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Chromium(III) Interactions with Nucleotides

J. J. FIOL, A. TERRON and V. MORENO*

Departamento de Química Inorgánica, Facultad de Ciencias, Universidad de Palma de Mallorca, Spain

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Some new chromium(III) complexes with nucleotides were obtained. For 5'AMP derivatives, different stoichiometries were observed for the complexes obtained at pH = 2, 3.5 and 5–7. The results provide more insight on the biological role of chromium(III).

Introduction

The interest on chromium(III) complexes with nucleotides arises from the use of these complexes as enzymatic labels by substitution of the activator or inhibitor [1, 2]. The biochemistry of chromium has recently become a topic of growing interest due to the presence of chromium(III) in the glucose tolerance factor [3–6]. Until now few complexes of chromium(III) were isolated in the solid state from Cr(III) with nucleotides, nucleosides or bases [7–9].

Experimental

The sources for 1,10-phenanthroline, nucleotides and chromium nitrate were Merck and Serva. Cr(urea)$_6$Cl$_3$·3H$_2$O was prepared according to described procedures [10].

Syntheses of $Cr_2(5'IMP)_3 \cdot 10H_2O$, $Cr_2(5'CMP)_3 \cdot 8H_2O$ and $Cr_2(5'GMP)_3 \cdot 5H_2O$

1 m*M* of nucleotide was dissolved in 5 ml of water and raised to pH = 3 with dilute nitric acid. 1 m*M* of chromium nitrate was dissolved in 5 ml of water. These two solutions were mixed and heated to 50 °C for 1–2 hours in a temperature-controlled bath. The complexes were precipitated by cooling or by addition of ethanol. They were filtered, washed with water and ethanol and dried in vacuum over P_2O_5. All the complexes were green.

Calculated for $Cr_2(5'IMP)_3 \cdot 10H_2O$: Cr(7.86), C(27.21), H(4.00), N(12.69), P(7.02). Found Cr(7.85), C(27.29), H(4.04), N(12.51), P(7.24). M.P.: 186–189 °C (d).

Calculated for $Cr_2(5'CMP)_3 \cdot 8H_2O$: Cr(8.58), C(26.75), H(4.29), N(10.40), P(7.67). Found: Cr(7.54), C(26.83), H(4.49), N(10.37), P(8.10). M.P.: 195–197 °C (d).

Calculated for $Cr_2(5'GMP)_3 \cdot 5H_2O$: Cr(8.13), C(28.17), H(3.60), N(16.43), P(7.27). Found Cr(7.30), C(28.02), H(4.05), N(16.12), P(8.10). M.P.: 157–160 °C (d).

Synthesis of $Cr_2(5'AMP)_3 \cdot 10H_2O$

Solutions of 1 m*M* chromium(III) nitrate and Na$_2$5'AMP were mixed and heated in a temperature-controlled bath to 50 °C for 2 hours. One solution of 1 m*M* 1.10-phenanthroline in 100 ml of water was added drop by drop to the first solution. The resultant solution had pH = 3.5. A green precipitate appeared; this was filtered, washed and dried over P_2O_5.

Calculated for $Cr_2(5'AMP)_3 \cdot 10H_2O$: Cr(7.85), C(27.17), H(4.23), N(15.85), P(7.02). Found: Cr(8.71), C(27.52), H(4.41), N(14.47), P(7.64). M.P.:210–215 °C.

Synthesis of $Na_2Cr(5'AMP)_2(OH) \cdot 8H_2O$

1 m*M* of Cr(OC(NH$_2$)$_2$)$_6$Cl$_3$·3H$_2$O was dissolved in 10 ml of water. 1 m*M* of Na$_2$5'AMP dissolved in 10 ml of water was added to this solution. The resultant solution had pH = 7.2. This solution was heated in a temperature-controlled bath to 50 °C for 1 hour. A green precipitate appeared, and was filtered, washed and dried as for the other complexes.

Calculated for $Na_2Cr(5'AMP)_2(OH) \cdot 8H_2O$: Cr(5.48), C(25.28), H(4.32), N(14.75), P(6.35), Na(4.32). Found: Cr(5.68), C(24.90), H(4.32), N(14.82), P(6.76), Na(5.33). M.P.: 210–215 °C(d).

Synthesis of $Cr(phen)(NO_3)_3 \cdot 2EtOH \cdot 3H_2O$

1 m*M* of 1,10-phenanthroline and 1 m*M* of chromium(III) nitrate were dissolved in 60 ml of

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*Author to whom correspondence should be addressed.

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TABLE I. Infrared Data for the Complexes (cm^{-1}).

