Conformational Change of Elongation Factor Tu (EF-Tu) Induced by Antibiotic Binding

CRYSTAL STRUCTURE OF THE COMPLEX BETWEEN EF-Tu-GDP AND AURODOX*

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Conformational Change of Elongation Factor Tu (EF-Tu) Induced by Antibiotic Binding

Aurodox is a member of the family of kirromycin antibiotics, which inhibit protein biosynthesis by binding to elongation factor Tu (EF-Tu). We have determined the crystal structure of the 1:1:1 complex of Thermus thermophilus EF-Tu with GDP and aurodox to 2.0-Å resolution. During its catalytic cycle, EF-Tu adopts two strikingly different conformations depending on the nucleotide bound: the GDP form and the GTP form. In the present structure, a GTP conformation-like conformation of EF-Tu is observed, although GDP is bound to the nucleotide-binding site. This is consistent with previous proposals that aurodox fixes EF-Tu on the ribosome by locking it in its GTP form. Binding of EF-Tu-GDP to aminoacyl-tRNA and mutually exclusive binding of kirromycin and elongation factor Ts to EF-Tu can be explained on the basis of the structure. For many previously observed mutations that provide resistance to kirromycin, it can now be understood how they prevent interaction with the antibiotic. An unexpected feature of the structure is the reorientation of the His-85 side chain toward the nucleotide-binding site. We propose that this residue stabilizes the transition state of GTP hydrolysis, explaining the acceleration of the reaction by kirromycin-type antibiotics.

Aurodox, an antibiotic excreted by Streptomyces goldiniensis, is the N-methyl derivative of kirromycin (occasionally also named mocimycin), which is the prototype of a large family of antimicrobial agents (1, 2). In all aspects investigated, aurodox (Fig. 1) shows identical effects to the more thoroughly studied kirromycin (3). These antibiotics have been shown to interfere with the role of bacterial elongation factor Tu (EF-Tu) in protein biosynthesis (4). EF-Tu is a vital component of the protein biosynthesis machinery. In its active GTP complex, the 44-kDa protein binds aminoacyl-tRNA (aa-tRNA) and delivers it to the A site of the messenger RNA-programmed ribosome.

Upon codon/anticodon interaction, GTP is hydrolyzed, leading to a major conformational change of EF-Tu (5–8). This in turn triggers the subsequent release of EF-Tu-GDP from the ribosome. Fast regeneration of active EF-Tu-GTP from the inactive GDP form requires catalysis by the nucleotide exchange factor EF-Ts.

Kirromycin interrupts the functional cycle of EF-Tu by inhibiting the release of the EF-Tu-GDP complex from the ribosome. The resulting stalled ribosome causes a polysomal traffic jam on the mRNA. In accordance with this mechanism, kirromycin sensitivity is dominant over kirromycin resistance. The disability of EF-Tu-GDP to dissociate from the ribosome in the presence of kirromycin is due to the formation of a quaternary complex with aa-tRNA and the antibiotic (1, 9). In agreement with this, it has been shown in vitro that EF-Tu-GDP has significant affinity (KD = 10 μM) for aa-tRNA in the presence but not in the absence of kirromycin (10). A possible explanation for this could be that in this complex, the EF-Tu adopts a conformation similar to its GTP form even when bound to GDP (11). Supporting evidence for this idea is provided by the electrophoretic behavior (12) and by sensitivity toward proteolytic cleavage (11).

Further activities of kirromycin (1) are (i) stimulation of the intrinsic GTPase activity of EF-Tu, (ii) decrease (by 3 orders of magnitude) of aa-tRNA binding to EF-Tu-GTP, (iii) inhibition of EF-Ts binding, (iv) increase of the EF-Tu nucleotide exchange rate for GDP (while affinity is largely unchanged), (v) increase of the affinity of EF-Tu for GTP by a factor of at least 100, and (vi) prevention of phosphorylation of EF-Tu (13). No crystal structure of an EF-Tu complex with kirromycin has been published to date, although crystals of a quaternary complex EF-Tu-GppNHpPhe-tRNAPhos-kirromycin diffracting to 3.5 Å have been reported (14). The crystal structure of a complex between EF-Tu-GDP and GE2270A, an antibiotic unrelated to kirromycin, has been published recently (15).

