ESR and Optical Studies on the Interaction between cis-Dichlorodiammine Platinum(II) and Tryptophan

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cis-Dichlorodiammine platinum(II), which is known to be an active anticancer compound, and tryptophan form paramagnetic complexes in aqueous solution. These can be detected by means of electron spin resonance spectroscopy (ESR) after a reaction time of several hours. The ESR spectra are interpreted to be due to Pt(III) in a low spin d^7 configuration. The optical results obtained are in accordance with the assumption of columnar structures of the paramagnetic complexes.

Introduction

There is great current interest in complexes formed between platinum ions and nucleic acids because of the anticancer activity of dihalodiammine platinum compounds discovered by Rosenberg [1]. It has been postulated that this activity is due to complex formation between the cis-dichlorodiammine platinum(II) compound and the purine and pyrimidine bases of DNA [2, 3]. Unfortunately, this platinum compound has a high toxicity, which limits its application [4]. For this reason, other platinum compounds have been investigated, the most promising of them seem to be the “platinum pyrimidine blues” [5]. These blue platinum complexes are, at least to a great extent, paramagnetic substances [6].

Another molecular site of action of the platinum complexes are amino acids and proteins [7, 8]. Several studies involving enzyme inhibition by platinum complexes have been reported [9]. The presence of amino acids or proteins result in a decrease of the association of cis-dichlorodiammine platinum(II) with DNA [10].

In this work it will be shown that paramagnetic species – similar to those attributed to “platinum pyrimidine blues” – are also obtained during the interaction of cis-dichlorodiammine platinum(II) with the amino acid tryptophan.

Experimental

cis-Dichlorodiammine platinum(II), cis-Pt(NH$_3$)$_2$(H$_2$O)$_2$Cl$_2$, and tryptophan were obtained from Serva.

Results

About 10 h after adding 0.1 M of tryptophan to a 0.1 M cis-diaquodiammine platinum(II) solution an ESR signal could be detected in the dark brown precipitate. The supernatant had pH 3. The ESR spectrum of this precipitate, after a reaction time of 5 days is shown Fig. 1 A. It is a typical spectrum of an axially symmetrical complex.

The g-values are $g_\parallel = 1.897 \pm 0.005$ and $g_\perp = 1.998 \pm 0.005$. The precipitate is nearly insoluble at pH 3. It can be dissolved, however, at pH 10. The optical spectrum of the alkaline solution is shown in Fig. 2B. It is quite different from that obtained with the filtrate of the original sample (Fig. 2A). As can be seen, in the solution at pH 3 a broad absorption band (Fig. 2Ab), and in the solution at pH 10 a shoulder is present at 280 nm, which is due to the π-system of tryptophan. A vibrational structure, however, which is typical for the tryptophan spectrum, is no longer observable. The spectrum in Fig. 2Aa still shows the typical
Fig. 1A. ESR spectrum of the precipitate of 0.1 M aqueous solution of \([\text{cis-Pt(NH}_3\text{)}_2(\text{H}_2\text{O})_2]^{2+}\) containing 0.1 M tryptophan, pH 3, after a reaction time of 5 days at 340 K, and measured at 77 K.

Fig. 1B. ESR spectrum of a powder prepared by dissolving the precipitate of the sample of Fig. 1A in aqueous solution, pH 10 (pH adjusted by KOH), followed by an evaporation at 340 K, and measured at 77 K.

Fig. 2A. Absorption spectra of aqueous solutions (pH 3, optical path 1 mm) of:
- a) filtrate of tryptophan (10\(^{-1}\) M) and \([\text{cis-Pt(NH}_3\text{)}_2(\text{H}_2\text{O})_2]^{2+}\) (10\(^{-1}\) M) after a reaction time of six days at 340 K, tenfold diluted,
- b) difference spectrum of a) and tryptophan (2.5 \times 10\(^{-4}\) M),
- c) tryptophan (2.5 \times 10\(^{-4}\) M).

