Intrinsic Tryptophan Fluorescence of Rat Liver Elongation Factor eEF-2 To Monitor the Interaction with Guanylic and Adenylic Nucleotides and Related Conformational Changes†

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ABSTRACT: Elongation factor 2 (eEF-2), which contains seven Trp residues, exhibited a tryptophan-characteristic intrinsic fluorescence with maximum excitation at 280 nm and an emission peak centered at 333 nm that suggested a hydrophobic environment of these tryptophans. Upon denaturation with 6 M guanidine hydrochloride, the maximum emission was shifted to 348 nm. Fluorescence quenching studies using acrylamide and iodide confirmed that the Trp residues were mainly buried in the native molecule and indicated an important heterogeneity, the fractional accessible fluorescence (f_a) values being 0.50 and 0.25, respectively. Partial quenching of eEF-2 fluorescence by nucleotides proved the existence of an interaction of the factor in the absence of ribosomes, not only with GDP but also with GTP, nonhydrolyzable analogs, GMP, and adenylic, but not cytidylic, nucleotides. Saturating binding plots showed different maximal changes of fluorescence depending upon the nucleotides, from 6.4% with ADP to 24.5% with GDP, and suggested the existence of more than one binding site for each nucleotide. Among all the nucleotides tested, only GTP at saturating concentration modified the f_a value obtained with acrylamide (−36%). The possibility that this modification is related to a conformational change of eEF-2 induced by GTP binding is discussed.

The eukaryotic elongation factor 2 (eEF-2)[1] catalyzes the translocation of peptidyl-tRNA from the ribosomal A site to the P site in the protein elongation cycle. This translocation step, whose molecular mechanism is not yet understood, involves specific interaction between three components: the factor eEF-2, the ribosome, and GTP. The precise role of GTP has been debated for a long time. It is now generally postulated that GTP induces a conformational change of eEF-2 responsible for its binding to the ribosome and that the hydrolysis of GTP to GDP and inorganic phosphate, which occurs after translocation, is responsible for the release of eEF-2 from the ribosomes (Brot, 1982; Moldave, 1985). The fact that native eEF-2 can interact with GDP in the absence of ribosomes is well established, but its interaction with GTP under the same conditions remains difficult to show experimentally (Crechet, 1985; Burns et al., 1986; Marzouki et al., 1991). The existence of specific conformational changes of eEF-2 induced by the binding of guanylic nucleotides was suggested by the observation that these nucleotides modify the susceptibility of eEF-2 to trypsin, with GTP and GDP having similar effects (Nilsson & Nygärd, 1985). More recently, we showed that the accessibility of two amino acid residues to exogenous reagents was different depending upon whether eEF-2 was included within a tertiary complex with ribosomes and either a nonhydrolyzable GTP analog or GDP, which could support the hypothesis that these two guanylic nucleotides induced different conformations of eEF-2 (Lavergne et al., 1990). Interaction of this factor with nucleotides other than the guanylic ones has never been demonstrated.

In this report, we show for the first time that the intrinsic tryptophan fluorescence, which is known to be very sensitive to a wide variety of environmental conditions (Lakowicz, 1983; Creed, 1984), can be used as an efficient probe to study the native conformation of eEF-2 and its interaction with various nucleotides, including the adenylic ones, and the specific conformational changes induced by nucleotide binding.

MATERIALS AND METHODS

Materials. All the nucleotides used and their derivatives, guanylyl 5′-(β,γ-imidotriphosphate) (GMP-PNP) and guanylyl 5′-(β,γ-methylenetriphosphate) (GMP-PCP), were from Boehringer, except for GTP which was obtained from Pharmacia as an ultrapure and stable solution. The purity of the nucleotides was controlled by chromatographic analysis on a Waters DEAE 5 PW column. KI, Gdn·HCl, and NATA were obtained from Sigma, and acrylamide was from Serva.

