Unique binding site for bis-benzimidazoles on transfer RNA

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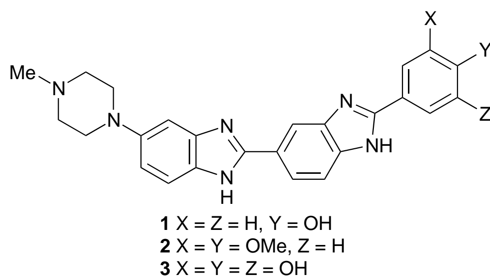
Ligands based on bis-benzimidazole structures related to Hoechst 33258 bind to transfer RNA with 1 : 1 stoichiometry and dissociation constants in the micromolar range, their interaction appearing significantly different in nature from that with duplex DNA, based on the ribonucleic acid-induced quenching of their fluorescence.

With ligand binding to RNA increasingly being recognised as a potential drug target, especially in the HIV field, an understanding of the molecular features which lead to strong, specific binding to RNA is a prerequisite to designing RNA-directed ligands as drug leads.[1,2] Use of *in vitro* selection of RNA sequences can determine preferred RNA sequences and structures (*e.g.* for tobramycin[3]), but this does not produce new RNA-binding structures. The complementary approach is to find novel RNA ligand structures and understand the basis of their binding interaction.[2]

Early studies indicated that netropsin and distamycin bind strongly to the minor groove of DNA but weakly to RNA (probably electrostatically to the phosphate ion sites on the backbone).[4] However, classical DNA-minor-groove ligands such as berenil[5] and benzimidazoles[6] have recently been reported to have strong interactions with RNA duplexes based on melting temperature determinations. However, these studies gave no indication of RNA-specifying interactions. The grooves of DNA and RNA differ in many respects. Thus, 4′,6-diamidino-2-phenylindole (DAPI) binds to AT stretches of B-DNA in the minor groove but intercalates into AU-RNA.[1] Strong evidence that DNA minor groove binders can bind in the tRNA groove is provided by an X-ray diffraction study with netropsin and distamycin, which shows specific hydrogen bonds to G51, U52 and G53 and electrostatic interactions with phosphates P61, P62 and P63 in this region,[7] but binding strengths were not measured. Porphyrins also bind brewer's yeast $tRNA^{Phe}$ in the TψC arm near the T54 and ψ55 residues.[8] Hoechst 33258 **1** binds strongly to B-DNA in AT-rich regions but except for specially designed synthetic oligonucleotides the absolute sequence specificity is low. Thus on calf thymus or plasmid DNA there are many sites for **1**. We now report a contrasting situation for RNA, namely specific binding to a unique site on tRNA of ligands based on **1**, *viz.* the 3,4-dimethoxy **2** and 3,4,5-trihydroxy **3** analogues.

Stock solutions of tRNA (from brewer's yeast, Boehringer Mannheim) were prepared in 0.1 m sodium phosphate buffer pH 7.80. Ligand stock solutions, initially in dimethyl sulfoxide (10 mm), were further diluted with phosphate buffer to the required concentration. Samples for fluorescence studies were filtered through a sterile Nalgene™ 0.2 μm syringe filter. Trihydroxy-Hoechst **3** was from a previous study[9] and 3,4-dimethoxy-Hoechst **2** was synthesised similarly by condensing ethyl 3,4-dimethoxybenzimidic hydrochloride (mp 143.5–145 °C, litr., 142–143 °C,[10] accurate mass, 210.1122. $C_{11}H_{16}NO_3$ requires 210.1130) with 2-(3,4-diaminophenyl)-6-(4-methylpiperazinyl)benzimidazole:[11] **2** was characterised by NMR and mass spectroscopy.‡ UV–VIS spectra were recorded at 25 °C using a Peltier-thermostatted cuvette holder in a Cary model 1E UV-Visible spectrophotometer using the Cary Base System Software and fluorescence emission spectra using a Hitachi F-2000 fluorescence spectrophotometer. The ligands were excited at wavelengths corresponding to their absorption maxima.



In the absorption spectra of a series of mixtures of tRNA (brewers' yeast) and **3** [Fig. 1(*a*)] the λ_{max} value of **3** was found to shift from 326 nm to longer wavelength as the proportion of tRNA increased, becoming approximately 338 nm (broad) for a 1 : 1 molar mixture. These UV–VIS spectra were recorded for a series of tRNA : **3** mixtures (prepared from 50 μm stock solutions of **3** and tRNA) varying the molar ratio of the components but keeping a constant total concentration of the components. The absorbance at 360 nm for each ratio, corrected

