ESR Studies on Platinum Uracil Blue

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ESR spectra of platinum uracil "blue" are discussed. Two main types of paramagnetic species have been found, one of which can be explained by assuming an unpaired electron spin delocalized over d_{z^2}-orbitals of two equivalent Pt centers, the other one is attributed to a more localized d^7 Pt(III) center. In both cases the hyperfine structure suggests an additional smaller probability density of the unpaired electron at one adjacent platinum center. The smallest possible oligomers to realize this facts are tetramers in the first case and dimers in the latter one.

Introduction

The platinum pyrimidine blues (PPB) have been the subject of considerable research effort eversince their antitumor potency was discovered [1, 2]. The PPB solutions which are derived from cis-dichlorodiammineplatinum(II) (PDD) or cis-diaquodiammineplatinum(II) (PDDa) consist of several species depending on the reaction time; an equilibrium seems to be reached after several days or weeks, depending on the procedure of the preparation. Attempts have been made to separate and crystallize the components, which are responsible for the blue color. Up to now, only a PDDa-α-pyridone blue complex has been studied by X-ray diffraction since it is available in the crystalline form [3]. However, the detailed structure as well as the mode of action of the compounds of the PPB solution are not yet known. Electron spin resonance (ESR) spectroscopy can be applied for investigating the structure of PPB complexes since many of them are paramagnetic. ESR spectra of several "blues" have been described [4 – 12] which support their mixed valence character as well as the assumption of an axial stacking of several Pt units in solution. It is generally assumed that their unpaired spin is located in an orbital with mainly 5 d_{z^2}-character. Concerning the nature of the electronic states, a delocalization of the unpaired spin over several Pt units in the stack has been suggested [4 – 6].

The investigation of the paramagnetism of the platinum uracil blue solution might be of a special interest, because animal test results indicate a dependence of the antitumor activity on the existence of the paramagnetic components [4].

In this paper it will be shown that different paramagnetic species can be observed in platinum blue solutions. Either a delocalization of the free spin over two equivalent Pt centers and two adjacent ones in a different surrounding, or an electron more localized at one Pt ion including a smaller interaction with a neighbouring one can be assumed for interpreting the ESR spectra, taking as example the PDD-6-methyluracil system.

Consequently tetrameric and dimeric species, resp., are the smallest possible paramagnetic oligomers in the platinum uracil blues solutions.

Experimental Section

The preparation of the platinum blue solutions has been performed according to procedures reported in the literature [1, 2, 4]. Solutions of *cis*-$Pt(NH_3)_2(H_2O)_2^{2+}$ (PDDa) were obtained by dissolving *cis*-$Pt(NH_3)_2Cl_2$ (PDD) in bidestilled water and subsequent addition of $AgNO_3$ (PDD: $AgNO_3$ = 1:2). The mixture was stored in the dark in a stoppered flask for about 12 – 15 h under constant stirring. AgCl was, then, filtered off. A pale yellow solution with pH 1.8 was retained. Various ligands (uracil, 1-methyluracil, 5-methyluracil (thymine), 6-methyluracil, 1.5-dimethyluracil) were added to appropriate volumes of this solution in order to obtain an equimolar ratio of ligand to platinum. All substances were of reagent grade quality. The samples were stored in the dark at 60 °C.

Oxidation and reduction of the samples were performed by adding solutions of H_2O_2 or of ascorbic

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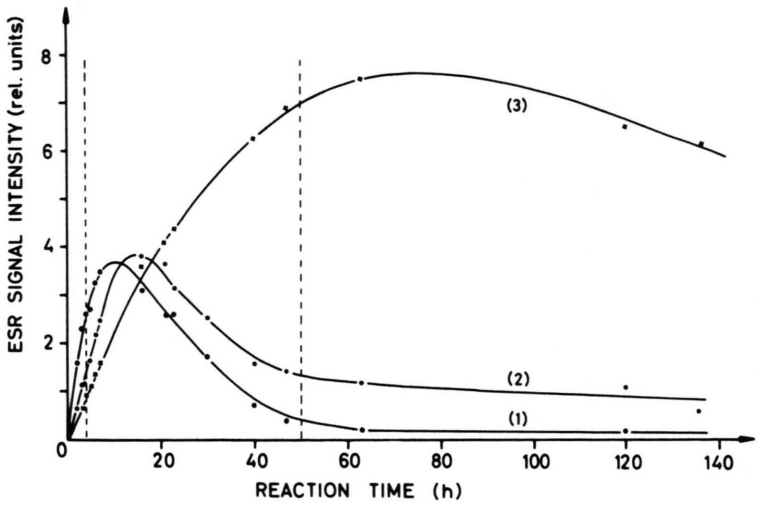