Tentative assignment	$H_25'AMP$	$Cr_2(5'AMP)(phen)(NO_3)_2(OH)_2 \cdot 7H_2O$	$Cr(5'AMP)(phen)NO_3 \cdot 3H_2O$
$\nu C{=}N + \delta NH_2$	1690s	1690s	1690s
δNH_2	1660sh	1660sh	1660sh
$\nu C{=}N + \nu C{=}C + \delta NH_2$	1645s	1645s	1645s
$\nu C{=}N + \nu C{=}C$	1612s	1610s	1605s
$\nu C{=}N + \nu C{=}C$	1550w	1575sh	1580sh
$\nu C{=}N + \delta C{-}H$	1460s	1480w	1475w
νring(phen)		1425vs	1425vs
νNO_3^-		1382vs	1382vs
$\nu C{=}N$	1340w	1330sh	1330sh
$\nu C{=}N + \delta C{-}H$	1280vw	1250sh	1250sh
$\nu C{=}N + \delta C{-}H$	1220s	1210m	1210m
$\nu C{-}O$, νPO_3^{2-} (deg)	1105, 1080–30br	1120–1060br	1100–1050br
νPO_3^{2-}	990s	1010–990s	990s
$\nu P{-}O$	810m	820m[a]	820m[a]
νring(phen)		725s	725s

[a] $\nu NO_3^- + \nu P{-}O$. s: strong, w: weak, m: medium, s: shoulder.

ethanol. The solution was heated with reflux for half an hour and then concentrated to 20–30 ml; the complex was precipitated by cooling, then filtered, washed with ethanol and dried over P_2O_5.

Calculated for $Cr(phen)(NO_3)_3 \cdot 2EtOH \cdot 3H_2O$: Cr (9.15), C(33.92), H(4.59), N(12.37). Found: Cr (9.22), C(34.28), H(3.66), N(11.38). This violet complex was used as a starting product for the synthesis of the ternary complexes.

Synthesis of $Cr_2(5'AMP)(phen)(NO_3)_2(OH) \cdot 7H_2O$

Solutions of 1 m*M* of 1,10-phenanthroline and chromium nitrate were mixed and heated to 50 °C for 2 hours in a temperature-controlled bath. One solution of 1 m*M* of $Na_25'AMP$ in 10 ml of water was added drop by drop to the first solution. The resultant solution was heated for 2 hours at 50 °C. The green complex was filtered, washed and dried as for the other nucleotide complexes.

Calculated for $Cr_2(5'AMP)(phen)(NO_3)_2(OH) \cdot 7H_2O$: Cr(11.39), C(28.91), H(3.93), N(13.80), P(3.39). Found: Cr(11.73), C(28.88), H(4.05), N(13.91), P(3.70). M.P.: 193 °C (d).

Synthesis of $Cr(5'AMP)(phen)NO_3 \cdot 3H_2O$ and $Cr(5'AMP)(phen)NO_3 \cdot 7H_2O$

Solutions of 1 m*M* of $Na_25'AMP$ and the chromium–phenanthroline complex were mixed and heated to 50 °C for 2 hours in a temperature-controlled bath. The initial pH was 2 for the first complex, and 5.5 for the second. The first complex was green and the second violet. Precipitation was obtained by addition of ethanol. The complexes were filtered, washed and dried over P_2O_5 in vacuum.

Calculated for $Cr(5'AMP)(phen)NO_3 \cdot 3H_2O$: Cr (7.50), C(38.10), H(3.75), N(16.16), P(4.47). Found: Cr(6.91), C(39.09), H(4.44), N(15.17), P(4.67). M.P.: 140–145 °C (d).

Calculated for $Cr(5'AMP)(phen)NO_3 \cdot 7H_2O$: Cr (6.80), C(34.51), H(4.44), N(14.64). Found Cr(6.16), C(34.43), H(4.24), N(14.61).

Carbon, hydrogen and nitrogen contents were determined by elemental analysis at the Institute of Bio-organic Chemistry of Barcelona using a Carlo-Erba analyzer. Phosphorus was determined using the phosphomolybdovanadate method. Chromium was detected by spectrophotometric methods and sodium by flame photometry.

Visible–U.V. spectra were recorded with a Perkin Elmer 552 spectrophotometer from aqueous solutions (concentration: 10^{-3} to 10^{-4} *M*). Infrared spectra (KBr pellets) were obtained using a Perkin Elmer 683 spectrophotometer connected with a Perkin Elmer 3600 data station. A Perkin Elmer 705 atomic absorption spectrophotometer was used in the determination of sodium.

Results and Discussion

The complexes were slightly soluble in water (to concentrations of 10^{-3} to 10^{-4} *M*) with the exception of $Cr(5'AMP)(phen)NO_3 \cdot 7H_2O$ which is easily soluble in water. All the complexes are insoluble in

TABLE II. Infrared Data for the Complexes (cm^{-1}).

