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Ethidium Bromide Binding to Transfer RNA: Transfer RNA as a Model System for Studying Drug–RNA Interactions†

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ABSTRACT: The interaction of ethidium bromide (EB) with tRNA has been examined by optical (absorption spectra, fluorescence intensity and lifetime) and ^{1}H nuclear magnetic resonance (^{1}H NMR) methods. The optical measurements show that the strongest EB binding site is intercalative. At higher EB levels about three additional nonintercalative sites are occupied. The ^{1}H NMR experiments gave the following information about the strong intercalative site. Results for mixed and pure species of transfer RNA (tRNA) showed that none of the residues involved in the tertiary structure are adjacent to, or disrupted by the strongly bound EB. The strong binding site in yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Val}_1$ is adjacent to the sixth base pair of the amino acid acceptor stem. Results for four other class I tRNAs are consistent with the strong binding site being located in the amino acid acceptor stem, but some other binding sites for these four tRNAs cannot be ruled out on the basis of the ^{1}H NMR results alone. Yeast $tRNA^{Leu}_3$, a class III tRNA, exhibits spectral changes on binding EB which are clearly different from those of the class I tRNA examined here. The results for yeast $tRNA^{Leu}_3$ are interpreted in terms of a unique EB binding site in the extra arm which stabilizes the base pairs of this stem. *E. coli* $tRNA^{fMet}$ appears to be different from the other tRNAs investigated here in that there seem to be several binding sites of similar binding strength. These results are consistent with the notion that the tertiary structure of tRNA restricts the binding of EB to a single site in the amino acid acceptor stem. Additional support for this notion is given by ^{1}H NMR results which show that EB and chloroquine, an intercalative drug, bind to the same unique site of *E. coli* $tRNA^{Glu}_2$.

The interaction of EB[1] with tRNA is of general interest in connection with questions regarding drug–nucleic acid interactions since EB is known to have a number of effects on the biological and biochemical properties of polynucleotides (Waring, 1965, 1975). For example, EB inhibits RNA (Kramer et al., 1974) and DNA (Loeb, 1974) polymerases and the digestion of DNA by DNase I (Eron and McAuslan, 1966). EB also has antibiotic properties and is mutagenic (Waring, 1975). Kramer et al. (1974) report that binding of EB affects the rate of in vitro replication of a small replicating RNA molecule and that mutational changes of only three bases eliminated the EB inhibition. Their results suggested that at least one important EB binding site was eliminated by the three base changes since the number of binding sites in the mutant RNA is smaller than in the wild type (Kramer et al., 1974). Several recent papers have illustrated the utility of EB in probing nucleosome structure (Angerer et al., 1974; Ide and Baserga, 1976; Ballestra et al., 1976). Lurquin and Buchet-Mathieu (1971) have demonstrated that binding of EB affects the rate of aminoacylation of some tRNA, and this raises interesting questions about the relationship between EB binding properties and the effect on the rate of aminoacylation.

The studies mentioned above serve to illustrate the important role of EB in polynucleotide studies and to emphasize the need for more information about the factors which control the binding of EB to polynucleotides.

Because much is known about the secondary and tertiary structure of tRNA molecules in the crystal and in solution, these molecules provide useful "model" systems for exploring some of the factors which may influence the binding of EB to RNA. Since tRNAs exhibits a variety of tertiary structural features not found in double helical systems previously investigated they are of special interest.

Previous studies of the optical melting curves of tRNA in the presence and absence of EB show there is competition between EB binding and formation of tertiary structure such that at high EB levels the tertiary structure is disrupted (Urbanke et al., 1973). This is to be contrasted with the stabilization of

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[1] Abbreviations used: EB, ethidium bromide; DSS, sodium 2,2-dimethylsilapentane-5-sulfonate.

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double helical nucleic acids by EB (Waring, 1974). A previous ^{1}H NMR investigation showed that EB intercalates to a unique site located in the acceptor stem of yeast tRNA^{Phe} (Jones and Kearns, 1975). Preliminary x-ray results suggest that the binding site on this tRNA is located elsewhere, at least in the crystal (Warrant et al., 1976; Stout and Sundaralingham, 1975).

To further understand the role of tertiary interactions on EB binding to tRNA, we have investigated the effect of EB on the ^{1}H NMR spectra of eight individual tRNA and mixed *E. coli* tRNA. The optical properties of EB–tRNA complexes were also investigated to determine the number of EBs bound per tRNA and the mode of binding in experimental conditions similar to those used in the ^{1}H NMR experiments.

