Ternary Chromium(III)–Histidine–Nucleotide Complexes

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Abstract

The first ternary chromium(III)–histidine–nucleotide complexes with purine and pyrimidine bases are described in the solid state. A histidine molecule of the starting chromium(III)–histidine complex is always removed during the reaction with the nucleotide. The starting histidine complexes and the ternary histidine–nucleotide–chromium(III) complexes have been characterized by elemental analyses, conductivity measurements, infrared and electronic spectroscopy, and by EPR and thermogravimetric analyses for the CMP derivatives. These results can afford more insight into chromium biochemistry.

Introduction

The presence of chromium(III) in the glucose tolerance factor [1] has increased interest in chromium biochemistry and the study of model chromium compounds. There are some recent studies of chromium(III)–amino acid complexes and also nicotinic and glutathione complexes. Some of these complexes [2–5] present activity as analogues of glucose tolerance factor (GTF). There is also great interest in preparing chromium(III) nucleotide complexes as labels of allosteric enzymes [6] and finding out the role of chromium(III) in transcription processes and RNA and DNA interactions [7].

Recently Theophanides and Harvey have pointed out the interest in knowing the reaction properties of metal urea complexes [8]. We have observed a total substitution of urea molecules from $Cr(urea)_6Cl_3 \cdot 3H_2O$ in reactions with nucleotides under mild conditions [9, 10].

The present paper studies the ternary system chromium(III)–histidine–nucleotide. Figure 1 gives the nucleotide structures and abbreviations used in this study.

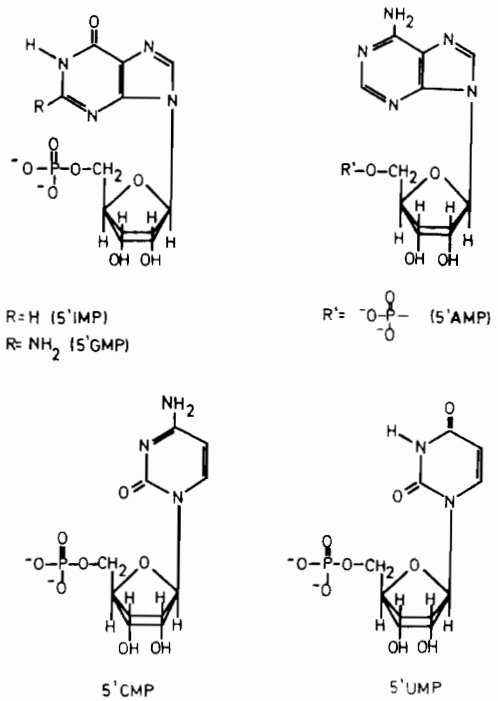

Fig. 1. Nucleotide structures and abbreviations used.

Experimental

The analyses of carbon, hydrogen and nitrogen were carried out with a Carlo Erba model 1106 microanalyzer at the Institute of Bio-organic Chemistry in Barcelona and with a Perkin-Elmer 240 B at the Faculty of Chemistry, Tarragona. The chlorine analysis was determined by the Schoniger method. Chromium was determined by using the colorimetric method for chromate [11]. The measurements were made with a Perkin-Elmer 552 UV–Vis spectrophotometer at 375 nm and 2 nm slit. The phosphorous content was determined by using the colorimetric method for phosphomolybdovanadate [12]. The measurements were carried out at 390 nm

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and 2 nm slit. The conductivities were measured with a Crison 525 conductimeter at 20.0 °C in 10^{-3} M complex solution. The infrared spectra were registered in the solid state (KBr pellets) on a Perkin-Elmer 683 IR spectrophotometer connected to a Perkin-Elmer 3600 data station. The reflectance spectra were recorded in the solid state on a Perkin-Elmer 552 UV–Vis spectrophotometer with an integrated sphere attachment. UV–Vis spectra were recorded in the same apparatus. The EPR spectrum of the CMP derivative was registered in the solid state at room temperature, on a Varian model E-12 in the X-band (Imperial College, London). The modulation frequency was 9.56 GHz.

The thermogravimetric analyses were recorded in a Mettler TA 3000 system at the Inorganic Chemistry Department, University of Granada (Spain).

The sources for nucleotides were Serva and Merck. The other products used were Merck. The starting $Cr(urea)_6Cl_3 \cdot 3H_2O$ complex was prepared according to procedure in the literature [13].

