Complexes of Co(II), Ni(II), Cu(II) and Zn(II) with 3′AMP and 2′AMP

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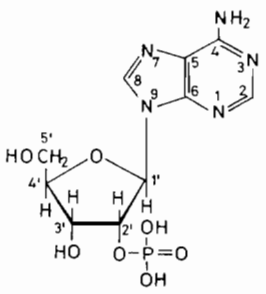
Abstract

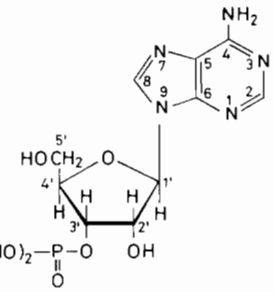
Derivatives of Co(II), Ni(II), Cu(II) and Zn(II) with 3′AMP and 2′AMP were synthesized and characterized by IR UV–Vis and fluorescence spectroscopy. There seems to be bonding of the metal ion to the base in all cases. The activation test, using the complexes as allosteric labels, was carried out with rabbit muscle glycogen phosphorylase b, but the enzyme was not activated, confirming that the phosphate group must necessarily be bonded to position 5′ of the ribose in order to activate this enzyme.

Introduction

Most of the studies carried out so far with 3d metal ions and 3′AMP and 2′AMP are studies in solution [1–5]. A study has recently been published of derivatives of Fe(III), Fe(II), Mn(II) and Cr(III) with these ligands [6]. These complexes may be used as analogues of 5′AMP in enzyme systems. As has already been suggested, the phosphate group must be esterified in position 5′ of the ribose ring in order to activate rabbit muscle glycogen phosphorylase b [7, 8]. These new complexes may be a good test of this opinion, as it was shown that some 5′AMP derivatives activated rabbit muscle glycogen phosphorylase b [9].



2′AMPH$_2$



3′AMPH$_2$

Experimental

Adenosine 3′-monophosphoric acid and adenosine 2′-monophosphoric acid (Serva), and nitrates of Co(II), Ni(II), Cu(II) and Zn(II) (Merck) were used as starting products. Glycogen and Mg(II) acetate were purchased from Merck; glycylglycine and tris-hydroxymethyl amine methane were purchased from Sigma. NADP, glucose-1-phosphate mutase and glucose-6-phosphate deshydrogenase from Boehringer-Mannheim and KH_2PO_4 from Fluka were used in the isolation and activity test of rabbit muscle glycogen phosphorylase b.

Synthesis of $Co_4(3'AMP)(OH)_6 \cdot 3H_2O$ and $Co_2(2'AMP)(OH)_2 \cdot 3H_2O$

1 mmol of Co(II) nitrate was added to 1 mmol of nucleotide dissolved in 40 ml of distilled water. The mixture was heated with stirring for 5 h in a thermostatic bath of 50 °C and a coloured solution was obtained. Sodium hydroxide 0.1 N was added to the solution to pH = 9.6; the resulting precipitate was filtered, washed repeatedly with water at 50 °C and vacuum dried over P_4O_{10}.

Synthesis of the other Derivatives

1 mmol of the nitrate of the corresponding metal ion was added to 1 mmol of nucleotide dissolved in 20 ml water, and the mixture was raised to an appropriate pH to avoid precipitation of hydroxides (4.5

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TABLE I. Analytical Data for the Complexes