Materials and Methods

Transfer RNA. The tRNA samples were purified as described elsewhere (Bolton et al., 1976). Yeast tRNA^{Phe} and tRNA$^{Leu}_3$ were supplied by Simon Chang and *E. coli* tRNA$^{Glu}_2$ was supplied by A. D. Kelmers. The ^{1}H NMR samples were prepared by vacuum dialysis against a solution containing 0.18 M NaCl, 10 mM $MgCl_2$, and 10 mM cacodylate at pH 7.0. Samples for which the methyl region of the spectrum was obtained were dialyzed against 0.1 M NaCl, 10 mM $MgCl_2$, and 10 mM KH_2PO_4 at pH 7.0; the tRNA samples were typically 1 mM. The yeast tRNA^{Phe} sample was prepared as described elsewhere (Jones and Kearns, 1975).

EB was dissolved in water and added directly to the samples in Wilmad 508 micro cells. The samples were then reduced to their original volume by a stream of dry, filtered nitrogen. In cases where both the low-field and methyl region spectra were obtained, both spectra were obtained after each addition of EB so as to allow comparison of spectra obtained in identical conditions. The intensities in both the low-field and methyl regions of the spectra were reproducible to within 5% after each addition of EB. The final stoichiometry of the EB–tRNA solution of some samples was checked by examination of the absorption spectrum of the solution using the extinction coefficient of EB at the isosbestic wavelength [4110 at 510 nM (Waring, 1965)] and taking the extinction coefficient of tRNA (260 nm) at 6×10^5 (about 1.6 nM/A_{260}).

^{1}H NMR Measurements. Most of the ^{1}H NMR spectra were obtained by slow field sweep of the spectra, except the spectra in Figures 2B and 4 which were obtained by correlation spectroscopy (Dadock and Sprecher, 1974). The resolution of the low-field spectra in Figure 4 was enhanced through use of convolution difference methods. All spectra were obtained with a Varian HR-300 spectrometer, and the temperature was controlled to within 1 °C during the course of an experiment. Chemical shifts were measured by use of side-band modulation of the water resonance which in turn was referenced to the usual standard sodium 2,2-dimethylsilapentane-5-sulfonate (DSS). All chemical shifts are in parts per million (ppm) downfield from DSS.

To determine the effect of binding of EB to tRNA on the position of the resonance of the methyl group of the ethyl side chain of EB, the chemical shift of this resonance was determined in the presence and absence of tRNA. There is a ~0.15 ppm upfield shift in the resonance position when the EB is bound to either *E. coli* mixed tRNA or *E. coli* tRNA$^{Val}_1$ relative to the resonance position in free EB. Patel and Canuel (1976) found a similar small shift in the position of this resonance when the EB was bound to self complementary deoxytetranucleotides.

Assignment of Imino Proton Resonances. The assignment of the resonances from secondary structure imino protons was based on assuming that the double-stranded regions of tRNA are made up of A form RNA helices (Kearns and Bolton, 1977; Kearns et al., 1977). The ring current shifts used are calculated using a dipolar expression fit to the Giessner-Prettre and Pullman (1970) calculations at 3.4 Å. The intrinsic positions of A·U and G·C base pairs were taken to be 14.5 and 13.6 ppm, respectively. This method has been used to predict the spectra of some 15 tRNA, and in almost every case, there is good agreement between the predicted and observed spectra (Kearns and Bolton, 1977; Kearns et al., 1977). We should point out that several other sets of ring current parameters were used in earlier work, but they lead to basically the same predicted spectra allowing for up to 0.2 ppm disagreements on some resonances (Kearns and Bolton, 1977; Kearns et al., 1977).

The identification and the assignment of the imino proton resonances from tertiary interactions have been discussed elsewhere (Bolton et al., 1976; Bolton and Kearns, 1975, 1977c; Kearns, 1976). Tertiary interactions are responsible for resonances at 14.3(9), 13.8, 13.0, and 11.5 ppm which have been assigned, respectively, to $(s^4)U_8$·A_{14}, A_{58}·T_{54}, G_{19}·C_{56} and the imino proton of U_{33} hydrogen bonded to phosphate 36 (Bolton et al., 1976; Bolton and Kearns, 1975, 1977c; Wong et al., 1975; Wong and Kearns, 1974; Reid et al., 1975; Daniel and Cohn, 1976; Kearns, 1976).

