 μM *Ss*EF-1α and 50 μM [$\gamma-^{32}$P]GTP (specific activity 184 cpm/pmol) in the absence (*open circles*) or in the presence of 5 μM (*filled circles*), 10 μM (*filled squares*), 20 μM (*filled diamonds*) or 50 μM (*filled triangles*) GE2270A in buffer C. The reaction was carried out at 60°C, and at the times indicated, 50-μl aliquots were withdrawn and analyzed for ^{32} P_i released as described in Materials and methods

GTPase of *Ss*EF-1α was calculated at about 7 μM for the GTPase^{Na} and 11 μM for GTPase^{r} (Fig. 7, inset).

Effect of GE2270A on the GTPase of engineered forms of *Ss*EF-1α

In order to identify the region of *Ss*EF-1α that might interact with GE2270A, we evaluated the effect of the antibiotic on the intrinsic GTPase activity of recombinant engineered forms of *Ss*EF-1α. As shown in Fig. 8, GE2270A was capable of inhibiting the NaCl-dependent GTPase of the isolated G and GM domains of *Ss*EF-1α. However, the concentration of antibiotic required to get half-inhibition of the GTPase of the isolated domain G of *Ss*EF-1α (14.8 μM) was 2.1- and 2.5-fold higher than that required for intact *Ss*EF-1α

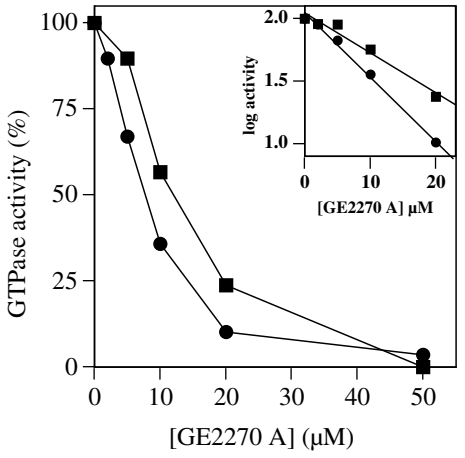

Fig. 7 Inhibition of the *Ss*EF-1α GTPase by GE2270A. The slope of the linear kinetics reported in Fig. 6 was plotted against GE2270A concentration as percentage of the activity measured in the absence of the antibiotic for NaCl-dependent (*filled circle*) or ribosome-dependent GTPase (*filled square*). *Inset* A graphical method used to calculate the concentration of antibiotic required to achieve half-maximum activity.

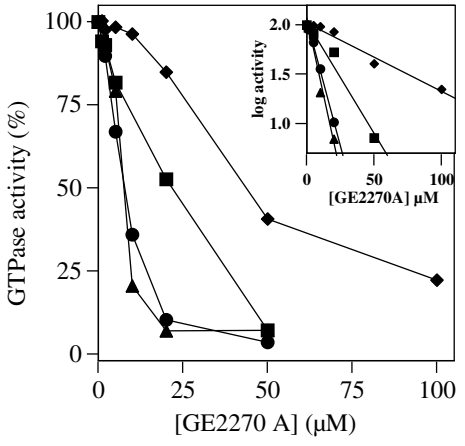

Fig. 8 Effect of GE2270A on the NaCl-dependent GTPase activity of engineered forms of *Ss*EF-1α. The experimental conditions are identical to those reported in Fig. 6a. The reaction was carried out by using 0.3 μM of *Ss*EF-1α (*filled circles*), *Ss*(G)EF-1α (*filled squares*), *Ss*(GM)EF-1α (*filled triangles*), or *Ss*(G) *Ec*(MC)EF-1α (*filled diamonds*)

(7 μM) and the GM domains of the factor (5.9 μM), respectively. Furthermore, we tested the effect of GE2270A on the GTPase of a chimeric EF containing the domain G of *Ss*EF-1α and the domains MC of *Ec*EF-Tu (Fig. 8). In this case, GE2270A was also able to inhibit the GTPase of the chimeric factor, but at a concentration 6.8-fold higher (46.3 μM, Fig. 8, inset) than that required for *Ss*EF-1α.

Effect of GE2270A on the thermostability of *Ss*EF-1α

The heat inactivation was tested by assaying the residual [^{3}H]GDP-binding ability of *Ss*EF-1α after 10-min

exposures to increasing temperatures in the absence or in the presence of the antibiotic (Fig. 9). We previously reported that *Ss*EF-1α was half-inactivated at 94°C (Arcari et al. 1999). However, when the protein solution contained a DMF concentration equal to that carried over by the antibiotic, the half-inactivation temperature lowered to 91°C. Under these conditions, the heat stability of *Ss*EF-1α was only a little affected by the antibiotic; in fact, the half-inactivation temperature became 90°C in the presence of GE2270A.