Compound	%Me	%C	%H	%N	%P	Colour	Melting point (°C)	μ_{eff} (M.B.)
$Co_4(3'AMP)(OH)_6 \cdot 3H_2O$	31.99[a]	16.28	3.25	9.49	4.20	violet	198	4.9
	(32.69)[a]	(16.97)	(3.30)	(9.38)	(4.64)			
$Co(3'AMP) \cdot 3H_2O$	12.86	26.19	3.92	15.27	6.76	purple	203	2.2
	(13.25)	(26.38)	(4.01)	(15.12)	(7.12)			
$Ni(3'AMP) \cdot 5H_2O$	11.88	24.29	4.45	14.17	6.27	pale	189	
	(11.31)	(24.11)	(4.12)	(13.80)	(6.51)	green		
$Cu(3'AMP) \cdot 5H_2O$	13.02	24.05	4.41	14.03	6.20	blue	187	
	(13.07)	(23.97)	(3.71)	(14.11)	(6.91)			
$Zn(3'AMP) \cdot 3H_2O$	14.06	25.82	3.87	15.07	6.66	white	189	
	(10.07)	(25.87)	(3.43)	(15.05)	(7.25)			
$Co_2(2'AMP)(OH)_2 \cdot 3H_2O$	21.38	21.77	3.63	12.70	5.62	violet	192(d)	3.88
	(21.94)	(21.93)	(3.99)	(12.44)	(5.75)			
$Co(2'AMP) \cdot 3H_2O$	12.86	26.19	3.92	15.27	6.75	violet	190(d)	5.11
	(12.36)	(25.80)	(4.00)	(14.82)	(6.40)			
$Ni(2'AMP) \cdot 6H_2O$	11.46	23.43	4.69	13.67	6.04	pale	187	
	(11.84)	(23.79)	(4.33)	(13.03)	(5.92)			
$Cu(2'AMP) \cdot 5H_2O$	13.02	24.60	4.51	14.31	7.58	blue	185	
	(13.27)	(24.32)	(3.65)	(14.21)	(7.73)			
$Zn(2'AMP) \cdot 4H_2O$	13.86	25.47	4.27	14.85	7.55	white	188	
	(13.20)	(25.20)	(4.31)	(14.64)	(7.70)			

[a]Calculated (found).

for Co(II), 6 for Ni(II), 5 for Cu(II) and 6 for Zn(II)). The mixture was heated with stirring for 5 h in a thermostatic bath at 50 °C. The complexes precipitated spontaneously on cooling. The precipitates were filtered, washed repeatedly with water at 50 °C and vacuum dried over P_4O_{10}.

The data from the elemental analyses appear in Table I.

The rabbit muscle phosphorylase b was prepared by the Fischer–Krebs method [10–12]. The enzyme was recrystallized three times and 5'AMP was removed by passage through a column of Sephadex G-25 gel and then treated with activated charcoal. The A_{260}/A_{280} ratios were always found to be between 0.53–0.54, as proof of total removal of 5'AMP from the enzyme. The concentration of the enzyme was determined spectrophotometrically using an extinction coefficient ($E^{1\%}_{1cm}$) at 280 nm of 13.2.

The activity of rabbit muscle glycogen phosphorylase b in the sense of glycogen degradation was determined spectrophotometrically using the procedure of Helmreich and Cori [13], and the increase in absorption at 340 nm of the final product NADPH was observed. The concentration of the enzyme was always 3.076×10^{-4} mg/ml. All the experiments were carried out in the presence of a metal-3'AMP or metal-2'AMP complex (the concentration of the complex in the test media was in the order of 1×10^{-4} M) and without free 5'AMP removed, as indicated above. The activity of the obtained rabbit muscle glycogen phosphorylase b in the presence of the allosteric activator was proved to be 100% relative activity. More details about the activity test are described in a previous paper [9].

The UV–Vis spectra were registered on a Perkin-Elmer 552 spectrophotometer with thermostatized cells from a Hetho-term 0.3T 623 batch of circulating water with a precision of ±0.02 °C. Infrared spectra (KBr pellets) were obtained using a Perkin-Elmer 683 spectrophotometer connected with a data station 3600 (Perkin-Elmer). Fluorescence was measured with a FICA MKII double beam spectrophotometer. Magnetic susceptibility was determined at the Institute of Applied Organic Chemistry of Barcelona by Faraday's method.

Carbon, hydrogen and nitrogen contents were determined with a Carlo Erba analyzer in the Institute of Bio-organic Chemistry of Barcelona. Phosphorous was determined by the phosphomolibdovanadate method, as was indicated previously [9]. Metals were detected using a Perkin-Elmer 705 flame atomic absorption spectrophotometer.

