Sensitivity of elongation factor Tu (EF-Tu) from different bacterial species to the antibiotics efrotomycin, pulvomycin and MDL 62879

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The sensitivity of elongation factor Tu (EF-Tu) from different species of bacteria to the EF-Tu-binding antibiotics efrotomycin, pulvomycin and MDL 62879 was tested by measuring the effect of these antibiotics on cell-free protein synthesis systems. EF-Tu from four different Gram-negative species was sensitive to all three antibiotics. Among Gram-positive bacteria, EF-Tu of *Bacillus subtilis*, *Staphylococcus aureus* and *Enterococcus faecalis* was resistant to efrotomycin and less sensitive to pulvomycin than EF-Tu of Gram-negative bacteria. EF-Tus from streptococci were significantly less sensitive than EF-Tus from Gram-negative bacteria to both efrotomycin and pulvomycin. All of the EF-Tus were sensitive to MDL 62879. The same sensitivity pattern emerged from GDP exchange assays, performed with partially purified EF-Tu from different bacterial species and pure *Escherichia coli* EF-Ts. These results suggest that the site of action of MDL 62879 is more conserved among bacterial species than those of efrotomycin and pulvomycin. Heterogeneity of EF-Tus from different bacterial species was also reflected in differences in their apparent molecular masses estimated by SDS-PAGE. EF-Tus from the Gram-positive species had higher molecular masses than those from all but one of the Gram-negative species.

Introduction

Elongation factor Tu (EF-Tu) is essential for bacterial protein synthesis and the gene encoding it (*tuf*) is highly conserved among eubacteria (Filer & Furano, 1980). Three different types of antibiotic have been found to be inhibitors of EF-Tu: the kirromycin class, pulvomycin and MDL 62879 (formerly GE 2270 A). These antibiotics differ in chemical structure (Fig. 1), in the nature of their interaction with EF-Tu (Fasano *et al.*, 1978; Wolf *et al.*, 1974; Anborgh & Parmeggiani, 1991) and in their spectrum of antibacterial activity (Selva *et al.*, 1991). All three antibiotics are natural products: kirromycins and pulvomycin are produced by different species of *Streptomyces*, MDL 62879 by *Planobispora rosea* (Parmeggiani & Swart, 1985; Akita *et al.*, 1963; Selva *et al.*, 1991).

Kirromycins stimulate the intrinsic GTPase activity of EF-Tu and block the protein in a non-dissociable ribosome-EF-Tu complex (Wolf *et al.*, 1974; Fasano *et al.*, 1978). MDL 62879 and pulvomycin inhibit the binding of aminoacyl-tRNA to EF-Tu. Pulvomycin increases the rates of association and dissociation of

GDP with EF-Tu; MDL 62879 does not affect these reactions (Anborgh & Parmeggiani, 1991; Pingoud *et al.*, 1982).

The antibacterial activities of the three antibiotics are quite different. MDL 62879 is active against Gram-positive bacteria (Selva *et al.*, 1991). Kirromycins are not active against some species of Gram-positive bacteria such as *Bacillus subtilis*, staphylococci and enterococci, but they are active against some Gram-negative bacteria such as *Neisseria gonorrhoeae* and *Haemophilus influenzae* (Frost *et al.*, 1976, 1979) on which MDL 62879 has little or no activity. Pulvomycin is also active against *N. gonorrhoeae* and *H. influenzae*; among Gram-positive species it is active against staphylococci and enterococci, but has very poor activity against streptococci (Akita *et al.*, 1963; Selva *et al.*, 1991).