Syntheses of Chromium(III)–Histidine Complexes

Synthesis of $Cr(L\text{-his})_2Cl_3 \cdot 3H_2O$

$Cr(urea)_6Cl_3 \cdot 3H_2O$ (1 mmol) was dissolved in 5 ml water and 2 mmol L-histidine in 10 ml water. The pH of the latter solution was raised to 8.97 with 2 N diluted NaOH (pK_3 histidine = 8.97). Both solutions were mixed and the resulting dissolution (pH = 8.85) was placed in a thermostatic bath at 45 °C for 8 h. At the end, a violet–red solution with pH = 3.6 was obtained. The final solution was concentrated in a rotavapor at 45 °C to 5 ml volume and was eluted through a Sephadex G-10 column (diameter = 1 cm, length = 40 cm). Two fractions F_1 and F_2 were obtained. A precipitate was obtained by evaporating the F_1 fraction. F_2 is a very unstable green complex which in a 24 h period changes to violet, and could not be isolated. The precipitate obtained from F_1 was vacuum dried over P_4O_{10} in a dessicator.

Anal. for $Cr(C_6H_9N_3O_2)_2Cl_3 \cdot 3H_2O$. Found (calc.): C, 28.21(27.56); H, 4.54(4.59); N, 16.51-(16.08); Cl, 18.42(20.38); Cr, 8.84(9.95)%. The complex is violet and decomposes at 248–255 °C. It is soluble in water and has a millimolar conductivity at 20.0 °C of 458 Ω^{-1} cm^2 mol^{-1}. This measurement agrees with a 1:3 type electrolyte.

Synthesis of $[Cr(L\text{-his})_2(H_2O)_2]Cl_3 \cdot 3H_2O$

This synthesis is similar to the one for the former complex, but the initial pH in this case was 5.9, the pK_2 value of L-histidine. The mixture was placed in a thermostatic bath at 45 °C for 14 h. The solution obtained was concentrated in a rotavapor to 5 ml and filtered through an analogous Sephadex G-10 column. A single fraction only was obtained in this case, and a precipitate was obtained by evaporating the solution. The precipitate was vacuum dried over P_4O_{10} in a dessicator.

Anal. for $[Cr(C_6H_9N_3O_2)_2(H_2O)_2]Cl_3 \cdot 3H_2O$. Found (calc.): C, 24.70(25.78); H, 4.75(5.01); N, 14.66(15.04); Cl, 21.68(19.07); Cr, 9.91(9.31)%. The complex decomposes at 165–170 °C and has a molar conductivity of 478 Ω^{-1} cm^2 mol^{-1}.

Working in the same way at initial pH 1.82 (pK_1 of L-histidine) a blue solution was obtained, but we failed in the attempt to isolate a well defined complex.

Synthesis of $Cr(L\text{-his})(OH)Cl_2 \cdot H_2O$

$Cr(urea)_6Cl_3 \cdot 3H_2O$ (2 mmol) was dissolved in 10 ml water and 2 mmol of L-histidine was dissolved in 10 ml water and the pH adjusted to 8.97 with 2 N diluted NaOH. Both dissolutions were mixed dropwise with continuous stirring and the resulting solution (pH = 8.56) was placed in a thermostatic bath at 45 °C for 8 h. The final solution (pH = 2.6) was concentrated in a rotavapor to 5 ml and eluted through a Sephadex G-10 column. Two fractions were eluted, firstly violet F_1, secondly blue–violet F_2. The second fraction was replaced in the thermostatic bath for 4 h and eluted again through the column; a single fraction was obtained. All the results indicated that it contained the same complex as F_1. The precipitate obtained by evaporating the F_1 solution was vacuum dried over P_4O_{10}.

Anal. for $Cr(C_6H_9N_3O_2)(OH)Cl_2 \cdot H_2O$. Found (calc.): C, 22.19(23.00); H, 4.32(3.83); N, 14.09-(13.42); Cl, 21.61(22.68)%. The complex decomposes at 375 °C, is grey–violet and soluble in water with a millimolar conductivity of 375 Ω^{-1} cm^2 mol^{-1} which agrees with a 1:2 type electrolyte.

Synthesis of $[Cr(L\text{-his})(H_2O)_4]Cl_3$

The complex was obtained in a similar way to the previous one adjusting initial pH at 5.97 (pK_2 of L-histidine). The solution was placed in a thermostatic bath and heated at 45 °C for 14 h (final pH = 2.36). The solution was concentrated to 5 ml in a rotavapor and eluted through a Sephadex G-10 column; a single fraction was obtained but evidence of very small blue unstable second fractions was detected. A precipitate was obtained by evaporating the single fraction, the complex was vacuum dried over P_4O_{10}.

Anal. data for $Cr(C_6H_9N_3O_2)(H_2O)_4Cl_3$. Found (calc.): C, 18.75(18.68); H, 4.02(4.41); N, 11.00-(10.89); Cl, 28.69(27.63); Cr, 13.72(13.49)%. The product decomposes at 235 °C, is red and soluble in water with a millimolar conductivity of 379 Ω^{-1} cm^2 mol^{-1}. This value is intermediate for the expected 1:2 and 1:3 type electrolyte.