Preparation of eEF-2. Rat liver eEF-2 (>95% pure) was prepared according to Marzouki et al. (1989), and its purity was confirmed by both 1D gel electrophoresis and titration of the Trp residues according to the method of Pajot (1976) as previously described (Divita et al., 1991). A value of 7.0 Trp residues/mol was found, in agreement with the theoretical value of 7 drawn from cDNA sequencing (Oleinikov et al., 1989); NATA was used as the standard, and the validity of the method was controlled by various model proteins: bovine serum albumin, ovalbumin, bovine trypsin, and egg lysozyme, found to contain 1.93, 3.0, 3.96, and 6.2 Trp residues/mol, respectively [theoretical values were 2, 3, 4, and 6 Trp residues/mol; for references see Divita et al. (1991)].

Biological Assays. Poly(U)-directed polyphenylalanine synthesis and GTPase activity were measured in the presence of rat liver 80S ribosomes as previously described (Conquet et al., 1987).

Fluorescence Measurements. They were performed at 25.0 ± 0.1 °C using an SLM—Aminco 8000 C spectrofluorometer equipped with a 450-W xenon lamp (excitation and emission

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[1] Abbreviations: eEF-2, eukaryotic elongation factor 2; Gdn·HCl, guanidine hydrochloride; GMP-PCP, guanylyl 5′-(β,γ-methylenetriphosphate); GMP-PNP, guanylyl 5′-(β,γ-imidotriphosphate); NATA, *N*-acetyltryptophanamide; TNP-ATP, 2′,3′-trinitrophenyladenosine triphosphate.

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slits set to 4 nm). Except when indicated, the excitation wavelength was set to 295 nm to limit fluorescence to tryptophan only. Emission spectra were collected from 310 to 410 nm. Experimental errors on the fluorescence measurements were estimated to be less than 5%. Both emission and excitation spectra were corrected for buffer blank, and the variations of the lamp emission power were automatically corrected with a rhodamine solution as standard in the reference channel. The eEF-2 concentration used was 0.1 μM, and the measurements were performed in buffer A: 20 mM Tris-HCl, pH 7.6, 8 mM $MgCl_2$, 100 mM KCl, 100 mM sucrose, 1 mM DTT, and 10% glycerol, in the presence or absence of 6 M Gdn·HCl. Additions of nucleotides or quenchers were made from concentrated stock solutions, the potassium iodide solution containing 0.1 mM sodium thiosulfate to prevent I_3^- formation. Fluorescence intensity values obtained in the presence of increasing concentrations of KI were corrected for the ionic strength effect by reference to controls performed with equal concentrations of KCl. Fluorescence was corrected for dilution. Corrections for inner-filter effect of nucleotides were made using NATA as standard and corrections for the absorption of acrylamide according to Calhoun et al. (1983).

The fluorescence quenching data using either acrylamide or iodide were analyzed according to the Stern–Volmer equation, which considers two types of quenching, collisional and static, and relates the decrease in fluorescence to the concentration of quencher as

$$F_0/F = (1 + K_{SV}[Q])e^{V[Q]} \quad (1)$$

where F_0 and F are the fluorescence intensities in the absence and in the presence of quencher, respectively, K_{SV} is the collisional Stern–Volmer constant, [Q] is the quencher concentration, and V is the static quenching constant (Eftink & Ghiron, 1976, 1981, 1987). If there is only collisional quenching (no static quenching), eq 1 becomes

$$F_0/F = 1 + K_{SV}[Q] \quad (2)$$

The plot of F_0/F vs [Q] is linear for a homogeneous population of emitting fluorophores but bends downward for multiple heterogeneous fluorophores. To such heterogeneous systems and assuming that the total fluorescence is partitioned into a f_a fraction quenchable with a constant K_Q and a fraction completely inaccessible to the quenching agents, Lehrer (1971) applied a modified Stern–Volmer equation:

$$F_0/(F_0 - F) = 1/[Q]f_aK_Q + 1/f_a \quad (3)$$

The plot of $F_0/(F_0 - F)$ vs $1/[Q]$ allows a graphical determination of f_a and K_Q.