For kirromycins, it has been reported that an intrinsically resistant EF-Tu is responsible for resistance in *B. subtilis* (Smith & Paress, 1978; Landini *et al.*, 1992), *Staphylococcus aureus* (Hall *et al.*, 1989) and some species of lactobacilli (Gloeckner & Wolf, 1984; Woerner & Wolf, 1982; Woerner *et al.*, 1983). We have studied the sensitivity to MDL 62879, efrotomycin (a member of the kirromycin class) and pulvomycin of EF-Tu from several bacterial species by using cell-free protein synthesis assays and GDP exchange reactions. The aim of our

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Fig. 1. Chemical structures of the antibiotics efrotomycin, pulvomycin and MDL 62879.

study was to further explore the extent to which the differences in the spectrum of antibacterial activity of the three antibiotics are mediated by EF-Tu or by other factors, such as penetration of the antibiotics into the bacteria, which is known to be the reason for resistance in *Escherichia coli* (Wolf *et al.*, 1974, 1978; Selva *et al.*, 1991).

Methods

Bacterial strains. The bacterial strains used for MIC determination and as sources of cell-free extracts are listed in Table 1.

Chemicals. MDL 62879 (1290 Da) and pulvomycin (838 Da) were prepared as described by Selva *et al.* (1991) at the Lepetit Research Center, Gerezano, Italy. Efrotomycin (1145 Da) was from Merck Sharp and Dohme. For MIC determination the antibiotics were dissolved in dimethylformamide (DMF) at 10 mg ml⁻¹ and diluted in water. For cell-free assays, MDL 62879 was dissolved at 50-fold the final concentration in 50% (v/v) DMF; efrotomycin and pulvomycin were dissolved in 10% DMF at 10-fold the final concentration. DMF (1%), always added to the controls, did not affect the assays. [2,6-³H]Phenylalanine and [8-³H]GDP were from Amersham. Electro-

phoresis reagents and molecular mass standards were from Bio-Rad. All other chemicals were from Sigma.

MIC determination. MICs were determined as in Selva *et al.* (1991).

Preparation of cell-free protein synthesis systems. Bacteria (1 litre culture) were grown to early exponential phase (OD₅₉₀ 0.2–0.6) and centrifuged at 13000 g for 10 min at 4 °C. The cells were resuspended in 20 ml of 0.85% NaCl and recentrifuged.

The bacteria were disrupted as follows. *B. subtilis*, *E. coli* and *Pseudomonas aeruginosa* were broken by sonication. The cell paste was suspended in 10 ml 10 mM-Tris/HCl, pH 7.7, 10 mM-MgCl₂, 10 mM-NH₄Cl, 3 mM-DTT, 0.25 mM-PMSF and leupeptin (10 µg ml⁻¹), and sonicated on ice (10 cycles of 30 s with intervals of 30 s). *N. gonorrhoeae* and *H. influenzae* cell pastes were resuspended in 10 ml protoplasting buffer (50 mM-Tris/HCl, pH 7.9, 5 mM-EDTA, 3 mM-DTT, 25%, w/v, sucrose, 0.25 mM-PMSF, 10 µg leupeptin ml⁻¹) and then incubated with lysozyme (100 µg ml⁻¹), lysostaphin (100 µg ml⁻¹) and mutanolysin (20 µg ml⁻¹) for 30 min at 37 °C followed by overnight incubation on ice. The protoplasts were pelleted by centrifugation at 30000 g for 30 min at 4 °C. The supernatant was collected, and the protoplasts were resuspended in 10 ml protoplasting buffer without sucrose and homogenized with a Dounce homogenizer (12 strokes on ice). The homogenate was incubated at 37 °C for 30 min and centrifuged at 30000 g for 30 min at 4 °C. The two supernatants were pooled and dialysed overnight against 10 mM-Tris/HCl, pH 7.5, 10 mM-MgCl₂, 0.25 mM-PMSF.

Staph. aureus and *Enterococcus faecalis* were lysed using the enzymic method described by Georgopapadakou *et al.* (1982). *Streptococcus pyogenes* and *Strep. gordonii* were lysed as described by Siegel *et al.* (1981), except that lysozyme (200 µg ml⁻¹) and lysostaphin (20 µg ml⁻¹) were added as well as mutanolysin (20 µg ml⁻¹).