Here we present the crystal structure of the 1:1:1 complex of Thermus thermophilus EF-Tu with GDP and aurodox at 2.0-Å resolution. The EF-Tu from this organism shares 82% sequence identity with the protein from Escherichia coli and has been shown to be similarly sensitive to kirromycin (16). The crystal structure presented here nicely explains the mutations reported to confer resistance to EF-Tu against the antibiotic (17–20).

EXPERIMENTAL PROCEDURES

Preparation of the Ternary Complex EF-Tu-GDP/Aurodox—Since aurodox was reported to be moderately sensitive to light (21), exposure was kept at a minimum. Because of its extremely low solubility in water, a 120 mM alcoholic solution was prepared. T. thermophilus EF-Tu was overexpressed and purified as a GDP complex as previously described (22). The protein was then dialyzed into buffer (20 mM sodium
Crystal Structure of EF-Tu-GDP-Aurodox

cacodylate, pH 7.0, 100 mM ammonium sulfate, 5 mM magnesium sulfate, 20 μM GDP) and concentrated to 9–12 mg/ml by ultrafiltration. The EF-Tu-GDP-aurodox complex was prepared by adding aurodox at a 5–7-fold molar excess to the protein solution, followed by incubation overnight. A minor precipitate was removed by centrifugation before crystallization set-ups were prepared. Nearly complete formation of the EF-Tu-GDP-aurodox complex was verified by native gel electrophoresis. As described by Mesters et al. (17), the EF-Tu-GDP-aurodox complex showed a clear band shift compared with the aurodox-free EF-Tu-GDP. Chemicals used were obtained from Sigma or Merck in pro analysis quality.

Crystallographic Data

The crystals were grown using the hanging drop vapor diffusion method. 2–4 μl of solution of this ternary complex were mixed with the same amount of reservoir solution (50 mM Tris, 200 mM sodium acetate, 33–35% polyethylene glycol 4000, final pH 7.8–9.0). Within 10 days in the dark at 19 °C, platelet-like crystals grew to a maximum size of 450 μm × 350 μm × 60 μm. Purification of the complex by gel filtration (Superdex 200 HR 10/30; Amersham Pharmacia Biotech) prior to crystallization did not improve the crystal quality.

Collection of Diffraction Data—Crystals were removed from the crystallization droplet with a nylon loop and immediately flash-cooled in a 100 K nitrogen stream generated by an Oxford Cryostream cooling system (Oxford Cryosystems, Oxford, United Kingdom). No further cryoprotectant was needed in addition to the polyethylene glycol in the mother liquor. Diffraction data were initially collected using an FR591 rotating copper anode x-ray generator (NOMIUS, Delft, The Netherlands) equipped with a 30-cm Mar Research image plate (X-ray Research, Hamburg, Germany). Data extending to 2.6 Å were collected over a crystal rotation range of 124° in two batches, as recommended by the program STRATEGY (23). A full data set to 2.0-Å resolution was subsequently collected using the Joint IMB Jena-University of Hamburg-EMBL synchrotron beamline X13 at Deutsches Elektronen-Synchrotron, Hamburg, at a wavelength of 0.913 Å. Diffraction intensities were recorded using a Mar Research CCD detector (X-ray Research, Hamburg, Germany). Data collection parameters are given in Table I.

Data Processing, Structure Solution, and Refinement—Diffraction data were indexed, integrated, and scaled using the HKL package (24). TRUNCATE (25) was used to derive structure amplitudes from the measured intensities. The space group was determined to be P2_1, with unit cell dimensions a = 69.15 Å, b = 101.00 Å, c = 79.15 Å, α = 90°, β = 113.47°. This unit cell has not been observed before for any EF-Tu crystal. The asymmetric unit was assumed to contain two ternary complexes, corresponding to a packing density of 2.9 Å³/Da and a solvent content of ~58% (26).

Using the diffraction data collected with CuKα radiation, molecular replacement was carried out using the program AMoRe (27). Based on the assumption that in the aurodox complex, a new EF-Tu conformation could exist as a consequence of a reorientation of domain 1 relative to the other two domains, domain 1 and domain 2/3 were treated as independent search molecules. In two different runs, the domains were taken from the structures of either Thermus aquaticus EF-Tu-GDP (8) or T. thermophilus EF-Tu-GppNHp (5), leading to equivalent solutions (R = 0.420 and 0.461, correlation coefficient 0.520 and 0.547, respectively, for the resolution range between 15.0 and 3.5 Å). The correctness of the solution was indicated by the equivalent arrangement of domain 1 and domain 2/3 in both noncrystallographic copies of the complex in the asymmetric unit. Furthermore, the C terminus of domain 1 was close to the N terminus of domain 2/3.