RESULTS

Intrinsic Fluorescence Properties of eEF-2. The excitation spectrum of eEF-2 fluorescence exhibited a maximum at a wavelength (λ_m exc) of 280 nm which was consistent with tryptophan fluorescence (not shown). Excitation at either 280 nm or 295 nm resulted in emission spectra with a maximum at a wavelength ($\lambda_m$em) of 333 nm and a bandwidth at half-height ($\Delta\lambda_{1/2}$) of 55 nm. Only a very small fraction of the emission fluorescence (near 310–320 nm) was due to the 21 tyrosine residues present in eEF-2 (Oleinikov et al., 1989), as deduced by normalizing the emission spectra relative to the emission at 360 nm where the fluorescence originated from tryptophan exclusively (Figure 1). The $\lambda_m$exc was not modified when eEF-2 was denatured, but its $\lambda_m$em was shifted to 348 nm, the $\Delta\lambda_{1/2}$ value being unchanged. A $\lambda_m$em value of 355 nm was observed under the same conditions with NATA, a solute-tryptophan model compound, whether Gdn·HCl was present or not.

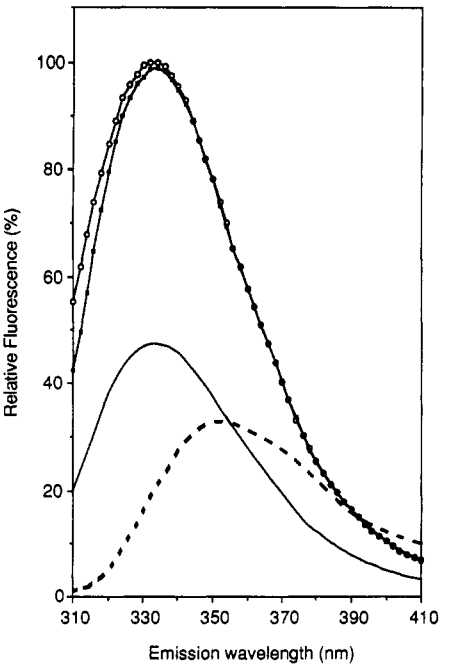

FIGURE 1: Fluorescence emission spectra of native and denatured eEF-2. Emission spectra of 0.1 μM native eEF-2 were determined in buffer A, the excitation wavelength being 280 (O—O) or 295 nm (—). The maximal fluorescence value measured by excitation at 280 nm was equal to 100. The calculated emission spectrum (■—■) is normalized by multiplying the fluorescence generated by excitation at 295 nm by a constant that is the ratio of the emission intensities at 360 nm produced by excitation at 280 and 295 nm. Emission spectrum of eEF-2 unfolded by overnight incubation in 6 M Gdn·HCl, the excitation wavelength being 295 nm (- - -).

Tryptophan Accessibility to Quenchers. Native or denatured eEF-2 was incubated with increasing concentrations of either acrylamide, a polar nonionic quencher that has access to all but the most buried residues, or iodide, a large polar anion considered to have access only to surface tryptophans. A progressive decrease of eEF-2 fluorescence intensity, more marked with acrylamide than with iodide at equal concentration, was observed. In the presence of 0.15 M acrylamide, the fluorescence of native eEF-2 at the $\lambda_m$em (331 nm) was reduced by 39%, the $\Delta\lambda_{1/2}$ value being 56 nm (Figure 2a). In the presence of 0.15 M iodide, the fluorescence at the $\lambda_m$em (which was also equal to 331 nm) was reduced by 17%, the $\Delta\lambda_{1/2}$ being decreased to 51 nm. The difference fluorescence spectrum of native versus quenched eEF-2 (Figure 2b) exhibited a maximum at 337 nm and a $\Delta\lambda_{1/2}$ of 54 nm (acrylamide) and a $\lambda_m$em at 344 nm with a shoulder at 323 nm and a $\Delta\lambda_{1/2}$ of 58 nm (iodide). The Stern–Volmer plots obtained using native eEF-2 in the presence of either acrylamide or iodide gave downward curves, indicative of Trp heterogeneity in regard to their accessibility to the quenchers (Figure 3a). The plots obtained using denatured eEF-2 and either acrylamide or iodide gave upward curvatures, which suggest that all residues became nearly equally accessible to the quenchers (Eftink & Ghiron, 1976). Modified Stern–Volmer plots (Lehrer, 1971) confirmed tryptophan heterogeneity in native eEF-2, since relatively small fractions of the total tryptophan emission were affected by iodide and acrylamide (f_a values = 0.25 and 0.50, respectively) (Figure 3b). The fact that both iodide and acrylamide had access to all tryptophan residues in denatured eEF-2 was confirmed by the value of f_a = 1.0, as was the case with NATA. The K_Q value corresponding to the fluorescence fraction (f_a) quenchable by acrylamide in native eEF-2 was half the values obtained