Alternative assignments have been proposed based on different intrinsic positions of the A·U and G·C base pairs (Robillard et al. (1976a,b)). Robillard and Kim (1976) used values of 14.35 and 13.54 ppm. In several earlier studies, intrinsic positions of 14.8 and 13.8 ppm were used (Hilbers and Shulman, 1974; Robillard et al., 1976a,b; Hilbers et al., 1976; Shulman et al., 1973), but the experimental support for such low-field values has been eliminated (Robillard et al., 1976a,b; Hilbers and Shulman, 1974; Hilbers et al., 1976; Geerdes and Hilbers, 1977; Kearns, 1976).

Fluorescence Measurements. Fluorescence intensity measurements were carried out using a homemade fluorimeter (Olmsted and Kearns, 1977), and lifetime measurements were obtained using a nanosecond lifetime instrument constructed by Professor J. Yguerabide. Absorption spectra were obtained with a Beckman Acta III spectrophotometer.

Results

Fluorimetric Studies of Ethidium Bromide Binding. The binding of EB to polynucleotides can be monitored by changes in the absorption spectrum, fluorescence quantum yield, and lifetime (LePecq and Paoletti, 1967; Tritton and Mohr, 1973; Waring, 1965). The changes in the absorption spectra indicate the number of EB which are bound, and the lifetime and quantum yield of fluorescence can be used to distinguish between intercalative and nonintercalative binding.

Optical absorption measurements of EB and tRNA in 0.17 M Na and 10 mM Mg showed that tRNA can bind up to ~4 EB/tRNA. Lifetime measurements showed that at low EB/tRNA levels the fluorescence decayed with a single lifetime of 28 ns, characteristic of intercalation. At higher EB/tRNA levels the decay was composed of the 28-ns component as well as shorter lived components. Thus, the lifetime measurements indicate that the most strongly bound EB is intercalated and that the other EB are not bound by intercalation as evidenced by their short lifetimes (LePecq and Paoletti, 1967). The steady-state fluorescence of EB in the presence of tRNA, shown in Figure 1, simply corroborates the lifetime measurements in showing that the most strongly bound EB is highly fluorescent, whereas the other EB which are bound fluoresce much more weakly. Intercalated EB is known to be much more fluorescent than nonintercalatively bound EB (LePecq and

TABLE I: Effect of EB on Spectra of Individual tRNA.

tRNA species	Positions of major loss in intensity (ppm)	Resonance(s) assigned within ±0.2 ppm[c]	Resonance(s) assigned within ±0.4 ppm[c]	Possible EB binding sites[a]
Yeast $tRNA^{Phe}$	14.4	6		5, 6
E. coli $tRNA^{Val}_1$	14.3		6	
	13.6	7, 12, 29	4, 6, 53	6, 7
E. coli $tRNA^{Glu}_2$	13.2	7, 28, 31, 49, 53		6, 7; 3, 31;
	13.0	1, 6, 28, 31, 50	4, 5, 7, 30, 32, 49, 51, 53	31, 32; 49, 50
E. coli $tRNA^{Phe}$	13.8	12, 50	7	
	13.3	11, 49, 53	2	2, 3; 6, 7; 11, 12; 28, 29;
	12.6	1, 3, 4, 27, 28, 29, 52	6	49, 50, 52, 53
Yeast $tRNA^{Asp}$	14.1	7, 11	1, 12	1, 2; 3, 4; 11, 12;
	13.2	28, 31, 52	4, 12, 29, 48, 51	27, 28; 48, 49; 50, 51
	12.4	2, 3, 49	27, 50	
E. coli $tRNA^{Met}$	13.2[b]	3, 4, 11, 54	2, 53	2, 3; 3, 4; 53, 54

[a] See text for details. [b] More than one resonance. [c] Of loss in intensity.

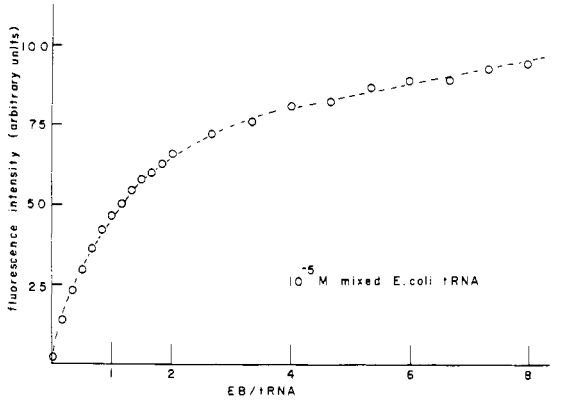

FIGURE 1: Plot of the fluorescence intensity of EB at different EB/tRNA ratios at a constant concentration of tRNA.

