GTP Binding to Elongation Factor eEF-2 Unmask a Tryptophan Residue Required for Biological Activity*

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Elongation factor eEF-2 from rat liver, which contains 7 tryptophan residues, was treated with increasing concentrations of N-bromosuccinimide (NBS) under conditions in which these residues were oxidized specifically. The reagent produced a characteristic lowering in both the absorbance at 280 nm and the intrinsic fluorescence at 332 nm of the factor. Fluorometric titration of tryptophans and correlation to eEF-2 residual activity on GTP hydrolysis and polyphenylalanine synthesis showed that modification of the two most reactive tryptophans completely inactivated the factor. These residues were identified as Trp343 and Trp221 after cleavage of the protein with cyanogen bromide, separation of the fragments by reversed-phase high-pressure liquid chromatography, and N-terminal sequencing of the two fragments which exhibited a decreased absorbance in the NBS-treated protein. Oxidation of the most reactive residue, Trp434, did not induce significant decrease of activity of the factor or of its ability to interact with GTP or GDP. On the contrary, oxidation of Trp221 inactivated the factor, whose residual fluorescence was still partially quenched by GDP but no longer by GTP. Preincubation of eEF-2 with GDP protected Trp221 against NBS oxidation and prevented concomitant inactivation of the factor, whereas preincubation of eEF-2 with GTP increased the sensitivity of the same Trp221 residue to the reagent. Our results show for the first time that Trp221, which is conserved and belongs to a well preserved domain in eukaryotic cells and archaeabacteria, plays an essential part in the catalytic activity of eEF-2. They strongly suggest that GTP induces a conformational change of the protein which unmask this residue, whereas GDP stabilizes a conformation which makes this residue much less accessible.

The eukaryotic elongation factor 2 (eEF-2) catalyzes the translocation of peptidyl tRNA from the ribosomal A site to the P site in the protein elongation cycle. Like its counterpart EF-G in the prokaryotic cells, it belongs to the large family of GTP-binding proteins with GTPase activity. The members of this family have several features in common. They are activated by GTP binding to consensus sequences, and the activated proteins interact with their effectors, the ribosome in the case of eEF-2. GTP hydrolysis inactivates the protein, which is released as a complex with GDP (Bourne et al., 1991). The mechanism of the translocation process catalyzed by eEF-2 (EF-G) is not yet understood, but it is assumed to be driven by conformational changes of the factor. Besides the GTP-binding domain, located near the N-terminal end (Kohno et al., 1986), eEF-2 possesses at least two specific sites which are not present in EF-G: a threonine residue located in the same region, which is phosphorylated by a specific kinase (Nairn and Palfrey, 1987; Ryazanov, 1987; Ryazanov et al., 1988) and a modified histidine, termed diphthamide, located in a region near the C-terminal end which is assumed to interact with ribosomes (Kohno et al., 1986). This diphthamide is specifically ADP-ribosylated by diphtheria toxin and NAD (Honjo et al., 1968 and 1971). Both phosphorylation and ADP-ribosylation inactivate the factor, but it is not known if these modifications produce conformational changes of the eEF-2 molecule.

We have recently shown that measurement of the intrinsic fluorescence of eEF-2, due to its Trp residues, can be used as an efficient probe to study the conformation of the protein and its interaction with nucleotides. Thus, we found that quenching of intrinsic fluorescence allows differentiation of GDP and GTP binding to eEF-2; in particular, only GTP binding modified the fractional fluorescence accessible to acrylamide (Sontag et al., 1993). The present work describes specific chemical modification of eEF-2 Trp residues by NBS in order to determine their respective reactivity and their role in the catalytic activity of the factor. Our results point out the special importance of 1 Trp residue, which has been identified as Trp221, for the function of eEF-2. This residue is protected in the presence of GDP and unmasked in the presence of GTP.

EXPERIMENTAL PROCEDURES

Materials—All nucleotides were purchased from Pharmacia. Their purity was controlled by anion-exchange chromatography on a Waters DEAE 5 PW column. NBS was from Sigma, and cyanogen bromide, guanidine hydrochloride, and the Micro BCA protein assay reagent were from Pierce Chemical Co. HPLC-grade acetonitrile and trifluoroacetic acid were from Solvents Documentation Sèmes. HPLC was performed on a Waters apparatus consisting of two M510 pumps, a U6K injector, and a 991 Photodiode Array Detector.

eEF-2 Preparation—Rat liver eEF-2 was purified as described previously (Marzouki et al., 1989) and its purity (>95% pure) checked by polyacrylamide gel electrophoresis (see Fig. 6, lane 2). Tryptophan residues were titrated according to the method of Pajot (1976). 7.9 ± 0.2 Trp residues/mol of eEF-2 were found, which is in agreement with the theoretical value drawn from cDNA sequencing (Oleinikov et al., 1988). Biological Activities and Protein Measurements—Poly(U)-directed polyphenylalanine synthesis, eEF-2-dependent GTPase activity, and [3H]GDP binding were measured as described previously (Conquet et al., 1987). Protein concentration was determined with the Micro BCA protein assay (Smith et al., 1985).