Synthesis of $Cr(L\text{-his})(urea)Cl_4 \cdot 2H_2O$

This complex was obtained in a similar way to the latter two but with an initial pH of 1.89 (pK_1 of L-

histidine). The histidine–urea complex solution was placed in a thermostatic bath at 45 °C for 26 h. A blue solution with pH = 1.32 was obtained at the end. The solution was concentrated to 5 ml, the pH was raised with diluted 2 N NaOH to 3 and eluted through a Sephadex G-10 column. A single fraction was obtained. A precipitate appeared after evaporation of the solution and was vacuum dried over P_4O_{10}.

Anal. for $Cr(C_6H_{10}N_3O_2)(CON_2H_4)Cl_4 \cdot 2H_2O$. Found(calc.): C, 18.79(18.88); H, 3.96(4.04); N, 15.88(15.73); Cl, 28.93(31.91); Cr, 10.63(11.69)%. The complex decomposes at 144–145 °C, it is deep blue, very hygroscopic with a millimolar conductivity of 590 Ω^{-1} cm^2 mol^{-1}, which agrees with a 1:4 type electrolyte.

Syntheses of Ternary Chromium(III)–Pyrimidine Nucleotide–Histidine Derivatives

Syntheses of Cr(5'CMP)(L-his)Cl$_2$·7H$_2$O and Cr$_3$(5'UMP)$_3$(L-his)(OH)Cl

A 5 ml water solution containing 1 mmol of Cr(L-his)(OH)Cl$_2$·7H$_2$O and a 5 ml water solution of 1 mmol disodium salt of 5'CMP or 5'UMP were mixed, pH of the mixture 3.5–3.6. The mixture was placed in a thermostatic bath at 45 °C for 11–16 h. The final solution was reduced to 5 ml in a rotavapor and eluted through a Sephadex G-10 column. Two fractions were obtained, F_1 blue–green and F_2 violet. By evaporating the solutions two precipitates were obtained. The second fraction complex appeared to be the starting complex and the first fraction complex contained the ternary derivatives that were dried over P_4O_{10}.

Anal. for Cr(5'CMP)($C_6H_9N_3O_2$)Cl$_2$·7H$_2$O. Found(calc.): C, 24.61(24.79); H, 4.25(4.96); N, 11.51(11.57); Cl, 10.30(9.78); Cr, 8.90(7.16); P, 5.33(4.27)%. The blue–grey CMP derivative is soluble in water, decomposes at 300 °C and has a millimolar conductivity of 422 Ω^{-1} cm^2 mol^{-1}. This value is greater than expected for a 1:2 type electrolyte. Protonation or dissociation equilibria either from histidine or nucleotide can increase the conductivity value.

Anal. for Cr$_3$(5'UMP)$_3$(L-his)(OH)Cl. Found(calc.): C, 27.22(27.57); H, 3.96(3.76); N, 9.76(8.77); Cl, 3.12(2.47); Cr, 10.27(10.86); P, 7.07(6.47)%. The grey UMP derivative is insoluble in water and unusual organic solvents and decomposes at 290 °C.

Attempts to Obtain Ternary Chromium(III)–Purine Nucleotide–Histidine Complexes

The syntheses using Cr(L-his)(OH)Cl$_2$·H$_2$O as starting product failed to obtain ternary complexes. Instead, binary compounds were obtained working under the same conditions as with the pyrimidine derivatives. The F_1 fraction eluted with 5'IMP was Cr$_2$(5'IMP)$_2$(OH)Cl·3H$_2$O. A precipitate is obtained with 5'GMP before elution: Cr$_2$(5'GMP)$_2$(OH)Cl·5H$_2$O and in the case of 5'AMP the complex previously described [10] Cr$_2$(5'AMP)$_3$·10H$_2$O.

Anal. for Cr$_2$(5'IMP)$_2$(OH)Cl·3H$_2$O. Found(calc.): C, 26.36(26.59); H, 3.98(3.21); N, 13.08(12.41); Cl, 3.21(3.93); Cr, 11.17(11.52); P, 6.99(6.87)%. The IMP derivative is grey, decomposes at 260 °C and is soluble in water with a millimolar conductivity value of 79 Ω^{-1} cm^2 mol^{-1} which agrees with a 1:1 type electrolyte.

Anal. for Cr$_2$(5'GMP)$_2$(OH)Cl·5H$_2$O. Found(calc.): C, 25.66(24.78); H, 4.05(3.61); N, 14.66(14.46); Cl, 2.63(3.67); Cr, 9.29(10.74); P, 6.34(6.40)%. The pale blue GMP derivative is insoluble in water and usual organic solvents.

Syntheses of Chromium(III)–Nucleotide–Histidine Ternary Complexes

Owing to the substitution of one histidine molecule from the coordination sphere of chromium(III) in the reaction with purine nucleotides, the complex Cr(his)$_2$Cl$_3$·3H$_2$O was used as starting product in the syntheses of ternary complexes.