All cell lysates were centrifuged at 30000 g for 30 min and the supernatant (S-30) was collected. The various S-30 obtained were centrifuged at 100000 g for 4 h at 2 °C. The ribosomal pellets were discarded, except for ribosomes from *E. coli*, which were resuspended in one-tenth of the S-30 volume in 10 mM-Tris/HCl, pH 7.7, 10 mM-MgCl₂ and washed with 0.6 M-NH₄Cl to remove adherent protein factors as described by Traub *et al.* (1971). The supernatants (S-100) were concentrated by ammonium sulphate fractionation at 30–65% of saturation (Ravel & Shorey, 1971). The 65% ammonium sulphate precipitate was resuspended in one-fifth of the S-100 volume in 10 mM-Tris/HCl, pH 7.7, 10 mM-MgCl₂, 0.25 mM-PMSF. This fraction (termed S-100') was used as the source of protein synthesis factors and for purification of EF-Tu.

Poly(U)-directed poly(Phe) synthesis. Poly(U)-directed poly(Phe) synthesis was performed as described by Traub *et al.* (1971), using a

Table 1. Antibacterial activity of efrotomycin, pulvomycin and MDL 62879

MIC determinations were performed in two independent experiments with the same results.

Species*	MIC (µg ml ⁻¹)		
	Efrotomycin	Pulvomycin	MDL 62879
<i>E. coli</i> K12 HfrC	> 128	> 128	> 128
<i>N. gonorrhoeae</i> L 997	0.25	0.13	32
<i>H. influenzae</i> ATCC 19418	4	16	128
<i>P. aeruginosa</i> ATCC 10145	> 128	> 128	> 128
<i>B. subtilis</i> ATCC 6633	> 128	2	0.13
<i>Ent. faecalis</i> ATCC 7080	> 128	16	0.25
<i>Staph. aureus</i> L 165	> 128	8	0.25
<i>Strep. pyogenes</i> L 49	16	128	0.25
<i>Strep. gordonii</i> Challis	8	128	0.13

* L, Lepetit Strain Collection; ATCC, American Type Culture Collection, Rockville, MD, USA.

mixed system in which NH_4Cl -washed ribosomes (5 pmol) were from *E. coli* and S-100' from different bacteria. The S-100' added for the cell-free assays contained 3–8 μg protein ml^{-1} . Assays were performed in 100 μl 30 mM-Tris/HCl, pH 7.7, 10 mM- MgCl_2 , 80 mM- NH_4Cl , 3 mM-DTT, 5 mM-GTP, 4 mM-ATP with 80 μg poly(U) and 50 pmol [^3H]phenylalanine [specific activity 12 Ci mmol^{-1} (1 Ci = 37 GBq)]. Incubation was for 15 min at 30 °C. Protein synthesis was stopped by adding trichloroacetic acid to a final concentration of 5% (w/v). The samples were heated for 10 min at 80 °C, cooled on ice and filtered on glass fibre filters using a cell-harvester (LKB).

Partial purification of EF-Tu. EF-Tu from the various species was partially purified by GDP-sepharose chromatography of the S-100' fraction. The S-100' fraction was made up to 0.35 M-NaCl and loaded on the column resin [2 mg protein (ml resin) $^{-1}$] which had been equilibrated with buffer A (20 mM-Tris/HCl, pH 7.9, 10 mM- MgCl_2 , 5 mM-DTT, 0.25 mM-PMSF, 0.35 M-NaCl). The column was washed with 5 vols buffer A and EF-Tu was eluted with buffer A containing 2 mM-GDP. The degree of purification was monitored by SDS-PAGE (20% acrylamide, 0.48% bisacrylamide) in a Phastgel Electrophoresis system (Pharmacia).

Purified EF-Tus from various species were tested for their ability to stimulate poly(Phe) synthesis in the presence of NH_4Cl -washed ribosomes and purified EF-Ts and EF-G from *E. coli*. The assay mixture contained 5 pmol ribosomes, 10 pmol EF-Ts, 10 pmol EF-G, 12 pmol [^3H]phenylalanyl-tRNA, charged and isolated as described by Ravel & Shorey (1971), and partially purified EF-Tu (0.25 μg , approx. 5 pmol) from various species. Otherwise, the assay conditions were the same as for poly(U) assays performed with S-100. EF-Ts and EF-G were purified as described by Leberman *et al.* (1980).