Fig. 1. ESR signal intensity of a frozen aqueous solution (77 K) of PDDa-6-methyluracil *vs.* reaction time (2) and after addition of 20 µl of either H_2O_2 (0.3%) (1) or 0.5 M of ascorbic acid (3) to 200 µl of the standard solution (2).

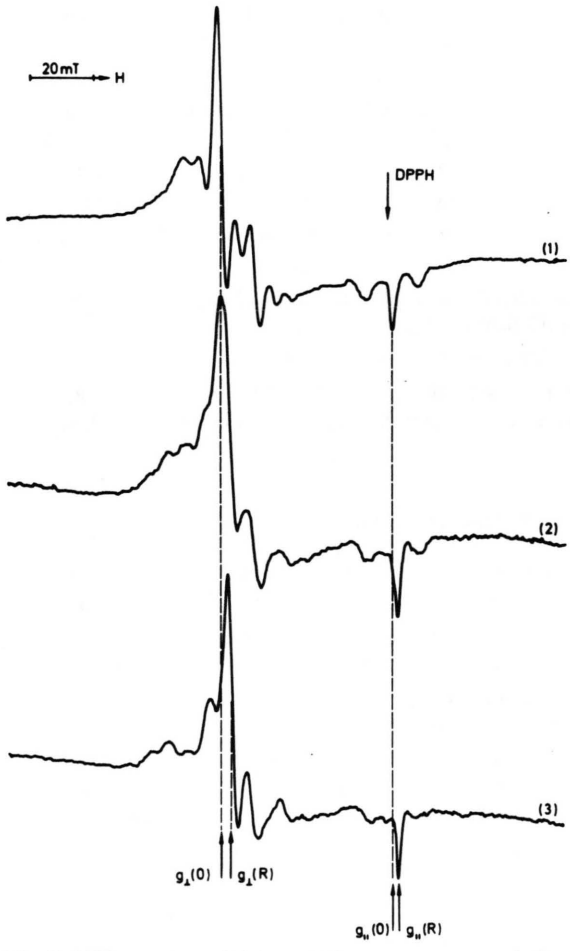

Fig. 2. ESR spectra of 0.1 M of frozen aqueous solutions (77 K) PDDa-6-methyluracil after a reaction time of 4 h at 60 °C (2). Spectra obtained after addition of 20 µl of either perhydrole (1) (30% in water, "type O" spectrum; $g_{\perp}(O)=2.394$, $g_{\parallel}(O)=1.991$)) or 0.1 M of ascorbic acid (3) ("type R" spectrum; g (R) = 2.375, $g_{\parallel}$ (R) = 1.983) to 300 µl of the standard solution (2).