Paoletti, 1967). Taken together, the optical results indicate that there is a single intercalative site for EB which is the major site occupied at one EB per tRNA. There are about three additional, weaker binding sites which are nonintercalative. Similar results were previously obtained by Urbanke et al. (1973) and Sakai et al. (1975).

^1H NMR Studies of Ethidium Bromide Binding. Evidence for a Unique Binding Site. The spectra of different tRNA samples in the presence and absence of EB are shown in Figure 2-7. The spectra of yeast $tRNA^{Phe}$ clearly show that the addition of EB induces localized changes in the spectrum. Since the spectra are very sensitive to tRNA conformation, this observation that the spectral changes are localized to a small number of spectral positions indicates that the binding is to a unique site. Similarly, the results for yeast $tRNA^{Leu}_3$, *E. coli* $tRNA^{Val}_1$, and $tRNA^{Glu}_2$ in Figures 4-6 show that EB induces discrete losses and gains in intensity in the spectra of these tRNA indicating that there is a unique binding site. For some of the other pure species of tRNA investigated, see Figure 5, the spectral changes are somewhat diffuse and these need to be discussed separately.

Evidence That EB Does Not Disrupt Tertiary Structure. The spectrum of *E. coli* $tRNA^{mixed}$ exhibits resonances from common tertiary interactions at 14.9, 13.8, 13.0, 11.5, and 1.1 ppm (Bolton and Kearns, 1975, 1977a,c). The spectra of mixed *E. coli* tRNA in Figure 2 show that the addition of 1 EB/tRNA does not affect any of these resonances. This indicates

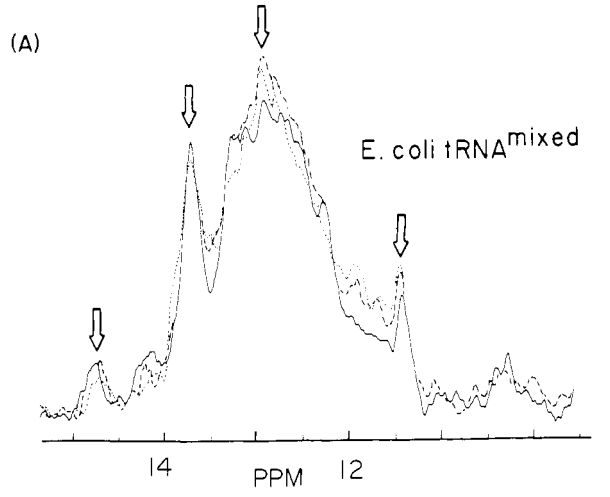

FIGURE 2: (A) The 300-MHz ^1H NMR spectrum of *E. coli* $tRNA^{mixed}$ in the presence and absence of EB. The dashed line is the spectrum for 1 EB/tRNA and the dotted line for 2 EB/tRNA. Arrows mark positions of resonances assigned to common tertiary interactions. (B) The 300-MHz ^1H NMR spectrum of *E. coli* $tRNA^{mixed}$ in the presence and absence of EB. The same sample was used for obtaining the low-field and methyl region spectra. The level of EB present in the sample is indicated on the figure and the spectra were obtained at 34 °C.

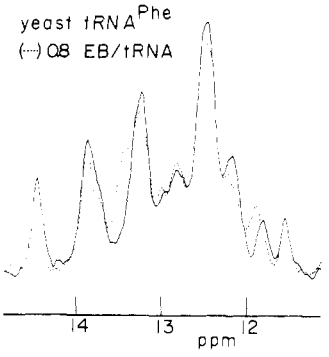

FIGURE 3: The 300-MHz ^{1}H NMR spectra of yeast tRNA^{Phe} in the presence and absence of 0.8 EB/tRNA (dashed line). The spectra were obtained at 10 °C and the spectra are taken from Jones and Kearns (1975).