GDP exchange assays. GDP exchange assays were performed as described by Fasano *et al.* (1978) using approximately 10 pmol EF-Tu-GDP (unlabelled) from various bacterial species, 2 pmol EF-Ts from *E. coli*, and 100 pmol [^3H]GDP (specific activity 10 Ci mmol^{-1}). Incubation was for 5 min at 0 °C in a final volume of 100 μl of reaction buffer (80 mM- NH_4Cl , 50 mM-Tris/HCl, pH 7.8, 10 mM- MgCl_2 , 3 mM-DTT). Reactions were stopped by the addition of 1 ml cold reaction buffer. Samples were immediately filtered on nitrocellulose filters (HAWP0045, Millipore) and the filters were washed with 6 ml of buffer.

Determination of radioactivity. Glass fibre filters were counted in a β -plate scintillation counter (Pharmacia). Nitrocellulose filters were dried and then counted by liquid scintillation.

Results

Antibacterial activity

MIC values of the three antibiotics, determined on aliquots of the cultures used to prepare S-100', are shown in Table 1. The results are in good agreement with data from the literature (Frost *et al.*, 1979; Selva *et al.*, 1991).

Cell-free protein synthesis

Table 2 shows the IC_{50} (the extrapolated antibiotic concentration at which the reaction is inhibited by 50%) of efrotomycin, pulvomycin and MDL 62879 against S-100' from Gram-negative bacteria. All of these cell-free protein synthesis systems were very sensitive to the three antibiotics, with little difference in the IC_{50} values.

The IC_{50} values of efrotomycin, pulvomycin and MDL 62879 in cell-free extracts of Gram-positive bacteria are shown in Table 3. Cell-free protein synthesis in extracts from kirromycin-resistant bacteria was unaffected by efrotomycin ($\text{IC}_{50} > 100 \mu\text{g ml}^{-1}$ for *B. subtilis* and *Staph. aureus*, and 50 $\mu\text{g ml}^{-1}$ for *Ent. faecalis*), suggesting that in these species resistance to efrotomycin is mediated by EF-Tu. Pulvomycin was less active in cell-free extracts from *Staph. aureus* and *Ent. faecalis* (IC_{50} 0.7 and 1.1 $\mu\text{g ml}^{-1}$, respectively) than in *B. subtilis* (IC_{50} 0.21 $\mu\text{g ml}^{-1}$) or in Gram-negative bacteria (Table 2). MDL 62879 was active at concentrations of 0.02–0.12 $\mu\text{g ml}^{-1}$ in cell-free systems from *Staph. aureus*, *Ent. faecalis* and *B. subtilis*.

The streptococcal cell-free protein synthesis systems responded differently as compared both to the Gram-negative and to the other Gram-positive bacteria. Both efrotomycin (IC_{50} 0.65 for *Strep. pyogenes* and 1 $\mu\text{g ml}^{-1}$

Table 2. Activity of efrotomycin, pulvomycin and MDL 62879 in cell-free poly(Phe) synthesis using S-100' from Gram-negative bacteria

IC_{50} values are the means of two independent experiments which gave very similar results. Ribosomes (5 pmol) were from *E. coli*. S-100' added contained 3–8 μg protein. The assay mixture also contained: 80 μg poly(U), 50 pmol [^3H]phenylalanine (12 Ci mmol^{-1}), 0.6 μg *E. coli* tRNA^{Phe}, 30 mM-Tris/HCl, pH 7.7, 10 mM- MgCl_2 , 80 mM- NH_4Cl , 3 mM-DTT, 5 mM-GTP and 4 mM-ATP.