Discussion

Drugs that specifically inhibit protein synthesis in eubacteria or in eukaryotes could be used as probes to investigate the phylogenetic relationship between these two living domains and the archaeal domain (Woese et al. 1990). Most of the drugs that inhibit eubacterial ribosome have no effect on a poly(U)-directed cell-free system of either the methanogenic or the hyperthermophilic archaebacteria, thus suggesting evolutionary rather than adaptive structural differences at the ribosome level (Cammarano et al. 1985). However, recent studies have shown that some eubacterial antibiotics can interact with purified archaeal EFs (De Vendittis et al. 2002; Masullo et al. 2002a, b). GE2270A is a cyclic, thiazolylpeptide antibiotic isolated from *Planobispora rosea* that inhibits bacterial protein synthesis by acting on EF-Tu (Anborgh and Parmeggiani 1991). The antibiotic binds to EF-Tu in its GDP- or GTP-bound form, thereby blocking the elongation cycle. We have used this antibiotic to compare functional features between the archaeal translation EF-1α isolated from *Sulfolobus solfataricus* with that of its homologous EF-Tu from *Ec*. It is known that GE2270A specifically binds to the M domain of *Ec*EF-Tu·GDP (Heffron and Jurnak 2000), and that it inhibits poly(U)directed-poly(Phe) synthesis with an IC_{50} of 0.4 μM. In the case of *Ss*EF-1α, poly (Phe) synthesis was only very weakly inhibited by GE2270A. In this case, the antibiotic did not affect, as instead occurred in the case of EF-Tu, the binding of *Ss*EF-1α·GTP to aa-tRNA. In fact, neither the amount of heterologous ternary complex *Ss*EF-1α·GTP·Phe-*Sc*tRNA^{Phe}, nor the ability of *Ss*EF-1α to protect [^{3}H]Phe- *Ec*tRNA^{Phe} against spontaneous deacylation was affected by the antibiotic. GE2270A might therefore interact with *Ss*EF-1α·GTP, without affecting its interaction with aa-tRNA. Therefore, as opposed to what was reported for *Ec*EF-Tu (Anborgh and Parmeggiani 1991; Heffron and Jurnak 2000), the formation of the ternary complex *Ss*EF-1α·GTP·aa-tRNA hindered the interaction between the EF and the antibiotic. It is likely that the affinity of *Ss*EF-1α·GTP for aa-tRNA is higher than that for GE2270A. However, a partial superimposition of antibiotic and aa-tRNA-binding sites cannot be excluded.

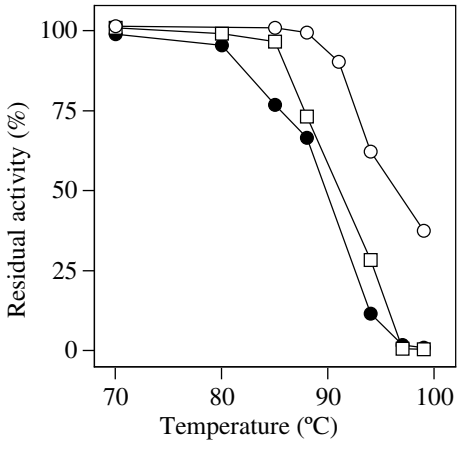

Fig. 9 Effect of GE2270A on the heat stability of *Ss*EF-1α. A sample of 4.0 μM *Ss*EF-1α was exposed for 10 min at the indicated temperatures in the absence (*open circle*) or in the presence (*filled circle*) of 50 μM GE2270A containing 6% dimethyl formamide (DMF), or 6% DMF alone (*open square*). The samples were chilled on ice for at least 30 min and then assayed for their residual [^{3}H]GDP-binding ability as reported in Materials and methods