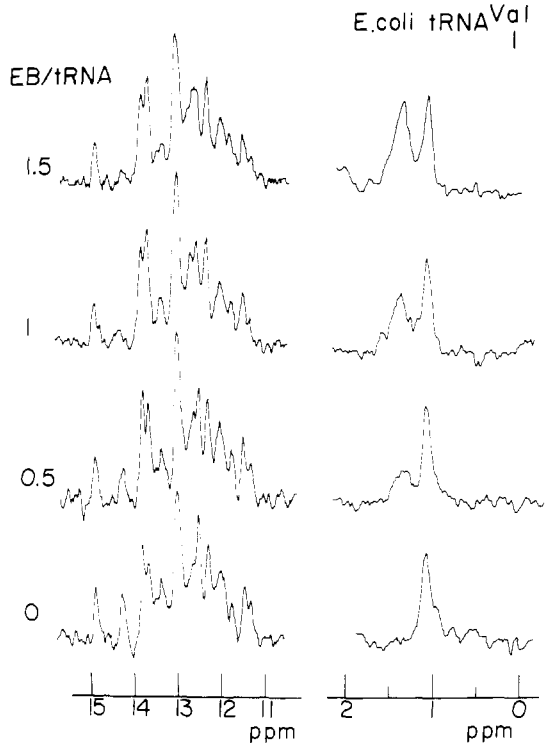

FIGURE 4: The 300-MHz ^{1}H NMR spectra of *E. coli* tRNA$^{Val}_1$ in the presence and absence of EB. The figure indicates the level of EB present in the samples and the spectra were obtained at 34 °C.

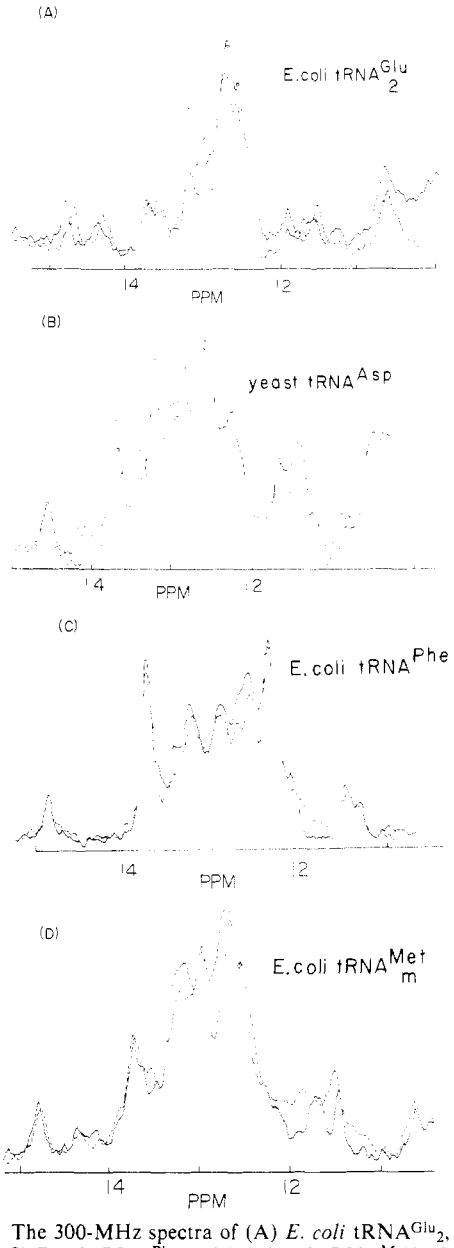

FIGURE 5: The 300-MHz spectra of (A) *E. coli* tRNA$^{Glu}_2$, (B) yeast tRNA^{Asp}, (C) *E. coli* tRNA^{Phe}, and (D) *E. coli* tRNA^{Met} in the presence and absence of EB. The dashed line is the spectrum for 1 EB/tRNA and the dotted line for 2 EB/tRNA. The spectra were obtained at 40 °C.

that EB does not bind adjacent to, or affect any of the common tertiary interactions. The spectra of *E. coli* tRNA$^{Val}_1$ in Figure 4 show that the addition of EB does not affect the resonance at 1.1 ppm from the methyl protons of T_{54}, a residue which is involved in the tertiary structure. At higher levels there are small changes at 14.9 and 13.8 ppm which could be due to partial occupancy of a binding site located near certain tertiary interactions.

Evidence That EB Does Not Decrease Extent of Base Pairing. The intensity of the low-field (11–15 ppm) spectra of tRNA is proportional to the number of base pairs and tertiary interactions which have slowly exchanging imino protons (extent of base pairing). The spectra in Figures 2–5 show that within experimental error EB has no effect on the total intensity of the spectrum. However, in the case of yeast tRNA$^{Leu}_3$ the addition of EB increases the total intensity of the low-field region, particularly at ~13.8 ppm. This indicates the binding of EB stabilizes additional base pairs in this tRNA.