Source of S-100'	Control (pmol)*	IC_{50} ($\mu\text{g ml}^{-1}$)		
		Efrotomycin	Pulvomycin	MDL 62879
<i>E. coli</i>	9.48	0.02	0.06	0.04
<i>P. aeruginosa</i>	9.93	0.04	0.06	0.04
<i>H. influenzae</i>	8.74	0.07	0.3	0.05
<i>N. gonorrhoeae</i>	2.88	0.08	0.02	0.02

* [^3H]Phenylalanine incorporated in 15 min at 30 °C. *E. coli* ribosomes without the addition of S-100' incorporated 0.03 pmol.

Table 3. Activity of efrotomycin, pulvomycin and MDL 62879 in cell-free poly(Phe) synthesis using S-100' from Gram-positive bacteria

IC_{50} values are the means of two independent experiments which gave very similar results. Conditions as described in the legend to Table 2.

Source of S-100'	Control (pmol)*	IC_{50} ($\mu\text{g ml}^{-1}$)		
		Efrotomycin	Pulvomycin	MDL 62879
<i>B. subtilis</i>	6.36	> 100	0.21	0.12
<i>Staph. aureus</i>	3.40	> 100	0.7	0.02
<i>Ent. faecalis</i>	0.67	50	1.1	0.02
<i>Strep. pyogenes</i>	4.55	0.65	2.45	0.18
<i>Strep. gordonii</i>	7.96	1.0	2.05	0.11

* [^3H]Phenylalanine incorporated in 15 min at 30 °C. *E. coli* ribosomes without the addition of S-100' incorporated 0.03 pmol.

Fig. 2. SDS-PAGE of partially purified EF-Tus from different bacterial species. Lanes A and M, molecular mass standards (phosphorylase *b*, 97.4 kDa; bovine serum albumin, 66; ovalbumin, 45; carbonic anhydrase, 30; soybean trypsin inhibitor, 21.4; lactalbumin, 14.2). Lanes B–L: EF-Tu (estimated molecular masses in parentheses) from B, *N. gonorrhoeae* (42.5 kDa); C, *H. influenzae* (47); D, *P. aeruginosa* (42.5); E, *E. coli* (42.5); F, *B. subtilis* (45); G, *Strep. pyogenes* (48); H, *Strep. gordonii* (48); I, *Ent. faecalis* (52); L, *Staph. aureus* (50.5).

Table 4. Effect of efrotomycin, pulvomycin or MDL 62879 (each at 20 µg ml⁻¹) on EF-Tu-GDP/[³H]GDP exchange mediated by EF-Tu from various bacterial species and EF-Ts from *E. coli*

Results are expressed as pmol [³H]GDP bound to EF-Tu in 5 min at 0 °C (means from two experiments, each with duplicate determinations; SD in parentheses). The experimental values are also shown as percentages of the control, calculated from the mean values of the four determinations.

Species	Control (pmol)	Efrotomycin		Pulvomycin		MDL 62879	
		(pmol)	(%)	(pmol)	(%)	(pmol)	(%)
<i>Staph. aureus</i>	0.64 (0.02)	0.62 (0.02)	97	0.51 (0.04)	80	0.63 (0.01)	98
<i>Ent. faecalis</i>	0.30 (0.01)	0.29 (0.01)	97	0.25 (0.01)	83	0.31 (0.01)	103
<i>Strep. pyogenes</i>	0.80 (0.04)	0.61 (0.02)	76	0.73 (0.06)	91	0.81 (0.04)	101
<i>Strep. gordonii</i>	0.76 (0.10)	0.54 (0.03)	71	0.62 (0.02)	82	0.73 (0.02)	96
<i>B. subtilis</i>	1.69 (0.14)	1.79 (0.06)	106	1.47 (0.02)	87	1.75 (0.16)	104
<i>E. coli</i>	2.26 (0.05)	0.87 (0.07)	39	1.07 (0.10)	47	2.23 (0.04)	99
<i>P. aeruginosa</i>	0.96 (0.07)	0.42 (0.02)	44	0.46 (0.04)	48	0.94 (0.11)	98
<i>N. gonorrhoeae</i>	2.96 (0.09)	1.03 (0.07)	35	1.41 (0.08)	48	2.78 (0.04)	94
<i>H. influenzae</i>	3.20 (0.16)	1.19 (0.01)	37	1.57 (0.18)	49	3.08 (0.25)	96

for *Strep. gordonii*) and pulvomycin (IC₅₀ 2.45 and 2.0 µg ml⁻¹) were less active here than in Gram-negative systems. In contrast, the IC₅₀ of MDL 62879 were similar to those obtained for other bacteria (0.18 and 0.11 µg ml⁻¹ for *Strep. pyogenes* and *Strep. gordonii*, respectively).

