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RELATION BETWEEN STRUCTURE AND THE DEGREE OF HYDRATION FOR COBALT(II) BONDED TO NUCLEOSIDE 5'-MONOPHOSPHATES

D.M.L. Goodgame, G.A. Leach, and Z. Warnke*

Chemistry Department, Imperial College, London SW7 2AY, U.K.

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SUMMARY: The variation of coordination geometry with degree of hydration has been studied for cobalt(II) bonded to 5'-AMP, 5'-GMP, 5'-IMP, 5'-UMP, and 5'-CMP. Pink compounds with octahedral coordination about the metal ions were isolated but partial dehydration results in conversion to blue compounds containing tetrahedral cobalt. The ease of conversion is markedly dependent on the identity of the nucleotide. In the case of Co^{II}-5'-AMP warming in water at 34°C is sufficient to cause the change to the tetrahedral form. Spectral results are given and some possible implications to the behaviour of cobalt-containing metalloenzymes, such as Co-RNA polymerase from E. coli, are briefly discussed.

INTRODUCTION: There is appreciable interest in the coordination of transition and other heavy metals to nucleic acids and their components (1,2). Much of this interest has centred on the determination of the bonding sites in the biological molecules and on the geometry around the metal ion. We have reported the structures of several cobalt(II) nucleotide derivatives isolated from aqueous solutions at pH ∿ 7. Cobalt is frequently used as a replacement for zinc in studies of, for example, RNA polymerase (3). We have observed (4) that in $[Co(5'\text{-GMP})(H_2O)_5]3H_2O$ and $[Co(5'\text{-IMP})(H_2O)_5].2H_2O$ cobalt binds to N7 of the purine, with five water molecules completing an octahedral coordination geometry. The metal ion in $[Co_2(5'\text{-UMP})_2(H_2O)_4]10H_2O$ is also octahedrally co-ordinated (5), but the nucleotide is bound to the metal via phosphate oxygens and a polymeric chain structure is adopted. In contrast, 5'-CMP forms a cobalt complex, $[Co(5'\text{-CMP})(H_2O)]$, in which the metal ion has a tetrahedral coordination geometry (6).

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* Present address: Institute of Chemistry, The University, 80216 Gdansk, Poland.

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This change in geometry with the lower hydration of the 5'-CMP compound coupled with our somewhat unexpected observation that cobalt(II) can adopt a tetrahedral geometry with 5'-AMP even in aqueous media has led us to investigate more closely the relationship between hydration and coordination geometry for a range of cobalt(II)-ribonucleotide compounds. Facile changes of geometry about a metal on changing from a water-rich to a more hydrophobic environment in cell processes might be expected to result in accompanying changes in the geometry of the biological molecule containing it and/or its substrate recognition.

MATERIALS AND METHODS: The disodium salts of 5'-AMP (Cambrian), 5'-GMP (Aldrich), 5'-IMP (Sigma), 5'-CMP and 5'-UMP (Koch-Light) and cobalt(II) nitrate hexahydrate (BDH) were used without further purification.

Except where stated below, the complexes were prepared as follows : A solution of the disodium salt of the nucleotide (1.0 mmole) in water (10 ml) was added, with stirring, to one of cobalt nitrate hexahydrate (0.291 g, 1 mmole) in water (5 ml) to produce a pink solution (or precipitate in the case of 5'-AMP and 5'-CMP) at pH 6-7. The solution (or mixture) was warmed at 60°C for 15 mins and then cooled to room temperature. The solid was filtered off, washed with a little ice-cold water and air dried. One half of the complex was kept and the remainder dehydrated by heating to 60°C *in vacuo* for 3 hours and then storing in a desiccator over silica gel. Upon dehydration, the complexes turned from pink to deep blue.

In the case of 5'-AMP, heating the suspension/solution of the pink complex produced a deep blue solid which remained blue on cooling. This was collected and dried as described above. When the reaction mixture was not heated a gelatinous pink solid was obtained, but this lost water so readily on exposure to the air that analyses could not be obtained for the original pink complex, but only for the resultant blue solid.