Comparison of EB and Chloroquine Binding. The spectra of *E. coli* tRNA$^{Glu}_2$ in the presence of EB and chloroquine are shown in Figure 7. The results show that both dyes induce essentially the same spectral changes and this indicates that EB and chloroquine bind to *E. coli* tRNA$^{Glu}_2$ at the same site.

Summary of Fluorimetric and ^{1}H NMR Results. The above results indicate that tRNA have a single strong site for intercalative binding of EB with several weaker sites which are probably due to outside binding. The strong site appears to be unique for several tRNA and is not located adjacent to any of the common tertiary interactions. EB binding to the intercalative site does not reduce the extent of base pairing and in at least one case it enhances the extent of base pairing. In addition, the binding of chloroquine appears to be very similar to that of EB. A summary of the effects of EB on the spectra of tRNA is given in Table I.

Discussion

Fluorescence experiments indicate that, when tertiary structure is absent (low salt, no magnesium) but secondary

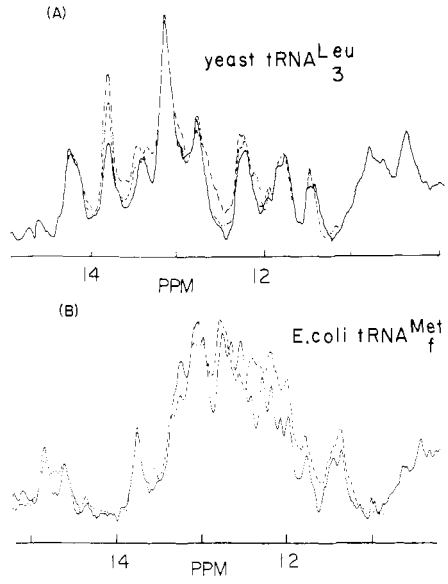

FIGURE 6: The 300-MHz ^{1}H NMR spectra of (A) yeast tRNA$^{Leu}_3$ and (B) *E. coli* tRNA^{Met} in the presence and the absence of EB. Figures are labeled as in caption to Figure 5 and the spectra were obtained at 40 °C.

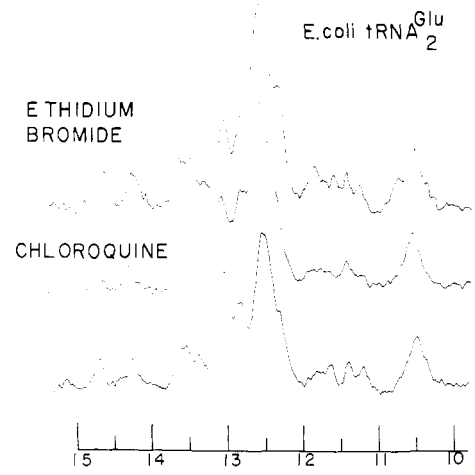

FIGURE 7: The 300-MHz ^{1}H NMR spectra of *E. coli* tRNA$^{Glu}_2$ and 1:1 complexes with ethidium bromide and chloroquine. The spectra were obtained at 40 °C.

structure is present, the majority of tRNA bind between 4 to 6 EB molecules by intercalation. However, when tertiary structure is present (10^{-2} M Mg^{2+}, $T < 50$ °C), tRNAs bind only a single EB molecule by intercalation. T-jump studies (Tritton and Mohr, 1973) indicate that the presence of excess EB stabilizes an early melting transition in yeast tRNA^{Phe} (in low salt, no magnesium) which has been assigned to thermal denaturation of tertiary structure. These, and other observations, show that when tRNA tertiary structure is present there is only one intercalation site per tRNA. For any given tRNA, the single binding site might be a unique one (the same in all molecules) or due to the fact that binding of EB at any one site excludes binding at other possible sites.

The ^{1}H NMR results presented in this paper, taken in conjunction with the optical results, permit us to draw the following conclusions concerning the drug binding properties of tRNA, the locations of the binding sites, and factors which are involved in controlling the binding. Since the various conclusions are not all of equal certainty, the important assumptions which are involved are also indicated.

(i) The tertiary structure of tRNA is unaffected by binding of one EB per molecule. This conclusion depends only on the general assignment of the resolved peaks in the spectra of *mixed* tRNA to *common* tertiary interactions, and there is strong experimental evidence supporting these assignments (Bolton et al., 1976; Bolton and Kearns, 1975, 1977a,c; Chao and Kearns, 1977). It is not dependent upon the assignment of the common resonances to specific tertiary interactions.