Although the two structural models obtained by the described molecular replacement approach were very similar, they were initially refined independently using CNS (28). 5% of the unique reflections were set aside for the calculation of Rmerge (29). After a first round of rigid body refinement, simulated annealing, and energy minimization in torsion angle space, the Rmerge values at 3.2-Å resolution for the starting models derived from the GDP and GTP forms could be reduced to 0.372 and 0.354, respectively. Thereafter, refinement was continued with the model derived from the T. thermophilus EF-Tu-GppNHp complex only. At this stage of refinement, the linker between the C terminus of domain 1 and the N terminus of domain 2/3 could be modeled into the electron density. There was no clear density, however, for residues 39–66, which were therefore removed from the model at this stage. In comparison with the structure of EF-Tu-GppNHp (5), the conformations of several side chains had to be altered, most notably that of Arg-124 (residue numbering is according to T. thermophilus EF-Tu). After this first round of refinement, the Rmerge dropped to 0.335, and the electron density for the GDP and aurodox molecules were very clear.

The program HyperChem (Hypercube Inc., Waterloo, Canada) was employed to build an initial atomic model for aurodox, which was then fitted into an F' – F difference density map (contoured at 2.5 σ above the mean), using the program O (30). This step improved the Rmerge to 0.305. Topology and parameter files for the antibiotic were generated with help of the program HIC-Up (31). Noncrystallographic symmetry restraints used for the first rounds of refinement were later removed, and refinement was continued against the synchrotron data that had become available in the meantime. The R and Rmerge of the final structural model are 0.191 and 0.222, respectively, for all data between 20.0- and 2.0-Å resolution. Refinement statistics are given in Table I.

For construction of several models for analyzing possible changes in EF-Tu-GDP or EF-Tu-GppNHp upon docking aurodox to its binding site, ALIGN (32) was used to superimpose the domains. For characterization of rigid body movements of domains (Table II), domains 1 of the pair of EF-Tu models to be compared were first superimposed (32). Then the rotation angle was calculated from the superposition operator of the analyzed elements.

RESULTS AND DISCUSSION

Crystallographic Data

Crystals of the EF-Tu-GDP-aurodox complex were readily and reproducibly obtained following the procedure described under “Experimental Procedures.” Washed and dissolved crystals showed only one band on native polyacrylamide gels, with an unambiguous shift compared with EF-Tu-GDP, proving that all EF-Tu in the crystal was in complex with aurodox (data not shown).

Structure Elucidation and Model Quality—The structure of the complex was solved by molecular replacement (27). Initial trials using the complete EF-Tu molecule as a search model failed, irrespective of whether the GTP or GDP form of the protein (5, 8) was used. On the other hand, both forms lead to the correct solution when domains 1 and 2/3 were used as two separate search models. Using synchrotron radiation data, the model was refined to 2.0-Å resolution (Table I). Electron density for the aurodox molecule is shown in Fig. 2. The two ternary EF-Tu-GDP-aurodox complexes in the asymmetric unit of the crystal show no significant differences (r.m.s. deviation for 557 Ca positions was 0.20 Å; 0.34 Å for all 379 Ca positions in the model). All Co–Ca deviations larger than 0.6 Å (i.e. at residues 233 (2.36 Å), 357 (1.36 Å), 296 (0.91 Å), 142 (0.86 Å), and 214 (0.83 Å) can be explained by crystal contacts. The model comprises a total of 770 amino acid residues, two GDP molecules, two Mg2+ ions, two aurodox molecules, and 598