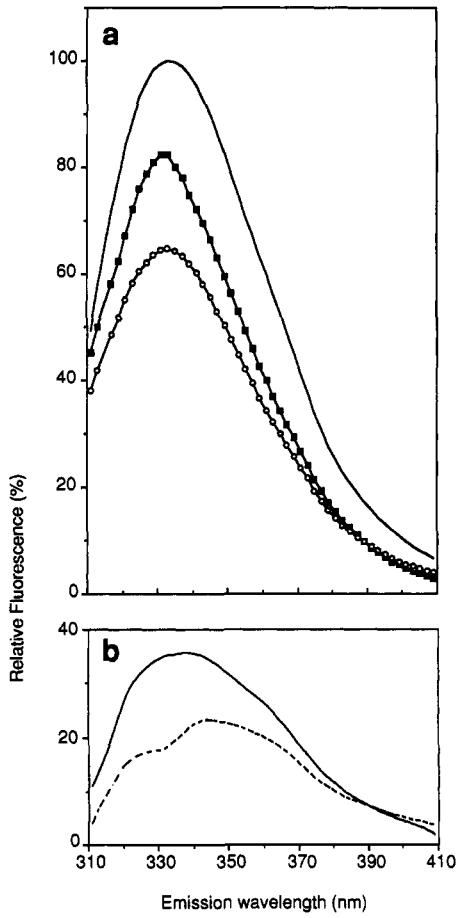

FIGURE 2: Effects of quenchers on the fluorescence emission spectrum of native eEF-2. All solutions contained 0.1 μM eEF-2 in buffer A. (a) Native eEF-2 (—); native eEF-2 plus 0.15 M acrylamide (O—O); native eEF-2 plus 0.15 M KI (■—■). The excitation wavelength was 295 nm. The fluorescence intensity corrected as indicated under Materials and Methods is expressed as a percentage of the value at the $\lambda_m$em of native eEF-2 (333 nm). (b) Difference fluorescence spectra of native eEF-2 vs native eEF-2 quenched by either 0.15 M acrylamide (—) or iodide (- - -).

using unfolded eEF-2 or NATA (7.7 M^{-1} for native eEF-2 vs 15.0 and 16.3 M^{-1} for denatured eEF-2 and NATA, respectively). The opposite was observed in the presence of iodide, with K_Q values of 12.5 M^{-1} for native eEF-2 vs 4.2 and 8.3 M^{-1} for denatured eEF-2 and NATA, respectively.

Control experiments have shown that eEF-2 preincubated with 0–0.15 M quenchers was fully active when assayed in poly(U)-directed polyphenylalanine synthesis and GTPase activity, i.e., in the presence of ribosomes and after such a dilution that the final quencher concentrations varied from 0 to 7.5 mM. This result precluded the possibility of irreversible eEF-2 structural changes induced by quenchers.