A solution containing 1 mmol of Cr(L-his)$_2$Cl$_3$·3H$_2$O dissolved in 5–10 ml water and a solution containing 1 mmol of the disodium salt of CMP, UMP, IMP, GMP and AMP were mixed (starting pH = 5.92, 5.92, 5.66, 5.56 and 5.80 repectively) and the mixture placed in a thermostatic bath at 45 °C for 21 h. A small amount of precipitate appeared and 50 ml of ethanol were added. The precipitate obtained was filtered off, washed with ethanol and vacuum dried over P_4O_{10}.

Anal. for Cr(5'CMP) ($C_6H_9N_3O_2$)(C_2H_6O)·2H$_2$O. Found(calc.): C, 33.60(33.50); H, 5.29(4.93); N, 14.60(13.75); Cr, 8.61(8.54); P, 4.72(5.09)%. The CMP ternary derivative is pink, decomposes at 270 °C and is soluble in water with a millimolar conductivity 70 Ω^{-1} cm^2 mol^{-1}. This value is greater than that expected for a non-electrolyte complex. Dissociation or protonation equilibria may be responsible for this conductivity value.

Anal. for Cr$_3$(5'UMP)$_2$(L-his)$_4$(EtOH)(OH)·H$_2$O. Found(calc.): C, 34.73(34.00); H, 4.95(4.57); N, 13.90(14.42); Cr, 10.64(10.05); P, 4.67(3.99)%. The pink UMP derivative decomposes at 245 °C and has a millimolar conductivity of 53 Ω^{-1} cm^2 mol^{-1}.

Anal. for Cr(5'IMP)($C_6H_9N_3O_2$)(EtOH)·4H$_2$O. Found(calc.): C, 31.27(30.77); H, 4.97(4.33); N, 15.55(15.71); Cr, 7.99(8.33); P, 4.69(4.97)%. The pink IMP derivative decomposes at 249–250 °C and is soluble in water with a millimolar conductivity of 76 Ω^{-1} cm^2 mol^{-1}.

Anal. for Cr(5'GMP)($C_6H_9N_3O_2$)(EtOH)·4H$_2$O. Found(calc.): C, 33.65(34.23); H, 4.72(4.44); N, 18.65(17.75); Cr, 7.86(8.24); P, 4.87(4.91)%. The

TABLE 1. Infrared Data for the Binary Complexes Chromium–Histidine (cm^{-1})

Tentative assignment	L-his	$Cr(L\text{-his})_2Cl_3\cdot 3H_2O$	$Cr(L\text{-his})_2(H_2O)_2Cl_3\cdot 3H_2O$	$Cr(L\text{-his})(OH)Cl_2\cdot H_2O$	$Cr(L\text{-his})(H_2O)_4Cl_3$	$Cr(L\text{-his})(urea)Cl_4\cdot 2H_2O$
ν_a-COO^-	1636vs	1660vs, br	1659vs, br	1660vs, br		
		1620vs, br	1623vs, br	1629vs, br	1628s	1630vs
δ-NH_2	1595m					
ν ring	1501w	1499m	1501s	1500s	1498m	1495m
ν_s-COO^-	1416s	1436s	1444s	1444s	1445s	1445m
δ C–H + ν ring	1317s	1309s	1295sh			
ν ring	1273s	1266s	1268w	1268m	1268w	1266w
	1253s	1214w				
	1174m					
	1065m	1029s	1032w	1030m	1028vw	1033w
ν Imz in plane	977s	991m	990w	991w	990sh	991sh
	969s					
νCCN + νCC +	838s	891s	894w	895vw		
ν ring		826m	828m	827m		822w
δ ring	808w		804m			
δ CH ring	653m	650w				
ν ring	625vs	621s	622s	622s	622s	622s
ρ_r-COO^- + νCr–O	541s	585s		535w, br		538w
νCr–N		508w		501sh		
		470w	466w	469vw	464w	460w
		342w	351w	350w	350w	

pink GMP derivative decomposes at 265–270 °C, is insoluble in water and usual organic solvents.

Anal. for $Cr(5'AMP)(C_6H_9N_3O_2)(EtOH)\cdot 5H_2O$. Found(calc.): C, 31.22(32.19); H, 4.87(5.37); N, 17.73(16.69)%. The pink AMP derivative decomposes at 238–240 °C and is soluble in water with a millimolar conductivity of 148 Ω^{-1} cm^2 mol^{-1}.

All the ternary complexes have the general formula $Cr(XMP)\cdot(L\text{-his})(EtOH)\cdot nH_2O$ and a molecule of histidine has been substituted from the coordination sphere of chromium during obtention. The UMP tends to produce unclearly defined ternary complexes.

