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The Synthesis and Characterization of Nucleotide Complexes Involving an Asymmetric Metal

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Abstract

The synthesis of cobalt and chromium complexes of H_4ATP and H_4GTP in which the metal is asymmetric are reported. These compounds were characterized by visible spectroscopy, fast atom bombardment mass spectroscopy (FAB MS), and ^{31}P NMR. The mass spectral data allow identification of the complexes to be made from ions in the molecular weight region. The effect of an asymmetric metal greatly alters the appearance of the ^{31}P NMR spectra in comparison to complexes which do not have this feature. Complexes of uridine diphosphoglucose, UDPG, are also reported. The effect of an asymmetric metal ion on the chromatographic and spectral properties of the complexes are discussed.

Introduction

The use of β,γ chromium(III) or cobalt(III) complexes of nucleotides, ML_4(nucleotide), (where L = H_2O or NH_3, see Fig. 1) to probe the active site of nucleotide-requiring enzymes has become commonplace in recent years [1]. These complexes are useful as probes because of their kinetically inert *d* electron configurations which prevent rapid ligand exchange. These complexes readily bind to active site areas but are generally resistant to hydrolysis of the triphosphate chain. The center phosphorus, P_β, of the triphosphate chain is prochiral and after complexation to a metal is chiral, being bound to four different groups. Since the nucleosides themselves are optically active the complexes which form are actually diastereomeric pairs. In principle these complexes should display different spectral and physical properties, such as solubility and chromatographic retention, which could result in the separation of the isomers.

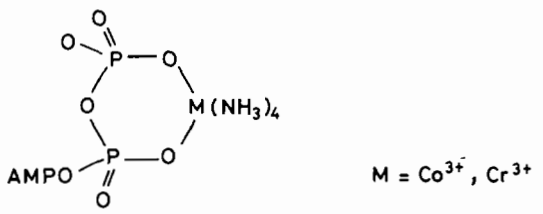

Fig. 1. Structure of a metal nucleotide complex.

Experimentally it has been observed that separation is achieved for the $Cr(H_2O)_4$(nucleotide) complexes under several conditions [2]. Further, the mechanism by which separation takes place is linked to hydrogen bonding interactions involving waters in the inner coordination sphere, as complexes without waters, such as $Cr(NH_3)_4$(HATP) or $Co(NH_3)_4$(HATP), do not separate under identical conditions [2a].

The purpose of this work was to synthesize and characterize new nucleotide complexes in which the metal is asymmetric. It was hoped that this additional element of asymmetry would cause some change in the spectral or chromatographic properties of these complexes. In addition we report the synthesis and preliminary characterization of complexes of the sugar nucleotide, uridine diphosphoglucose (UDPG).

It was found that the chromatographic properties of the $Co(en)_2$(nucleotide) complexes did not differ significantly from those reported previously for $Co(NH_3)_4$(HATP), in which no separation of isomers was achieved [2a]. The columns investigated included a gravity driven cycloheptylamylose column and a C_{18} HPLC column. Apparently neither of these stationary phases are sensitive to an asymmetric metal. The behavior of the $Cr(en)(H_2O)_2$(HATP) complex is similar to that reported [2b] for $Cr(H_2O)_4$(HATP) on a C_{18} column except that the peaks are somewhat broader. These observations tend to support the postulation that the separation mechanism, on these columns, depends in part on the presence of at least some waters in the inner coordination sphere. We have also observed that the ^{31}P NMR spectral properties are highly sensitive to the presence of an asymmetric metal.

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Experimental

General

$[Cr(NH_3)_4(H_2O)Cl]Cl_2$ [3] and $[Co(en)_2(CO_3)]$-Cl [4] were prepared by the literature procedures. $[Cr(en)H_2O)_2Cl_2]Cl$ and $[Cr(en)(H_2O)_4]^{+3}$ were prepared from the reaction of $[Cr(en)(H_2O)(O_2)_2]\cdot H_2O$ with HCl and $HClO_4$ respectively as described previously [5]. The nucleotides, UDPG, and UDPG pyrophosphorylase were purchased from Sigma. The Dowex 50W-X2 ion exchange resin (100-200 mesh) was obtained from Bio-Rad. All of the complexes are isolated from the ion exchange resin as aqueous solutions. Attempts to precipitate any nucleotide complexes of this type result in their decomposition [6]. The chromium complexes are stored at 4 °C and the cobalt complexes at −20 °C.

