# Metal Ion Catalysis of Proton Exchange Between Purine Nucleosides or Nucleotides and Solvent Water

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*Summary* Co-ordination of the methylmercury(II) cation at N-7 of inosine and guanosine-5′-phosphate catalyses proton exchange between the proton bound to C-8 and the solvent water leading to disappearance of the $^1$H n.m.r. signal in $D_2O$.

WHILE studying the binding of heavy metal cations to nucleosides and nucleotides using $^1$H n.m.r. and Raman difference spectrophotometry[1,2] we have discovered that co-ordination of a metal ion can catalyse exchange of protons between the base and solvent. The Raman difference spectrum of 50 mM-inosine + 50 mM $MeHg^{II}$ *vs.* 50 mM-inosine, both at pH 8 (Figure) shows that a reaction occurs. Carbonyl stretching modes are the best-defined group vibrations of nucleic acid bases. Such a vibration gives rise to the band observed for inosine (I) at 1692 cm$^{-1}$. Co-ordination of a metal ion at N-1 with displacement of the proton causes the disappearance of this band as shown by the negative feature in the difference spectrum, similar to what has been observed for uridine.[1] A similar change is observed with solutions for which the pH is appreciably greater than the p*K* for this proton, 8·8. Examination of a series of solutions in $D_2O$ containing 25 mM-inosine and varying concentrations of $MeHg^{II}$ at pD 8 shows that the integrated intensity of the carbonyl band decreases linearly with $MeHg^{II}$ concentration, vanishing entirely at a 1 : 1 mol ratio. At higher $MeHg^{II}$ : inosine ratios, further changes are observed in the spectra which could arise from mercuriation at other sites.

$^1$H N.m.r. spectra of inosine have been studied thoroughly.[3] We observed resonances at 5·14 (8-H), 5·02 (2-H), and 2·89 p.p.m. (1′-H) downfield from $Me_4N^+$ as internal reference. As the concentration of $MeHg^{II}$ is increased with [inosine] = 25 mM, pD = 8, the chemical shifts decrease slightly and reach limiting values of 5·10 (8-H), 4·92 (2-H), and 2·86 p.p.m. (1-H) at $MeHg^{II}$ : Ino = 1. No substantial change occurs in the position of the 2- or 1′-H resonances at higher ratios, but the 8-H resonance vanishes entirely. If solutions are examined a short time after preparation, the disappearance of the 8-H resonance can be followed. The constancy of the Raman spectrum and the 2-H and 1′-H chemical shifts as the 8-H resonance disappears indicate that this is due to exchange with solvent, not substitution of 8-H by MeHg. Heating for at least 1 h at 100 °C is necessary to effect complete exchange.[3] In all these experiments, controls were run which contained no $MeHg^{II}$ to ensure that effects caused by the metal were being observed.

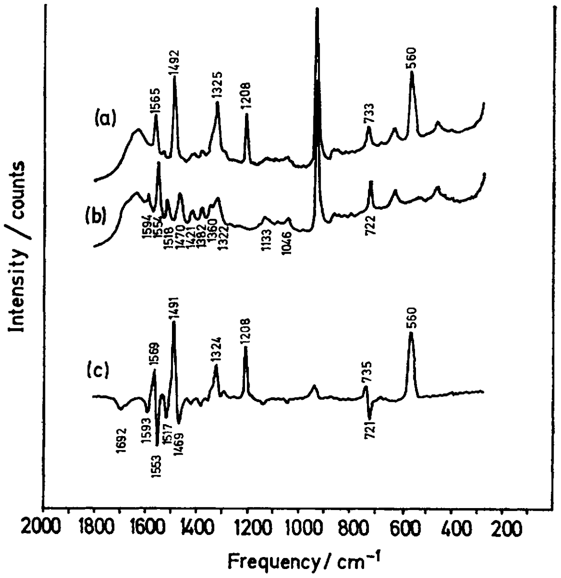
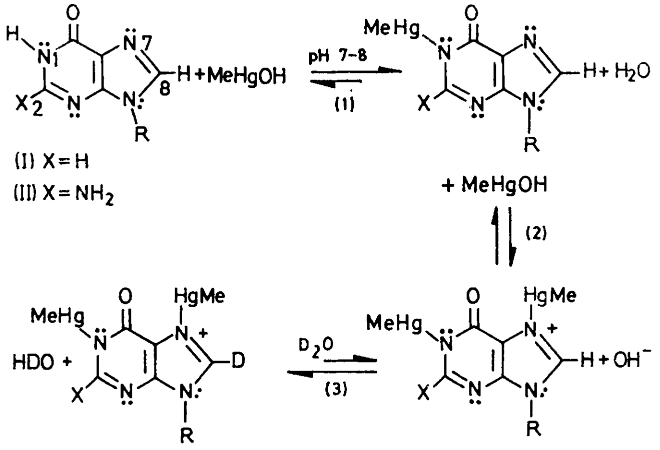


FIGURE. Raman spectra of $MeHg^{II}$-inosine binding. (a), 50 mM $MeHg^{II}$ + 50 mM inosine; (b), 50 mM inosine; (c), difference spectrum. All solutions at pH 8.

The rapid exchange noted at $MeHg^{II}$ : inosine ratios $>1$ seems similar to the behaviour observed for 7-methylinosine.[4] The 8-H resonance cannot be found in $D_2O$ for this compound because of rapid exchange with the solvent. At neutral pH, the compound exists in the neutral betaine form, and it has been assumed that the formal charge of $+1$ imposed on N-7 by alkylation increases the acidity of 8-H, leading to rapid exchange with solvent. Examination of $MeHg^{II}$–guanosine-5′-phosphate (II) solutions shows that a similar exchange reaction occurs in $D_2O$ leading to the disappearance of the 8-H resonance. Exchange does not occur with adenosine because of the weak interaction with $MeHg^{II}$.

Metal ion catalysis of this exchange is to be expected on the basis of the mechanism suggested for the exchange of tritium bound at C-8. Kinetic data indicate that the exchange occurs by $OH^-$ abstraction of 8-H hydrogen from the 7-protonated form giving rise to an ylide-type intermediate which then is reprotonated at C-8 by the medium.[5]

These results support the assumption of Simpson[6] that $MeHg^{II}$ can bind to both N-1 and N-7 of inosine and guanosine. Rapid exchange of 8-H can be taken as evidence for co-ordination at N-7, and $^1H$ n.m.r. spectroscopy can be used to determine such co-ordination. Binding to N-1 for which the equilibrium constant is much larger than for N-7 does not lead to any measurable exchange of 2-H.



Other metal species which are strong electrophiles, *e.g.* $Hg^{II}$, $Au^{III}$, and $Pt^{II}$, can be expected to have a similar labilizing effect. Recently, pyrimidine and, in low yields, purine nucleotides have been mercuriated at the 5 and 8 positions, respectively, by reaction with $Hg(OAc)_2$ under mild conditions.[7] Displacement of the *N*-bonded mercury(II) occurs during the product work-up, and the Hg–C bonds are inert. These reactions probably are catalysed by co-ordination to ring nitrogen atoms.

Binding sites of paramagnetic cations, especially $Cu^{2+}$, have been assigned on the basis of broadening and disappearance of proton resonances caused by the cation.[8] The broadening generally is assumed to arise from dipolar-dominated spin–lattice relaxation.[9] The metal ion catalysis of exchange with solvent described here is another mechanism which can lead to the disappearance of nucleoside resonances.

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