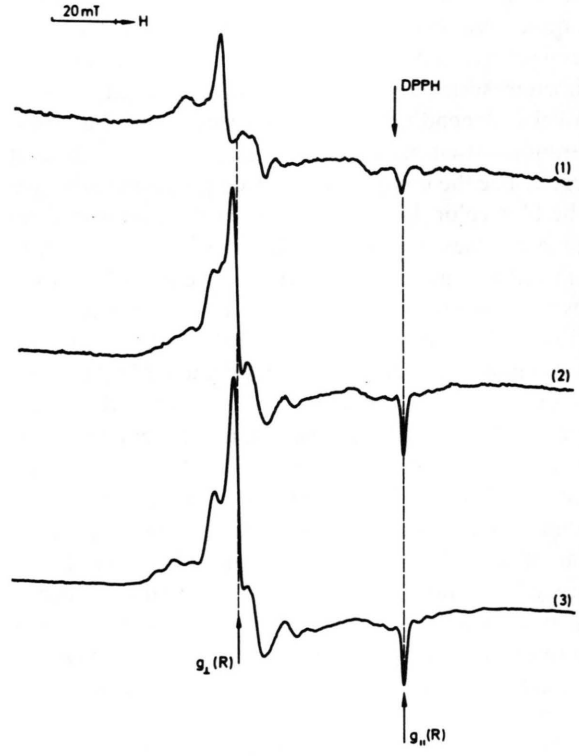

Fig. 3. ESR spectra of 0.1 M of frozen aqueous solutions (77 K) of PDDa-6-methyluracil after a reaction time of 50 h at 60 °C (2). Spectra obtained after addition of 20 µl of either perhydrole (30% in water) (1) or 0.1 M of ascorbic acid (3) to 300 µl of the standard solution (2); $g_{\perp}$ (R) = 2.375, $g_{\parallel}$ (R) = 1.983.

acid, respectively. The electron spin resonance (ESR) measurements were taken on a conventional X-band spectrometer, at a microwave power of 1 mW and modulation amplitude of 0.5 mT.

The relative ESR signal intensities have been calculated by determining the moment of the ESR spectra by a planimeter.

PDDa-6-methyluracil solutions turned out to contain the highest concentration of paramagnetic species under the experimental conditions used. The experimental results for these samples are, therefore, reported in the figures of the following section.

Results

Depending on the reaction time between PDDa and 6-methyluracil in aqueous solution, types as well as yield of the paramagnetic species in the colored solution vary. Under the experimental conditions used, the yield reaches a maximum value at about 15 h (Fig. 1(2)). Addition of a redox agent drastically changes the yield of the paramagnetic species.

In the case of oxidation (Fig. 1(1)) it is enlarged at short reaction times and diminished at longer reaction times. In the case of reduction (Fig. 1(3)) the opposite effect occurs.

Furthermore, the type of the paramagnetic species has been changed, too. The spectra obtained after two significantly different reaction times of 4 h and 50 h are shown in Fig. 2 and Fig. 3. The transition between the different ESR signals can be monitored by a titration curve. Repeated oxidation and reduction of the solution can be performed without any significant change in either the signal pattern or in intensity of the corresponding ESR spectra.

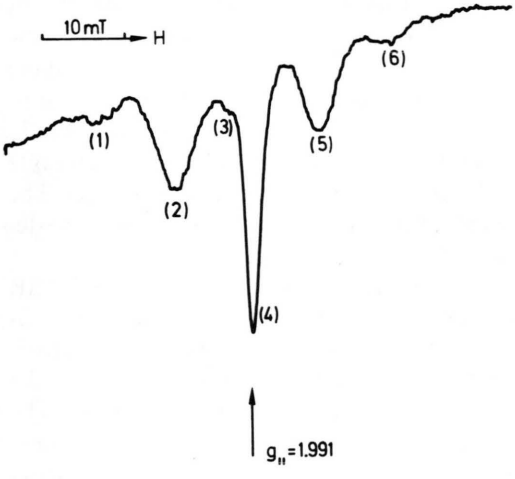

Fig. 4. $g_{\parallel}$ -line pattern of spectrum (1) of figure 2 ("type O") registered with enlarged amplification (×3.2) on an extended magnetic field range.

Of special interest is the spectrum obtained after short reaction times (several hours) and addition of oxidizing agents to the solution: "type O" spectrum. It is very similar to that one obtained with solutions containing platinum-α-pyridone blue complexes, the compound which has already been characterized structurally. This spectrum allows a relatively detailed interpretation.

With this type of ESR spectrum present, the solution exhibits a strong absorption band at 730 nm, which is mainly responsible for the blue-green color of the solution. This absorption band disappears completely after addition of a reducing agent, leading to the "type R" ESR-spectrum. In this case, the lowest energy absorption band is positioned at about 650 nm [13].