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FIG. 1. Chemical structure of aurodox.
Two segments of the polypeptide chain, switch I (residues 50–64) and switch II (residues 83–99), act as sensors for the presence or absence of GTP and transmit this signal into the other two domains (5, 7). In the GTP form, switch I forms an α-helix, which unfolds to a β-hairpin in the GDP complex (7, 8). Concomitantly, the switch II helix changes both its orientation and length upon GTP hydrolysis (5, 7). Domain 2 (residues 212–310) and domain 3 (residues 311–405) are β-barrels of seven and six antiparallel β-strands, respectively, that share an extended interface. Characteristic for each of the known EF-Tu structures is the relative arrangement of domains 2/3 to domain 1. So far, in all known complexes with GTP analogues, the EF-Tu exists in a compact form with tight interfaces between domains 1 and 2 as well as 1 and 3 (Fig. 3A (5)). This conformation is also seen in the ternary complexes of EF-Tu-GppNHp with aa-tRNAs (34, 35). In contrast, in all GDP complex structures determined so far, a large hole opens between domains 1 and 2, making the molecule much less compact (Fig. 3C (7, 8, 33)).

Certainly, the most striking feature of the ternary complex between EF-Tu-GDP and aurodox presented here (Fig. 3B) is its overall similarity to that of the binary complex EF-Tu-GppNHp, the active conformation of EF-Tu (Fig. 3A). The current structure supports the proposal that kirromycin induces a conformation of EF-Tu-GDP that is similar to the GTP form, thus preventing its release from the ribosome after and despite hydrolysis of GTP to GDP and P. Aurodox binds to the interface between domains 1 and 3 (Fig. 3B). To accommodate the antibiotic in this binding site, domains 2 and 3 are rotated about 9.7 and 15.4°, respectively (averaged values for molecules A and B), relative to their position in the GppNHp form (5). This rotation implies a small but unambiguous movement of domain 3 with respect to domain 2. The Ca r.m.s. deviations between EF-Tu-GDP-aurodox and either EF-Tu-GppNHp (5) or EF-Tu-GDP (8) are listed in Table II. To force EF-Tu-GDP into this GTP complex-like conformation, the antibiotic has to provide more favorable interactions between itself and domains 1 and 3 than these domains alone would have in the GDP complex. This is achieved by strong interactions of the antibiotic with both domains 1 and 3, effectively gluing them together (see Figs. 3 and 4 and Table III).

Another feature that emphasizes the overall strategy of the antibiotic of coercing the GDP-bound form into a GTP complex-like conformation is the rearrangement of switch II into an intermediate form, which, however, is more similar to the conformation found in the GTP form. Nevertheless, there are important deviations, such as a different positioning of the thoroughly scrutinized residue His85 (36); in addition to a somewhat different conformation of the main chain, the conformation over the Ca–Cβ bond of the side chain is synclinal in the EF-Tu-GDP-aurodox complex but antiperiplanar in the GppNHp complex (−44° and −164°, respectively, for χ1). This change brings the His85 side chain close to the nucleotide-binding site (see Fig. 5). The implications of this are discussed below, in context of the GTPase activity.

Only very weak electron density was observed for residues 42–52, and no density was observed for residues 53–64 (switch I). Based on the structure of EF-Tu-GppNHp (5), residues 42–52 were modeled into molecule A, but nevertheless they retain poor electron density after refinement. Both the α-helical conformation of switch I as seen in EF-Tu-GppNHp and the β-hairpin conformation observed in EF-Tu-GDP would be compatible with the structure of the protein in the aurodox complex. However, in both copies in the asymmetric unit, the electron density for Thr-65 and Ala-66 clearly indicates that...
FIG. 3. Tertiary structure of EF-Tu. A, EF-Tu from T. thermophilus with GppNHp bound (GTP form); B, EF-Tu from T. thermophilus with GDP and aurodox bound (aurodox form; this work); C, EF-Tu from T. aquaticus with GDP bound (GDP form).2
deduced increase of the GDP exchange rate (37), analogous to the EF-Ts action on EF-Tu-GDP by which the Asp-81/Wat/Mg2+ interaction, among others, is perturbed. The nearly perfect octahedral coordination of the Mg2+ ion in the current structure is largely identical to the one seen in the binary EF-Tu-GDP complex (7, 38). The metal is in contact with the hydroxyl group of Thr-25, the β-phosphate, and four water molecules.