(ii) The binding of EB does not affect the s^4U_8·A_{14} interaction and, therefore, the binding site cannot be located adjacent to this tertiary structure base pair. This conclusion depends upon the assignment of the 14.9-ppm resonance in *E. coli* tRNA to the 8–14 tertiary interaction, and experimental evidence supporting this assignment is quite strong (Bolton and Kearns, 1975; Wong et al., 1975; Wong and Kearns, 1974; Reid et al., 1975; Daniel and Cohn, 1976). The one exception to this general conclusion is *E. coli* tRNA^{Met} for which there is ^{1}H NMR evidence for a slight perturbation of the resonance from the s^4U_8·A_{14} tertiary interaction.

(iii) Binding of EB adjacent to the tertiary base pair between A_{58} and T_{54} can also be ruled out as EB has no effect on the resonance of the methyl protons of T_{54}. This was shown to be the case for mixed *E. coli* tRNA and *E. coli* tRNA$^{Val}_1$.

Both of the above mentioned sites have been suggested as possible EB binding sites on the basis of x-ray diffraction studies on tRNA crystals which had been grown in the absence of EB and to which EB was subsequently added by diffusion into the crystal (Warrant et al., 1976; Stout and Sundaralingham, 1975; Liebman et al., 1977). The crystal and solution studies do not necessarily have to agree as to the location of the EB binding site since the structure of tRNA in the crystal is subject to constraints not present in solution.

(iv) For several tRNAs the binding is clearly to a unique site. This follows from the observation that the spectral changes which occur on binding a single EB are sufficiently pronounced and localized that binding to a unique site is required. If binding occurred to many different sites, addition of one EB per tRNA would not induce the complete loss of a resonance at one position without affecting other resonances. These considerations are independent of the assignments of resonances in the low-field spectrum.

(v) In *E. coli* tRNA$^{Val}_1$ and yeast tRNA^{Phe} the intercalation site is located between two secondary structure A·U base pairs. This conclusion depends on the assumption that any resonance which occurs to lower fields than 13.6 ppm must be due to an A·U base pair (secondary or tertiary interaction) and the demonstration that none of the tertiary interactions which give rise to resonances in this region of the spectrum are affected by binding one EB. The experimental evidence that the intrinsic position of the resonances from G·C base pairs is 13.5–13.6 ppm is quite strong (Geerdes and Hilbers, 1977) and this rules out the possibility that a resonance from a G·C base pair could be located below 13.6 ppm. In both of these tRNA, binding sites which contain two adjacent A·U base pairs are located at the base of the amino acid acceptor stem. In yeast tRNA^{Phe} the site could be between base pairs 5 and 6 or 6 and 7, but in *E. coli* tRNA$^{Val}_1$ only the latter site is possible. In *E. coli* tRNA$^{Glu}_2$, the binding site is adjacent to at least one G·C base pair since the major spectral effects are located near 13.2 ppm.

(vi) The ^{1}H NMR results offer no evidence that the binding of EB is sequence specific. For yeast tRNA^{Phe} and *E. coli* tRNA$^{Val}_1$, the binding is between A·U base pairs, whereas in the other tRNA the binding is either between G·C base pairs or between one A·U and one G·C base pair.

(vii) Comparison of the effect of EB and chloroquine dem-

onstrates that both drugs bind at the same site of *E. coli* $tRNA^{Glu}_2$. Further characterization of the EB binding sites requires detailed assignments of the low-field spectra. Our interpretation of the spectra is based on use of A form RNA helices for the tRNA secondary structure and ring current shift calculations (see Materials and Methods). Although this method gives good agreement with the observed spectra of some 15 tRNA, it must be realized that the proposed assignment of any particular resonance could be in error. With this note of caution we consider the interpretation of the results obtained for individual tRNA which are summarized in Table I.

