Dyson, H. J., Holmgren, A., & Wright, P. E. (1988) *FEBS Lett. 228*, 254–258.
Eklund, H., Cambillau, C., Sjöberg, D. M., Holmgren, A., Hoog, J.-O., & Brandén, C.-I. (1984) *EMBO J. 3*, 1443–1449.
Ernst, R. R., Bodenhausen, G., & Wokaun, A. (1987) *Principles of Nuclear Magnetic Resonance in One and Two Dimensions*, Clarendon Press, Oxford, U.K.
Hiraoki, T., Brown, S. B., Stevenson, K. J., & Vogel, H. J. (1988) *Biochemistry 27*, 5000–5008.
Holmgren, A. (1985) *Annu. Rev. Biochem. 54*, 237–271.
Jeener, J., Meier, B. H., Bachmann, P., & Ernst, R. R. (1979) *J. Chem. Phys. 71*, 4546–4553.
Kelley, R. F., & Stellwagon, E. (1984) *Biochemistry 23*, 5095–5102.
LeMaster, D. M., & Richards, F. M. (1988) *Biochemistry 27*, 142–150.
Macura, C., Huang, Y., Suter, D., & Ernst, R. R. (1981) *J. Magn. Reson. 43*, 259–281.
Marion, D., & Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun. 113*, 967–974.
Marion, D., & Bax, A. (1988a) *J. Magn. Reson. 80*, 528–533.
Marion, D., & Bax, A. (1988b) *J. Magn. Reson. 79*, 352–356.
Mueller, L. (1987) *J. Magn. Reson. 72*, 191–196.
Neuhaus, D., Wagner, G., Vasák, M., Kägi, J. H. R., & Wüthrich, K. (1985) *Eur. J. Biochem. 151*, 257–273.
Pardi, A., Billeter, M., & Wüthrich, K. (1984) *J. Mol. Biol. 180*, 741–751.
Piantini, U., Sørensen, O. W., & Ernst, R. R. (1982) *J. Am. Chem. Soc. 104*, 6800–6801.
Plateau, P., & Guéron, M. (1982) *J. Am. Chem. Soc. 104*, 7310–7311.
Rance, M., Sørensen, O. W., Bodenhausen, G., Wagner, G., Ernst, R. R., & Wüthrich, K. (1983) *Biochem. Biophys. Res. Commun. 117*, 479–485.
Redfield, A. G., & Kunz, S. D. (1975) *J. Magn. Reson. 19*, 250–254.
Reutimann, H., Luisi, P.-L., & Holmgren, A. (1983) *Biopolymers 22*, 107–111.
Söderberg, B.-O., Sjöberg, B.-M., Sonnerstam. V., & Brändén, C.-I. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 5827–5830.
Thomas, R. M., Boos, R., & Luisi, P.-L. (1986) Folding of Fragments of Thioredoxin from *Escherichia coli*, in *Thioredoxin and Glutaredoxin Systems: Structure and Function*, A. (Holmgren, A., et al., Eds.) pp 87–92, Raven Press, New York.
Wollman, E. E., d'Auriol, L., Rimsky, L., Shaw, A., Jacquot, J.-P., Wingfield, P., Graber, P., Dessarps, F., Robin, P., Galibert, F., Bertoglio, J., & Fradelizi, D. (1988) *J. Biol. Chem. 263*, 15506–15512.
Wüthrich, K. (1986) *NMR of Proteins and Nucleic Acids*, Wiley, New York.
Wüthrich, K., & Wagner, G. (1979) *J. Mol. Biol. 130*, 1–18.
Wüthrich, K., Billeter, M., & Braun, W. (1984) *J. Mol. Biol. 180*, 715–740.

Conformation and Reactivity Changes Induced by *N*-Methylkirromycin (Aurodox) in Elongation Factor Tu

