

Some New Complexes with Adenine Nucleotides

A. TERRON and V. MORENO*

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

Received June 24, 1981

Introduction

The study of the interaction of metallic ions with nucleotides is a topic of increasing and many-sided interest for several reasons. Among this can be cited the search for anti-carcinogenic compounds and a clarification of their action [1] and the study of the interaction of the divalent metals in nucleic acids as well as the environmental poisoning caused by heavy metals [2]. The clarification of the action of certain enzymes by substitution of the activator or allosteric inhibitor, generally a magnesium complex with a nucleotide by a more inert complex [3] could be added to the list. The recent use of a vanadium complex in the separation of messenger RNA should increase interest in the topic [4].

Since 1970 a considerable number of papers have been published whose aim has been to isolate 3d

metallic complexes with adenine nucleotides so that they may be studied [5–12].

Experimental

Adenosine-5'-monophosphoric acid disodium salt and adenosine-5'-triphosphoric acid disodium salt (Merck), nitrates of Cr(III) and Mn(II), sulphate of Fe(II) and vanadyl sulphate obtained from vanadium pentoxide and purified by recrystallisation were used as starting products.

5 to 10 ml of 1 mM solutions of the metallic salt were mixed with 5 to 10 ml of 1 mM nucleotide and raised to an appropriate pH to avoid precipitation of the hydroxides. The solutions were heated in a temperature-controlled bath to 40–50 °C for 1–2 hours. With Mn(II) and Fe(II), a nitrogen atmosphere was used to prevent oxidation.

The complexes were precipitated by cooling or by the addition of ethanol. They were filtered, washed with water and ethanol and dried in vacuum over P₂O₅. They are then stable at room temperature in the dessicator.

Carbon, hydrogen and nitrogen contents were determined by elemental analysis at the Institute of Bio-organic Chemistry of Barcelona by a Carlo Erba analyzer. Phosphorous was determined using the phosphomolybdovanadate method. Metals were detected by atomic absorption or spectrophotometric methods and sodium by flame photometry. A Beckman Acta III spectrophotometer and a Perkin Elmer

* Author to whom correspondence should be addressed.

TABLE I. Analytical Data for the Complexes.

Compound	Metal	C	H	N	P	Na	Colour	μ_{eff} (B.M.)	M.P. (°C)																																																																																																												
UO ₂ (5'AMPH)NO ₃ ·3H ₂ O	c ^a	16.39	2.59	11.47	4.32		yellow		160 (d)																																																																																																												
	f	16.27	2.32	10.69	4.32					VO(5'AMP)·3H ₂ O	c	25.74	3.86	15.02	6.64		light blue		190 (d)	f	25.64	3.86	14.18	7.01		Mn(5'AMP)·5H ₂ O	c	24.48	4.49	14.28	6.32		light pink	5.35	190 (d)	f	24.39	3.58	14.99	5.27		Cr(5'AMP)NO ₃ ·4H ₂ O	c	9.80	22.60	3.76	15.81	5.83	green	3.07	220 (d)	f	9.32	22.67	3.59	16.17	5.84	Fe(5'AMP)·3H ₂ O	c	12.27	26.37	3.96	15.38	6.86	grey	4.09	195 (d)	f	12.70	25.96	4.00	14.63	6.47	Fe(5'AMPH)(NO ₃) ₂ ·4H ₂ O	c	9.40	20.10	3.52	16.41	5.22	yellow	5.06	170 (d)	f	9.32	20.95	3.57	15.69	5.38	Cr(5'ATP)Na·6H ₂ O	c	7.78	17.96	3.29	10.47	13.92	green	3.80	170 (d)	f	8.47	18.00	3.81	9.93	13.71	Fe(5'ATPH)·5H ₂ O	c	8.59	18.47	3.54	10.77	14.31	yellow	6.07	150 (d)	f	8.40
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^ac: calcd. f: found.

TABLE II. Infrared Data for the Complexes.

	ν C-N	ν P-O	PO_4^{3-}	ν C-O-P
$\text{UO}_2(5' \text{ AMPH})\text{NO}_3 \cdot 3\text{H}_2\text{O}$	1690 vs	1145 s	980 s	820 m
$\text{VO}(5' \text{ AMP}) \cdot 3\text{H}_2\text{O}$	1700 vs	1110 s	1010 s	820 m
$\text{Mn}(5' \text{ AMP}) \cdot 5\text{H}_2\text{O}$	1690 vs	1130 s	990 s	825 m
$\text{Cr}(5' \text{ AMP})\text{NO}_3 \cdot 4\text{H}_2\text{O}$	1700 vs	1140 s	990 s	825 m
$\text{Fe}(5' \text{ AMP}) \cdot 4\text{H}_2\text{O}$	1700 vs	1130 s	br	815 m
$\text{Fe}(5' \text{ AMPH})(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	1690 vs	1130 s	br	825 m
$\text{Cr}(5' \text{ ATP})\text{Na} \cdot 6\text{H}_2\text{O}$	1695 vs	1075 s	980 s	803 m
$\text{Fe}(5' \text{ ATPH}) \cdot 5\text{H}_2\text{O}$	1695 vs	1095 s	993 s	807 m
$5' \text{ AMPNa}_2$	1650 vs	1100 s	980 s	800 m
$5' \text{ AMPH}_2$	1690 vs	1140 s	970 s	810 m
$5' \text{ ATPNa}_2$	1695 vs	1050 s	965 s	800 m

TABLE III. Spectroscopic Properties of the Complexes.

