

# INVESTIGATION OF NUCLEOTIDE-METAL ION SYSTEMS, III. STUDY OF THE ATP—Ni(II) SYSTEM

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The Ni(II) complex of ATP-5' [ATP] with composition  $\text{NiCl}_2 \cdot \text{ATPH}_2 \cdot 11 \text{H}_2\text{O}$  has been prepared. The spectral and electrochemical behaviours of this compound have been studied and conclusions drawn on the site of coordination, supporting that in solution the Ni(II) ion is bound to the  $\beta$ - and  $\gamma$ -phosphate groups and a distorted octahedral  $\text{NiO}_6$  microsymmetry is formed.

## Introduction

In previous papers [1, 2] we surveyed the possible species which can be formed by means of interaction between the transition metal ions and nucleotides. The biological importance of these compounds is a proved fact, but the views relating to the structures of these compounds and to the coordination sites of the metal ions are very different [3—6].

In contrast to the earlier investigations our aim was to isolate the Ni(II)-ATP compound in a crystalline form and to contribute to a better knowledge of this system by physico-chemical study of the product.

## Results and discussion

A 1:1 mixture of concentrated aqueous solutions of  $\text{NiCl}_2 \cdot 6 \text{H}_2\text{O}$  and  $\text{ATP} \cdot \text{Na}_2$  was poured slowly under constant stirring into acetone cooled to about 278 K. The palegreen microcrystalline product was filtered, washed with ethanol and dried over  $\text{P}_2\text{O}_5$  in vacuo. On the basis of the analytical data [%C<sub>calcd.</sub>: 14.42 (14.30); %H<sub>calcd.</sub>: 1.69 (1.63); %N<sub>calcd.</sub>: 8.41 (8.53); %Ni<sub>calcd.</sub>: 7.05 (7.08, titrimetric)], we regard the composition  $\text{NiCl}_2 \cdot \text{ATPH}_2 \cdot 11 \text{H}_2\text{O}$  as probable. By alcoholic precipitation from a neutral mixture (buffered with NaOH to pH=7), BHATTACHARYYA ET AL. [7] isolated a product of formula  $\text{Na}_2\text{NiATP} \cdot 4 \text{H}_2\text{O}$ .

Details of the physico-chemical experimental methods are given in a previous paper [1]. In the pH-metric titration curve of an aqueous mixture of the Ni(II) salt and  $\text{ATP} \cdot \text{Na}_2$ , the second buffer region — characteristic of the ligand — disappears, indicating formation of the 1:1 complex. The stability constants of this compound,  $\lg K_1[\text{NiATPH}] = 2.72 \pm 0.02$  and  $\lg K_2[\text{NiATP}] = 5.02 \pm 0.02$ , are known from the literature [8].

The solid sample is paramagnetic, with  $BM=3.12$  ( $T=298$  K), indicating the presence of two unpaired electrons and of a six-coordinated, strongly distorted octahedral structure.

The molar conductivity ( $\Lambda_M=185 \Omega^{-1} \text{ cm}^{-1}$ ) differs slightly from the value measured for the Co(II)-ATP compound [2]. For a millimolar solution of  $\text{Na}_2\text{NiATP} \cdot 4 \text{ H}_2\text{O}$  the value  $\Lambda_M=218 \Omega^{-1} \text{ cm}^{-1}$  is given by BHATTACHARYYA et al. [7]. The  $\Lambda$  vs.  $\sqrt{c}$  relationship indicates a weak 1:2 type electrolyte, which is consistent with the composition suggested above.

The polarographic behaviour of the Ni(II)—ATP complex is different from that of the Co(II), Cu(II) and Zn(II) complexes. In  $0.1 \text{ mol dm}^{-3}$   $\text{KNO}_3$  supporting electrolyte, the half-wave potential of the Ni(II)  $\rightarrow$  Ni(O) reduction step ( $-1.043$  V vs. N.C.E.) is shifted in the positive direction in the presence of  $\text{ATP} \cdot \text{Na}_2$  ( $-0.950$  V vs. N.C.E.). In both cases the electrode reactions are irreversible. Further investigations relating to this phenomenon are in progress.

The reflectance spectrum of the crystalline product, the absorption spectrum of a solution made from it, and that of the mixture containing the components in the ratio 1:1 are practically identical, and indicate unambiguously the presence of a six-coordinated species.

The three characteristic bands can be assigned to the  ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$  [ $\nu_1$ ],  ${}^3T_{1g}(F)$  [ $\nu_2$ ],  ${}^3T_{1g}(P)$  [ $\nu_3$ ]  $d-d$  transitions [9]. The spectrum does not change in the presence of an excess of  $\text{ATP} \cdot \text{Na}_2$ , indicating that dissociated or hydrated species are not present in considerable amount. The measured and the calculated parameters are listed in Table I.