C. Balestrieri, A. Giovane, L. Quagliuolo, and L. Servillo*
Department of Biochemistry and Biophysics, University of Naples, Via Costantinopoli 16, 80138 Napoli, Italy
G. Chinali
Institute of Biological Structures, 2nd Medical School, University of Naples, Via Pansini 5, 80100 Napoli, Italy
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ABSTRACT: Kirromycin and related antibiotics inhibit protein synthesis in bacteria by acting on elongation factor Tu (EF-Tu). We have studied the effects of *N*-methylkirromycin (aurodox) on some molecular properties of this protein. The binding of the antibiotic causes a dramatic variation in the protein fluorescence emission spectrum with the appearance of a new maximum at around 340 nm. Addition of aurodox to trypsinized EF-Tu resulted in an emission spectrum similar to that of the denatured intact factor. Fluorescence lifetime analysis performed by a multifrequency phase fluorometer indicated that the fluorescence emission of the factor is heterogeneous with the major component having a lifetime near 4.8 ns in the absence and 6.6 ns in the presence of the antibiotic. These results were interpreted in terms of an antibiotic-induced environmental modification of the unique tryptophan residue of the protein leading to an increase in its quantum yield. However, aurodox did not modify the solvent exposure of this residue, as judged by fluorescence quenching experiments. Moreover, 1-anilino-8-naphthalenesulfonate (ANS) binding studies, as well as analysis of the protein reactivity toward the sulfhydryl group reagent 5,5′-dithiobis(2-nitrobenzoate) (DTNB), showed that, in the presence of aurodox, the behavior of the EF-Tu·GDP complex nears that of EF-Tu·GTP. These results strongly support the hypothesis that aurodox not only confers a "GTP-like" conformation to the EF-Tu·GDP complex but also produces a less stable folding of the protein around the tryptophan residue that may contribute to the multiple functional effects of this antibiotic.

The first step of each elongation cycle in protein biosynthesis is promoted by elongation factor Tu (EF-Tu)[1] which forms a ternary complex, with GTP and aa-tRNA, that binds to mRNA-programmed ribosomes. As a result, GTP is hydro-

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lyzed, and EF-Tu is released from the ribosome as a complex with the GDP, leaving the aa-tRNA positioned at the A site ready for peptide bond formation (Miller & Weissbach, 1977).

The antibiotic kirromycin binds to EF-Tu in a 1:1 ratio and inhibits protein biosynthesis by blocking the dissociation of the EF-Tu·GDP complex from the ribosome after GTP hydrolysis [Wolf et al., 1974, 1977; for a review, see Parmeggiani and Sander (1980)]. In the presence of kirromycin, EF-Tu undergoes noticeable functional changes. The factor displays a marked GTPase activity, even in the absence of aa-tRNA and ribosomes (Chinali et al., 1977). Moreover, kirromycin stimulates the exchange between free and factor-bound GDP and increases by 2 orders of magnitude the affinity of EF-Tu for GTP up to a value close to that for GDP (Chinali et al., 1977; Fasano et al., 1978). Finally, EF-Tu kirromycin can form stable complexes with aa-tRNA and ribosomes not only in the presence of GTP but also in the presence of GDP or without these nucleotides at all (Wolf et al., 1974).

In this paper, we have investigated the effects of aurodox (*N*-methylkirromycin) on some molecular properties of EF-Tu complexed with guanyl nucleotides. The results indicate that the antibiotic induces an altered EF-Tu conformation which has some features in common with those of the factor complexed with GTP.

Materials and Methods

Crystalline EF-Tu was purified as a complex with GDP from *Escherichia coli* A-19 strain at mid-log phase as described in Arai et al. (1972). The complex concentration was determined by the absorbance at 280 nm with $\epsilon = 41\,600\ M^{-1}\ cm^{-1}$ (Abrahamson et al., 1985).

Aurodox (*N*-methylkirromycin) Na^+ salt was a gift of Dr. Maehr, Hoffmann-La Roche. The antibiotic concentration was determined spectrophotometrically at 325 nm using a value of $\epsilon = 36\,280\ M^{-1}\ cm^{-1}$ (Berger et al., 1973). Aurodox Na^+ salt was used in place of kirromycin because of the higher purity (>95%): the two antibiotics have the same activity and identical affinity for EF-Tu in the *E. coli* in vitro system (Chinali, 1981). DTNB and ANS were purchased from Pierce. ANS, obtained as the magnesium salt, was crystallized 4 times from hot water as described in Weber and Young (1964). An $\epsilon = 5000\ M^{-1}$ at 350 nm was used to determine its concentration.

All measurements were made in buffer solutions containing 0.1 M KCl, 20 mM Tris-HCl (pH 7.4), 10 mM $MgCl_2$, and 0.1 mM dithiothreitol. Dithiothreitol was omitted from solutions used to study the DTNB reaction.