[Co(en)$_2$(nucleotide)]

The carbonato complex, $Co(en)_2(CO_3)^+$ (0.2 mmol in 10 ml of water) was converted to the cis diaquo complex by the addition of 0.4 ml of 1 N HCl followed by gentle warming. This solution was then added to 0.2 mmol of Na_2H_2ATP or Na_3-HGTP in 10 ml of water and the mixture was heated at 80 °C for 8–10 minutes. The solution was cooled, adsorbed onto Dowex 50W-X2 and eluted with 0.1 M lithium formate followed by 0.3 M aniline as described previously [1a].

[Cr(en)(H$_2$O)$_2$(HATP)]

This complex was prepared by the reaction of Na_2H_2ATP (0.2 mmol) with either $Cr(en)(H_2O)_2$-$(Cl)_2^{+1}$ or $Cr(en)(H_2O)_4^{+3}$ in a 1:1 fashion at 80 °C for 10 minutes. The product was isolated as described above. The solution magnetic moment of this material was determined by the Evans method [7]. The diamagnetic susceptibility was calculated from Pascal's constants as -0.353×10^{-3} cm^3/mol. $\chi_M = 5.91 \times 10^{-3}$ cm^3/mol at 308 K; $\mu = 3.81$ B.M.

[Cr(NH$_3$)$_4$(UDPG)]$^+$ and [Co(en)$_2$(UDPG)]$^+$

These complexes were prepared by the reaction of the appropriate transition metal salt with Na_2-UDPG in a 1:1 fashion at 80 °C for 8 minutes. After cooling to room temperature the solution is adsorbed onto Dowex 50W-X2 and washed with water to remove unreacted sugar nucleotide. The product is separated from the transition metal starting material by elution with 0.1 M lithium formate or 0.5 M perchloric acid. The latter method is to be preferred since the formate anion may enter the inner coordination sphere in some cases. Preliminary experiments [8] conducted with Cr-$(NH_3)_4(UDPG)^+$ indicate that this complex is an inhibitor of UDPG pyrophosphorylase. Attempts to prepare $Co(NH_3)_4(UDPG)^+$ by this procedure resulted in the precipitation of cobalt hydroxide during the heating process.

NMR

The ^{31}P NMR spectra were recorded on a Nicolet Magnetics Corp. NT-360/Oxford NMR spectrometer at 145 MHz, at 24 °C with a 10 mm broadband probe and an internal ^2H lock (sample volume ~2.4 ml. 30–40% D_2O). Proton-band (modulated) decoupling was used. Chemical shifts are referenced to 85% phosphoric acid. Positive values of chemical shifts denote an upfield shift. The ^1H NMR of $Cr(en)(H_2O)_2$(HATP), to determine the magnetic moment, was recorded on a 90 MHz instrument. Tertiary butyl alcohol was employed as the proton reference.

Mass Spectra

The spectra were recorded on a V.G. Micromass 7070H instrument equipped with a FAB probe. The beam gas was xenon and the ion gun was operated at 6–8 kV and 1–2 mA. The samples were introduced into the glycerol matrix by pipetting several microliters of the nucleotide complex solution onto the glycerol. Spectra were recorded in the positive ion mode.

Results and Discussion

The syntheses of these nucleotide complexes follow procedures which have been developed for related complexes and the formulation of the products is the same. The $Cr(NH_3)_4(UDPG)^+$ and $Co(en)_2(UDPG)^+$ are slightly different from the nucleotide complexes however. The nucleotides have four ionizable P–OH groups and in forming a complex to a trivalent metal can undergo loss of three protons to give a neutral complex, ML_4-(nucleotide)^0. This is the case in the preparation of the isoionic complexes described here. UDPG has only two P–OH groups and therefore yields a monovalent cation on complexation to a trivalent metal. The UDPG complexes can not be isolated as isoionic materials and as far as charge is concerned are probably closer to complexes of the nucleoside monophosphates [9], Co(AMP) or Cr(AMP), than the nucleotides. The visible spectra maxima for these complexes are given in Table I.

A useful technique for characterizing these complexes is Fast Atom Bombardment Mass Spectroscopy, FAB MS. The suitability of this technique for metal nucleotide complexes has recently been demonstrated [10]. Ions in the molecular weight region are listed in Table II. In general a protonated molecular ion, MH^+, is observed. The molecular ion fragments by the successive loss of neutral ligands. This results ultimately in the appearance

TABLE I. Visible Spectral Maxima for Complexes.