	UV-visible maxima (nm)		Fluorescent data		
$\text{UO}_2(5' \text{ AMPH})\text{NO}_3 \cdot 3\text{H}_2\text{O}$	265	300 s	ex 300	em 390	pH: 6.3
$\text{VO}(5' \text{ AMP}) \cdot \text{H}_2\text{O}$	285	750	ex 285	em 405	pH: 6.3
$\text{Mn}(5' \text{ AMP}) \cdot 5\text{H}_2\text{O}$	260		no fluorescence		
$\text{Cr}(5' \text{ AMP})\text{NO}_3 \cdot 4\text{H}_2\text{O}$	260	310 (e: 19,02) 610 (e: 15,39)	ex 260 ex 310	em 370 em 410	pH: 3
$\text{Fe}(5' \text{ AMP}) \cdot 4\text{H}_2\text{O}$	260	350 s	ex 260 ex 350	em 325 em 410	pH: 6.3
$\text{Fe}(5' \text{ AMPH})\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	260	350 s 450 s	no fluorescence		
$\text{Cr}(5' \text{ ATP})\text{Na} \cdot 6\text{H}_2\text{O}$	257	300	no fluorescence		
$\text{Fe}(5' \text{ ATPH}) \cdot 5\text{H}_2\text{O}$	260	302	no fluorescence		

703 atomic absorption spectrophotometer were used. Results are shown in Table I. Magnetic susceptibility was measured by Faraday's method at the Institute of Applied Organic Chemistry of Barcelona. Infrared spectra were obtained using a Perkin Elmer 577 spectrophotometer. Dust X-rays diagrams were taken on a Siemens diffractometer. Fluorescence was measured with a double beam Fica MK II spectrophotometer.

Discussion

Since the complexes are only slightly soluble in water (to concentrations of the order of 1×10^{-3} to 1×10^{-4}), molecular weight determinations are not feasible. All complexes display 1:1 stoichiometry, with a microcrystalline structure. $\text{Fe}(5' \text{ AMPH})$

$(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ crystals have been observed but are quite unstable. Dust X-ray diagrams show chromium complexes to have an amorphous structure.

In the infrared spectrum of $5' \text{ AMP}$, the most characteristic bands are those due to the adenine ring in the zone of 1600 – 1700 cm^{-1} , those of phosphoric ester from 1100 to 900 cm^{-1} and those of phosphoric ester from 800 to 810 cm^{-1} [5–7, 13, 14]. The data are tabulated in Table II. The bands due to the adenine ring change their form and are slightly shifted when their spectrum is compared to the $5' \text{ AMP}$ spectrum obtained by precipitation from solutions with the same pH as solutions used for the synthesis of the complexes for $\text{VO}(\text{II})$, $\text{Mn}(\text{II})$ and $\text{Fe}(\text{II})$. No change was observed in the other complexes. In the former cases, the shift of the band from 1650 cm^{-1} seems to indicate union with the adenine ring, possibly by N(7) as certain X-ray

studies indicate [7]. This is in agreement with the fluorescent data which will be discussed later in this paper.

The 1100 and 980 cm^{-1} bands for 5'AMP are shifted towards higher frequencies for all the complexes which seems to indicate coordination with phosphate [13]. The shifting of the phosphoric ester band indicates a strengthening C—O—P bond. As expected, the shift is greater for the hard metallic ions.

5'ATP complexes show only notable variations for some of the phosphate bands, which would indicate the interaction between the metal and the adenine ring. Neither 5'AMP nor 5'ATP showed fluorescence except at very low pHs. Any shift of the band from 260 nm due to the adenine ring or fluorescent emission from by excitation in the area of 260 to 285 nm would indicate metal—adenine interaction. 5'ATP complexes with Cr(III) and Fe(III) show no fluorescence which confirms that they only form bonds with the phosphates. This is in agreement with previous experience [16].

On the other hand, complexes with Fe(II) and VO(II) show fluorescence at nearly neutral pHs which clearly indicates metal adenine interaction. The Fe(III)—5'AMP complex shows no fluorescence over a wide pH range, but Cr(III) with the same ligand does at a pH of 3. At this pH the adenine ring is already fluorescent and any conclusion drawn should be based on different spectra. Uranyl is a unique case. It shows no fluorescence at 260 or 285 nm, yet it does at 300 nm, where it shows a shoulder in its ultraviolet spectrum. This can be interpreted as a joining of the cation with the OH groups in ribose, which is in agreement with Agarwal [17], although

it may be also due to fluorescent phenomena of the uranyl cation itself.

Magnetic susceptibility studies (Table I) are in agreement with an octahedral geometry for the 3d metals with the exception of the Fe(II)—5'AMP complex. The abnormally low value for this complex could be due to a very distorted octahedral symmetry or some other symmetry such as trigonal bipyramid or a distorted tetrahedral symmetry. These magnetic susceptibility studies show results very similar to those for complexes of Fe(II) with 2'AMP and 3'AMP synthesized and currently under study in our department.

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