Chemical Modification by NBS—eEF-2 (1 μM, 100 μl) was dialyzed for 90 min against buffer containing 50 mM Tris acetate, 0.1 mM EDTA, and 200 mM NBS.
identified by N-terminal sequencing and mass spectroscopy.

Aquapore guard column (2.1 x 30 mm) using a linear gradient of ace-
owidebreve{r}thanol-water (80:20, v/v) at 1 ml/min. The lyophilized AMP samples were divided into two aliquots. One was used to measure the residual fluorescence emission at 350 nm of AMP which had been incubated overnight at 25 °C in the presence of 6 μM guanidine hydrochloride. Free l-trypophan was used for the calibration curve. CNBr Cleavage—AMP (0.5 mg of protein) was incubated in 500 μl of 70% (v/v) formic acid with 25 mg of CNBr in the dark during 16 h at room temperature. The samples were lyophilized and then submitted to three washing-lyophilization cycles with Milli-Q water to eliminate formic acid and CNBr as much as possible. After solubilization in 0.5 ml of 50 mM Tris-HCl, pH 8.8, containing 6 M guanidine hydrochloride and 10% (v/v) acetonitrile in 0.1% trifluoroacetic acid, the samples were separated by reversed-phase HPLC on a Vydac C18 column (300 Å, 5 µm, 4.6 x 300 mm) equipped with a C8 Aquaprep guard column (2.1 x 30 mm) using a linear gradient of ace-
onitride in 0.1% trifluoroacetic acid. The fragments corresponding to the main peaks were lyophilized, analyzed by gel electrophoresis, and identified by N-terminal sequencing and mass spectroscopy.

Electrophoresis Analysis—Sample preparation, electrophoresis, and Coomassie Blue G-250 staining were performed as described by Schagger and von Jagow (1987) using a Mini-protein gel (0.75 mm thickness) from Bio-Rad: 40 mm height separating gel (16.5% acrylamide, 10% NBS-modified protein were recorded at 25.0 ± 0.1 °C on a SLM 6000 C8 automatic fluorometer equipped with a 450-watt xenon lamp. The cuvette contained 1 ml of 0.1 μM AMP-2 in buffer A. The samples of AMP-2 were excited at 295 nm, and the emission spectra were recorded in the wavelength range of 310–410 nm. Emission and excitation bandpasses were set at 4 nm to ensure that only Trp residues were excited (Werber et al., 1972). Variations of the lamp emission power were automatically corrected with a rhodamine solution used as a standard in the reference channel. Both emission and excitation spectra were corrected for buffer blank. The nucleotide-induced quenching of AMP-2 fluorescence was deduced from the decrease of fluorescence intensity at 392 nm in the presence of increasing amounts of either GTP or GDP (Sontag et al., 1991; Geourjon and DelBage, 1993). Molecular mass of the CNBr fra-
gments was measured by electrospray mass spectroscopy on a VG Trio 11 spectrometer. The lyophilized peptides were dissolved in 50% acetoni-
trile for their amino acid composition. The CNBr fragments of AMP-2 were sequenced by automatic Edman degradation using a 473A liquid-
phase sequencer (Applied Biosystem). Identification of the fragments was achieved by comparison of their N-terminal sequences with the theoretical ones predicted using the Antheprot program (Geourjon et al., 1993). Oxindole groups, produced by NBS oxidation of Trp residues, were oxidized the most exposed residues, whereas the other proteins.