Fluorescence Measurements. Steady-state emission measurements were made on a Perkin-Elmer MPF-2A spectrofluorometer at 20 °C. Due to the high absorption of aurodox at the excitation wavelengths, some measurements were made in cuvettes with inner dimensions of 3 × 3 mm. In all cases, conditions were used in which the absorbance of the solutions never exceeded 0.2 unit. Fluorometric titrations of EF-Tu with ANS in the presence of the various ligands were made as described in Arai et al. (1975). The Scatchard analysis was performed directly from the fluorometric ANS titration data, by assuming that the free ANS fluorescence is negligible as compared to that of bound ANS and by determining the relative molar fluorescence of the bound ANS from the plateau of a fluorometric titration curve of a diluted ANS solution with an excess of EF-Tu. Addition of aurodox to this solution did not modify the relative molar fluorescence of ANS bound to EF-Tu.

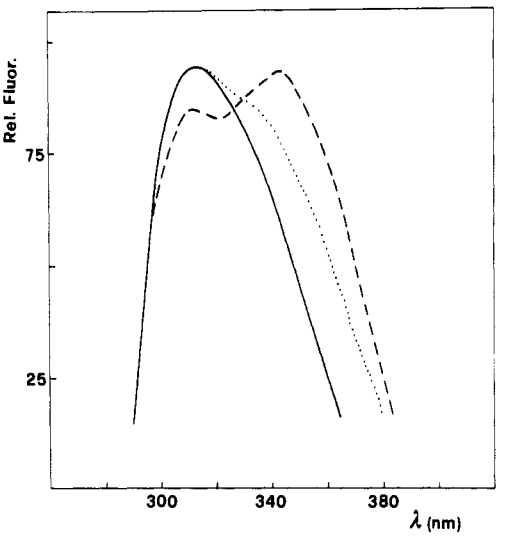

FIGURE 1: Normalized emission spectra of EF-Tu·GDP (—), EF-Tu·GTP (···), and EF-Tu·GDP in the presence of aurodox (- - -) upon excitation at 280 nm. The complex EF-Tu·GTP was obtained directly in the cuvette by adding to the EF-Tu·GDP solution (0.28 mg/mL) phosphoenolpyruvate (4.5 mM) and pyruvate kinase (3 μg/mL). The spectrum was recorded after 1 h of incubation at 25 °C. The aurodox concentration was 12 μM.

Time-Resolved Measurements. Lifetime measurements were performed by using an ISS GREG-200 multifrequency cross-correlation phase and modulation fluorometer equipped with a xenon lamp. The emission was observed through a long-wave pass filter with a cutoff wavelength at 330 nm to avoid Raman emission. The modulation frequency was varied from 10 to 110 MHz. A solution of *p*-terphenyl (from Kodak) in cyclohexane was placed in the reference cell to correct the "color error" (Lakowicz et al., 1981). A lifetime of 1.000 ns was assigned to the reference solution. At each frequency, the data were collected until the standard deviations of phase and modulation were below 0.2° and 0.004, respectively. The temperature of the sample compartment was maintained at 20 °C by an external bath circulator and was controlled prior to and after each measurement. Phase and modulation data were analyzed by a nonlinear least-squares program described in Jameson et al. (1984), assuming a two-exponential fluorescence decay model.

Results

The fluorescence emission spectrum of EF-Tu·GDP complex, upon excitation at 280 nm, is rather unusual for a tryptophan-containing protein. In fact, the spectrum of EF-Tu, which contains a single tryptophan and 10 tyrosine residues, is dominated by the tyrosine contribution, with an emission maximum at around 308 nm (Arai et al., 1977; Jameson et al., 1987). In contrast, tryptophan-containing proteins show an emission maximum at higher wavelengths, with the tyrosine contribution overwhelmed by that of tryptophan or sometimes, appearing as a shoulder on the blue side of the spectrum.