Results and Discussion

The complexes are only slightly soluble in water (in concentrations of 10^{-3} M) and are insoluble in the usual organic dissolving agents, so molecular weight determinations were not feasible. The complexes are microcrystalline, but crystals suitable for studying X-ray diffraction could not be obtained.

TABLE II. Infrared Data for the 3'AMP Complexes (cm^{-1})

Tentative assignment	Na_2-3'AMP pH = 4.5	Co_4(3'AMP)-$(OH)_6 \cdot 3H_2O$	Co(3'AMP)$\cdot 3H_2O$	Ni(3'AMP)$\cdot 5H_2O$	Cu(3'AMP)$\cdot 3H_2O$	Zn(3'AMP)$\cdot 3H_2O$
$\nu_{C=N} + \delta_{NH_2}$	1660s[a]	1660vs	1660vs	1660vs	1660vs	1660vs
$\nu_{C=N} + \nu_{C=C} + \delta_{NH_2}$	1650vs	1649s	1649vs	1643vs	1648vs	1642vs
$\nu_{C=N} + \nu_{C=C}$	1605s	1606m	1604s	1600s	1608s	1600vs
$\nu_{C=N}$, –C=C–	1575m	1579m	1584m	1580s	1580s	1580s
$\nu_{C=N} + \delta_{C-H}$	1486m	1483m	1484m	1480m	1486m	1480m
$\nu_{C=N}$	1426w	1424w	1426m	1420m	1429m	1420m
ν_{Ring}	1308w	1304w	1303w	1300w	1304w	1297m
$\nu_{PO_2^-}$(asi)	1215vs	1212w	1212m	1210w	1216m	Overlaps
$\nu_{PO_3^{2-}}$(deg) + ν_{C-O}	1110s	1135vs	1128–1027vs,br	1140–80vs,br	1133vs	1160vs
		1090vs				1140vs
$\nu_{PO_2^-}$(si)	1062s	1069vs	–	–	1063vs	1080vs
$\nu_{PO_3^-}$(si)	980m	978vs	986vs	980vs	992vs	1003vs
				970vs	988vs	985vs

[a]s = strong; vs = very strong; m = medium; w = weak; br = broad.

TABLE III. Infrared Data for the 2'AMP Complexes (cm^{-1})

Tentative assignment	Na_2-2'AMP pH = 4.5	Co_2(2'AMP)-$(OH)_2 \cdot 3H_2O$	Co(2'AMP)$\cdot 3H_2O$	Ni(2'AMP)$\cdot 6H_2O$	Cu(2'AMP)$\cdot 5H_2O$	Zn(2'AMP)$\cdot 4H_2O$
$\nu_{C=N} + \delta_{NH_2}$	1685vs[a]	1678vs	1655vs	1659vs	1660vs	1680vs
$\nu_{C=N} + \nu_{C=C} + \delta_{NH_2}$	1636vs	1649vs	1651vs	1647vs	1645vs	1625vs
$\nu_{C=N} + \nu_{C=C}$	1620s	1608vs	1602vs	1607s	1605vs	1610s
$\nu_{C=N} + \nu_{C=C}$	1575m	1573m	1580m,sh	1582m	1580m	1575m
$\nu_{C=N} + \delta_{C-H}$	1503m	1505m	1490m	Overlaps	1483m	1450m
$\nu_{C=N}$	1433m	1421m	1430w	1429m	1425m	1425m
ν_{Ring}	1306w	1296s	1305w	1309m	1335m	1315m
$\nu_{PO_2^-}$(asi)	1220vs	1206m	1200w	1218m	1210m	–
$\nu_{PO_3^{2-}}$(asi)	1115vs	1141–1057vs,br	1140vs	1134–1090s,br	1140–1070vs,br	1100–1150vs,br
	1093vs		1060vs			
	1073vs		1055vs			
$\nu_{PO_3^{2-}}$(si)	980s	991–981vs	990vs	948vs	990vs	1000vs
ν_{M-L}			560m		280w	
			503m		260w	

[a]s = strong; vs = very strong; m = medium; w = weak; br = broad, sh = shoulder.