We have also investigated the effect of GE2270A on the interaction of *Ss*EF-1α with GDP and GTP. As for EF-Tu, *Ss*EF-1α also displayed a higher affinity for GDP rather than for GTP (K_ds equal 1.6 and 35 μM, respectively, Masullo et al. 1991); although in this case, the difference in the affinity was only one order of magnitude higher. GE2270A moderately increased the [^{3}H]GDP/GTP exchange rate on *Ss*EF-1α by a factor of 1.6, and it had almost no effect on the [^{3}H]GDP/GDP exchange. Regarding the [^{3}H]GDP/GTP nucleotide exchange, the result obtained was opposite to what was observed for *Ec*EF-Tu (Anborgh and Parmeggiani 1991). However, the destabilizing effect of GE2270A was only marginal, and it was abolished when other macromolecular components of protein synthesis were present. In fact, under these conditions the nucleotide exchange on *Ss*EF-1α was very fast (not shown). The selectivity of GE2270A for *Ss*EF-1α·GTP was also evident on native PAGE. Indeed, the mobility of the *Ss*EF-1α·GTP complex was slightly retarded compared to *Ss*EF-1α·GDP in the presence of the antibiotic. This behavior was less evident with respect to that observed for *Ec*EF-Tu (Anborgh and Parmeggiani 1993; Möhrle et al. 1997), probably because the separation conditions used were different (pH 4.5 instead of pH 8 for *Ss*EF-1α and *Ec*EF-Tu, respectively). It is likely that the binding of GE2270A to *Ss*EF-1α affects the net charge of the EF only when complexed with GTP.

The increase in the GTP exchange rate on *Ss*EF-1α induced by GE2270A was not associated with an increase in the catalytic activity of the enzyme as the antibiotic inhibited both the *Ss*EF-1α GTPase^{Na} and the GTPase^{r}. In both cases, the effect observed was similar to that reported for *Ec*EF-Tu regarding the GTPase stimulated by 400 mM NH_4Cl in the absence or in the presence of ribosome (Anborgh and Parmeggiani 1991). However, both *Ss*EF-1α GTPase^{Na} and GTPase^{r} resulted in greater antibiotic sensitivities than those of

*Ec*EF-Tu because these activities were almost totally inhibited at a 50 μM concentration of antibiotic. In this condition, GE2270A should drastically reduce the rate of poly(Phe) incorporation catalyzed by *Ss*EF-1α. Therefore, the interactions of *Ss*EF-1α·GTP with the other macromolecular components involved in protein synthesis abolished the inhibitory effect of GE2270A on the GTPase^r. In particular, these interactions could induce on the EF conformational changes that did not allow the interaction with the antibiotic.

The availability of two truncated forms of *Ss*EF-1α corresponding to the G domain and the GM domains (Masullo et al. 1997) allowed an investigation of the region of the EF probably involved in the interaction with the antibiotic. GE2270A showed a stronger inhibitory effect on intact *Ss*EF-1α and on *Ss*EF-1α lacking the C-terminal domain, whereas it inhibited to a lesser extent the isolated domain G. These results suggested that the M domain of *Ss*EF-1α appear to be the region of the enzyme most responsible for the effects caused by GE2270A. However, the finding that the intrinsic GTPase of the isolated domain G was inhibited to a lesser extent by GE2270A could indicate that the site of the interaction between the antibiotic and *Ss*EF-1α also involved the G domain of the factor. In addition, these results suggested that the C domain of *Ss*EF-1α was not involved in the interaction with the antibiotic. Concerning the less marked inhibition by GE2270A of the GTPase of the chimeric EF, the effect can be ascribed to its higher catalytic efficiency as compared to the intact and truncated *Ss*EF-1α (Arcari et al. 1999). The analyses of the results obtained in this work are in good agreement with the reported 3-D structure of the *Ec*EF-Tu·GDP·GE2270A complex (Heffron and Jurnak 2000). In fact, GE2270A binds to the second domain of *Ec*EF-Tu·GDP, and on the basis of the sequence alignment between EF-Tu and *Ss*EF-1α (Arcari et al. 1994), only three out of nine residues in *Ec*EF-Tu that make the strongest interaction with the antibiotic are conserved in *Ss*EF-1α. These residues are Glu259, Asn273, Gly275 (that in *Ss*EF-1α correspond to Glu279), Asn293 and Gly295, respectively. In addition, there are two other similar positions, Phe261 and Val274 in *Ec*EF-Tu (that in *Ss*EF-1α correspond to His281 and Ile294, respectively). It is noticeable that all these residues are located in the M domain of *Ss*EF-1α. However, two amino acid residues that are crucial for the interaction between *Ec*EF-Tu and GE2270A (Arg223 and Gly257) are, in eukaryal and archaeal EF-1α, different and correspond to semi-invariant positions in these two living domains (Val/Ile and Ser/Thr) (Creti et al. 1994). These residues correspond in *Ss*EF-1α to Val245 and Ser277.

In conclusion, this study showed that although *Ss*EF-1α is more similar to eukaryal rather than eubacterial EF-Tu, it was able to interact with the inhibitor of eubacterial protein synthesis GE2270A. However, although the overall 3-D structure of *Ss*EF-1α·GDP resembles that of *Ec*EF-Tu, most of the structural requirements important for the interaction with GE2270A are changed in *Ss*EF-1α, probably because in the course of evolution it underwent very different environmental constraints.

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