Tentative assignment	$Na_25'AMP$	$Cr_2(5'AMP)_3 \cdot 10H_2O$	$Na_2Cr(5'AMP)_2(OH) \cdot 8H_2O$	$Cr(5'AMP)(phen)NO_3 \cdot 7H_2O$
$\nu C{=}N + \delta NH_2$	1650s	1650s	1650s	1690s, 1680sh, 1660s, 1640s 1636sh
$\nu C{=}N + \nu C{=}C$	1600s	1610s	1610s	1607s
$\nu C{=}N + \nu C{=}C$	1580m	1580m	1580m	1570sh
$\nu C{=}N + \delta C{-}H$	1480m	1480m	1480m	1480m
$\nu C{=}N$	1420m	1420m	1420m	1430vs (phen)
νNO_3^-				1382vs
νring	1310m	1300m	1340m, 1300m	1305m, 1300w
$\nu C{=}N + \delta C{-}H$	1250m	1250sh	1270vw, 1240m	1255sh, 1240m
$\nu C{=}N + \delta C{-}H$	1210m	1210m	1210m	1210w
$\nu C{-}O + \nu PO_3^{2-}$(d)	1100–1030br	1120–1050br	1130–1060br	1100–1040br
νPO_3^{2-}(sym)	980s	990s	990s	990s
$\nu P{-}O$	790s	815m	820m	820m[a]
νring (phen)				725vs

[a] $\nu P{-}O + \nu NO_3^-$. s: strong, m: medium, w: weak, sh: shoulder, br: broad.

TABLE III. Infrared Data for the Complexes (cm^{-1}).

Tentative assignment	$Na_25'CMP$	$Cr_2(5'CMP)_3 \cdot 8H_2O$	Na_2GMP	$Cr_2(5'GMP)_3 \cdot 5H_2O$	$Na_2(5'IMP)$	$Cr_2(5'IMP)_3 \cdot 10H_2O$
$\nu C{=}O + \delta NH_2$	1710s	1730s	1695s	1695s	1680s	1685s
$\delta NH_2 + \nu C{=}N$	1660sh	1660sh	1660sh	1645sh	1640sh	1640sh
νring	1530m	1530w	1605m	1600s	1590m	1590m
νring	1495s	1490m	1535m	1530m	1545m	1555m
νring	1410m	1410m	1480m	1480m	1520w	1520m
νring	1375m	–	1415m	1410m	1480m	1460m
νring	1290m	1280m	1360m	1360m	1420m	1420m
νring			1230m	br	1380m	1380m
νring					1344m	1350m
νring					1330w	1320w
νring					1213s	1210s
νPO_3^{2-}(sym)	972s	985s	970s	980m	972s	990s
$\nu P{-}O$	780m	780m	800m	790m	790m	780m

s: strong, m: medium, w: weak, sh: shoulder, br: broad.

usual organic solvents. The compounds are microcrystalline but no suitable crystal for X-ray structural studies was obtained.

Slight variations in the ring bands were observed for 5'AMP derivatives obtained at pH = 2 (Table I) (These bands must be compared with the acid H_25'-AMP). The complexes isolated at pH greater or similar to the pK's nucleotide presented shifts from the frequencies of the disodium salt of 5'AMP (Table II) [11–15]. The complex $Cr(5'AMP)(phen)NO_3 \cdot 7H_2O$ presents a band at 1690 cm^{-1} that can tentatively be assigned as a coupling of the bending vibration of the $-NH_2$ group and the C=N stretching vibration [11]. There were also two more bands at 1650 and 1640 cm^{-1} and some shoulders, whereas the disodium salt presents a broad band at 1650

TABLE IV. Ultraviolet–Visible Data for the Complexes (nm).

Compound	λ_{max} (ϵ)	λ_{max} (ϵ)	λ_{max} (ϵ)	λ_{max} (ϵ)
$Cr(5'AMP)NO_3 \cdot 4H_2O$ [9]	260(15400)	310(19)		610(15)
$Cr_2(5'AMP)_3 \cdot 10H_2O$	280(400)	326(75)	403(105)	
$Na_2Cr(5'AMP)_2(OH) \cdot 8H_2O$	275sh(20)	306(20)	418(25)	578(20)
$Cr_2(5'IMP)_3 \cdot 10H_2O$	250			
$Cr_2(5'CMP)_3 \cdot 8H_2O$	270			
$Cr_2(5'GMP)_3 \cdot 5H_2O$	250, 270			
$Cr_2(5'AMP)(phen)(NO_3)_2(OH)_2 \cdot 7H_2O$	267(308)	396(40)	445(20)	572(20)
$Cr(5'AMP)(phen)NO_3 \cdot 3H_2O$	270(740)	395(40)		580(20)
$Cr(5'AMP)(phen)NO_3 \cdot 7H_2O$	280(12)	362(96)	415(53)	590(20)

cm^{-1}. In the three complexes (Table II) the ring band at 1600 cm^{-1} [11] was shifted. In the ring band zone between 1500–1200 cm^{-1}, a splitting for the band of 1250 cm^{-1} [11, 13] was observed in the complex $Na_2Cr(5'AMP)_2(OH) \cdot 8H_2O$ (1270 vw, 1240m cm^{-1}). In this complex a new ring band appeared at 1380 cm^{-1}. For these three complexes (Table II) the ring band from 1220 cm^{-1} [12, 13] was shifted to lower frequencies.