Complex	Color	λ_{max}	λ_{max}
$Co(en)_2(HGTP)$	red	509(69)	367(53)
$Co(en)_2(HATP)$	red	509(73)	367(48)
$Cr(en)(H_2O)_2(HATP)$	violet	550(42)	406(35)
$Co(en)_2(UDPG)^+$	pink	506(104)	380(74)
$Cr(NH_3)_4(UDPG)^+$	pink	532	398

of a metal–nucleotide ion. These ions will sometimes adduct glycerol from the matrix resulting in the observation of a $[MH - L + Hglycerol]^+$ ion. Few peaks are observed in the spectrum outside the molecular ion region in accordance with the general low energy conditions associated with FAB MS. Thus molecular ions are observed for both $Co(en)_2$-(HATP) and $Co(en)_2(HGTP)$. Each of these molecules undergoes two successive losses of ethylenediamine. These fragments and the adducts of the fragments with glycerol are observed. It is interesting to note the order of the loss of the netural ligands in Cr(en)-$(H_2O)_2(HATP)$. The waters are obviously not bound to the chromium as tightly as the ethylenediamine. This is based on the fact that $[Cr(H_2O)_2(HATP) - en]^+$ ion is not observed. The loss of ethylenediamine occurs only after two successive water losses. Attempts were made to obtain a spectrum of Cr-$(NH_3)_4(UDPG)^+$ under the same conditions employed for the other complexes. Unfortunately a suitable spectrum could not be obtained. In general however FAB MS offers a convenient and characteristic method for the identification of complexes of this type. The observation of a molecular ion and ions corresponding to the loss of neutral ligands allow easy identification of the complexes to be made.

The use of ^{31}P NMR on the diamagnetic cobalt complexes enables identification of the phosphorus atoms bound to the cobalt to be made. On the average a ^{31}P resonance shifts −10 ppm on coordination to a cobalt [11]. Table III contains the chemical shift values for these complexes and a few related molecules. The chemical shifts reported for the $Co(en)_2$(nucleotide) complexes do not differ greatly from those observed for $Co(NH_3)_4(HATP)$ as expected. However the present examples show greatly enhanced resolution of the resonances due to the fact that these complexes have an element of asymmetry not found in $Co(NH_3)_4(HATP)$.

TABLE III. ^{31}P NMR Chemical Shifts of Selected Complexes.

Compound	^{31}P Chemical Shifts		
	α	β	γ
ATP	10.9	21.3	6.0
$Co(NH_3)_4(HATP)$	10.5	9.8	−4.6
$Co(en)_2(HATP)$	10.5	9.8	−4.7
$Co(en)_2(HGTP)$	10.4	9.8	−4.8
UDPG[a]	11.7	12.8	–
$Co(en)_2(UDPG)$	2.8	3.6	–
ADP	9.7	5.3	–
$Co(NH_3)_4(ADP)$[b]	−1.1	−4.3	–

[a]Ref. 12. [b]Ref. 11.

Consider P_β of $Co(NH_3)_4(HATP)$. It should appear as a doublet of doublets as it is split by the two non equivalent phosphorus atoms, P_α and P_γ. Further P_β is chiral and present in solution as both enantiomers. The complex itself is actually diastereomeric as adenosine is optically active. Thus P_β should appear as eight resonances, a doublet of doublets for each enantiomer. A multiplet of six peaks is actually observed due to overlapping of some of the resonances. A similar situation exists for P_β of $Co(en)_2(HATP)$. In this case though both P_β and the cobalt are optically active. This brings the number of optical isomers of this complex to four. The corresponding resonance should consist of a total of sixteen lines, a doublet of doublets for each of the four isomers. This pattern was observed

TABLE II. Ions in the Molecular Weight Region. Relative Abundances Given in Parentheses.

Ion	$Co(en)_2(HGTP)$	$Co(en)_2(HATP)$	$Cr(en)(H_2O)_2(HATP)$
$[MH]^+$	700(27)	684(31)	653(27)
$[MH_2 - en]^+$	641(100)	625(100)	–
$[MH_2 - en + S]^+$	733(14)	–	–
$[MH_2 - 2en]^+$	581(32)	565(95)	–
$[MH_2 - 2en + S]^+$	673(36)	657(8)	–
$[MH - H_2O]^+$	–	–	635(100)
$[MH - H_2O + S]^+$	–	–	727(36)
$[MH - 2H_2O]^+$	–	–	617(90)
$[MH - 2H_2O + S]^+$	–	–	709(31)
$[MH_2 - 2H_2O - en]^+$	–	–	558(56)