Fig. 2B. Absorption spectrum of a saturated aqueous solution of the Pt/tryptophan-complex (preparation see text), pH 10, optical path 1 mm.

tryptophan fine structure due to free or complexed tryptophan. In the latter case, however, the atoms of the \(\pi\)-system should not be involved in the bonding.

It was impossible to detect any ESR spectrum of the alkaline solution. The powder obtained after evaporation of this solution at 340 K, however, is also paramagnetic. It exhibits an ESR spectrum of an axially symmetrical complex with \(g_{||} = 1.905 \pm 0.005\) and \(g_{\perp} = 2.006 \pm 0.005\) (Fig. 1B).

Qualitatively, the same optical and ESR results are obtained if the samples are prepared with aqueous solutions of \(\text{cis-Pt(NH}_3\text{)}_2\text{Cl}_2\). In comparison to the aquo compound a longer reaction time is, however, required for the formation of the paramagnetic complex.

**Discussion**

Four-coordinate square planar complexes of Pt(II) exhibit low spin \(d^8\) configurations. The predominant feature of the interaction of Pt(II)(NH\(_3\))\(_2\)Cl\(_2\) and tryptophan, however, is the appearance of paramagnetism.

There are only a few investigations on paramagnetic platinum ions [12–18]. Part of the experiments were done on platinum in garnet [14] or in different other host lattices [12]. It was also observed in Magnus’ green salt [15, 16], in gamma- or electron-irradiated K\(_2\)PtCl\(_4\) crystals [13], in \(\text{cis-Pt(SCN)}_2(\text{NH}_3)_2\) oxidized with iodine [17], in dithiolene complexes of platinum [18], and in some “platinum pyrimidine blue” compounds [6]. In all
of these investigations the existence of Pt(III), Pt(V), or holes extended over several Pt sites have been postulated to be responsible for the paramagnetism observed.

It is known that under certain conditions square planar \( d^8 \) complexes often crystallize in columnar stacks with metal-metal interactions between adjacent metal ions [19-21]. Partial oxidation should stabilize such a chain resulting in paramagnetism detectable by means of ESR spectroscopy if suitable sensitivity and relaxation conditions are fulfilled. The platinum blue compounds have mixed oxidation numbers [22] also, and therefore should be detectable by means of ESR [23]. The “platinum pyrimidine blues” are reported to have mixed valence states, too [6, 24, 25]. It is reasonable to assume columnar structures in all of these cases.

In the case of the Pt-tryptophan complexes investigated the ESR spectrum might be due to either Pt(I) or Pt(III). Therefore, \( d^8 \) or \( d^7 \) low spin states must be responsible for this paramagnetism.

The ESR-spectra as shown in Fig. 1 are typical for axially symmetrical complexes with \( g_{\perp} > g_{||} \geq 2 \). The spectra can be interpreted by the following spin Hamiltonian:

\[
\begin{align*}
H &= \beta g_{||} S_z H_z + \beta g_\perp (S_x H_x + S_y H_y) + \\
&= A_{||} S_z I_z + A_\perp (S_x I_x + S_y I_y)
\end{align*}
\]

with spin \( S = 1/2 \). This is consistent with the unpaired spin being in the \( d^{2} \)- or in the \( d_{xy} \)-orbital, if the existence of square planar complexes is assumed. If the unpaired electron is in the \( d_{z^2} \)-orbital perturbation calculations to the first order result in [13, 26]:

\[
\begin{align*}
g_{||} &= 2 + \frac{6 \xi}{E (d_{x^2-y^2}) - E (d_z^2)} \\
g_\perp &= 2 + \frac{8 \xi}{E (d_{x^2-y^2}) - E (d_{xy})}
\end{align*}
\]

In the case of a \( d_{xy} \)-ground state, the \( g \)-values are:

\[
\begin{align*}
g_{||} &= 2 + \frac{8 \xi}{E (d_z^2) - E (d_{xy})} \\
g_\perp &= 2 + \frac{2 \xi}{E (d_{x^2-y^2}) - E (d_{xy})}
\end{align*}
\]

\( \xi \) is the parameter of the spin orbit coupling; \( E (d_{x^2-y^2}) \), \( E (d_z^2) \), \( E (d_{x^2-y^2}) \), and \( E (d_{xy}) \) are the energy levels of the respective \( d \)-orbitals. The \( g \)-values measured are not consistent with a \( d^8 \) configuration.