RESULTS

Effect of NBS Modification on the Spectral Properties of eEF-2—Treatment of eEF-2 by NBS at pH 5.0 produced characteristic changes in its spectral properties, depending on the NBS/eEF-2 molar ratio (Fig. 1). The ultraviolet absorbance peak at 206 nm was considerably decreased, whereas the absorbance at 250 nm was significantly increased (Fig. 1A). Intrinsic fluorescence emission of eEF-2 excited at 295 nm, which is almost exclusively due to the 7 tryptophan residues (Sontag et al., 1993), was also considerably diminished by NBS (Fig. 1B). In addition, there was a slight blue shift of the maximal emission wavelength, which varied from 332 nm for the control to 327 nm for eEF-2 treated with a 120 molar excess of NBS under the same conditions, the bandwidth at half-height being diminished from 55 to 46 nm. Increasing concentrations of NBS oxidized the most exposed Trp residues in priority, whereas those buried in less polar regions of the molecule exhibited a lower reactivity, as shown by Spande and Witkop (1967a) with other proteins.

Fig. 2 compares the variation of the residual fluorescence of eEF-2 modified with increasing concentrations of NBS (open circles) to that of the unmodified Trp residues titrated after denaturation of eEF-2 (closed squares). The titration curve indicated the existence of two distinct classes of Trp residues: a first set of 4 residues, relatively accessible to NBS up to a molar ratio NBS/eEF-2 = 80, and a second set of 3 less reactive residues. The titration curve of unmodified Trp residues did not coincide with the curve of the residual intrinsic fluorescence of the factor, indicating that the contribution of each of the 7 Trp residues to eEF-2 fluorescence was not equivalent, as esti-
imated from data of Fig. 2. Numbering the residues according to their susceptibility to NBS oxidation, the contribution of each of the three most accessible Trp residues was approximately the same (10–13% each), whereas residues 4 and 5 appeared to contribute two times more (21–24%), and the last two residues 6 and 7 showed the lowest contribution (9–11%). These results are in agreement with the classification of Burstein et al. (1973) who noted a decreasing quantum yield for the three following classes of Trp residues: those in limited contact with water (here, residues 4 and 5), those completely exposed (here, residues 1–3), and last, those buried in nonpolar regions of proteins (here, residues 6 and 7).

**Dependence of eEF-2 Activities on Tryptophan Modification by NBS**—The treatment of eEF-2 with increasing amounts of NBS progressively inactivated the factor. Fig. 3 represents the inactivation curves as functions of the number of Trp residues modified. These curves were not identical for the different activities tested. Modification of the first Trp residue had almost no effect on any of the activities. In contrast, modification of the second Trp residue almost completely abolished both poly(U)-directed polyphenylalanine synthesis and GTase activities, measured in the presence of 80 S ribosomes, whereas the formation of [3H]GDP-eEF-2 ribosome complex was only partly affected (less than 40%). After modification of the third Trp residue, no activity could be detected, no matter which test was used. These results prove that the second Trp residue plays the essential part in the process of eEF-2 inactivation by NBS.

**Correlation between Tryptophan Modification and eEF-2-Nucleotide Interactions**—The binding of guanylic, and also adenylic, nucleotides to eEF-2 induces a partial quenching of the intrinsic fluorescence of eEF-2 Trp residues (Sontag et al., 1983). In the present experiments, a maximal quenching of 8 and 16% of eEF-2 intrinsic fluorescence was reached after GDP and GTP binding, respectively (Fig. 4A). Under conditions in which the first Trp residue was oxidized by NBS (25 molar excess, Fig. 4B), approximately the same proportions of eEF-2 residual fluorescence were quenched by GTP and GDP as in controls without NBS. When the second Trp residue was oxidized (45 molar excess of NBS, Fig. 4C), no fluorescence quenching was observed with GTP, whereas GDP quenched 5.7% of the residual fluorescence, amounting to 78% of the initial fluorescence of intact eEF-2 (Fig. 4C). When a 60-molar excess of NBS was used to oxidize the third Trp residue (Fig. 4D), neither GDP nor GTP produced any detectable quenching of the residual fluorescence of eEF-2 (Fig. 4D).

In other experiments (Fig. 5A), eEF-2 was first incubated with either GDP or GTP and then treated with increasing concentrations of NBS. Preincubation of eEF-2 with both nucleotides shifted the polyphenylalanine synthesis inactivation curve obtained in the absence of nucleotide, but in opposite directions; GDP protected eEF-2 against inactivation by NBS (closed circles), whereas GTP increased the sensitivity of eEF-2 to NBS inactivation (open circles). This conclusion was confirmed by the experiment illustrated in Fig. 5B, in which eEF-2 was preincubated with different concentrations of nucleotides and then treated with NBS. A 33 molar excess of NBS produced a 40% inhibition of polyphenylalanine synthesis in control eEF-2. Under these conditions, the eEF-2 residual activity was increased after GDP binding, but decreased after GTP binding.