Purification of EF-Tu

The EF-Tus of all of the species considered were partially purified by affinity chromatography on GDP-Sepharose. SDS-PAGE of the partially purified EF-Tus, with their apparent molecular masses, is shown in Fig. 2. Gram-

positive bacteria appear to have EF-Tus with higher molecular masses than those of most of Gram-negative bacteria. *E. coli*, *P. aeruginosa* and *N. gonorrhoeae* had an EF-Tu with the same molecular mass (42.5 kDa); *H. influenzae* was the only Gram-negative species whose EF-Tu (47 kDa) appeared to be larger than those of some of the Gram-positive species.

These EF-Tu preparations stimulated cell-free poly-(Phe) synthesis in the presence of washed ribosomes, EF-Ts and EF-G from *E. coli*, suggesting that the bands seen on SDS-PAGE do represent the EF-Tu of the various species. The system without EF-Tu incorporated 0.06 pmol phenylalanine. The degree of stimulation obtained with approximately 5 pmol EF-Tu from different species ranged from sevenfold with EF-Tu from *Ent. faecalis* to about 100-fold for EF-Tu from Gram-negative bacteria (data not shown).

GDP-exchange assays

The effect of efrotomycin, pulvomycin and MDL 62879 on EF-Tu-GDP/[³H]GDP exchange (GDP-exchange) in the presence of EF-Ts from *E. coli* was tested using partially purified EF-Tu from the various species (Table 4). MDL 62879, at a concentration of 20 µg ml⁻¹, had no effect on the GDP-exchange reaction mediated by any of the EF-Tus. With EF-Tu from Gram-negative bacteria, efrotomycin inhibited GDP-exchange by about 60%. With streptococcal EF-Tu, the reaction was only slightly inhibited by efrotomycin (by 24% for *Strep. pyogenes* and 29% for *Strep. gordonii*), and there was no inhibition when EF-Tus from *B. subtilis*, *Staph. aureus* or *Ent. faecalis* were used. With EF-Tu from Gram-negative bacteria, pulvomycin inhibited GDP-exchange by about 50%, whereas it only slightly inhibited the reaction with EF-Tu of Gram-positive species (10–20%).

Discussion

EF-Tu from *E. coli* had already been reported to be sensitive to efrotomycin, pulvomycin and MDL 62879, indicating that these antibiotics do not penetrate into *E. coli* cells (Wolf *et al.*, 1974, 1978; Selva *et al.*, 1991). Our results suggest that the mechanism of resistance is the same for all three antibiotics in the case of *P. aeruginosa*, and for MDL 62879 in *N. gonorrhoeae* and *H. influenzae*. Our data also indicate that the binding sites of the three inhibitors are highly conserved among Gram-negative species.

The situation is more complex in Gram-positive bacteria. MDL 62879 is the only EF-Tu inhibitor which was active against all of the Gram-positive species tested, inhibiting both cell growth and cell-free protein synthesis

(Tables 1 and 3). Thus the binding site of MDL 62879 appears to be highly conserved in EF-Tu from Gram-positive bacteria as well as in Gram-negative species.

Efrotomycin did not inhibit cell-free protein synthesis in cell extracts from *B. subtilis*, *Staph. aureus* or *Ent. faecalis*, which are resistant to this antibiotic. For *B. subtilis* and *Staph. aureus* (as well as for some species of lactobacilli) similar observations have been reported for the kirromycins (Smith & Paresse, 1978; Woerner & Wolf, 1982; Hall *et al.*, 1989). Therefore, naturally occurring resistance to kirromycins in Gram-positive bacteria seems to be mediated by a resistant EF-Tu. Cell-free systems from streptococci were also somewhat less sensitive to efrotomycin than those from Gram-negative bacteria. Thus, the kirromycin binding site on EF-Tu of Gram-positive bacteria appears to be less conserved than that of Gram-negative species.