Switch II (residues 83–99) of EF-Tu is known to display considerable conformational variability, depending on whether GTP, GDP, or EF-Ts is bound. In the aurodox complex, we find yet another conformation for this segment (Fig. 5). The trigger for the conformational changes is the peptide between Pro-83 and Gly-84. With GppNHp bound, Gly-84 N hydrogen-bonds to the γ-phosphate. In both structures with bound GDP (EF-Tu-GDP and EF-Tu-GDP/aurodox), the peptide between Pro-83 and Gly-84 is flipped relative to the GTP form, and the carbonyl group points toward the empty γ-phosphate position. When GTP is bound to the aurodox complex, we would expect the peptide 83–84 to flip back in order to avoid steric and electrostatic repulsion between the carbonyl of Pro-83 and the γ-phosphate.

In the structure of EF-Tu-GppNHp, the γ-phosphate of the nucleotide as well as the “nucleophilic water” (which is responsible for the attack on the γ-phosphorus atom during GTP hydrolysis; see Berchtold et al. (5)) are shielded from bulk solvent by a hydrophobic gate consisting of the side chains of Val-20 and Ile-61. Berchtold et al. (5) have proposed that in the ribosome-mediated GTPase reaction, one or both wings of the hydrophobic gate could open, providing access to the γ-phosphate for His-85 and partly explaining the acceleration of the GTP hydrolysis rate on the programmed ribosome. In the ternary EF-Tu-GDP-aurodox complex described here, Ile-61 is disordered, along with the entire switch I. The observed effect on His-85 is the same as proposed for ribosome binding of EF-Tu-GTP. Concomitant with the opening of the hydrophobic gate, an altered orientation of His-85 is observed. This side chain is now oriented toward the nucleotide-binding site and could easily contact the γ-phosphate when presented (Fig. 5). Thus, its role could be similar to that of Gln-61 in the

TABLE II

<table>
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<tr>
<th>Molecule</th>
<th>Domain 1</th>
<th>Domain 2</th>
<th>Domain 3</th>
<th>Domain 2/3</th>
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<td>B</td>
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<td></td>
<td>B</td>
<td>9.4°</td>
<td>15.2°</td>
<td>11.8°</td>
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<tr>
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<tr>
<td>Rotation</td>
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<td>11.8°</td>
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<td>B</td>
<td>9.4°</td>
<td>15.2°</td>
<td>11.8°</td>
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FIG. 4. Residues involved in resistance mutations. With the exception of Tyr-161 and Leu-121, most of these residues cluster around the goldinoic acid.

these residues deviate from the conformations found in either the GppNHp or the GDP form. Therefore, switch I appears to have a new, highly mobile conformation in the aurodox complex.

Nucleotide Binding and Hydrolysis—The $K_d$ for GTP decreases more than 100-fold upon aurodox binding (37), which is most probably due to stabilization of the GTP complex-like structure of EF-Tu induced by the antibiotic. Ktromycin has also been shown to increase the intrinsic GTPase rate of EF-Tu by 1 order of magnitude (3). In the present structure, the electron density for the nucleotide, GDP, is clear, indicating full occupancy. The binding mode of GDP is the same as in the binary EF-Tu-GDP complex (7, 8, 38), although the high flexibility of the effector region (including switch I) in the current structure appears to preclude the existence of strong interactions between Tyr-47 OH and the α-phosphate or between Asp-51 and the magnesium ion (via water). The side chains of residues 47 and 51 (as modeled in molecule A) are only partially visible in the electron density. The weakening of the Asp-51/Wat/Mg2+ interaction could very well explain the observed increase of the GDP exchange rate (37), analogous to the EF-Ts action on EF-Tu-GDP by which the Asp-81/Wat/Mg2+ interaction, among others, is perturbed. The nearly perfect octahedral coordination of the Mg2+ ion in the current structure is largely identical to the one seen in the binary EF-Tu-GDP complex (7, 38). The metal is in contact with the hydroxyl group of Thr-25, the β-phosphate, and four water molecules.

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The E. coli numbering scheme is given for amino acids involved in resistance mutations (17–20).

<table>
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<th>Molecule B</th>
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<tr>
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<td>Aur C39–Gln-125 CB</td>
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<td></td>
<td>Aur C39–Val-126 CB</td>
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Ras-GDP-AlF$_3$-GAP complex (39) (i.e. stabilization of the transition state). However, the acceleration of the GTP hydrolysis rate by aurodox is only ~10-fold rather than 10$^5$-fold as in the Ras-GAP complex, because a residue analogous to the so-called arginine finger (Arg-789 of GAP) is not present in EF-Tu-GTP-aurodox.