Results and Discussion

Chromium–Amino Acid Complexes

The chromium–amino acid complexes obtained were soluble and it was possible to measure their molar conductivities in water. The conductivity values are consistent with non-coordination of the Cl^- to chromium, except for the complex $Cr(L\text{-his})(H_2O)_4Cl_3$ which has a millimolar conductivity value of 379 Ω^{-1} cm^2 mol^{-1}, intermediate value for electrolytes 1:2 and 1:3. Coordination of one chloride to the metal cannot be ruled out for this complex.

Table 1 records the infrared data for chromium amino acid complexes. Tentative assignments [14–18] have been carried out according to the literature.

The infrared band that appears at 1636 cm^{-1} in histidine, tentatively assigned as ν_a-COO^-, appears in all the complexes as a strong band overlapping with the δ-NH_2(assym) band which appears at 1595 cm^{-1} in histidine. A frequency increase of 20 cm^{-1} is observed in ν_a-COOO for the complexes $Cr(L\text{-his})_2Cl_3\cdot 3H_2O$, $[Cr(L\text{-his})_2(H_2O)_2]Cl_3\cdot 3H_2O$ and $Cr(L\text{-his})(OH)Cl_2\cdot H_2O$, which seems to indicate a strengthening of the carboxylic group. The ν_s-COO^- band (at 1416 cm^{-1} in histidine) increases its frequency in all the complexes, appearing between 1436–1445 cm^{-1}, indicating coordination of chromium to the carboxylic group in all cases. Also ρ_r-COO^- (in histidine at 541 cm^{-1}) shows changes in frequency or overlaps with other bands.

The imidazole group bands show important changes. The histidine bands at 1273 and 1174 cm^{-1} appear in the complexes as a single band in the area 1268–1266 cm^{-1}. The ring band at 1065 cm^{-1} shifts to 1033–1028 cm^{-1}; the band that appears at 1033 cm^{-1} in $Cr(L\text{-his})(urea)Cl_2\cdot 2H_2O$ may also be due to a urea molecule band. The stretching ring histidine band at 977 cm^{-1} shifts also to higher frequencies. The histidine bands at 808 and 838 cm^{-1} also show notable shifts in some cases.

In the 470–460 cm^{-1} area, weak bands are observed and tentatively assigned as Cr–N stretching bands, either from the amino or imidazole groups. In the 350–340 cm^{-1} area, weak bands are tentatively assigned as Cr–N stretching bands from the imidazole. No evidence of the presence of Cr–Cl stretching bands is observed. These results agree with the conductivity measurements.

Table 2 records the electronic spectra data. The average values of 10 Dq and B' are calculated using the d^3 Tanabe–Sugano diagram [19]. $Cr(urea)_6Cl_3\cdot 3H_2O$ has a 10 Dq = 16 100 cm^{-1} and B' = 651 cm^{-1} [19] and $Cr(H_2O)_6^{3+}$ 10 Dq = 17 400 and B' = 695 cm^{-1}. The green F_2 unstable phase isolated during the obtention of $Cr(L\text{-his})_2Cl_3\cdot 3H_2O$ presents maxima at 617 and 450 nm, leading to 10 Dq = 16 400 cm^{-1} and B' = 548 cm^{-1}. These results seem to indicate coordination of chromium(III) to O donor and N donors with an important nephelauxetic effect. These 10 Dq and B' values are similar to complex $Cr(L\text{-his})(urea)Cl_4\cdot 2H_2O$ values. A reaction mechanism of substitution of urea molecules where the carboxylic group of histidine is mainly responsibe for the substitution can perhaps be inferred.

The 10 Dq values increase with greater pH obtention values and B' decreases. The coordination sites of chromium(III) with histidine seem to be N amino, N imidazole and carboxylic groups in complexes obtained at pH 8.97 as starting pH, while the complexes obtained at starting pH = 5.97 seem to be coordinated only to N imidazole and carboxylic groups. The complex $Cr(L\text{-his})(urea)Cl_4\cdot 2H_2O$ obtained at starting pH 1.82 has an average B' value of 547 cm^{-1}. This result is in accordance with the

TABLE 2. Electronic Data of the Binary Complexes Chromium–Histidine

Complexes	λ_1; ϵ_1 (nm)	λ_2; ϵ_2 (nm)	Δ_0 (cm^{-1})	B' (cm^{-1})
$[Cr(L\text{-his})_2]Cl_3\cdot 3H_2O$	517; 54.5	387; 58.8	19300	580
$[Cr(L\text{-his})_2(H_2O)_2]Cl_3\cdot 3H_2O$	557; 38.5	405; 40.2	17900	608
$Cr(L\text{-his})(OH)Cl_2\cdot H_2O$	556; 23.9	410; 24.9	18000	570
$[Cr(L\text{-his})(H_2O)_4]Cl_3$	579; 30.4	413; 34.8	17300	675
$Cr(L\text{-his})(urea)Cl_4\cdot 2H_2O$	579; 28.4	427; 27.8	17300	547

bonding of chromium to the carboxylic group of one urea molecule which has a significant nephelauxetic effect, greater than coordinated water. Comparison of these average values with those of $[Cr(L\text{-his})(H_2O)_4]Cl_3$ is clearly significant.