**Identification of the Trp Residues Modified by NBS**—In order to identify the Trp residue which was responsible for eEF-2 inactivation, control and NBS-treated eEF-2 were submitted to CNBr cleavage. Gel electrophoresis verified that (i) NBS oxidation of eEF-2 did not induce any cleavage of the protein (Fig. 6, lane 3) and (ii) CNBr cleavage of NBS-modified or unmodified eEF-2 produced identical fragment patterns (Fig. 6, lanes 4 and 5). Amino acid analysis confirmed that no significant oxidation of amino acid residues other than the Trp residues had occurred (data not shown). During the separation of the fragments by reverse-phase HPLC, six main peaks were detected at 280 nm, which corresponded to peptides containing

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**Fig. 3.** Decrease of eEF-2 activities as a function of the number of modified Trp residues. eEF-2 samples were treated with different concentrations of NBS and divided into three aliquots. One (A) was equilibrated with a buffer containing 10 mM HEPES, pH 7.0, 10 mM MgCl₂, 80 mM KCl and used for measuring poly(U)-directed polyphenylalanine synthesis in the presence of 80 S ribosomes (Reboud et al., 1972). The other aliquots were equilibrated with a buffer containing 20 mM Tris, pH 7.4, 10 mM MgCl₂, 10 mM KCl, 5 mM 2-mercaptoethanol and used for measuring GTase activity (B) and [3H]GDP binding (C) in the presence of 80 S ribosomes as described previously (Conquet et al., 1987).

**Fig. 4.** Effect of Trp modification on the nucleotide-induced quenching of eEF-2 intrinsic fluorescence. eEF-2 (1 μM) was incubated either in the absence of NBS (A) or in the presence of a 25 (B), 45 (C), or 60 (D) molar excess of NBS. MgCl₂ (8 mM) was added, and the samples were analyzed for fluorescence emission at 332 nm upon excitation at 295 nm, in the presence of increasing concentrations of GTP (C) or GDP (D). ΔF represents the fluorescence quenched by the nucleotides.
Chemical Modification of eEF-2 Tryptophan Residues

**Fig. 5.** Effects of nucleotides on NBS-induced inactivation of eEF-2. eEF-2 (1 μg) was preincubated with GDP (●) or GTP (○) for 10 min in buffer A containing 8 mM MgCl₂ before being incubated with NBS. Poly(U)-directed polyphenylalanine synthetic activity of 10-μl aliquots was measured as in Fig. 3A. A, nucleotide concentration (30 μM) was constant whereas the NBS/eEF-2 molar ratio varied as indicated. A control preincubated without nucleotide (△) was tested under the same conditions. B, nucleotide concentration was increased, whereas the NBS/eEF-2 molar ratio (33) remained constant.

**Fig. 6.** Effects of NBS and CNBr on the electrophoretic pattern of eEF-2. Lane 1, myoglobin fragments used as markers (3 μg). Lane 2, purified eEF-2 from rat liver (3 μg). Lane 3, purified eEF-2 (3 μg) treated with a 100 molar excess of NBS. Lanes 4 and 5, CNBr cleavage products of eEF-2 (7 μg) unmodified or modified by a 100 molar excess of NBS, respectively. Lanes 6 and 7, CNBr fragments (2 μg of each) repurified from peaks 6 and 5 of Fig. 7, respectively.

