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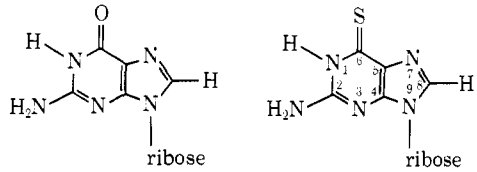
Metal Binding by Thionucleosides

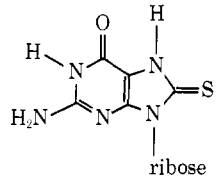
Sir:

Metal ions and complexes are potentially powerful tools for studying the structure and base sequence of polynucleotides.[1,2] Since naturally occurring polynucleotides have many available binding sites,[3] it appeared useful to investigate the interaction of metals with less common, modified nucleosides, especially thiolated nucleosides. By exploiting the "class b"[4] (or "soft"[5]) nature of the sulfur donor atom, selective and quantitative metal binding was anticipated. In this preliminary report we present equilibrium constants for metal–thionucleoside binding, the synthesis and structure determination of a metal–mercaptopurine complex, and evidence for the metalation and denaturation of a 4-thioU residue, effected during the denaturation and renaturation, respectively, of *E. coli* $tRNA^{Val}$.



1, G

2, 6-thioG



3, 8-thioG

Using nmr, Kan and Li[6] had determined the equilibrium constant, $K_1 = 5.9$ l./mol, for the binding of mercuric chloride to guanosine (**1**) in DMSO. A similar study in our laboratory with 6-thioguanosine (**2**) indicates the formation of species with a 6-thioG:$HgCl_2$ ratio greater than unity. Assuming a 2:1 complex, the lower limit for the 6-thioG binding constant is 50 times greater than the constant for guanosine.

The binding of sodium *p*-hydroxymercuribenzoate (PHMB) to thionucleosides **2** and **3** was also studied by uv spectroscopy in Tris buffer solutions of pH 7.4–7.6. Continuous variation (Job) plots[7] indicate the formation of 1:1 complexes. Examination of these plots (inset, Figure 1) shows the curve for 6-thioG to break more sharply than that for 8-thioG. This result suggests the binding constant for 6-thioG to be substantially greater than that for 8-thioG. Equilibrium constants, evaluated spectrophotometrically (Figure 1) by the method described in ref 8, confirm the qualitative results of the Job's plots. For 8-thioG, using data at 310 nm (C=S chromophore), K_1 was found to be $4.8 \pm 1.0 \times 10^5$ l./mol, whereas the analysis of similar data taken at 340 nm for the 1:1 PHMB–6-thioG complex gives a K_1 value of $7.2 \pm 2.6 \times 10^6$ l./mol.[9]

The higher equilibrium constant for the 6-thioG complex may be due to a chelate effect. PHMB bound to sulfur in 6-thioG (**2**) can form a stable five-membered ring by coordinating to N(7) simultaneously. For 8-thioG (**3**), a four-membered ring would result from chelation to N(7). In order to investigate the possibility of chelation directly, the crystal structure of a 6-mercaptopurinepalladium complex was determined. To simulate a riboside, N(9) was blocked by a benzyl substituent. The complex bis(6-mercapto-9-benzylpurine)palladium(II)-dimethylacetamide was synthesized by allowing 1-propanol vapors to diffuse into the reaction solution prepared from the ligand[10] and $PdCl_2$ in dimethylacetamide. The molecular structure, determined in an X-ray crystallographic study to be reported in detail elsewhere, is shown in Figure 2.

The coordination geometry is a slightly distorted square in which two chelating ligands contribute both sulfur and N(7) donor atoms. Distortions of the ligand from the geometry found in 6-mercaptopurine monohydrate[11] result from the *ca.* 0.3-Å decrease in the S---N(7) "bite" distance upon chelation. This structure may be contrasted with the results for a copper complex of 9-methylhypoxanthine (6-oxo-9-methylpurine) in which the metal is bound only to N(7).[12]

Having established that thionucleosides are good ligands for binding class b metal ions,[13] it was of interest to study the interaction of mercury(II) with naturally occurring thiolated bases. Solutions of valine tRNA from *Escherichia coli*[14] were dialyzed to

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(2) F. J. Schmidt, R. M. Bock, and S M. Hecht, *Biochem. Biophys. Res. Commun.*, **48**, 451 (1972), and references therein.
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(8) J. N. Armor and A. Haim, *ibid.*, **93**, 867 (1971).
(9) The K_1 value of 6-thioG was computed over the concentration range $0.6 \lesssim$ [PHMB]/[6-thioG] $\lesssim 1.2$, the very small slope of the line accounting for the uncertainty in the constant. Outside this range anomalous behavior was observed, which is attributed to the large value of the equilibrium constant.
(10) J. A. Montgomery and C. Temple, Jr., *Syn. Proc. Nucl. Acid Chem.*, **1**, 47 (1968).
(11) (a) E. Sletten, J. Sletten, and L. M. Jensen, *Acta Crystallogr., Sect. B*, **25**, 1330 (1969); (b) G. M. Brown, *ibid.*, **25**, 1338 (1969).
(12) E. Sletten, *Chem. Commun.*, 558 (1971).
(13) Parallel results have been previously obtained with other thiolated bases and metals; *e.g.*, (a) E. R. Garrett and D. J. Weber, *J. Pharm. Sci.*, **59**, 1383 (1970); **60**, 845 (1971); (b) B. C. Pal, *J. Org. Chem.*, **36**, 3026 (1971).