Table I  
Measured and calculated parameters  
of the complex

	Refl. spectrum	Soln. spectrum
$\nu_1$	8 750 $\text{cm}^{-1}$	8 850 $\text{cm}^{-1}$
$\nu_2$	14 200 $\text{cm}^{-1}$	14 500 $\text{cm}^{-1}$
$\nu_3$	23 400 $\text{cm}^{-1}$	25 600 $\text{cm}^{-1}$
Dq	875 $\text{cm}^{-1}$	885 $\text{cm}^{-1}$
B	757 $\text{cm}^{-1}$	903 $\text{cm}^{-1}$
$\beta$	0,70	0,83
LFSE	10 500 $\text{cm}^{-1}$	10 620 $\text{cm}^{-1}$

of the  $[\text{Ni}(\text{H}_2\text{O})_6]^{2+}$  ion, proving that the ligand field strengths of ATP and of  $\text{H}_2\text{O}$  are approximately the same.

The solution spectrum is only slightly influenced by the pH. In the range  $\text{pH}=2-10$  the positions of the bands remain almost unchanged. With increasing pH, there are almost no changes in the intensities of the  $\nu_1$  and  $\nu_2$  bands, whereas the  $\nu_3$  band changes to a greater extent (in  $4 \cdot 10^{-2} \text{ mol dm}^{-3}$  solution  $E=0.252-0.340$ ).

By means of the magnetic moment and the 10 Dq value a spin — orbital coupling constant of  $\lambda=-233 \text{ cm}^{-1}$  can be calculated for the crystalline substance [e.g. 11]. Considering the value  $\lambda_{\text{ion}}=-315 \text{ cm}^{-1}$  and the relationship  $\alpha^2=\lambda/\lambda_{\text{ion}}$ , we obtain  $\alpha=0.84$ , which is identical with the  $\beta$  value calculated only from the spectral data.

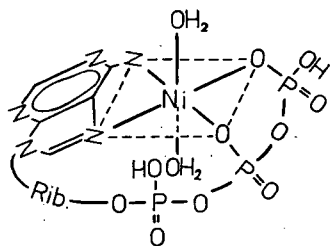
The spectrum and the calculated parameters suggest that the binding sites of the Ni(II) ion are in the phosphate chain. Even if there is a weak interaction with

the  $N_7$  atoms of the purine ring in the crystalline state, it ceases to exist in solution. If Ni—N bonds were present, a more covalent bond character (*i.e.* a larger  $Dq$  and a smaller  $\beta$  value) and band splitting (low symmetry) would be expected.

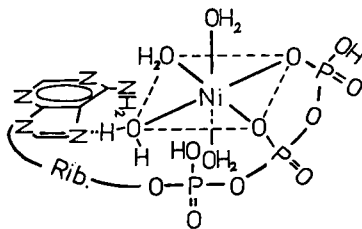
The infrared spectrum of the Ni(II) complex differs slightly from that of free ATP·Na<sub>2</sub>. The main frequencies are: 3380 s ( $\nu_{NH} + \nu_{OH}$ ), 1711 s (?), 1664 m ( $\nu_{C=N}$ ), 1628 w, 1528 w, 1247 s ( $\nu_{P=O}$ ),  $\sim 1097$  s, 1012 s (P—O + C—O), 924 m (P—O—P), 834 w, 640 w,  $\sim 535$  m. Unfortunately the  $\nu_{OH}$  and  $\nu_{NH}$  bands totally overlap, so that the possible  $C_6-NH_2-Ni(II)$  interaction can not be observed.

On the above basis, we have taken the formation of a low-stability high-spin 1:1 complex into consideration. We assume that the binding sites of the Ni(II) ion are primarily on the  $\beta$ - and  $\gamma$ -phosphate groups. It is unlikely that the metal ion forms a direct bond with the  $N_7$  atom of the adenine part or with the  $C_6-NH_2$  group, though from a steric point of view this possibility can not be excluded. On the basis of <sup>1</sup>H-NMR investigations some authors [12] consider possible the formation of an outer sphere complex [Ni—HOH<sup>+</sup>N<sub>7</sub>].

In this case, with the linkage of a further three H<sub>2</sub>O molecules the Ni(II) ion becomes six-coordinated, having a NiO<sub>6</sub> microsymmetry. This is the conformation which is mostly in accordance with the experimental data. Therefore, as regards the possible structures I and II, we prefer the latter.



I.



II.

Japanese authors [13] suggest that the binding of metal ion on the phosphate chain is the likeliest, based on LCAO calculations. This is supported by the facts that for a given metal ion the stability constants increase in the sequence AMP < ADP < ATP, and for ATP, CTP and ITP these constants remain the same within experimental error, indicating the unimportant role of the ring. It is worth mentioning that the calculated stability constants values are of the same order as is characteristic of M—O systems, but are considerably lower than the values calculated for M—N, O systems [14, 15].

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ИССЛЕДОВАНИЕ СИСТЕМ НУКЛЕОТИДЫ-ИОНЫ МЕТАЛЛОВ, III  
 ИЗУЧЕНИЕ СИСТЕМЫ АТФ—Ni(II)

Й. Чапар и Л. Кисс

Синтезирован комплекс АТФ—5' (АТФ) с Ni(II) состава  $\text{NiCl}_2 \cdot \text{ATP} \cdot 11 \text{H}_2\text{O}$ . Изучены спектроскопические и электрохимические свойства соединений и сделаны заключения относительно строения координационной структуры, основываясь на том, что Ni(II) ионы в растворах связаны с  $\beta$ - и  $\gamma$ -фосфатными группами и образуются деформированные октаэдрические микросимметрии группы  $\text{NiO}_6$ .