Trp residues due to their high A₂₂₀/A₂₈₀ ratio (Fig. 7, A and B). Oxidation of the first two Trp residues with a 45 molar excess of NBS almost completely suppressed the absorbance of peaks 5 and 6 at 280 nm, whereas the other four peaks remained unchanged (Fig. 7C). The A₂₂₀/A₂₈₀ ratios of peaks 3, 5, and 6 were expressed as functions of the number of modified Trp residues (Fig. 8A). The ratio of peak 6 (open circles) was decreased with a NBS concentration which oxidized one Trp residue. A higher NBS concentration, producing the oxidation of a second Trp residue, was needed to decrease the ratio of peak 5 (closed squares). The ratio of peak 3 remained constant. Therefore, it is clear that the Trp residue which is the most sensitive to NBS oxidation is located in peak 6, and the second one in terms of NBS sensitivity is located in peak 5. In order to see if there was a correlation between the decrease of peak 5 or 6 and the protein synthesis activity of eEF-2, the variation of the A₂₂₀/A₂₈₀ ratio was expressed as a function of the inhibition of synthetic activity. Fig. 8B shows that there was an almost
linear relationship between the variation of the $A_{280}/A_{290}$ ratio of peak 5 and the inhibition of protein synthesis, which was not the case for peak 6. These results confirm the involvement of the second oxidized Trp residue in eEF-2 activity. The contents of peaks 5 and 6 were analyzed by gel electrophoresis in the presence of sodium dodecyl sulfate. There was only one band in peak 6, but two bands in peak 5 (data not shown). The contents of peaks 5 and 6 were submitted to a second HPLC run using a 2-fold less steep gradient. Two peptides were separated from peak 5: one was a contaminant that did not contain any Trp residue. The re-purified peptides from peaks 5 and 6, which gave unique bands on gel electrophoresis (Fig. 6, lanes 6 and 7, respectively), were analyzed for their N-terminal sequences (Table I). The peptides could be localized without any ambiguity in the published amino acid sequence of rat liver eEF-2 (Oleinikov et al., 1989). The molecular ratio of the two main peptides from peaks 6 and 5 was determined by electrospray mass spectroscopy (see “Experimental Procedures”). The experimental values (1499 ± 4 and 2849 ± 1, respectively) were very close to the theoretical values (1497 and 2848), which confirmed that the entire sequences of our purified peptides were identical to those of the predicted CNBr fragments. From these results, it was concluded that the Trp residues in peaks 6 and 5 are TrpZz1 and Trp343, respectively.

Effects of GTP and GDP Binding on TrpZz1 Modification—Since GTP binding increased the sensitivity of eEF-2 to NBS inactivation and GDP binding protected the factor against inactivation, the effects of nucleotide binding on NBS oxidation of TrpZz1 were tested. After treatment of eEF-2 with a 35 molar excess of NBS, cleavage by CNBr and separation of the fragments by reverse-phase HPLC, the absorbance of peaks 5 and 6 at 280 nm were diminished of 37 and 79%, respectively (Table III). When eEF-2 was preincubated with GDP before being treated with NBS, peak 5 value was identical to that observed in eEF-2 in the absence of GDP and NBS treatment, whereas peak 6 value remained very low. These results show that GDP binding prevented the modification of TrpZz1 completely but did not affect the oxidation of Trp343. On the other hand, when eEF-2 was preincubated with GTP, peak 5 was diminished by 70% as compared with the control and peak 6 kept the same low level as that observed without preincubation. This means that GTP increased the extent of Trp221 modification 2-fold but had no effect on the modification of Trp343. The levels of the other peaks were not affected by either NBS treatment or preincubation with nucleotides.

**DISCUSSION**

Chemical modification of eEF-2 Trp residues by NBS is shown here to bring original information on the contribution of each of these residues to the intrinsic fluorescence of the factor and on the role of one of them in the biological activity of the factor and its interactions with nucleotides. The specificity of the observed Trp oxidation, which was carried out at acidic pH in order to increase both reactivity (Spande and Wickop, 1967b) and selectivity (Lundblad and Noyes, 1984), was proved by our observations that NBS did not produce any significant modification of amino acids other than Trp, nor any cleavage of eEF-2, nor any difference in the peptide pattern obtained after CNBr treatment. The successive oxidation of the different Trp residues by increasing concentrations of NBS showed that each Trp residue contributed differently to eEF-2 intrinsic fluorescence, depending upon its location in the eEF-2 molecule.

We focused our study on the 2 most exposed Trp residues, since after their oxidation, eEF-2 lost its biological activity completely as shown by both GTPase activity and polyphenylalanine synthesis. The 2 residues were identified after CNBr cleavage of the eEF-2 molecule and alignment of the N-terminal sequences of the corresponding peptides with the known sequence of rat liver eEF-2 (Oleinikov et al., 1989). After oxidation of the most reactive residue, TrpZz1, eEF-2 kept its biological activity and still interacted with both GTP and GDP. Therefore, a direct participation of this residue in the catalytic site of eEF-2 could be excluded. The fluorescence of TrpZz1 should not be quenched by GTP or GDP binding to intact eEF-2; the fact that the quenching of the residual fluorescence of eEF-2 by GTP or GDP binding was not increased after oxidation of this residue is probably related to a small modification of the second tryptophan, as shown in Fig. 8A. The consequences of the oxidation of TrpZz1 were quite different from those observed with Trp343. Modification of TrpZz1 completely inactivated eEF-2 in GTP hydrolysis and polyphenylalanine synthesis, suggesting that this residue is located in a strategic position of the molecule. Under these conditions, eEF-2 fluorescence was no longer quenched by GTP. The absence of quenching can be due to one of the following reasons. First, GTP binding could not occur after modification of the factor. Second, GTP bound to the factor but did not quench its fluorescence, because the fluorescence of TrpZz1 in intact eEF-2 is specifically quenched by GTP binding. Under the same conditions, addition of GDP partially quenched the residual fluorescence of eEF-2. This meant that GDP still bound to the modified factor, in agreement with the result illustrated by Fig. 3C. Moreover, the GDP-quenchable fluorescence of eEF-2 was only partly related to TrpZz1.