Among the Gram-positive bacteria considered, *B. subtilis* was the most sensitive to pulvomycin in terms of MIC (2 µg ml⁻¹) and in sensitivity of cell-free protein synthesis (IC₅₀ 0.21 µg ml⁻¹). *Staph. aureus* and *Ent. faecalis* were less sensitive to pulvomycin (MIC 8 and 16 µg ml⁻¹, respectively) and also showed a reduced sensitivity in cell-free assays (IC₅₀ 0.7 and 1.1 µg ml⁻¹, respectively). In streptococcal cell-free assays the activity of pulvomycin (IC₅₀ 2.0 and 2.45 µg ml⁻¹) indicates a lower sensitivity of their EF-Tu. However, the MIC of pulvomycin for these bacteria is particularly high (128 µg ml⁻¹) and may not be totally explained by reduced interaction of pulvomycin with EF-Tu. In any event, our data suggest that the pulvomycin-binding site is not highly conserved among Gram-positive bacteria.

The effects of efrotomycin, pulvomycin and MDL 62879 on the GDP-exchange reaction have been described for EF-Tu and for EF-Tu:EF-Ts (EF-T) from *E. coli*. Efrotomycin and pulvomycin stimulate GDP exchange on EF-Tu (Van der Meide *et al.*, 1980; Wolf *et al.*, 1974, 1978). EF-Ts enhances the rate of GDP-exchange, but this effect disappears in the presence of efrotomycin or pulvomycin, possibly because these antibiotics compete with EF-Ts in binding to EF-Tu (Fasano *et al.*, 1978; Parmeggiani & Swart, 1985).

We performed GDP-exchange assays in the presence of pure EF-Ts from *E. coli*. MDL 62879 does not affect this reaction either with *E. coli* EF-Tu alone (Anborgh & Parmeggiani, 1991) or with EF-Tu from different bacterial species in the presence of EF-Ts (Table 4). The effects of efrotomycin and pulvomycin on the GDP-exchange reaction with EF-Tu from different species and *E. coli* EF-Ts paralleled in most cases the sensitivity patterns of the various EF-Tus in the poly(Phe) synthesis assays. Thus, efrotomycin did not inhibit exchange with EF-Tu from the efrotomycin-resistant bacteria, and only slightly inhibited EF-Tu from *Strep. pyogenes* or *Strep.*

gordonii. Pulvomycin inhibited GDP-exchange with EF-Tu from Gram-negative species, but only slightly in Gram-positive species; even EF-Tu from *B. subtilis*, which was the most sensitive to pulvomycin among Gram-positive bacteria in the protein synthesis assay, was only slightly inhibited in GDP-exchange experiments.

It has already been reported that there is some degree of heterogeneity in the molecular masses of EF-Tu from different bacterial species as estimated by SDS-PAGE (Woerner *et al.*, 1983). The EF-Tus of the Gram-positive bacteria we studied had apparent molecular masses ranging from 45 to 52 kDa, whereas those from the Gram-negative bacteria ranged from 42.5 to 47 kDa (Fig. 1). Previous SDS-PAGE estimates of the molecular mass of EF-Tu from *B. subtilis* and *E. coli* were 47 and 44 kDa, respectively (Woerner *et al.*, 1983), values similar to those estimated by us (45 and 42.5 kDa). In the case of *E. coli* EF-Tu, the SDS-PAGE estimates are close to the molecular mass calculated from the amino acid sequence (43 225 Da; Arai *et al.*, 1980). Hall *et al.* (1991) reported a molecular mass of 41 kDa for the EF-Tu of *Staph. aureus*, which is much lower than our estimate of 50.5 kDa. It is possible that Hall *et al.* (1991) encountered degradation problems during their purification of *Staph. aureus* EF-Tu.

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