Ethidium Binding To Yeast tRNA^Phe: A New Perspective On An Old Bromide
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Abstract
We have reinvestigated the binding of ethidium bromide (EB) to yeast tRNA^Phe using frequency domain fluorometry and Global analysis. Previous fluorescence investigations of EB - tRNA interactions have been interpreted qualitatively. We have performed experiments using frequency domain fluorometry in which we measure the lifetime decay of EB bound to tRNA. In addition to the "strong" binding site, which has a lifetime of 17.7 ns, there is a "weak" binding site, which has a lifetime of 5.4 ns. Interestingly, though, this second binding event reduces the lifetime of the first EB to 3.3 ns. Global analysis of the data was consistent with a model in which a second EB molecule binds, which has a lifetime of only 5.4 ns. We also noticed that the fluorescence enhancement of the first EB bound and that the lifetime of this EB should be monotonic with the EB/tRNA ratio. However, we noticed that the fluorescence enhancement of the second site increases as the EB/tRNA ratio increases, while the lifetime of the first EB decreases as the EB/tRNA ratio increases.

Introduction
The interaction of ethidium bromide with nucleic acids has been studied extensively for more than four decades. The interaction of EB with nucleic acids has been studied using optical spectroscopy, NMR and X-ray crystallography. The consensus of the literature is that the "strong" interaction is between EB and the DNA helix, while the "weak" interaction is between EB and the DNA minor groove. The "strong" binding site is ~1-2 nm from the major groove, while the "weak" site is ~4 nm from the major groove. The "strong" binding site has a dissociation constant in the range of 0.1 to 0.25 µM, while the "weak" binding site has a dissociation constant in the range of 50 to 100 µM. We next tried to link this long lifetime component with the NMR model of Chu at al (mentioned in the introduction).

Sample Preparation
Total tRNA^Phe was obtained from Saccharomyces cerevisiae and used without further purification. EB was obtained from Molecular Probes. The buffer utilized was 20 mM HEPES, 8 mM MgCl2, 0.1 mM EDTA, 100 mM KCl at pH 7 unless otherwise specified. The EB/tRNA ratio was varied from 0.5 to 100.

Time-Resolved Measurements
Intensity decay data were obtained using an ISS model 3005 multichannel phase and modulation fluorometer utilizing a Spectra-Physics model 2017 argon laser as the excitation source. Samples were excited at 568 nm and emission was collected over a frequency range of 500 kHz to 80 MHz. The data collected were analyzed using Global software. The time-domain fluorescence lifetime of the EB bound to tRNA was 17.7 ns. The fractional intensities of the 4 lifetime components of EB for the "weak" binding component is virtually constant. The "strong" binding component is largely constant, while the "weak" binding component decreases as the EB/tRNA ratio increases. In both of these traditional analyses the long lifetime component decreases as the EB/tRNA ratio increases. However, even as the lifetime decreases one would expect that some of the EB molecules only have one EB bound and that the lifetime of the EB should be monotonic. We next tried to link this long lifetime component with the NMR model of Chu at al.

New Analysis
In both of these traditional analyses the long lifetime component decreases as the EB/tRNA ratio increases. However, even as the lifetime decreases one would expect that some of the EB molecules only have one EB bound and that the lifetime of the EB should be monotonic. We next tried to link this long lifetime component with the NMR model of Chu at al. We also noticed that the fluorescence enhancement of the first EB bound and that the lifetime of this EB should be monotonic with the EB/tRNA ratio. However, we noticed that the fluorescence enhancement of the second site increases as the EB/tRNA ratio increases, while the lifetime of the first EB decreases as the EB/tRNA ratio increases. The small influence of salt concentration on the "weak" binding site was observed. When the second EB binds to tRNA, the lifetime of the EB in the "strong" binding site decreases to 17.7 ns. In both of these traditional analyses the long lifetime component decreases as the EB/tRNA ratio increases. However, even as the lifetime decreases one would expect that some of the EB molecules only have one EB bound and that the lifetime of the EB should be monotonic. We next tried to link this long lifetime component with the NMR model of Chu at al.

Summary
The lifetime data are consistent with a model in which a single EB bound to the "strong" binding site of tRNA has a lifetime of 27.6 ns. When a second EB binds to tRNA, the lifetime of the EB in the "strong" binding site decreases to 17.7 ns. If the NMR model of Chu at al (mentioned in the Introduction) is correct then the two EB binding sites may be quite close to one another and the observed decrease in the EB lifetime could be due either to a change in the DNA conformation near the "strong" binding site or energy transfer from EB in the "strong" site to EB in the "weak" site. The analysis and interpretation of steady-state fluorescence titration data must take into account the decrease in the fluorescence enhancement of the first EB bound when the second site is occupied.

The small influence of salt concentration on the "weak" binding site is interesting since it has been mentioned in the literature that high salt decreases the fluorescence enhancement of the first EB bound when the second site is occupied.

Magnesium
Chloride
EB
[NaCl] in mM

Fractional Intensity
Lifetime
[EB] / [tRNA]

Fractional Intensity
Lifetime
[EB] / [tRNA]