Figure 1 illustrates the corrected emission spectra of the EF-Tu·GDP, EF-Tu·GDP·aurodox, and EF-Tu·GTP complexes. The modifications of the spectrum induced by the antibiotic are relevant: the tryptophan residue emission now becomes prominent, as indicated by the emission maximum at around 340 nm, while the contribution of tyrosine residues produces a shoulder at 310 nm. In agreement with previous

[1] Abbreviations: EF-Tu, elongation factor Tu; ANS, 1-anilino-8-naphthalenesulfonate; DTNB, 5,5′-dithiobis(2-nitrobenzoate); Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

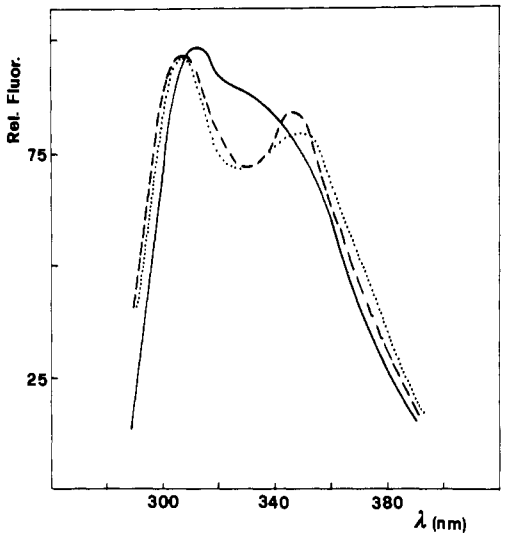

FIGURE 2: Normalized emission spectra of trypsin-treated EF-Tu·GDP (—), of trypsin-treated EF-Tu·GDP in the presence of aurodox (---), and of EF-Tu·GDP in 6 M guanidine hydrochloride (···) upon excitation at 280 nm. The EF-Tu·GDP solution (0.28 mg/mL) was added with trypsin (5 μg/mL); the spectrum was recorded after 20 h of incubation at 25 °C. Aurodox was then added to this solution to a final concentration of 12 μM.

Table I: Phase and Modulation Lifetime Values

frequency (MHz)	EF-Tu·GDP		Ef-Tu·GDP·aurodox		EF-Tu·GTP	
	τ^P (ns)	τ^M (ns)	τ^P (ns)	τ^M (ns)	τ^P (ns)	τ^M (ns)
10	2.823	4.142	3.836	6.999	3.078	4.588
20	2.667	4.127	2.859	5.859	2.764	4.144
30	2.159	3.954	2.464	4.968	2.388	4.168
50	1.730	3.275	1.759	3.920	1.755	3.607
60	1.483	2.978	1.556	3.682	1.533	3.341
90	1.244	2.684	1.151	3.189	1.130	2.700
110	1.013	2.354	0.955	2.709	0.989	2.395

observations (Arai et al., 1977), conversion of EF-Tu·GDP to EF-Tu·GTP results in a shoulder at the red edge of the spectrum.

It is known that trypsin cleaves EF-Tu in a few fragments (Arai et al., 1976) that remain tightly associated and can be separated only under denaturing conditions. The nicked EF-Tu retains many functional properties of the native protein (Wittinghofer et al., 1980). The fluorescence emission spectra of the EF-Tu·GDP complex incubated 20 h with trypsin, alone and in the presence of aurodox, are reported in Figure 2. The emission maximum of the nicked EF-Tu·GDP is still at 310 nm, but a shoulder appears at the red edge of the spectrum. The addition of aurodox to the nicked complex causes a dramatic effect: the emission spectrum now displays two well-resolved peaks at 310 and 345 nm. This emission spectrum is very similar to that observed when the intact protein was denatured in 6 M guanidine hydrochloride (Figure 2). Additional evidence for the aurodox-induced environmental change around the tryptophan residue comes from the analysis of the fluorescence lifetime data. In Table I, the phase and modulation lifetime values of the EF-Tu·GDP complex at various frequencies are compared to the corresponding values observed in the presence of the antibiotic. As a reference, the values of the EF-Tu·GTP complex are also reported. In all cases, the difference between phase and modulation lifetime values indicates that the fluorescence decay cannot be described by a single exponential (Spencer & Weber, 1969). We analyzed the data by fitting them to a two-exponential decay model as described in Jameson et al. (1984). The results for

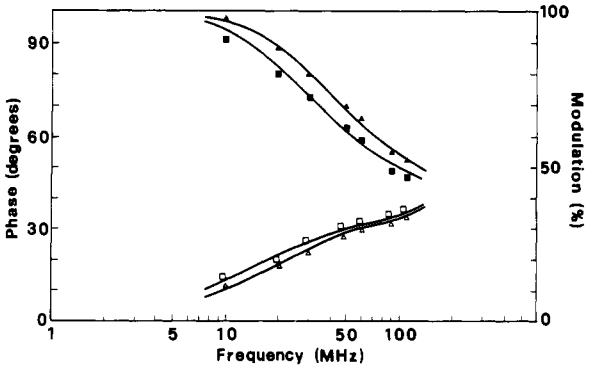

FIGURE 3: Multifrequency phase (open symbols) and modulation (closed symbols) data obtained for EF-Tu·GDP alone (triangles) and in the presence of aurodox (squares). The excitation was at 280 nm. The solid lines come from the two-component fit.