In the ternary complexes the ring bands due to phenanthroline appeared shifted from 1240 and 740 cm^{-1} for phenanthroline, to 1430 and 725 cm^{-1} for the complexes.

The phosphate group bands were shifted to higher frequencies for all the complexes [12]. This may be due to inductive effects of the chromium(III) coordinated to the phosphate group. A new band at 350 cm^{-1} appeared in the complex $Cr(5'AMP)(phen)NO_3 \cdot 7H_2O$ that can be tentatively assigned as a Cr–N stretching vibration [16].

The nitrate group bands correspond to a free nitrate (ν_3: 1382 cm^{-1} and ν_2: 820 cm^{-1}). This latter band overlaps with the P–O stretching band in the ternary complexes [16].

The 5'IMP, 5'CMP and 5'GMP derivatives present shifts in the phosphate group bands that indicate coordination with the phosphate group. The ring bands do not display changes in their frequencies except for the carbonyl stretching vibration coupled with the C=C vibration in the 5'CMP derivative (Table III).

The ultraviolet data showed notable influence for pH in the interaction of chromium(III) with the bases. Table IV shows the maxima of U.V.–visible spectra. In the 5'AMP derivatives isolated at pH greater than or similar to the pK's nucleotides, a shift in the maximum of the adenine ring at 260 nm, and a decrease of molar extinction coefficient were observed. This case is for derivatives of stoichiometry 2:3 and 1:2. For the complex of stoichiometry 1:1 isolated at pH = 2, no shift was observed in this band, possibly because no interaction of the metal ion–adenine ring occurs [9].

No changes were observed in the ultraviolet bases bands between the complexes and the ligands for 5'IMP, 5'CMP and 5'GMP derivatives.

Bands due to phenanthroline at 280 nm overlap with those due to the adenine ring at 260 nm for the ternary complexes. The most notable variations appear for the complex $Cr(5'AMP)(phen)NO_3 \cdot 7H_2O$.

This complex can be a good enzymatic label and also a good model for an enzymatic system (enzyme–metal–nucleotide). 1,10-phenanthroline is similar to a histidine residue for the protein chain and a model for glucose tolerance factor GTF. In fact, the infrared spectrum of this complex is very similar to the proper GTF described by Mertz [3].

The stoichiometry is a function of the pH. For example, at pH = 2 the complex with 1:1 stoichiometry was obtained [9]; at pH = 3.5 the 2:3 derivative, and at pH between 5 to 7 the 1:2 derivative were obtained. This behaviour is similar for chromium(III) aminoacid complexes [17]. No relationship can be established for the IMP, GMP and CMP derivatives because the nucleotides have different bases in each case.

The synthesis of the 1:2 stoichiometry complex from $Cr(urea)_6Cl_3 \cdot 3H_2O$ deserves more attention. The synthesis reaction was: $Cr(urea)_6Cl_3 \cdot 3H_2O$–$Na_25'AMP$—$Na_2Cr(5'AMP)_2(OH) \cdot 8H_2O$.

The synthesis was designed to obtain a ternary complex by partial substitution of the urea molecules coordinated to chromium(III). The total urea molecules substitution was not expected due to the inert character of chromium(III) complexes. The reaction temperature was 50 °C but the obtaining temperature for $Cr(urea)_6Cl_3 \cdot 3H_2O$ was 70 °C. There may be an intermediate reaction complex that presents certain lability and makes feasible the total substitution of urea molecules. It was verified recently that Cr(III)–EDTA type complexes are labile [18]. The coordination geometry of $Cr(H_2O)(EDTA)$ is a distorted octahedral, which seems to be determinant for the lability of the complex. The lability occurs in front of anionic ligands, the way 5′AMP occurs. A substitution inert complex would appear inappropriate as a biological catalyst, but its function in GTF need not be restricted to a structural support in tertiary structure. An explanation of the results may be that the resultant complex by substitution of one or two urea molecules by one 5′AMP molecule presents distorted octahedral coordination geometry which makes feasible the reaction with the anion, the second 5′AMP molecule, and the total substitution of urea molecules.

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