Binding Site of Aurodox—Clear density was also seen for the antibiotic (Fig. 2). It fits perfectly into its binding site at the interface of domains 1 and 3. This site shows no overlap with the one for the unrelated antibiotic GE2270A (15), in agreement with the observation that EF-Tu can bind both kirromycin and GE2270A simultaneously (40). The overall location of the aurodox binding site has been predicted by us previously, on the basis of the location of resistance mutations (17). A schematic drawing of aurodox in its binding site is shown in Fig. 4. The three ring systems of aurodox, namely the pyridone, the tetrahydrofuran, and the goldinoic acid, are arranged in an extended way with the aliphatic tail attached to the goldinoic acid bending backwards. Hydrophobic contacts clearly dominate over hydrophilic ones, especially at the goldinoic acid end of aurodox. The pyridone and the tetrahydrofuran rings bind to domain 1, and the aliphatic tail on the goldinoic acid is embedded in a pocket formed by the hydrophobic faces of the following residues from both domains 1 and 3: Thr-343, Ala-387, Arg-389, and Thr-394. The hydrophobic linker between the goldinoic acid and the tetrahydrofuran ring interacts with Leu-323, Thr-394, and Gln-125. The third hydrophobic part, the linker between the tetrahydrofuran ring and the pyridone ring, is in contact with Leu-121, Tyr-161, and Glu-327. Hydrogen bonds are formed between the side chain of Tyr-161 and the hydroxyl group of the pyridone ring and between its main-chain oxygen and one of the hydroxyl groups of the tetrahydrofuran ring. The other hydroxyl group hydrogen-bonds to the Arg-124 guanidinium group. The amide nitrogen of aurodox hydrogen-bonds to the main-chain oxygen of Gln-125. Most other heteroatoms make hydrogen bonds to the protein via water molecules (see Table III), with the weakest interactions made by the goldinoic acid oxygens.

Inspection of the known structures of EF-Tu-GDP and EF-Tu-GppNHp shows that neither has the binding crevice for aurodox preformed. The domain 1–3 interface is very different from the one for the unrelated antibiotic GE2270A (15), in agreement with the observation that EF-Tu can bind both kirromycin and GE2270A simultaneously (40). The overall location of the aurodox binding site has been predicted by us previously, on the basis of the location of resistance mutations (17). A schematic drawing of aurodox in its binding site is shown in Fig. 4. The three ring systems of aurodox, namely the pyridone, the tetrahydrofuran, and the goldinoic acid, are arranged in an extended way with the aliphatic tail attached to the goldinoic acid bending backwards. Hydrophobic contacts clearly dominate over hydrophilic ones, especially at the goldinoic acid end of aurodox. The pyridone and the tetrahydrofuran rings bind to domain 1, and the aliphatic tail on the goldinoic acid is embedded in a pocket formed by the hydrophobic faces of the following residues from both domains 1 and 3: Thr-343, Ala-387, Arg-389, and Thr-394. The hydrophobic linker between the goldinoic acid and the tetrahydrofuran ring interacts with Leu-323, Thr-394, and Gln-125. The third hydrophobic part, the linker between the tetrahydrofuran ring and the pyridone ring, is in contact with Leu-121, Tyr-161, and Glu-327. Hydrogen bonds are formed between the side chain of Tyr-161 and the hydroxyl group of the pyridone ring and between its main-chain oxygen and one of the hydroxyl groups of the tetrahydrofuran ring. The other hydroxyl group hydrogen-bonds to the Arg-124 guanidinium group. The amide nitrogen of aurodox hydrogen-bonds to the main-chain oxygen of Gln-125. Most other heteroatoms make hydrogen bonds to the protein via water molecules (see Table III), with the weakest interactions made by the goldinoic acid oxygens.