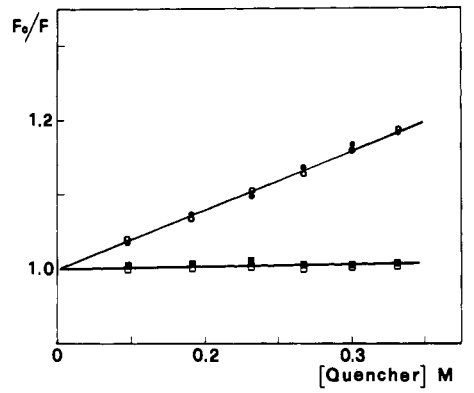

FIGURE 4: Stern-Volmer plot of iodide (squares) and acrylamide (circles) fluorescence quenching of EF-Tu·GDP alone (open symbols) and in the presence of aurodox (closed symbols). The excitation was at 295 nm; the fluorescence intensity was measured at 345 nm.

EF-Tu·GDP and for EF-Tu·GTP·aurodox complexes are reported in Figure 3. The lifetime values obtained for the EF-Tu·GDP complex were 4.85 and 0.57 ns with fractional intensities of 0.55 and 0.45 for the two decay components, respectively, and 6.65 and 0.61 ns with fractional intensities of 0.55 and 0.45, respectively, for the EF-Tu·GDP·aurodox complex. The EF-Tu·GTP complex showed intermediate lifetime values of 5.36 and 0.57 ns with the same fractional intensities. The longer lifetime values of the fluorescence emission observed in the presence of the antibiotic are indicative of a modified situation around the tryptophan residue which probably leads to an increase of its quantum yield. It is noteworthy that aurodox is a good acceptor of the tryptophanyl residue fluorescence, having an intense absorption spectrum centered at around 330 nm. Therefore, hypothetical changes in the fluorescence parameters due to the antibiotic, as nonradiative energy transfer from the tryptophan residue to the factor-bound antibiotic, would cause a reduction rather than an increase in the fluorescence emission lifetimes. The environmental modifications induced by aurodox do not seem, however, to affect the solvent exposure of the tryptophan residue. It has recently been demonstrated by fluorescence quenching experiments that the tryptophan residue in the EF-Tu·GDP complex is only marginally accessible to the solvent (Jameson et al., 1987). We carried out fluorescence quenching experiments on the EF-Tu·GDP complex in the presence of aurodox using iodide and acrylamide as quenchers. The results were equivalent to those reported in the absence of the antibiotic (Jameson et al., 1987); i.e., the iodide quenching was ineffective, while the acrylamide quenched the EF-Tu·GDP complex with the same efficiency both in the

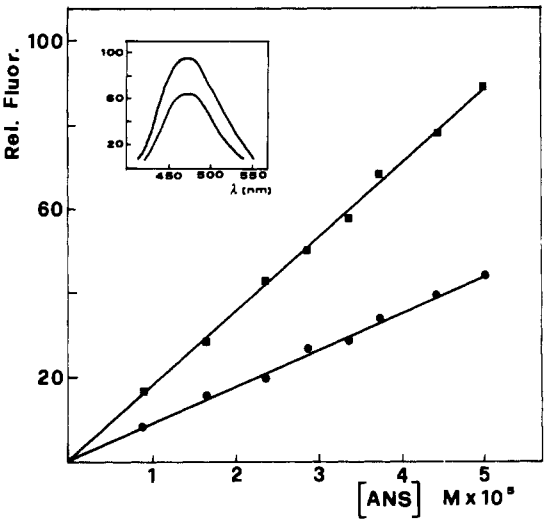

FIGURE 5: ANS fluorometric titration of EF-Tu·GDP alone (closed circles) and in the presence of aurodox (closed squares). The initial protein concentration was 0.28 mg/mL; the initial aurodox concentration was 12 μM. The fluorescence intensity was measured at 470 nm upon excitation at 380 nm. Inset: Emission spectra of the solution containing EF-Tu·GDP in the presence of ANS (30 μM) before (lower curve) and after (upper curve) aurodox addition.