The same experiments have been performed with other PDDa-pyrimidine systems. If uracil, 1-methyluracil, 5-methyluracil (thymine), or 1,5-dimethyluracil are used as ligands, the same "type O" ESR spectra are obtained as in the case of 6-methyluracil. Differences are found, however, for their "type R" spectra. Therefore, the type of ligands seems to influence the ESR pattern of the "type R" spectra, whereas the "type O" ESR spectra seem to be influenced to a minor extend only.

Discussion

Only a few ESR spectra of blue platinum complexes have been presented in the literature. Paramagnetic complexes have been obtained with the ligand molecules uracil, uridine, α-valerolactam [4], α-pyridone [5, 6], thymine, cytidine [7], 6-methyluracil [8, 9], and 5-uridinemonophosphate [10]. The only detailed interpretation has been given for platinum-α-pyridone blue solutions and single crystals [5, 6]. In order to explain the frozen solution spectra it has been assumed that the unpaired electron is delocalized over four platinum centers, arranged in a linear chain.

In general, the formation of one-dimensional structures, starting from a d^8-system like Pt(II), is strongly influenced by electron donors or acceptors,

since the one-dimensional structures are partially oxidized [14]. This applies also for platinum blue complexes [4, 5]. It is, therefore, not surprising that the presence of redox systems in platinum blue solutions, which are known to consist of several different components, changes its composition drastically, in particular the yield of paramagnetic Pt(III) is affected.

Such a behavior of the solution, as characterized in Fig. 1, might be explained as follows: The formation of the blue solution, starting from Pt(II) complexes, occurs concomittantly with an increase in the oxidation number of the solution. After several days it will be greater than 3. At the same time, mixed valence complexes are also formed, as can be concluded from the optical absorption spectra of the solution [13].

The presence of a redox agent causes, therefore, an oxidation or reduction of certain components of the platinum blue solution depending on the value of the redox potential of the component and the redox agent as well. It is suggested, therefore, that the paramagnetic species responsible for the ESR spectrum and which appears on oxidation is formed from a Pt(II) containing oligomer. On the other side, Pt(II) – Pt(IV) mixed valence oligomers might be the reducable precursor of the "type R" species, which is the dominant one after long reaction times.

The ESR spectra (Fig. 2, 3) imply axial symmetry for the paramagnetic species as reasonable approximation. The observation that $g_\perp > 2 > g_\parallel$ is consistent with the assumption that the unpaired spin of the d^7 Pt(III) occupies a d_{z^2}-orbital. The observed relation between the g-values is in accordance with ligand field calculations [15, 16]

$$g_\parallel = 2.002 - 3\,(\xi_{\mathrm{eff}}/\Delta E_{\mathrm{xz,yz} \to z^2})^2$$

$$g_\perp = 2.002 + 6\,(\xi_{\mathrm{eff}}/\Delta E_{\mathrm{xz,yz} \to z^2}) - 6\,(\xi_{\mathrm{eff}}/\Delta E_{\mathrm{xz,yz} \to z^2})^2.$$

The effect of covalency appears indirectly in altered values of the spin-orbit interaction parameter ξ_{eff}.

The characteristic feature of the "type O" spectrum (Fig. 2(1)) can be seen in the $g_\parallel$-region, which consists of six lines (Fig. 4).

Considering second order hyperfine (hf)-splitting [15, 17] ($I(^{195}Pt) = 1/2$, natural abundance 33.7%), the line distribution shown results from a structure consisting of two equivalent Pt centers stacked in z-direction. The relative line intensities should be 1 : 7.9 : 1 : 16.5 : 7.9 : 1. If line (3) of Fig. 4 is disregarded, a five line pattern results. Such a line distribution can be explained by assuming first order hf interaction of the unpaired spin with two equivalent Pt centers. To account for the $g_\perp$-line pattern of the "type O" spectrum, the ligand hf-type interaction with one additional Pt nuclear spin has to be invoked resulting in an additional 1 : 4 : 1 splitting of each line. This hf interaction is not resolved in the $g_\parallel$-part of the spectrum. In this case, it results only in a broadening of the lines 1, 2 and 5, 6 and with the central line remaining narrow (s. Fig. 4). Taking into account these considerations it seems reasonable to suggest the following Hamiltonian