It is very improbable to have a \( d^8 \) configuration. In such a system, a hole in either the \( d_{z^2} \)- or the \( d_{xy} \)-orbital indicate that these orbitals are positioned higher in energy than the \( d_{z^2} - d_{xy} \)-orbital. This, however, cannot be assumed in the case of nearly planar complexes. Therefore, it is suggested that the paramagnetic species is Pt(III) with its \( d^7 \) configuration. The \( d^7 \)-state of Pt seems to be stable only in di- or polymeric structures [6, 13, 15, 16, 19]. Such a structure can be formed either by an interaction in the \( z \)-direction of the complex or by bridging through the ligands in the plane of the single complex. From the experiments presented a decision cannot be made. Due to the bulky ligands used the interaction within the plane seems to be improbable.

The hyperfine splitting observed can be attributed to the interaction of the unpaired spin with a \(^{195}\)Pt nucleus (I = 1/2 and a natural abundance of 33.7 per cent). Unfortunately, the hf-splitting cannot be determined very precisely. A value of \( A_\perp \approx 160 \) G has been obtained for the doublet (s. Fig. 1B). In the spectrum of the precipitate of the acidic solution (Fig. 1A) one additional hf line can be seen, others can be assumed to be present but cannot be noticed because of the broadness of the lines. Therefore, no explanation can be offered for this hf splitting as yet. A second complex might exist with different metal-metal interactions [21, 27] or these lines might belong to a triplet being due to the interaction between two or more \(^{195}\)Pt nuclei.

The optical spectrum of the filtrate (Fig. 2A) contains of course no information on the precipitated complexes. Characteristic is the broad absorption band centered at about 280 nm. It doesn’t show the typical vibrational structure of indole. The spectrum of the complexes dissolved in alkaline solution (Fig. 2B) does not exhibit the indole structure either. There is a shoulder at about 320 nm with a relatively high extinction. In accordance with optical absorption spectra of columnar structures [28] it might be suggested, that this absorption is due to charge-transfer transitions between adjacent platinum ions.

It is impossible to decide if these spectra are identical with those obtained with paramagnetic complexes in the solid state. The concentrations of the saturated solutions are not high enough in order to decide, if the paramagnetism is also present in the solution or if it is a solid state effect only.
The possible coordination sites of transition metal ions are the amino and the carboxyl group of tryptophan \[7, 8, 29\]. The nitrogen of the indole residue might also be involved, although this position is usually not bound to a metal ion \[8, 30\]. From the optical spectra obtained it might be concluded that an atom is involved in the complexation which contributes electrons to the \(\pi\)-system of the indole ring. This is supported by the change in the indole absorption band. The \(\pi\)-system seems to be involved in a stacking of the planes of the single platinum complexes.

This suggestion is supported further by the observation that alanine, which does not possess the indole ring, does not produce paramagnetic species during an investigation time of several weeks.

In summary, one can say that tryptophan and Pt can form paramagnetic complexes; probably, more than one paramagnetic species are formed. The paramagnetism seems to be due to Pt(III) with a hole in a \(d_{x^2-y^2}\) or \(d_{y^2}\)-orbital. The formation of dimer or polynuclear Pt-complexes seems to be necessary in order to observe stable paramagnetic Pt(III). The change in the absorption of the \(\pi\)-system of the indole ring might be caused by its involvement in the stacking between the platinum complexes.