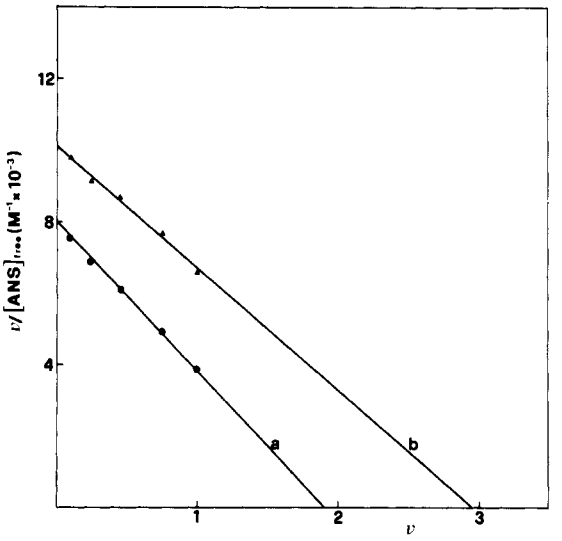

FIGURE 6: Scatchard plot of ANS binding to EF-Tu·GDP alone (closed circles) and in the presence of aurodox (closed triangles). The analysis was performed on ANS fluorometric titration data as reported under Materials and Methods.

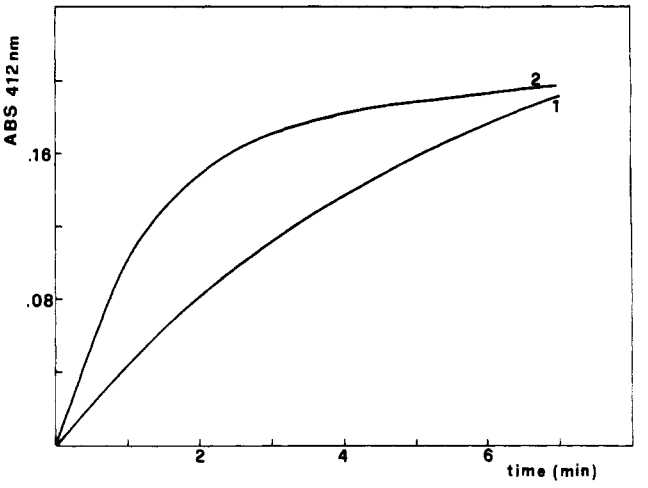

FIGURE 7: Reaction time course of EF-Tu·GDP alone (curve 1) and in the presence of aurodox (curve 2) with DTNB. The protein concentration was 0.28 mg/mL; the aurodox concentration was 12 μM. The reaction was started by adding DTNB (final concentration 0.5 mM) in the reference and sample cuvettes at 25 °C followed by continuously recording the absorbance at 412 nm.

presence and in the absence of the antibiotic (see Figure 4).

An increase of the tryptophan contribution to the emission spectrum of the factor, although not as great as in the case of the kirromycin interaction, was also observed when the EF-Tu·GDP complex is converted to EF-Tu·GTP complex (Arai et al., 1977; Figure 1). The analogy between the two phenomena led us to seek other similarities between the kirromycin EF-Tu·GDP complex and the EF-Tu·GTP complex. With this aim, we studied the binding of ANS to the aurodox·EF-Tu·GDP complex, since it was previously demonstrated that this fluorophore forms a 2:1 complex with EF-Tu·GDP and a 3:1 complex with EF-Tu·GTP (Arai et al., 1975). The fluorometric titrations of the EF-Tu·GDP complex in the presence or in the absence of aurodox are reported in Figure 5. The inset of Figure 5 shows the effect of aurodox addition to a solution containing the EF-Tu·GDP complex in the presence of ANS excess. The fluorometric data were analyzed as described under Materials and Methods, and the results of the Scatchard analysis are reported in Figure 6. It was found that the aurodox·EF-Tu·GDP complex binds 3 mol of ANS, like the EF-Tu·GTP complex.