Interaction of eEF-2 with Nucleotides. Addition of GTP or GDP to native eEF-2 produced a decrease of the intrinsic fluorescence of the factor, which remained centered at 333 nm ($\Delta\lambda_{1/2}$ unchanged at 55 nm) (Figure 4a). A fluorescence decrease was also observed after addition of ATP, which indicated that, as GTP and GDP, ATP also interacted with eEF-2 (Figure 4b). Addition of 50 μM ATP to eEF-2 broadened its fluorescence spectrum ($\Delta\lambda_{1/2}$ 60 nm, $\lambda_m$em remaining equal to 333 nm). ADP addition up to 5 μM concentration did not change the eEF-2 emission spectrum. Beyond this concentration, ADP most likely modified the environment of Trp residues since it shifted the $\lambda_m$em to 338 nm, decreased the fluorescence at wavelengths under 347 nm, and, unlike the other nucleotides, slightly increased the fluorescence at higher wavelengths, the fluorescence spectrum

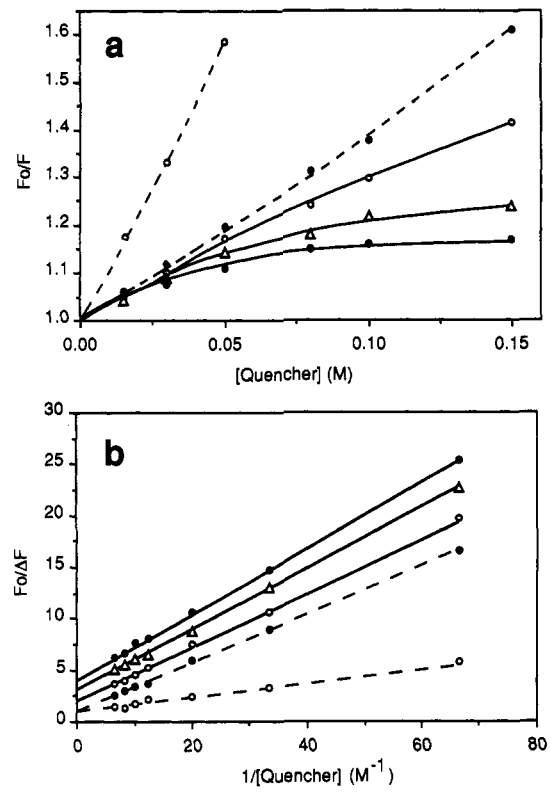

FIGURE 3: Quenching of eEF-2 intrinsic fluorescence. (a) Stern–Volmer plots of the quenching of the fluorescence emission of 0.1 μM native (—) or denatured (- - -) eEF-2 by either acrylamide (O) or iodide (●). In parallel experiments, 50 μM GTP was added to native eEF-2 prior to the assay with acrylamide (△). F_0 and F are total fluorescence values determined upon integration of the emission spectrum (from 310 to 410 nm) in the absence and in the presence of quenchers, respectively. (b) Modified Stern–Volmer plots according to Lehrer (1971) from the data of (a). $\Delta F = F_0 - F$. The intercepts represent $1/f_a$ and the slopes $1/f_aK_Q$.

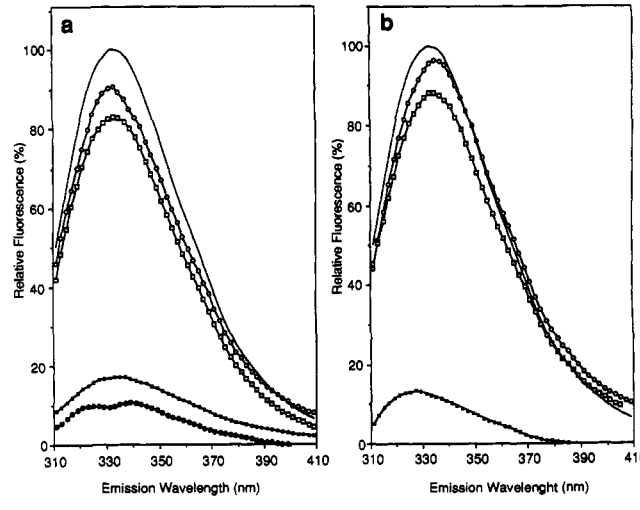

FIGURE 4: Effect of nucleotides on the intrinsic fluorescence of native eEF-2. 0.1 μM native eEF-2 in buffer A was incubated in the presence or the absence of 50 μM nucleotides for 15 min at 25 °C prior to being assayed. (a) Top spectra: native eEF-2 (—); native eEF-2 plus GTP (□—□); native eEF-2 plus GDP (O—O). Bottom spectra: difference fluorescence spectra of native eEF-2 vs eEF-2 quenched by either GTP (■—■) or GDP (●—●). (b) Top spectra: native eEF-2 (—); native eEF-2 plus ATP (□—□); native eEF-2 plus ADP (O—O). Bottom spectrum: difference fluorescence spectrum of native eEF-2 vs eEF-2 quenched by ATP (■—■). The excitation wavelength was 295 nm.