The Co(5'-CMP) complex showed similar behaviour on heating but, in this case, the pink colour slowly returned as the aqueous mixture was allowed to cool. However, elemental analyses showed that the pink solid contained a very large quantity of occluded water and even air drying was found to remove sufficient water to cause conversion to the blue form.

Infrared spectra were recorded as Nujol mulls between caesium iodide plates using a Perkin-Elmer 257 spectrophotometer (4000-625 cm^{-1}). Diffuse electronic reflectance spectra were obtained using a Beckmann DK2 spectrophotometer (350-2500 nm) and electron paramagnetic resonance spectra on a Varian E12 e.p.r. spectrometer.

Pregl analyses were carried out by the Microanalytical Laboratory, Imperial College.

RESULTS AND DISCUSSION: The formulae and the analytical results of the complexes we have isolated are given in Table I. Thermogravimetric experiments were also used to confirm the water contents of the compounds with 5'-GMP, 5'-IMP, and 5'-UMP. The samples of the pink compounds with these three nucleotides and that of the blue complex with 5'-CMP were shown to be the same as those on which full X-ray structural studies had previously been carried out, by comparisons of their X-ray powder patterns and infrared spectra.

Because the new compounds could not be obtained in crystalline form suitable for single-crystal X-ray studies, information about their geometry was obtained by spectral methods. Cobalt(II) ions are particularly suitable for this approach as there is a clear distinction between the electronic spectra of the metal in octahedral as compared with tetrahedral environments.

The reflectance spectra of all the pink, fully hydrated compounds are typical of essentially octahedral geometries. A representative spectrum (of $[Co(5'\text{-IMP})(H_2O)_5]2H_2O$) is shown in Figure 1 and the numerical results for all the cobalt nucleotide compounds are given in Table II, together with the band assignments and derived parameters.

In contrast, the spectra of the blue, partly dehydrated compounds have band energies and the high intensities associated with a tetrahedral geometry (see the spectrum of blue $Co(5'\text{-IMP})H_2O$ in Figure 1). Numerical results for these compounds are also given in Table II.

The environments of the metal ions in these compounds were further investigated by examining the e.p.r. spectra of manganese(II) ions doped (nominal 1% doping) into the host cobalt compound. Manganese(II) ions in the pink complexes of 5'-GMP and 5'-IMP gave e.p.r. spectra showing hyperfine splitting of several transitions close to the $g_{eff} = 2$ region (Figure 2). Such a spectrum is expected when the effects of zero-field splitting are finite but small, as would be the case for the environment of one N7 of the purine unit and five water molecules, shown by the X-ray studies (4).

Upon partial dehydration and formation of the analogous blue tetrahedral complexes, the manganese hyperfine signals collapsed to give a broad,

Table I. Microanalyses of the Cobalt(II)-Nucleotide Compounds

	COLOUR	% FOUND		% CALCULATED	
		C	H	C	H
Co(5'-AMP)H_2O	Blue	28.04	2.88	28.45	3.34
Co(5'-CMP).$2H_2O$	Blue	25.72	3.92	25.97	3.88
Co(5'-GMP).$8H_2O$	Pink	21.76	4.88	21.29	5.00
Co(5'-GMP).$2H_2O$	Blue	27.61	3.97	26.33	3.54
Co(5'-IMP).$7H_2O$	Pink	22.50	4.37	22.61	4.74
Co(5'-IMP).H_2O	Blue	28.74	3.21	28.38	3.10
Co(5'-UMP).$8H_2O$	Pink	20.43	5.11	20.58	5.18
Co(5'-UMP).$3H_2O$	Blue	24.94	3.84	24.84	3.94

Table II. Electronic Reflectance Spectra of the Cobalt(II)-Nucleotide Compounds

Pink (Octahedral) Complexes

NUCLEOTIDE	$^4T_{1g}(P) \leftarrow {}^4T_{1g}(F)$ cm^{-1}	$^4A_{2g} \leftarrow {}^4T_{1g}(F)$ cm^{-1}	$^4T_{2g} \leftarrow {}^4T_{1g}(F)$ cm^{-1}	Dq cm^{-1}	B cm^{-1}	β
5'-AMP	19230	15630	-*	835	868	0.90
5'-CMP	19050	15870	7690	818	789	0.81
5'-GMP	19230	15870	7550	833	830	0.86
5'-IMP	19610	16260	7690	857	852	0.88
5'-UMP	19230	14600	6850	775	885	0.91