Another point of similarity between the aurodox·EF-Tu·GDP complex and the EF-Tu·GTP complex was found in the reactivity of their cysteine residues toward the sulfhydryl reagent DTNB. EF-Tu has three cysteine residues which display different reactivity. Two of them are titrated, under native conditions, by sulfhydryl group reagents, like DTNB or mercurials, while the third group becomes titratable only upon denaturation of the protein (Arai et al., 1974). Moreover, the rate of the DTNB reaction is strongly dependent on the ligands interacting with the factor, EF-Tu·GTP reacting more rapidly than EF-Tu·GDP (Arai et al., 1974). Figure 7 illustrates the kinetics of the DTNB reaction of the EF-Tu·GDP complex in the presence and in the absence of aurodox. It clearly appears that the antibiotic increases the reaction rate. We found that the EF-Tu·GTP complex has a reaction kinetic superimposable on that of the EF-Tu·GDP·aurodox complex.

Discussion

The activity of EF-Tu during the elongation cycle of protein biosynthesis is regulated by a precise sequence of allosteric mechanisms mediated by interactions with GDP, aminoacyl-tRNA, ribosome, and EF-Ts.

Kirromycin and related antibiotics break down this ordered series of allosteric effects which is associated with the sequential interaction of the factor with the various effectors. These antibiotics block protein biosynthesis by preventing the release of EF-Tu from the ribosome after GTP hydrolysis [for a review, see Parmeggiani and Sander (1980)]. It was also shown that the free EF-Tu·GDP·kirromycin complex interacts with aminoacyl-tRNA with an affinity lower than that of the EF-Tu·GTP complex but much higher than that of the EF-Tu·GDP complex (Wolf et al., 1977; Johnson et al., 1985). Moreover, in the presence of the antibiotic, the rates of both trypsin cleavage and GTP exchange are indistinguishable from those seen with EF-Tu·GTP. Thus, it has been postulated that the antibiotic induces a "GTP-like" conformation of the factor even when bound to GDP (Wolf et al., 1977; Douglass & Blumenthal, 1979). Our data on ANS binding and on the reactivity with DTNB are consistent with this conclusion. However, the effects induced by aurodox on EF-Tu are not

simply limited to the change from a "GDP-like" to a "GTP-like" conformation. In fact, kirromycin-like antibiotics appear to alter most of the conformation-based control mechanisms that regulate the activity of EF-Tu. These antibiotics can mimic, completely or in part, the action of all the EF-Tu effectors: (1) aminoacyl-tRNA, in stabilizing GTP binding; (2) ribosomes and aminoacyl-tRNA, in inducing GTP hydrolysis; (3) GTP, in the enzymatic binding of aminoacyl-tRNA to the ribosomal A site; (4) GTP and GDP, in preventing EF-Tu from forming a complex with EF-Ts; and (5) EF-Ts, in stimulating the release of GDP (Wolf et al., 1977; Chinali et al., 1977; Fasano et al., 1978). In agreement with these multiple functional effects, a large difference between the fluorescence emission spectrum of EF-Tu·GTP and EF-Tu·GDP aurodox complexes was observed. The contribution of the single tryptophan residue, which is irrelevant to the emission spectrum of EF-Tu·GDP and is present as a shoulder in the spectrum of the EF-Tu·GTP complex, becomes the major emission peak in the presence of aurodox both with EF-Tu·GDP and with EF-Tu·GTP complexes. The binding of aurodox to EF-Tu·GDP also causes an increase of fluorescence emission lifetimes much larger than that produced by the replacement of GDP by GTP. Moreover, the observation that addition of the antibiotic to trypsinized EF-Tu GDP induces a fluorescence emission spectrum similar to that of the intact denatured EF-Tu suggests that aurodox induces a less tight folding of the protein around the tryptophan residue.

The observed spectral effects appear to be specifically due to environmental modifications around the unique tryptophanyl residue which is located in position 184 near the end of the N domain of EF-Tu (Arai et al., 1980; Jurnak, 1985). The extensive changes of the contribution of this residue to the fluorescence emission spectrum of EF-Tu induced by GTP and aurodox indicate that the tryptophan is located in a region of the molecule subjected to extensive conformational changes upon binding of guanine nucleotides and kirromycin.

EF-Tu from *E. coli* contains three domains: the N, middle, and C domains (La Cour et al., 1985). The N domain (residues 1–200) contains the guanine nucleotide binding site and the catalytic center of GTPase activity (Parmeggiani et al., 1987). Removal of the middle and C-terminal domains eliminates the ability of the N domain to distinguish between GDP and GTP. Moreover, the isolated N domain (also called the G domain) can catalyze GTP hydrolysis, and this reaction is further stimulated by ribosomes (Parmeggiani et al., 1987). All these effects were interpreted as a consequence of the removal of the control mechanisms exerted by the middle and C domains on the N domain. The fact that the same effects are also produced by kirromycin-like antibiotics on intact EF-Tu suggests that these antibiotics may also act by affecting the controls exerted on the N domain by the other two domains of the factor.