also being broadened ($\Delta\lambda_{1/2}$ 58 nm). The $\lambda_m$em and $\Delta\lambda_{1/2}$ values of the differential spectra of native eEF-2 versus eEF-2 quenched by 50 μM nucleotides (Figure 4a,b, bottom spectra)

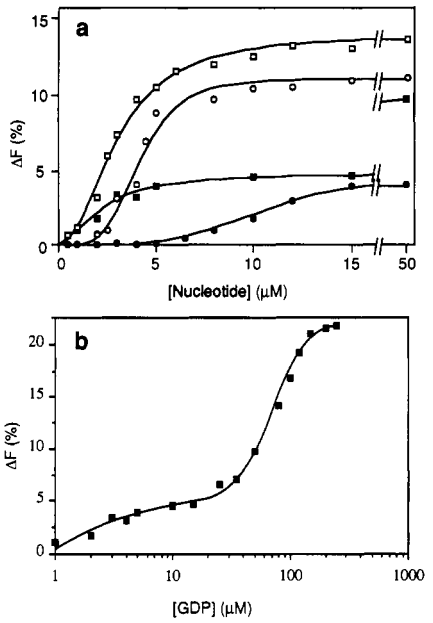

FIGURE 5: eEF-2 fluorescence decrease versus nucleotide concentration. The total fluorescence decrease ($\Delta F = F_0 - F$) of 0.1 μM native eEF-2 determined upon integration of the emission spectrum (from 310 to 410 nm) and expressed as percent of F_0 is plotted as a function of increasing concentrations of nucleotides. (a) Saturation curves of GTP (□), GDP (■), ATP (○), and ADP (●) are fitted to the Hill equation as indicated in the legend of Table I. (b) GDP saturation curve up to a 250 μM concentration on a semi-log scale.

Table I: Apparent Parameters of Nucleotide Binding to eEF-2[a]

ligand	$[S]_{1/2}$ (μM)	ΔF_{max} (%)
GTP	3.0	14.0
GDP	2.5	5.3
	68	24.5
ATP	4.1	11.1
ADP	13.2	6.4

[a] Binding constants were determined from the saturation curves (Figure 5) fitted to the Hill equation which can be formulated: $\Delta F = \Delta F_{max}[S]^n/(K' + [S]^n)$ using the Multifit 2.0 program (Day Computing, Cambridge, U.K.). ΔF is the fluorescence decrease of eEF-2 in the presence of nucleotides, [S] represents the nucleotide concentration, n is the Hill coefficient, and K' is a constant from which the nucleotide concentration yielding half-maximal quenching, $[S]_{1/2}$, was calculated by the relation $[S]_{1/2} = K'^{1/n}$.

were reproducibly found to be the following: GTP, 333 nm, 55 nm; GDP, 339 and 325 nm, 55 nm; ATP, 329 nm, 48 nm. In the case of ADP, the differential spectrum was nondeterminable due to the shift of the emission spectrum.

Figure 5 shows the curves of the eEF-2 fluorescence decrease versus nucleotide concentration, fitted to the Hill equation. The binding parameters determined from these curves are listed in Table I. Large variations in the values of these parameters were observed, in particular the ΔF_{max}, depending upon the nature of the nucleotide used. The saturation curves obtained with GTP, ATP, and ADP were sigmoidal, with Hill coefficient values ≥2. The $[S]_{1/2}$ values for GTP and ATP were not very different, whereas that for ADP was significantly higher. Nonhydrolyzable GTP analogs, GMP-PCP and GMP-PNP, had the same binding constants as GTP. In the case of GDP, the curve was best described by a combination of two phenomena: saturable ligand binding with relatively high affinity and binding to a second site of low affinity, which was filled during incubation of eEF-2 with GDP concentration higher than 25 μM. Saturation curves were also obtained with GMP and AMP, but, in contrast, no quenching was observed after addition of cytidylic nucleotides (50 μM CTP) (results not shown).