Blue (Tetrahedral) Complexes

NUCLEOTIDE	$^4T_1(P) \leftarrow {}^4A_2$ cm^{-1}	$^4T_1(F) \leftarrow {}^4A_2$ cm^{-1}	Dq cm^{-1}	B cm^{-1}	β
5'-AMP	17700	6850	393	849	0.88
5'-CMP	17390	6860	395	827	0.85
5'-GMP	17390	7140 †	412	811	0.84
5'-IMP	17390	7140	412	811	0.84
5'-UMP	17860	7140 †	411	844	0.87

* Not observed.

† Value only approximate due to the broad nature of the band.

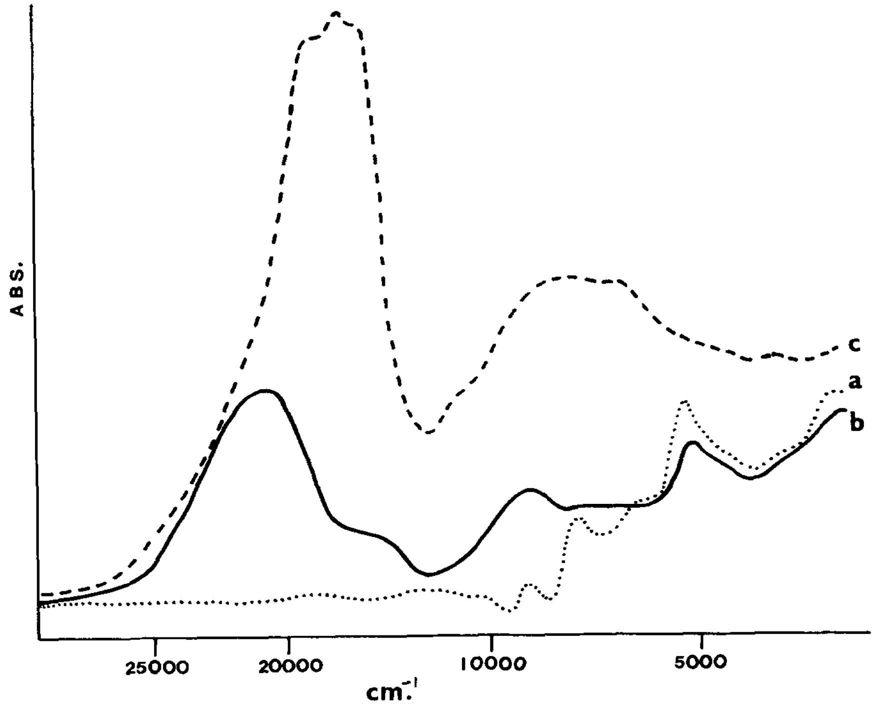

Figure 1. Electronic (reflectance) spectra of: (a) $Na_2(5'\text{-IMP})$; (b) $Co(5'\text{-IMP}).7H_2O$; (c) $Co(5'\text{-IMP}).H_2O$.

relatively featureless absorption - an effect which indicates strong dipolar interactions between the manganese(II) ions and the host, paramagnetic cobalt ions. It seems probable, therefore, that the tetrahedral geometry in the blue complexes results from a change from a monomeric, octahedral structure in the original pink compounds to a polymeric structure on removal of some of the water molecules. Such a polymeric structure has been found (6) for the blue compound $[Co(5'\text{-CMP})(H_2O)]$, in which the metals are linked by nucleotides binding via N3 and phosphate groups.