Resistance Mutations in EF-Tu and Phosphorylation of Thr-394—Kirromycin resistance mutations have so far only been studied with elongation factors Tu from E. coli and Salmonella typhimurium (17–20). Due to the absolute conservation of the affected residues and the high structural similarity between E. coli and T. thermophilus EF-Tu, observations made with the present structure should also apply to the homologous proteins. The methyl group that distinguishes aurodox from kirromycin does not make any contacts (shorter than 4.4 Å) to the protein. This agrees with the observation that kinetic data show no evidence for the two compounds (3) reacting at the GTP binding site of EF-Tu.
previously published resistance mutations (Q341H, ΔI310, and E390K (18)) and the cross-linking of kirromycin to Lys-357 (41) cannot be explained on the basis of direct interactions evident from our structure. Residue 310 resides within the linker between domains 2 and 3. Its deletion might interfere with the slight mutual rearrangement of domains 2 and 3 observed in the aurodox complex, thus preventing strong binding of the antibiotic. Mutations of Gln-341 and Glu-390, which are involved in aa-tRNA binding (34, 35) will decrease the affinity of EF-Tu-GppNHp/EF-Tu-GDP-aurodox for aa-tRNA such that EF-Tu-GDP-aurodox cannot create a polysomal traffic jam.

EF-Tu-GDP complexes of various mutants were reported to be more resistant to kirromycin than the corresponding EF-Tu-GTP complexes (17). As an explanation, resistant variants of EF-Tu were postulated to display two lines of defense against antibiotic binding: one before and one after GTP hydrolysis. With the current structure at hand, we can elaborate on the latter idea a bit further. Since the conformation of EF-Tu in its GTP form is much more similar to EF-Tu-GDP-aurodox than is the binary GDP complex, less than perfect binding of the antibiotic may be sufficient to support the small conformational changes required. On the other hand, when starting from the binary GDP complex, ideal interaction between the antibiotic and EF-Tu is required to drive the accompanying huge conformational change. Therefore, kirromycin can still bind to some resistance mutants such as G328D in their GTP but not their GDP form; i.e. the defense against binding the antibiotic becomes apparent only after GTP hydrolysis on the ribosome (17). The most resistant variants of EF-Tu, A375T and Q124K, already perturb aurodox binding to the GTP form (17). If this first line of defense breaks down at very high antibiotic concentrations, aurodox will be evicted after GTP hydrolysis, as a second line of defense.

**Mutually Exclusive Binding of EF-Ts and Aurodox**—It has long been known that binding of kirromycin and of the nucleotide exchange factor EF-Ts to EF-Tu are mutually exclusive (2). Based on our EF-Tu-GDP-aurodox structure and that of EF-Tu-EF-Ts (42, 43), we point out several structural features for an explanation. Phe-82 of EF-Ts is critical in the EF-Tu/EF-Ts complex, aurodox cannot induce the conformational change. Therefore, kirromycin can still bind to some resistance mutants such as G328D in their GTP but not their GDP form; i.e. the defense against binding the antibiotic becomes apparent only after GTP hydrolysis on the ribosome (17). The most resistant variants of EF-Tu, A375T and Q124K, already perturb aurodox binding to the GTP form (17). If this first line of defense breaks down at very high antibiotic concentrations, aurodox will be evicted after GTP hydrolysis, as a second line of defense.

**aa-tRNA Binding**—One of the most important effects induced by the antibiotic is the ability of EF-Tu-GDP-aurodox (and even nucleotide-free EF-Tu-aurodox) to bind aa-tRNA (44), a property normally associated with EF-Tu-GTP. In both EF-Tu-GppNHp-aa-tRNA complex structures determined so far (34, 35), the conformation of the protein is very similar to that in the GppNHp complex (5), with the most prominent deviations occurring in switch I (residues 53–64). Obviously, EF-Tu-GDP-aurodox can bind aa-tRNA because the protein adopts a GTP-like conformation. r.m.s. deviation values for Ca atoms in domains 1–3 between EF-Tu-GDP-aurodox and EF-Tu-GppNHp-Phe-tRNA are only ~0.6 Å each. Although aurodox increases the affinity of EF-Tu-GDP for aa-tRNA, it de-
creases it in case of EF-Tu-GTP by about 3 orders of magnitude (10). The disorder of switch I in the aurodox complex disturbs the interaction between G1 of the aa-tRNA and Asn-64. Together with the somewhat different orientations of domains 2 and 3, this could contribute to the decreased affinity for aa-tRNA.

To conclude, the effect of kirromycin on EF-Tu can thus be explained in general terms by the GTP complex-like form the protein is forced to adopt and, in more detail, by the specific residues that are in contact with the antibiotic and the nucleotide.

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REFERENCES
Conformational Change of Elongation Factor Tu (EF-Tu) Induced by Antibiotic Binding: CRYSTAL STRUCTURE OF THE COMPLEX BETWEEN EF-Tu-GDP AND AURODOX

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