Organothallium(III) Reagents for Specific Modification of Transfer RNA from *Escherichia coli*

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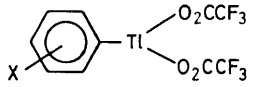
The thiouridine site in transfer RNA from *Escherichia coli* specifically binds *p*-tolylthallium(III) bis(trifluoroacetate), providing a new class of organometallic labels in nucleic acid chemistry.

We have recently used organothallium(III) derivatives (**1**) to specifically inactivate enzymes, *e.g.* lactate dehydrogenase,[1] phosphoglycerate phosphokinase.[2] Organothallium(III) reagents offer a number of advantages (over organomercurials, for example) in biochemistry. Thus, they are bifunctional *at thallium* and thus potential cross-linkers; they may be expected to exert redox reactivity ($Tl^{III} \rightleftharpoons Tl^{I}$) in suitable circumstances: the stable ^{205}Tl isotope (spin $\frac{1}{2}$, 70.48% natural abundance) is 19.2% as sensitive an n.m.r. nucleus as ^{1}H and Tl–H coupling constants are extremely large.[3] There have been reports of effects of Tl^{+} (K^{+} replacement) on ribosomes, aminoacyl–transfer RNA binding, and peptidyl transferase activity.[4] We report here the specific complexation of (**1**) by transfer RNA (tRNA) from *Escherichia coli*. This shows a number of features which are different from the interactions of (**1**) with protein systems.[1,2]

E. coli tRNA was obtained from the Sigma Chemical Company. The most obvious evidence of binding of (**1**) to *E. coli* tRNA comes from the changes in the absorption spectrum of tRNA on addition of (**1**) in 0.1 M Tris buffer, pH 7.50 (containing 0.1 M NaCl and 0.01 M $MgCl_2$). Addition of (**1a**) or (**1b**) caused a progressive diminution in the intensity of the band at 335 nm, a characteristic[5] of the thiouridine base, s^4U_8. The extent of change in the absorbance at 335 nm depended on the ratio of [(**1a**)]/[tRNA]. In contrast, there was no detectable change in the spectral region around 260 nm, which reflects the environment of the bases (*e.g.* their involvement in stacking interactions). A 10^4-fold molar excess of thallium(I) acetate had no discernible effect at 260 or 335 nm. Use of a [^{14}C]-labelled form of (**1a**), incubated with tRNA as above followed by passage over Sephadex G-25 to separate large tRNA (-derived) molecules from unbound (**1a**), showed that binding of (**1a**) was *completely reversible* by this criterion (both from scintillation counting of eluted fractions and spectral



(**1**) **a**; X = 4-Me
b; X = 2-CO_2^-

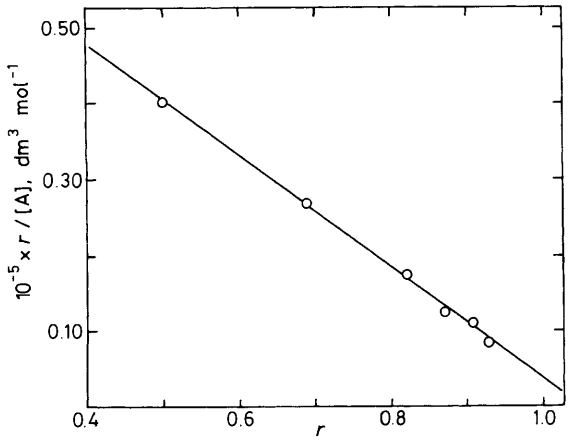


Figure 1. Scatchard plot of $r/[A]$ *vs.* r for binding of *p*-tolylthallium(III) bis(trifluoroacetate) with *E. coli* tRNA at 25 °C in 0.1 M Tris buffer (pH 7.50), containing 0.1 M NaCl and 0.01 M $MgCl_2$. The concentration of tRNA was 3.5×10^{-5} M; r is equal to [bound (**1a**)]/[tRNA] total and [A] is the [free (**1a**)].

regeneration in the 335 nm region). A Job type plot for (**1a**) with *E. coli* tRNA showed complexation, confirmed by the spectral method (335 nm) of Armor and Haim,[6] which gave a value of $7.69 \pm 1.02 \times 10^4$ dm^3 mol^{-1} for the binding constant (Scatchard analysis, see Figure 1, also gave a binding constant of $7.25 \pm 0.19 \times 10^4$ dm^3 mol^{-1}). We could find no evidence of tRNA cross-linking using gel electrophoresis.

The spectral evidence (the change in absorbance at 335 nm) is in line with modification of the s^4U_8 residue of *E. coli* tRNA, reversibly (G-25 evidence) and selectively. It was argued for protein systems[1] that (**1**) would have an affinity for 'softer' sites, *e.g.* S and N side chains of amino-acids and this appears to be borne out for this tRNA. The tRNA system differs from the enzymes studied to date[1,2] in that reaction with (**1**) abolishes enzyme activity in an irreversible manner (by the above G-25 criterion, and others). The molecular origins of the differences between the protein and nucleic acid systems remain to be elucidated.

However, this readily synthesised[7] new class of heavy metal-based labels of biomolecules is clearly of wide applicability for studies of biomacromolecules and may serve as useful probes (*e.g.* by n.m.r.), labels (for *X*-ray diffraction, electron microscopy, *etc.*), or structure–function aids (redox, cross-linking activities).

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