S = glycerol

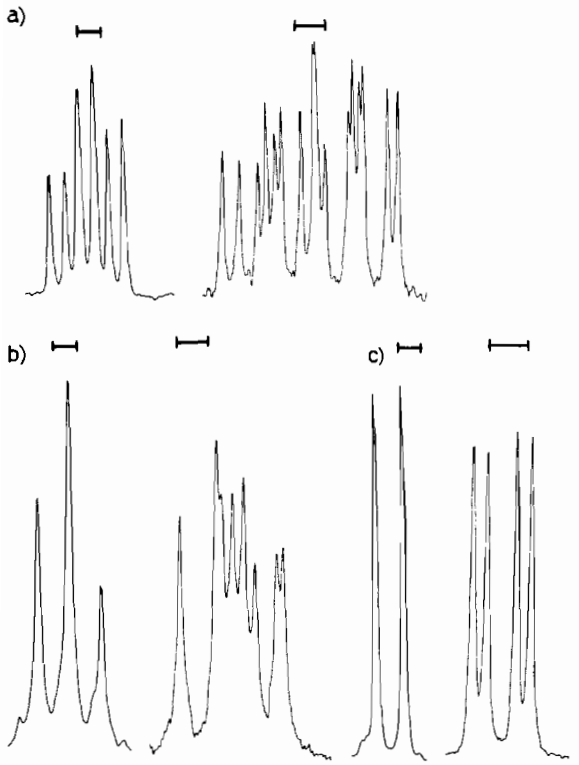

Fig. 2. ^{31}NMR spectra of $Co(NH_3)_4(HATP)$ (left) and $Co(en)_2(HATP)$ (right). The horizontal bars indicate 0.1 ppm. a) P_β, b) P_α, c) P_γ.

in $Co(en)_2(HATP)$ and is compared in Fig. 2a with the identical resonance of $Co(NH_3)_4(HATP)$.

A similar situation exists for P_α, which should appear as a doublet being coupled only to P_β. Four resonances should be observed for P_α in $Co(NH_3)_4(HATP)$ a doublet for each of the two isomers, and eight in $Co(en)_2(HATP)$, a doublet for each of the four isomers. The resonances are shown in Fig. 2b. In $Co(NH_3)_4(HATP)$ only three peaks are observed whereas all eight are visible in $Co(en)_2(HATP)$.

A somewhat different situation exists for P_γ. In $Co(NH_3)_4(HATP)$ it appears as a doublet, showing little sign of the chirality of the adjacent P_β. This is in contrast to P_α, which is not chiral itself, but shows the effects of being coupled to the chiral P_β by appearing as more than a simple doublet. This difference between P_α and P_γ is due to the relative position of their chemical shifts. P_α resonates very close to P_β and is thus sensitive to its chirality. P_γ on the other hand resonates ~15 ppm from P_β and is thus only slightly affected by its chirality. The same situation exists for P_γ of $Co(en)_2(HATP)$ except that in this complex P_γ is bound to a chiral metal. It appears as two doublets, one for each of the metal isomers. This is shown in Fig. 2c. $Co(en)_2(HGTP)$ gives a spectrum which illustrates these same points except that some of the resonances of P_α and P_β are not quite as well resolved.

The spectrum of $Co(en)_2(UDPG)^+$ is shown in Fig. 3. The assignment of the resonances in Table III is based on the assignment of free UDPG [12]. The phosphorus atoms of this complex are each chiral as is the cobalt. Each isomer will give rise to a doublet for each phosphorus atom. Clearly the spectrum indicates that more than one isomer is present but the resolution here does not allow for observation of all of the resonances.

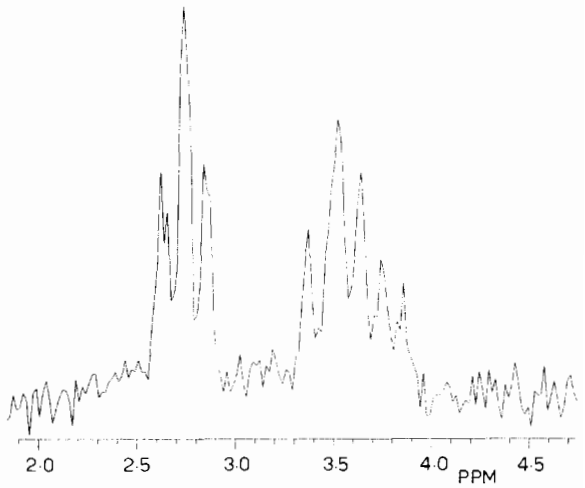

Fig. 3. ^{31}P NMR spectrum of $Co(en)_2(UDPG)^+$.

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