The number of histidine molecules coordinated to chromium also increases 10 *Dq* and decreases *B'* values as expected.

Derivatives Obtained from $Cr(L\text{-his})(OH)Cl_2 \cdot H_2O$ in Reaction with Nucleotides

The CMP derivative is the only chromium–nucleotide–histidine ternary complex obtained from $Cr(L\text{-his})(OH)Cl_2 \cdot H_2O$. The nucleotide 5'UMP leads to an insoluble, perhaps polymeric, complex of no structural interest. A total substitution of L-his coordinated to chromium is observed for the purine nucleotides. These results are similar to those previously described using $Cr(urea)_6Cl_3 \cdot 3H_2O$ as starting product reacting with nucleotides [8, 9].

The conductivity measurements agree with a 1:1 type electrolyte for the IMP derivative. The value of 422 Ω^{-1} cm^2 mol^{-1} in the conductivity measurement for $Cr(5'CMP)(L\text{-his})Cl_2 \cdot 7H_2O$ is close to that expected for a 1:3 electrolyte owing to protonation or dissociation equilibria either from the nucleotide or amino acid, or owing to the coordination of CMP to the chromium outer sphere through stacking interactions between the pyrimidine and imidazole rings: Masuda and Yamauchi [20] working with $[Pt(bipy)(en)]^{2+}$ and 5'AMP [20] have established these stacking interactions by NMR, CD and X-ray diffraction studies.

Table 3 contains the infrared data for pyrimidine nucleotide derivatives. Histidine bands not overlapping with nucleotides appear at 1502, 1320, 1279 and 617 cm^{-1} in the CMP derivative. The carboxylic group assymmetric stretching band shifts 6 cm^{-1} to lower frequency indicating coordination to the carboxylic group. The stretching C=O band appears in the CMP complex at 1728 increasing its frequency due to hydrogen bonding [21–24] or to a histidine free carboxylic group [25].

The stretching symmetric phosphate band shifts to higher frequency. This result agrees with coordination of chromium to the phosphate group [21–24].

The UMP derivative clearly shows histidine bands at 1391 cm^{-1} (1416 cm^{-1} histidine, carboxylic group stretching) and 1465 cm^{-1} (1465 cm^{-1} in histidine, deformation of $-NH_2$ group). The phosphate group bands also show shift to higher frequency.

Table 4 records the electronic data for these complexes. The d–d transitions present splittings owing to a distorted octahedral geometry. The shift for the $\pi \rightarrow \pi^*$ band in the CMP ternary complex seems to indicate interaction of the base residue, either with the metal or a stacking interaction with the imidazole ring. The 10 *Dq* average values of the order of 17 100 cm^{-1} agree with a preferent coordination to O donors.

TABLE 3. Infrared Data for the Ternary Complexes Chromium–Nucleotide–Histidine (cm^{-1})

Tentative assignment	L-his	$Na_2$5'CMP	$Na_2$5'UMP	$Cr(5'CMP)(L\text{-his})Cl_2 \cdot 7H_2O$	$Cr_3(5'UMP)_3(L\text{-his})(OH)Cl$
$\nu C{=}O$				1728s	1709s
$\nu C_2{=}O$		1663vs	1704s, br	1680s	1694s
			1689s, br		
$\nu C_4{=}O$			1679s, br		1682s
ν_a-COO^-	1636vs			1630s	1633s
ν ring	1501w	1531m		1502m	
		1498s	1478m		
δ_s-NH_2	1465s				1465s
ν_s-COO^-	1416s			1393m	1391s
$\nu C{-}H + \nu$ ring	1317s			1320sh	1317sh
ν ring	1273s		1284m	1279s	1271s
	1253s		1267m		
ν_a-$PO_3^{2-} + \nu C{-}O$ (sugar)		1115vs, br	1125s, br	1100vs, br	1118s, br
		1082vs, br	1092s, br		1086s, br
			1081s, br		
ν_s-$PO_3^{2-} + \nu$ ring	977s	977vs	981s, br	999vs, br	1013s, br
	969s			905w	1001s, br
					905sh
$\nu CCN + \nu CC + \nu Imz$	838s			805m	818m
	808w				
ν ring	625vs			617w	624w
ρ_r-$COO^- + \nu Cr{-}O$	541s			583w	562w, br
					428w
					396w