Influence of Nucleotide Binding on Tryptophan Accessibility to Quenchers. At saturating concentrations of nucleotides, only GTP markedly influenced the tryptophan accessibility of eEF-2 to quenching by acrylamide. Prior incubation of eEF-2 with GTP increased the curvature of the Stern–Volmer plot, which indicated a decrease of the effect of acrylamide particularly at high concentrations (Figure 3a). In this case, the f_a value deduced from the modified Stern–Volmer plot was reduced from 0.50 for native eEF-2 (and fully-formed complexes with GDP, ATP, or ADP) to 0.32 for fully-formed eEF-2–GTP complex, the K_Q values for the interaction with acrylamide not being significantly modified with any of these nucleotides. In contrast, prior incubation of eEF-2 with each nucleotide did not change the f_a value obtained with iodide (0.25), the K_Q value of 12.5 M^{-1} for native eEF-2 and fully-formed complexes with GTP or GDP being increased to 18.6 M^{-1} for fully-formed complexes with ATP or ADP (data not shown).

DISCUSSION

In this paper, we show for the first time that measurements of the intrinsic fluorescence of eEF-2, which is almost entirely due to its Trp residues, can give useful information on both its native conformation and its interaction with nucleotides.

Most of the Trp residues appear deeply buried inside the native eEF-2 as deduced from the rather low $\lambda_m$em (333 nm) [see the classification of Trp residues in the proteins of Burstein et al. (1973)] and from the 50% decrease of the K_Q value in the presence of acrylamide determined with native eEF-2 compared to the values determined with denatured eEF-2 and with NATA. This is in agreement with results showing the compactness of native eEF-2: when this large molecule (MW ≃ 95 000) is treated with trypsin, most of the basic residues are not accessible to the enzyme (Nilsson & Nygård, 1985, 1988; Lavergne et al., 1990). The relatively high K_Q value observed with native eEF-2 in the presence of iodide was most likely due to a local environment of the fluorophores, rich in positively charged residues, as illustrated by Lehrer (1971) using synthetic polypeptides. Indeed, three among the seven Trp residues (Trp_{261}, Trp_{343}, and Trp_{650}) are inside regions rich in Arg or Lys residues. The shift of the $\lambda_m$em to 348 nm when eEF-2 was unfolded indicated a displacement of fluorescent Trp residues to a more polar environment. The loss of fluorescence (–44%) as a result of unfolding can be explained by the quenching property of this environment (Luisi & Favilla, 1970; Burstein et al., 1973). The downward curvature of the Stern–Volmer plots obtained in the presence of both quenchers and the low f_a values (0.25 and 0.50) indicate a high heterogeneity of Trp residues due to differential environment polarity in native eEF-2. Iodide was especially useful to topographically differentiate Trp residues; the separation between the two maxima of residual and differential fluorescence spectra was 13 nm with iodide compared to only 6 nm with acrylamide. The decrease of $\Delta\lambda_{1/2}$ of the eEF-2 emission spectrum in the presence of 0.15 M iodide (55 nm vs 51 nm) may reflect a decrease in this heterogeneity due to differential quenching of the Trp residues although iodide effects upon eEF-2 structure or interaction with bound water cannot be excluded.