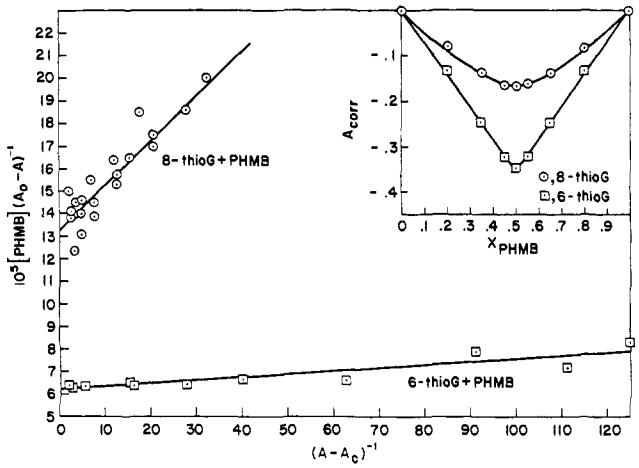


Figure 1. Spectrophotometric titration of 8-thioG (6×10^{-5} and 5×10^{-5} *M*) and 6-thioG (5×10^{-5} *M*) with PHMB. The symbols in the plot are A_0, the absorbance of the free nucleoside, A_c, the absorbance of the fully complexed nucleoside, and A, the absorbance of a given solution containing PHMB and nucleoside. The equilibrium constant, K_1, is obtained from the inverse slope of the plot (see ref 8 for details). The inset shows Job plots[7] for PHMB and thiolated nucleoside under similar conditions.

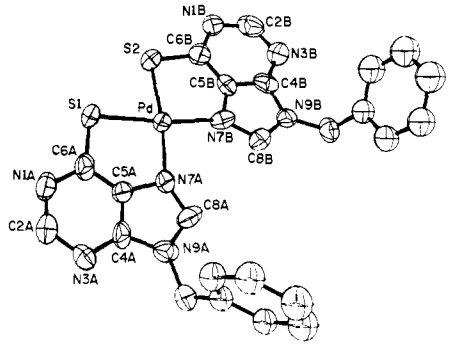
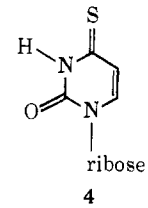


Figure 2. Molecular structure of bis(6-mercapto-9-benzylpurine)-palladium(II)-dimethylacetamide. The solvent, which is not coordinated, is omitted from the drawing. Dimensions of the coordination geometry are distances of Pd–S1, 2.305 (3), Pd–S2, 2.311 (3), Pd–N7A, 2.08 (1), Pd–N7B, 2.05 (1) Å; angles of S1–Pd–S2, 85.8 (1), S1–Pd–N7A, 88.1 (3), S2–Pd–N7B, 87.8 (3), N7A–Pd–N7B, 98.3 (4)°.

give desired buffer and salt conditions. When PHMB was added to a solution of $tRNA^{val}$ in 0.05 *M* Tris (pH 7.2, 0.1 *M* NaCl, 0.01 *M* $MgCl_2$), no change in the absorption due to 4-thioU at 338 nm was observed (Figure 3, curve A).[16] When $HgCl_2$ was added under these conditions (curve B, Figure 3), the 338-nm absorption

(14) This tRNA contains 4-thiouridine (**4**) in the eight position from the 5′ end.[15]



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(16) This result may be compared with the report by M. N. Lipsett and B. P. Doctor, *J. Biol. Chem.*, **242**, 4072 (1967), in which $tRNA^{tyr}$ containing two adjacent 4-thioU residues per molecule is quantitatively titrated with PHMB. Although experimental conditions are scantily reported, the titration appears to have been performed in the absence of Mg^{2+} ions.

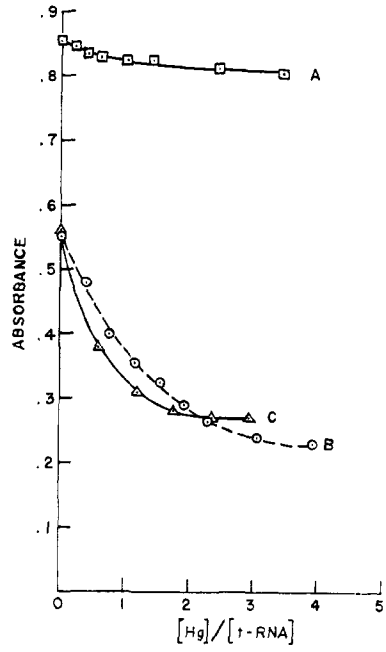

Figure 3. Titration of (A) $tRNA^{val}$, 1.6×10^{-5} *M*, with PHMB; (B) $tRNA^{val}$, 1.05×10^{-5} *M*, with $HgCl_2$; (C) "denatured" (see text) $tRNA^{val}$, 0.86×10^{-5} *M*, with PHMB.

decreased as a function of mercury concentration, indicating that $HgCl_2$, but not PHMB, binds the sulfur atom of 4-thioU in $tRNA^{val}$.

To test the hypothesis that PHMB does not bind because the phenyl group makes it too bulky to reach a sterically hindered sulfur atom, the tertiary structure of the tRNA was opened by removing the magnesium ions (dialysis against EDTA, followed by dialysis against 0.05 *M* Tris, pH 7.2, containing 0.1 *M* NaCl) and heating to 40°.[17] Under these conditions, the absorption maximum is shifted slightly, to 335 nm, and increases in intensity. As PHMB is added, the band decreases to less than its original intensity (Figure 3, curve C), showing that the 4-thioU sulfur atom can be made accessible to PHMB if the native tertiary structure of the $tRNA^{val}$ is broken.

The spectrum does not change upon cooling to 20°, but when Mg^{2+} ions are added back to the solution, the band at 335 nm is restored to its original intensity implying that PHMB has become unbound and the tRNA has resumed its native tertiary structure.[18]

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