The above ^1H NMR data suggest that there is a unique binding site for EB on each tRNA. Since tRNAs have many common structural features, it is reasonable to assume that the EB intercalation site might be the same for all tRNA. The ^1H NMR results indicate that the binding site for *E. coli* $tRNA^{Val}_1$ and yeast $tRNA^{Phe}$ is located in the acceptor stem. While the ^1H NMR results are consistent with the notion that EB only binds to the acceptor stem of class I tRNA, the results for four tRNAs are also consistent with binding to the TΨC stem, two are consistent with binding to the anticodon stem and two with binding to the hU stem (Table I). Therefore, except in the cases of yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Val}_1$, we cannot rule out the possibility of binding to sites other than the acceptor stem. After this paper was submitted Wells and Cantor (1977) published a report in which they examined the energy transfer between EB and a fluorescent dye bound to the 3′ end of tRNAs. Their results are consistent with binding of EB to the acceptor stem. Yeast $tRNA^{Leu}_3$ is different from the other tRNAs examined in that it has a large extra arm, and in this case the binding of EB causes a selective *increase* in intensity near 13.8 ppm without any corresponding loss of intensity elsewhere in the spectrum. This rather unusual behavior can be interpreted in terms of stabilization of the small 3 base pair helix of the extra arm by binding of the EB. This indicates that EB can bind at different sites on different tRNAs. Binding of EB to *E. coli* $tRNA^{fMet}$ is somewhat different than other tRNAs as suggested on the basis of optical results (Surovaya and Borissova, 1976). The ^1H NMR results on *E. coli* $tRNA^{fMet}$ are not consistent with a unique intercalation site and suggest that there are either several intercalation sites or outside binding sites or both. In addition, EB affects the resonance position of the $s^4U_8 \cdot A_{14}$ base pair making *E. coli* $tRNA^{fMet}$ the only tRNA for which there is ^1H NMR evidence that EB affects the tertiary structure.

While the ^1H NMR results do not exclude binding of EB to the TΨC, hU, and anticodon stems in some tRNA, they indicate a preference for binding to the acceptor stem. Examination of the models that have been used to interpret the x-ray diffraction data of yeast $tRNA^{Phe}$ (Ladner et al., 1975; Quigley et al., 1975; Sussman and Kim, 1976; Stout et al., 1976) suggests a reasonable explanation for these observations. Because of the many tertiary interactions connecting the hU and TΨC arms, intercalation of an EB molecule in either of these stems could disrupt the tertiary structure. The anticodon stem is also constrained (at least partially) through its junction with the main core of the molecule, by tertiary interactions involving U_{33} and by stacking interactions in the anticodon loop. Only the amino acid acceptor stem is relatively unaffected by formation of the tertiary interactions, and in the cases where the EB binding site can be unambiguously assigned, it is located in the acceptor stem. Wells and Cantor (1977) have suggested that the binding of EB is specific to the acceptor stem since the conformations of some of the riboses in this stem are favorable for intercalation (Tsai et al., 1975).

Although the above considerations apply specifically to the binding of ethidium bromide, they may apply to other dyes and drugs which bind by intercalation. The comparison of the chloroquine and EB binding to *E. coli* $tRNA^{Glu}_2$ supports this notion. We are not able to make any predictions regarding the effects which tertiary interactions have on nonintercalative binding on the basis of these results.

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Physical Characterization of Myosin Light Chains†

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ABSTRACT: This paper reports the results of an investigation into the size and shape of the low molecular weight subunits (light chains) of myosin from several animal species. Hydrodynamic, analytical gel filtration, and fluorescence anisotropy decay measurements indicated that these light chains could be represented by a general ellipsoidal model having a longest axis of about 100 ± 20 Å. Investigation into the stability of the internal structure of the scallop regulatory light chain was carried out by studying the effect of pH, ionic strength, temperature, and guanidine hydrochloride on its circular dichroic spectrum. The nearly complete insensitivity of the circular dichroic spectrum to pH, ionic strength, and temperature variations from 4 to 70 °C indicated that this subunit contained regions of very stable structure which probably exist when it is bound to myosin.

The myosin molecule is a hexameric enzyme comprised of two large polypeptide chains of about 200 000 and two pairs of low molecular weight polypeptide chains ranging from 17 000 to 25 000. The low molecular weight subunits can be divided into two classes on the basis of their chemical structure, electrophoretic mobilities, and the methods used to dissociate them from myosin.

Light chains of one class can be removed only with concomitant loss of ATPase activity and have been called alkali-light chains in rabbit (Gazith et al., 1970; Weeds, 1969) and SH-light chains in the myosin regulated adductor muscle of scallop (Szent-Györgyi et al., 1973). On the basis of the loss of ATPase activity with removal of light chains by various agents, a direct role of the myosin light chains in the ATPase activity and ADP binding of myosin was postulated by Gershman et al. (1968, 1969), Dreizen et al. (1967), Dreizen and Gershman (1970), and Dreizen and Richards (1973). More recently, it has been demonstrated that the two different types of alkali-light chains (alkali-1 and alkali-2) have no effect

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