This conclusion is consistent with the large environmental modifications produced by aurodox around the unique tryptophan residue located in the N domain of EF-Tu, as revealed by our fluorescence studies.

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Registry No. Aurodox, 12704-90-4.

References

Abrahamson, J. K., Lane, T. M., Miller, D. L., & Johnson, A. E. (1985) *Biochemisty 24*, 692–700.

Arai, K., Kawakita, M., & Kaziro, Y. (1972) *J. Biol. Chem. 247*, 7029–7037.

Arai, K., Kawakita, M., Nakamura, S., Ishikawa, I., & Kaziro, Y. (1974) *J. Biochem. 76*, 523–534.

Arai, K., Arai, T., Kawakita, M., & Kaziro, Y. (1975) *J. Biochem. 77*, 1095–1106.

Arai, K., Nakamura, S., Arai, T., Kawakita, M., & Kaziro, Y. (1976) *J. Biochem. 79*, 69–83.

Arai, K., Arai, T., Kawakita, M., & Kaziro, Y. (1977) *J. Biochem. 81*, 1335–1346.

Arai, K., Clark, B. F. C., Duffy, L., Jones, M. D., Kaziro, Y., Laursen, R. A., L'italien., J., Miller, D. L., Nagarkatti, S., Nakamura, S., Nielsen, K. M., Petersen, T. E., Takahashi, K., & Wade, M. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1326–1330.

Berger, J., Lehr, H. H., Teitel, S., Meehr, H., & Grunberg, E. (1973) *J. Antibiot. 26*, 15–22.

Chinali, G. (1981) *J. Antibiot. 34*, 1039–1045.

Chinali, G., Wolf, H., & Parmeggiani, A. (1977) *Eur. J. Biochem. 75*, 55–65.

Douglass, J., & Blumenthal, T. (1979) *J. Biol. Chem. 254*, 5383–5387.

Fasano, O., Bruns, W., Crechet, J. B., Sander, G., & Parmeggiani, A. (1978) *Eur. J. Biochem. 89*, 557–565.

Jameson, D. M., Gratton, E., & Hall, R. D. (1984) *Appl. Spectrosc. Rev. 20*, 55–106.

Jameson, D. M., Gratton, E., & Eccleston, J. F. (1987) *Biochemistry 26*, 3894–3901.

Johnson, A. E., Janiak, S., Dell, V. A., & Abrahamson, J. K. (1985) in *Structure, Function and Genetics of Ribosomes* (Hardesty, B., & Kramer, G., Eds.) p 551, Springer-Verlag, New York.

Jurnak, F. (1985) *Science* (Washington, D.C.) *230*, 32–36.

La Cour, T. F. M., Nyborg, J., Thirup, S., & Clark, B. C. F. (1985) *EMBO J. 4*, 2385–2388.

Lakowicz, J. R., Cherek, H., & Balter, A. (1981) *J. Biochem. 15*, 131–146.

Miller, D. L., & Weissbach, H. (1977) in *Nucleic Acid Protein Recognition*, pp 409–440, Academic Press, New York.

Parmeggiani, A., & Sander, G. (1980) in *Topics in Antibiotic Chemistry* (Sammes, P. G., Ed.) Vol. 5, pp 159–221, Ellis Horwood Ltd., New York.

Parmeggiani, A., Swart, G. W. M., Mortensen, K. K., Jensen, M., Clark, B. F. C., Dente, L., & Cortese, R. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 3141–3145.

Spencer, R. D., & Weber, G. (1969) *Ann. N.Y. Acad. Sci. 158*, 361–376.

Weber, G., & Young, L. B. (1964) *J. Biol. Chem. 239*, 1415–1423.

Wittinghofer, A., Frank, R., & Laberman, R. (1980) *Eur. J. Biochem. 108*, 423–431.

Wolf, H., Chinali, G., & Parmeggiani, A. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4910–4914.

Wolf, H., Chinali, G., & Parmeggiani, A. (1977) *Eur. J. Biochem. 75*, 67–75.