TABLE 4. Diffuse Reflectance Spectra of the Ternary Complexes Chromium–Nucleotide–Histidine

Complexes	$^4T_{2g} \leftarrow {}^4A_{2g}$ (nm)	$^4T_{1g} \leftarrow {}^4A_{2g}$ (nm)	$\pi \rightarrow \pi^*$ (nm)	Δ_0 (cm^{-1})
Cr(5'CMP)(L-his)$Cl_2 \cdot 7H_2O$	610, 562	430, 363	324, 271	17100
Cr_3(5'UMP)$_3$(L-his)(OH)Cl	608, 564	430, 389	292, 260	17100
Cr(5'CMP)(L-his)(EtOH)$\cdot 2H_2O$	602, 558	430, 388	301, 267	17300
Cr_3(5'UMP)$_2$(L-his)$_4$(OH)(EtOH)$\cdot H_2O$	599, 556	430, 388	280, 261	17300
Cr(5'IMP)(L-his)(EtOH)$\cdot 4H_2O$	608, 559	432, 376	296, 268	17200
Cr(5'GMP)(L-his)(EtOH)$\cdot H_2O$	604, 558	430, 385	310, 268	17200
Cr(5'AMP)(L-his)(EtOH)$\cdot 5H_2O$	606, 560	430, 386	264	17200
$Na_2$5'CMP			308, 260	
$Na_2$5'UMP			292, 250	
$Na_2$5'IMP			290, 240	
$Na_2$5'GMP			305, 242	
$Na_2$5'AMP			293, 252	

TABLE 5. Thermogravimetric Data

Temperature range (°C)	Weight loss (%)		Tentative assignment
	Calc.	Found	
Complex Cr(5'CMP)(L-his)$Cl_2 \cdot 7H_2O$			
0–90	4.96	4.88	$2H_2O$
90–210	7.44	7.32	$3H_2O$
210–240	9.85	9.76	$2H_2O$ + Cl
240–465	26.24	26.02	L-his + Cl
465–525	33.47	34.15	cytidine
Residue			phosphate of chromium
Complex Cr(5'CMP)(L-his)(EtOH)$\cdot 2H_2O$			
41.6–87	5.05	5.33	EtOH
191–612	73.06	71.67	L-his + $2H_2O$ + cytidine
Residue			phosphate of chromium

The CMP ternary complex has a *g* value of 1.98 of chromium with an octahedral surrounding. The broad signal does not permit more information to be obtained. The thermogravimetric data for the CMP derivative (Table 5) are consistent with the ternary character of this complex.

Ternary Chromium(III)–Nucleotide–Histidine Complexes Obtained from Cr(L-his)$_2$Cl$_3 \cdot 3H_2O$

The general formula for these complexes is Cr(5'XMP)(L-his)(EtOH)$\cdot n H_2O$ where 5'XMP is a purine or pyrimidine nucleotide. One of the histidine molecules of the starting complex has been substituted.

All the ternary complexes with the exception of the GMP derivative are water soluble, and conductivity measurements lead to millimolar conductivity values between 53 and 148 Ω^{-1} cm^2 mol^{-1}. These values are greater than expected for a non-electrolyte and may be explained as protonation or dissociation equilibria either from the nucleotide or the amino acid, or due to coordination of the nucleotide to the outer sphere of chromium through stacking interactions between the bases and the imidazole ring [20].

Table 6 records the infrared data for pyrimidine nucleotide derivatives and Table 7 the purine nucleotide derivative.

The histidine bands at 1317, 653 and 625 cm^{-1} appear with small shifts and the same shape in the ternary complexes.

The phosphate group stretching bands increase their frequency owing to interaction with the phosphate group. The carbonyl stretching bands appear in the CMP derivative at 1726 and 1717 cm^{-1}, this may be due either to indirect coordination of the carbonyl group from the nucleotide through hydrogen bonding or to a free carboxylic group of histidine [25]. The ring band at 1531 cm^{-1} in the free Na_2CMP shifts changing its intensity to 1529 cm^{-1}. The other ring bands show no clear information owing to the overlapping with histidine bands.

A weak band that appears at 351 cm^{-1} has the same shape and intensity as the tentatively assigned stretching chromium–N imidazole band in the starting complex.

The doublet at 650, 625 cm^{-1} of histidine appears in all three ternary purine nucleotide derivatives. Phosphate group bands also show a 15 cm^{-1} increase in the frequencies. The C_6=O carbonyl stretching band shows no significant change for IMP and GMP derivatives. The other strong ring bands show no change or overlap with histidine bands. The symmetric stretching of the carboxylic group band from histidine appears at 1413, 1419 and 1386 cm^{-1} in the ternary GMP, IMP and 5'AMP derivatives. All the complexes present a weak band near 390 cm^{-1} tentatively assigned as Cr–N stretching band.