The IR spectra bands were assigned by analogy with those of 5'AMP [14–17]. The IR data from the 3'AMP derivatives are found in Table II. The complex bands were compared with those of the disodium salt precipitated at pH = 4.5. The ν(C=C), (C=N) at 1605 cm^{-1} ring band in the disodium salt and the ν ring band at 1308 cm^{-1} show shifting in all cases. The other ring bands also undergo slight variations, which seems to indicate bonding of the metal ion to the adenine ring in all cases.

The phosphate group bands appear in the 1200–900 cm^{-1} area. Splitting, indicating bonding of the metal ion to the phosphate group, is observed in the derivatives of Cu(II), Zn(II), and Co(II) in basic medium on the $\nu(PO_4^{2-})$ (deg) band. In the derivatives of Co(II) and Ni(II), the band shifts at higher frequencies, which also seems to indicate bonding to the phosphate group, but this is not easily observed as this band is very broad. The symmetric stretching band of the phosphate group shows shifting

TABLE IV. Spectroscopic Properties of the Complexes

Compound	λ_{max} (UV–Vis) (nm)	λ_{max} (fluorescent data) (nm)
$Co_4(3'AMP)(OH)_6 \cdot 3H_2O$	259, 397–401sh	pH: 7.0 Ex. 295 Em. 370
$Co(3'AMP) \cdot 3H_2O$	259	pH: 7.0 Ex. 290 Em. 325
$Ni(3'AMP) \cdot 5H_2O$	261	pH: 7.0 Ex. 298 Em. 333
$Cu(3'AMP) \cdot 5H_2O$	260	pH: 7.0 Ex. 295 Em. 328
$Zn(3'AMP) \cdot 3H_2O$	260	pH: 7.0 Ex. 294 Em. 325
$Co_2(2'AMP)(OH)_2 \cdot 3H_2O$	258	pH: 7.0 Ex. 290 Em. 320
$Co(2'AMP) \cdot 3H_2O$	259, 396–400sh	pH: 7.0 Ex. 290 Em. 325
$Ni(2'AMP) \cdot 6H_2O$	258	pH: 7.1 Ex. 290 Em. 350
$Cu(2'AMP) \cdot 5H_2O$	259	pH: 7.0 Ex. 290 Em. 360
$Zn(2'AMP) \cdot 4H_2O$	259	pH: 7.0 Ex. 300 Em. 290

in all cases, confirming bonding of the metal to the phosphate group. In the case of Ni(II), there is one band at 980 cm^{-1} and another at 970 cm^{-1}.

The IR data for the derivatives of 2'AMP are presented in Table III. The 2'AMP ring bands show important variations in frequency in all cases, which seems to indicate bonding of the metal ion to the adenine ring as for the 3'AMP derivatives.

The symmetric stretching band of the phosphate group shifts in all cases. The $\nu(PO_4^{2-})$ (deg) band splits significantly in the derivatives of Co(II) (pH = 4.5) and Zn(II). In the other complexes, the band shifts at higher frequencies, which seems to indicate bonding with the phosphate group. In the derivative of Co(II) obtained in basic medium, a new band appears at 991 cm^{-1}, assignable as due to a splitting of the phosphate group stretching band. New bands appearing in the derivatives of Co(II) and Cu(II) are tentatively assigned as ν(M–N) in the case of Cu(II) and ν(M–O) in the case of Co(II) [19].

The UV–Vis bands (Table IV) show the characteristic maximum of $\pi \rightarrow \pi^*$ of the adenine ring. Fluorescent emission [20, 9] confirms bonding of the metal to the adenine ring, perhaps through N(7) according to the results of infrared spectra.

The enzyme activity of these complexes was tested with rabbit muscle glycogen phosphorylase b as a substitute for 5'AMP, the normal allosteric activator. None of the complexes activate the enzyme, confirming the hypothesis that the phosphate group must be esterified in position 5' in order to produce activation [7, 8].

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