$$H = \beta\, \boldsymbol{H} \cdot \boldsymbol{g} \cdot \boldsymbol{S} + \boldsymbol{S} \cdot \boldsymbol{A} \cdot (\boldsymbol{I}^{(1)} + \boldsymbol{I}^{(2)}) + \boldsymbol{S} \cdot \boldsymbol{B} \cdot \boldsymbol{I}^{(3)}$$

for a trimeric species, in which β is the Bohr magneton, $\boldsymbol{g}$ the g-tensor, and $\boldsymbol{A}$ and $\boldsymbol{B}$ the hyperfine tensors concerning the interaction of the electron spin with the two equivalent Pt centers and the interaction with an additional Pt center in a trimeric species, respectively. The expression is practically also valid for a tetrameric species due to the very small probability of finding a second ^{195}Pt adjacent to the two equivalent Pt ions. From the experiments, $A_\perp = 22\ (\pm 1)$ mT, $A_\parallel = 17\ (\pm 1)$ mT, $B_\perp = 11\ (\pm 1)$ mT, $B_\parallel$ not resolved, are obtained.

On the other side, at least a tetrameric species must be required because of the equivalence of the two Pt centers and the observable hf splitting of an additional Pt atom located axially in regard to one of the two other Pt atoms. Equivalence of the two Pt centers, however, can exist only, if number and type of ligands are the same for both of them, *e.g.* there are two other platinum ions bound to the two equivalent Pt centers, bound axially to the binuclear unit. A tetrameric species is the smallest conceivable structure for the "type O" paramagnetic species. The actual length of this species in solution cannot be determined by these experiments.

The basic characteristics of the "type R" ESR spectra (Figs 2 and 3, spectra 3) obtained after addition of a reducing agent, are significantly different from the "type O" spectrum. Again, it exhibits the pattern of an axially symmetrical species. The $g_\perp$-part of the spectrum, however, consists of three triples of lines, in which the central triple has about 8 – 10 times the intensities of the outer triples. The low field one is relatively best resolved.

The hf coupling of the electronic spin to one Pt nuclear spin results in a triple of lines of relative intensities of about 1 : 4 : 1. The interaction with the second Pt center results in a subsequent triplet. This scheme suggest a Hamiltonian of the form

$$H = \beta \boldsymbol{H} \cdot \boldsymbol{g} \cdot \boldsymbol{S} + \boldsymbol{S} \cdot \boldsymbol{A} \cdot \boldsymbol{I}^{(1)} + \boldsymbol{S} \cdot \boldsymbol{B} \cdot \boldsymbol{I}^{(2)}.$$

According to this explanation, the central triple should, however, exhibit only half of the observed intensity. It might, therefore, be suggested that the "type R" spectrum consists of two spectra with nearly identical g-values; only one of them exhibits the nine line pattern in its $g_\perp$-part ($A_\perp = 41(\pm 1)$mT, $B_\perp = 12(\pm 1)$mT). Such a spectrum requires a binuclear platinum complex as the smallest possible unit.

The spectrum obtained by addition of an oxidizing agent to the platinum blue solution after a long reaction time (50 h) can be explained similarly (Fig. 3(1)).

Among the different paramagnetic complexes which can be detected in the platinum uracil blue solutions there is only one with an electronic configuration of an unpaired electron spin delocalized over two equivalent platinum centers ($A_\perp = 22$ mT). The other ESR spectra, not resolved too well, can be explained by a more localized Pt(III) center ($A_\perp = 41$ mT). In both cases additionally a diminished probability density of the free electron spin at an adjacent axially located Pt center is apparent, as is indicated by the hf structure of the ESR spectra ($B_\perp = 11$ mT (12 mT)). The ESR experiments give the lower limit of the number of platinum centers in the paramagnetic components present in the solution and some indications on their electronic configuration. Other methods such as optical absorption studies are necessary in order to get additional information on these components of the platinum uracil blue solution.

Acknowledgements

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