TABLE 6. Infrared Data for the Ternary Complexes Chromium–Nucleotide–Histidine (cm^{-1})

Tentative assignment	L-his	$Na_2$5′CMP	$Na_2$5′UMP	Cr(5′CMP)(L-his)(EtOH)·$2H_2O$	Cr_3(5′UMP)$_2$(L-his)$_4$(OH)(EtOH)·H_2O
$\nu C=O$				1726–1717m	
$\nu C_2=O$		1663vs	1704s, br	1663s	1695s, br
			1689s, br		
$\nu C_4=O$			1679s, br		1673s, br
δ-NH_2 + $\nu C=N$ + $\nu C=C$		1650vs		1647s	1648s
ν_a-COO^-	1636vs				1633s, br
ν ring	1501w	1531m		1529w	1502w
		1498s	1478m	1490s	1470m
ν_s-COO^-	1416s			overlapped	overlapped
$\nu C-H$ + ν ring	1317s			1318sh	1317w
ν ring	1273s		1284m	1289m	1272s
ν_a-PO_3^{2-} + $\nu C-O$ (sugar)		1115vs, br	1125s, br	1110s, br	1114s, br
		1082vs, br	1092s, br	1083s, br	1086s, br
			1081s, br		
ν_s-PO_3^{2-} + ν Imz	977s	977vs	981s	994s	993s
	969s				
δ CH ring Imz	653m			650w	649sh
ν ring	625vs			624m	626m
ρ_r-COO^-	541s			520w	521w
$\nu Cr-N$				351w	345vw

TABLE 7. Infrared Data for the Ternary Complexes Chromium–Nucleotide–Histidine (cm^{-1})

Tentative assignment	$Na_2$5'GMP	$Na_2$5'IMP	Cr(5'IMP)(L-his)-(EtOH)·H_2O	Cr(5'GMP)(L-his)-(EtOH)·$4H_2O$	Tentative assignment	$Na_2$5'AMP	Cr(5'AMP)(L-his)-(EtOH)·H_2O
$\nu C_6{=}O$	1691s, br	1692s	1692s	1692s	δ-NH_2 + νC=N	1646vs	1645vs
δ-NH_2	1671sh		1674s			1608s	
ν_a-COO^-			1645s	1645s	ν_a-COO^-		1635s
			1633s	1635s, br	νC=C + νC=N	1584s	1575sh
δ-NH_2 + νC–N + νC–C	1577m	1594m	1604w	1592w	ν ring	1506w	1504w
		1551m		1552m		1484s	1482m
ν_s-COO^-			1413sh	1419w		1425m	1422w
ν ring		1383m	1380w	1381w	ν_s-COO^-		1386w, br
	1366m	1371m	1370w		ν ring	1307m	1314w
ν_a-PO_3^{2-} + νC–O (sugar)	1181m					1253m	1254w
	1142s, br	1143s, br			ν_a-PO_3^{2-} + νC–O (sugar)	1120s, br	1106s, br
	1117s, br	1119s, br	1114s, br	1122s, br		1094s, br	1093s, br
	1085s, br	1097s, br	1095s, br	1092s, br	ν_s-PO_3^{2-} + νImz	977vs	992m
ν_s-PO_3^{2-} + νImz	979s	979s	994s	992m	νCCN + νCC + νImz +	901m	908w
νPO	785m	793m	783w	793w	νC–O–P	879m	826m
δ NH	633m	647m	645sh	650w		820sh	
ν ring			623m	625m	νPO	797s	799m
ρ_r-COO^-			overlapped	561sh	ν ring		624w
νCr–N			391w	390w	ρ_r-COO^- + δ ribose	541s	535br
					νCr–N		392w

The electronic data show average 10 Dq values of 17 200 cm^{-1} and B' values of 750 cm^{-1} (Table 4). These results agree with a mixed coordination to O and N donors indicating coordination to carbonyl groups of phosphate groups in the nucleotides. On the other hand, stacking interactions between bases and no direct coordination of chromium(III) to the nucleotide cannot be disregarded owing to the conductivity measurements and the lack of significant shifts of purine bands. Splitting of d–d bands owing to a distorted octahedral geometry was observed.

For the 5'CMP derivative a thermogravimetric study was performed (Table 5), clearly confirming the ternary character of the complex.

General Comment

The synthesis of ternary chromium(III)–nucleotide–histidine complexes is difficult owing to the tendency of nucleotides to substitute histidine molecules. This tendency was confirmed in our laboratory with other amino acids [26]. These facts may be important in understanding the behaviour of chromium(III) in biological systems. A chromium(III)–protein transport system may be changed by interactions with nucleotides, and the ternary Cr–protein–nucleotide complex may be responsible for biological activity. The study of other ternary systems Cr(III)–nucleotide–amino acid is in progress.

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