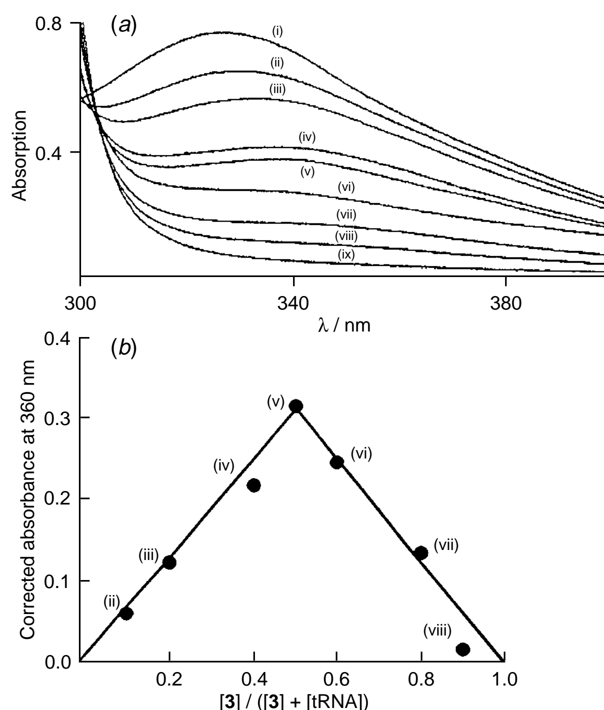


Fig. 1 (*a*) UV–VIS absorption spectra of 3, 4, 5-trihydroxy-Hoechst **3** and brewer's yeast tRNA (combined concentration 50 μm) in 0.1 m sodium phosphate buffer pH 7.80. Mole fraction of **3**: (i) 1, (ii) 0.9, (iii) 0.8, (iv) 0.6, (v), 0.5, (vi) 0.4, (vii) 0.2, (viii) 0.1 and (ix) 0. (*b*) Job plot of the data from (*a*), corrected as described in the text.

for the contribution from unbound **3** or tRNA assuming 1 : 1 tight binding, plotted against the mole fraction of **3** to produce a Job plot [Fig. 1(*b*)] showed 1 : 1 complex formation and tight binding.

Addition of tRNA to a solution of **2**, **3** or related ligands leads to extensive fluorescence quenching, which is easier to study for some ligands than others depending on the initial fluorescence intensity of the free ligand. A sample of **2** (1 ml of a 10 μm solution in 0.1 m sodium phosphate buffer, pH 7.80) was excited at 338 nm and its fluorescence emission spectrum recorded between 300 and 600 nm after each addition of a series of aliquots of $tRNA^{Phe}$ (20 μl from 100 μm stock). A plot of the change in the fluorescence intensity, corrected for dilution *vs.* [$tRNA^{Phe}$] added was fitted by the equation for a single-site binding [eqn. (1)],

$$\text{Fluorescence } (F) = F_{max}[tRNA^{Phe}]/(K_{diss} + [tRNA^{Phe}]) \quad (1)$$

with a value for K_{diss} of 14.8 + 0.9 μm. There is also evidence of a much weaker binding site(s). The binding site for ligands in this class remains to be identified but the binding is both specific, as shown by the Job analysis for **3**, and reasonably strong based on the dissociation constant determined for **2**. The environment of these bis-benzimidazole ligands on the RNA is clearly different in detail from that on DNA as the fluorescence of Hoechst 33258 is highly enhanced on binding to the minor groove of B-DNA[12,13] in contrast to the strong quenching seen here with tRNA. This may be an indirect effect, as seen for bromouridine quenching in DNA, or may indicate that the angles between the planes of the benzimidazole rings and the terminal aryl groups and their molecular environments differ substantially on DNA and RNA for such compounds. However, in spite of the detailed knowledge of binding interactions and such angles for Hoechst and its congeners in the DNA groove from NMR[12,14,15] and X-ray diffraction[16,17] studies, there is no information on the basis of RNA recognition by this family of structures. The molecular frameworks of **2** and **3** and their specific sequestration of tRNA (selectively occupying a unique run of 4–5 base-pairs out of approximately 75) provides a new lead for analogue design to increase the strength of binding to RNA-based grooves and to understand some aspects of ligand–RNA recognition at an atomic level.

Footnotes

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‡ *Selected data* for **2**: δ_H [270 MHz, $(CD_3)_2SO$], 2.24 (s, 3 H, NCH_3), 3.12 [m, 4 H, $N(CH_2)_2$], 3.60 (m, 4 H, $N(CH_2)_2$], 3.84 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 6.93 (d, 1 H, Ar-H, *J* 8.6 Hz), 7.00 (s, 1 H, Ar-H), 7.13 (d, 1 H, Ar-H, *J* 8.6 Hz), 7.43 (d, 1 H, Ar-H, *J* 8.25 Hz), 7.65 (d, 1 H, Ar-H, *J* 8.25 Hz), 7.81 (d, 1 H, Ar-H, *J* 7.9 Hz), 7.82 (s, 1 H, Ar-H), 7.96 (d, 1 H, Ar-H, *J* 8.6 Hz), 8.27 (s, 1 H, Ar-H); FAB MS: Calc. for $C_{27}H_{29}N_6O_2$, 469.2352. Found, 469.2354. % (M + 1): Calc., 32.7; Found, 31.9%. % (M + 2): Calc., 4.91; Found, 4.83%.

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