Partial dehydration of the cobalt(II) derivative of 5'-AMP, and its conversion to the blue form, occurs very much more readily than with the other purine nucleotides studied. Indeed the formation of the blue, polymeric species takes place even in water on gentle warming (∿ 34°C) whereas virtually all tetrahedral cobalt(II) complexes with other organic or inorganic ligands

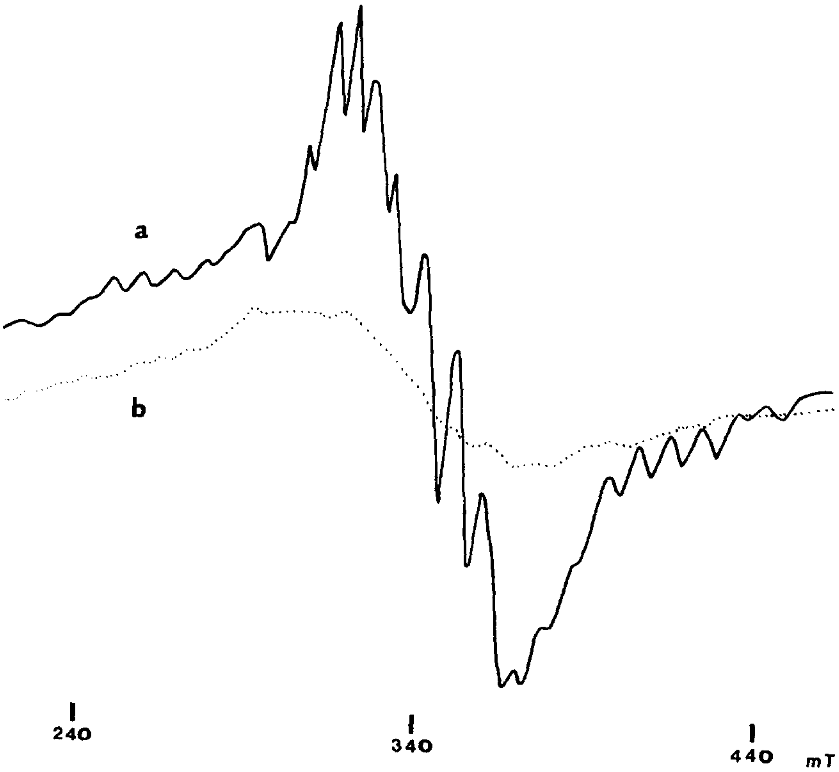

Figure 2. X-Band e.p.r. spectra of: (a) Co(Mn)(5'-IMP).$7H_2O$; (b) Co(Mn)(5'-IMP).H_2O [instrumental gain 5 x that of (a)] (1% nominal Mn doping in each case).

are relatively unstable in water and are converted to octahedral species either by simple addition of water molecules or by a combination of ligand replacement and water addition.

Some further qualitative tests were made of the ease of dehydration by suspending samples of the pink cobalt-nucleotide compounds at 36°C in 1-methylpentane-2,4-diol (chosen as a reasonable model of a non-aqueous environment). The ease of conversion to the blue form was: 5'-AMP >> 5'-UMP > 5'--IMP > 5'-GMP ∿ 5'-CMP (with typical conversion times of several minutes for cobalt-5'-AMP, ca. 3-4 hrs for 5'-UMP and ca. 1 day for 5'-IMP, for samples of ∿ 30 mg).

The spectral and other observations we report here have relevance to more complex biological systems in several respects. Electronic spectroscopy of the metal centres has been frequently used in studies of cobalt-containing enzymes (7) and a 'nearly tetrahedral' coordination geometry has been proposed in many cases. Moreover changes in such spectra on enzyme-substrate or enzyme-template interaction have been demonstrated. For example the spectrum of cobalt enriched RNA-polymerase from E. coli shows bands at $\sim$17,100 and $\sim$ 14,200 cm^{-1} both of which were found (8) to be perturbed by a template analogue, $d(pT)_{10}$, but only the 17,100 cm^{-1} band was influenced by the addition of nucleoside triphosphates.

As these intrinsic metals (Co or Zn in the RNA-polymerase quoted above) presumably have structural and functional roles in gene transcription (3) it is important to consider the extent to which such processes will be influenced by changes in the nature of the solvation medium. The particularly facile way in which the cobalt-nucleotide compounds we have studied change their geometry from octahedral to tetrahedral on partial dehydration demonstrates the ease with which these coordination changes could occur at the metal centres of such metalloenzymes. Secondly, the potential for such changes in coordination geometry and the attendant structural changes in the organic moiety are clearly dependent upon the identity of the nucleotide unit under given hydration conditions. If this selectivity also prevails in the biological system then this might well provide at least one factor in, for example, a recognition process.

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