The most interesting results concern the interaction of eEF-2 with nucleotides. Indeed, the relatively large number of Trp residues in eEF-2 molecule allow nucleotide binding to be detected with a higher sensitivity than by the methods previously used. Thus, partial quenching of eEF-2 fluorescence observed in the presence of nucleotides proved that, in the absence of ribosome, eEF-2 specifically interacts not only with

GDP, which was already well established, but also with GTP, which was difficult to show experimentally (Crechet, 1985; Burns et al., 1986), with GTP nonhydrolyzable analogs, and with GMP. They also established, for the first time, the existence of a specific interaction of eEF-2 with adenylic nucleotides: ATP, ADP, AMP. In contrast, no interaction was detected with CTP used as a control. It is worth mentioning that yeast elongation factor eEF-3 has been shown to exhibit an ATPase activity, in addition to the GTPase one; both activities are displayed by the same active site, and ATPase activity seems essential to the function of eEF-3 (Uritani et al., 1988).

The quenching of eEF-2 Trp residues observed in the presence of nucleotides can be explained in two ways, which are not exclusive of each other. First, quenching is related to the spectral overlap of the base absorption red-edge with the fluorescence spectrum of some eEF-2 Trp residues which produces a Förster energy transfer, as proposed in the case of creatine kinase (Vasak et al., 1979) and mitochondrial F_1-ATPase (Divita et al., 1992). Such a mechanism would be in agreement with our observation that guanylic nucleotides, in which the overlap is large, produce a higher ΔF_{max} value than adenylic nucleotides, in which the overlap is significantly smaller. Second, nucleotide binding produces a conformational change of the factor, which brings some Trp residues in a higher-quenching environment. In the case of ADP binding at a concentration higher than 5 μM, Trp residues became more accessible to solvent as shown by the red shift and the increase in bandwidth of the eEF-2 emission spectrum. The differences observed between the differential fluorescence spectra using GTP, GDP, and ATP might be due to differences either in the relative location of the binding sites and the quenched Trp residues or in conformational changes induced by the binding of these nucleotides. Among all nucleotides tested, GTP was the only one to decrease the f_a value obtained with acrylamide. Such a decrease could be due to a conformational change induced specifically by GTP binding in the eEF-2 molecule, possibly related to that occurring during translocation. However, one cannot completely exclude the possibility of a direct and specific shielding by GTP of Trp residues from the quencher nor even an alteration of the lifetime/quantum yield of specific Trp residues by GTP, which could modify their "apparent" accessibility to acrylamide.

Because of the complexity of our system, it is difficult to draw a definite conclusion about the number of sites of eEF-2 which interact with the nucleotides. That saturation curves fitted to the Hill equation were sigmoidal and gave n apparent values ≥ 2 in the case of GTP, ATP, and ADP suggests the presence of at least two cooperative binding sites, while in the case of GDP, results agree with the existence of two noncooperative binding sites with different affinities. The concentrations yielding half-maximum quenching ($[S]_{1/2}$) were similar for GTP and GDP (high-affinity site) under our conditions using a relatively high Mg^{2+} concentration. We cannot discard the possibility that a difference between the affinity for GTP and GDP might be found at very low Mg^{2+} concentration, as was noted in the case of ras p21 protein (Skelly et al., 1990). Recent experiments performed with fluorescent ATP (ADP) analogs, TNP-ATP (ADP), strongly suggest that 2 mol of analog binds to 1 mol of eEF-2 (Sontag et al., unpublished results), which strengthens the hypothesis of the presence of more than one nucleotide binding site on eEF-2. In connection with this, it is interesting to note that in the eEF-2 primary structure (Oleinikov et al., 1989), one can distinguish twice the sequence AXXXXGK (residues 26–32 and 277–283) required for binding of the β- and γ-phosphate groups of nucleotide and present in most GTP and ATP binding proteins (Saraste et al., 1990). Thus, the presence of two such sequences in eEF-2 could agree with the existence of two nucleotide binding sites, especially as the first sequence is located in the G1 region necessary for the GTPase activity (Kohno et al., 1985, 1986) and the second one belongs to a domain which cross-links oxidized GTP (Nygård & Nilsson, 1986). The question could be asked whether the interaction between eEf-2 and adenylic nucleotides could be related to the requirement of ATP and NAD^+ involved in the two known specific modifications, phosphorylation and ADP-ribosylation, which inactivate eEF-2 (Palfrey, 1983; Ryazanov & Spirin, 1990; Nygård & Nilsson, 1990).

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