Preincubation of eEF-2 with nucleotides before NBS treatment yielded important information concerning the relationship between TrpZz1 accessibility and nucleotide binding. It is clear from the results of both Fig. 5 and Table II that GTP binding increased the sensitivity of TrpZz1 to NBS oxidation. The most likely explanation is that GTP induced a conformational change in the eEF-2 molecule, which exposed the strategic TrpZz1 to the external medium. This conformation could correspond to the activated form of eEF-2, which would make the binding of the factor to the ribosome possible. It would not correspond to a relaxed state of the entire molecule, since our

**TABLE I**

| Fragment      | Sequence                  | Predicted CNBr fragment |
|---------------|---------------------------|--------------------------|
| From peak 6   | RRWLPAGDA...             | R^aRRWLPAGDALQ[M]_523    |
| From peak 5   | IDPVLGTVGPG...           | P^aIDPVLGTVGPGSC         |
| Contaminating fragment of peak 5 | VNFTVD... | V^bVNFTVDQIRAIM_13 |

**TABLE II**

| Treatment    | Peaks | 1 | 2 | 3 | 4 | 5 | 6 |
|--------------|-------|---|---|---|---|---|---|
| None         | 0.28  | 0.88 | 1.00 | 1.36 | 1.56 | 1.63 |
| NBS          | 0.31  | 0.92 | 1.00 | 1.39 | 0.98 | 0.35 |
| GDP, then NBS| 0.30  | 0.89 | 1.00 | 1.38 | 1.61 | 0.35 |
| GTP, then NBS| 0.30  | 0.88 | 1.00 | 1.39 | 0.47 | 0.30 |

*Note: a and b indicate the number of Trp residues.*
previous experiments suggested that GTP binding decreased the overall accessibility of the Trp residues in the factor to acrylamide (Sontag et al., 1993). Therefore it seems likely that the conformational change of the molecule in the presence of GTP, which is shown here, is restricted to a small domain surrounding TrpZ1.

Alignments of the EF-2 sequences surrounding TrpZ1 reveal that GDP binding to eEF-2 protected amino acid residues 20916 were much less accessible to the reagent. Another possibility is that this shielding was due to GDP binding directly or in close proximity to this residue. The latter hypothesis seems less likely than the former, although it cannot be excluded; TrpZ1 belongs neither to the consensus GTP/GDP-binding sequences (A2HVDHGC2, D2HSGP107, N156KMD164) of Knoho et al., 1986 nor to the sequence of the fragment Gly233-Trp243 which has been cross-linked with oxidized GTP byNilsson and Nygård (1988).

Alignment of the consensus sequences of eEF-2 from rat and from other eukaryotic cells and archaeabacteria using the data from Swiss Prot 24 Bank and the Antheprot program (Geourjon et al., 1991; Geourjon and Delage, 1993) reveals that TrpZ1 is conserved in all factors (see Table III). Moreover, the amino acid sequence surrounding this Trp residue is also remarkably conserved, with only a few variations, mainly in archaeabacteria. Alignment of the sequence of rat eEF-2 with that of EF-G from Escherichia coli (Zengel et al., 1984) could not be achieved, because the EF-G molecule is smaller. Except for the motifs characteristic of the GTP-binding site, there are relatively few consensuses sequences with many gaps, making alignment unsafe. The conservation of TrpZ1 and its surrounding domain in all EF-2 molecules we examined, in addition to the fact that oxidation of this residue in rat eEF-2 completely inactivates the factor, strongly suggests that this Trp residue is essential for the function of the factor. An attractive hypothesis is that TrpZ1, when unmasked by GTP binding to eEF-2, interacts with the universal purine-rich sequence of the large subunit rRNA, which is modified by ricin and α-sarcin toxins and which has been assumed to trigger translocation through an allosteric transition of rRNA (Wool et al., 1992).

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