Aggregation of platinum–uridine blue as a function of concentration in aqueous solution by small angle X-ray scattering

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Abstract

Aqueous solutions of amorphous platinum–uridine blue were studied by the small angle X-ray scattering method in the concentration range 1.4–140 mg/ml. The results showed a dependence on concentration, which was interpreted with the aid of computer simulations as arising from aggregation of dinuclear Pt units. The radii of gyration of the aggregates varied between 6 and 15 Å.

Key words: X-ray scattering; Platinum blue complexes; Uridine complexes

1. Introduction

'Platinum blues' [1] is a generic name for highly colored platinum coordination complexes synthesized from Pt(II) reagents like $Pt(NH_3)_2Cl_2$, $Pt(NH_3)_2(H_2O)_2SO_4$ or $Pt(NH_3)_2(NO_2)_2$ and [–N–C=O] or [O=C–N–C=O] ligands [2–5]. The syntheses have given crystalline polynuclear products mainly with simple amide ligands. With polyfunctional ligands like pyrimidine nucleosides amorphous products [2–4] are obtained, which show remarkable antitumoral activity [6–8]. The shortest Pt–Pt distance of 3 Å of amorphous Pt–uridine blue [1, 9, 10] was determined for the first time by means of the extended X-ray absorption fine structure method [11]. We have studied the structures of amorphous platinum pyrimidine nucleoside complexes both in the solid state and in aqueous solution by X-ray scattering and EXAFS methods [12–16]. Results of our wide angle X-ray scattering (WAXS) [13, 15] and EXAFS [16] studies indicated that Pt–uridine blue has a tetranuclear basic Pt structure in the solid state. Our synthesis of Pt–uridine blue differs from that used by Teo *et al.* [11] in respect of the counter-ion and the work-up. Since Pt–uridine blue is prepared in acidic solution, $(H_3N)_2OPt(II)$ behaves as a bifunctional electrophile toward uridine, and N- and O-coordination to give *cis*-double bridged Pt_2 units regioselectively is possible. These Pt_2 units may then undergo association and oxidation into hydrogen bonded and partially Pt–Pt bonded mixed-valence tetranuclear complexes.

In this small angle X-ray scattering (SAXS) study the average particle sizes in aqueous solutions of Pt–uridine blue were determined in the concentration range 1.4–140.0 mg/ml. It has been proposed that there are more than one oligomeric species in aqueous solutions of Pt blues [17, 18]. For instance, studies on crystalline tetranuclear Pt–α-pyridone blue indicated that it maintains its mixed-valent oligomeric structure upon initial dissolution in water, but disproportionates and decomposes gradually (4 h) into diamagnetic dinuclear Pt units [17]. Our aim is to indentify the Pt units that dominate the scattering from the SAXS data with the aid of computer simulations.

2. Experimental

2.1. Samples

Microanalysis was done by Dornis und Kolbe, Mikroanalytisches Laboratorium, Germany. Vis spectra were taken by means of a Shimadzu UV-200 double beam spectrometer and IR spectra on KBr pellets pressed at 9 tons by a Biorad FTS-7 FTIR spectrometer. All the reagents were used as obtained from commercial sources. The gel was from Merck, Fractogel TSK HW-

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50(S) and the elution was carried out with 0.012 M H_2SO_4. The diaquadiammine platinum(II) sulfate, $[Pt(NH_3)_2(H_2O)_2]SO_4$ was prepared and used as a 0.1 M aqueous solution via K_2PtCl_4 (Aldrich) and K_2PtI_4 in a reaction with Ag_2SO_4 (AnalaR). Pt blue was synthesized by a modification of the original method [10]. We used the acidic aqua complex (pH = 2–3) instead of the neutral, a higher reaction temperature, and the reaction mixture was not light-protected. The product was stored at room temperature in a brown desiccator over P_2O_5, and measurements were made within a month.

Synthesis of Pt–uridine blue

A mixture of $[Pt(NH_3)_2(H_2O)_2]SO_4$ (30 ml, 3.0 mmol) and uridine (Sigma, 0.7326 g, 3.0 mmol) was heated in an oil bath at 70–73 °C in a transparent flask for 16 h with magnetic stirring. The deep blue mixture was gel filtered, and the only visible zone, the blue one, was taken directly into a 20-fold amount of acetone. The precipitation was finalized at 8 °C for 12 h to give a loose and lumpy product. The product was filtered with a sintered glass funnel (G4) and dried under vacuum (0.01 mmHg) for 10 h to give 1.1100 g (66.8% of theory based on Pt) of a blue powder. The sample for elemental analysis was synthesized in the same way except that the mixture was stirred at room temperature for 7 h after the reaction and the product was washed with acetone during the filtration. *Anal.* Found (original method [10]): C, 18.90 (20.55); H, 3.53 (3.02); N, 9.20 (8.20); S, 3.88 (2.64); Pt, 35.22 (37.96)%. Vis (3 mg/ml H_2O): 566 nm (broad, unsymmetric); IR (KBr): 1641 cm^{-1} (C=O). Treatment of an aqueous solution of Pt blue with hydrogen peroxide results irreversibly in a yellow solution.

2.2 SAXS measurements and data evaluation

The small angle X-ray scattering experiments were carried out with Cu Kα radiation monochromatized by means of a total reflecting mirror (Huber small-angle chamber 701) and Ni filter. Radiation for SAXS measurements was obtained from a sealed Cu anode fine focus X-ray tube powered by a Siemens Kristalloflex 710H unit. The scattered radiation was detected by a linear one-dimensional position sensitive proportional counter (MBraun OED-50M). A narrow slit (1 mm) was installed in front of the detector window to minimize background scattering. The primary beam was narrow (FWHM $\leqslant 0.008$ $Å^{-1}$) compared to its length (FWHM ≈ 0.16 $Å^{-1}$). The reliable range of measurements was roughly $k = 0.04$–0.70 $Å^{-1}$, where $k = 4\pi/\lambda \sin\theta$ is the magnitude of the scattering vector, 2θ the scattering angle and λ the X-ray wavelength.

The sample container was a narrow cavity (0.8 mm) in a steel frame with thin stretched polypropylene foils (6 μm) as flat faces. The samples were made in deionized water 0.5–1.0 h before the measurements. The temperature was 25 ± 1 °C in all measurements.

The measurements were repeated several times for each concentration (Table 1). For a reasonable statistical accuracy a longer measuring time was needed for the most dilute concentration. For concentrations of 9.8–140 mg/ml the results were reproducible, but the most dilute concentration, 1.4 mg/ml, gave dissimilar results in consecutive scans. The background due to the solvent and the foils was subtracted [19] and the intensity curves were corrected for beam-length smearing [20].

3. Results

The intensity curves are shown in Fig. 1. The particle sizes were estimated by means of an independent particle model (Guinier's approximation [21]). The intensity curves obeyed Guinier's approximation at two ranges of k. The determined radii of gyration (R_{g1} and R_{g2}) are given in Table 2 with a precision of about 10%. The larger of the two radii, R_{g1}, depended strongly on concentration and at a concentration of 1.4 mg/ml also on time.

Figure 2 shows the distance distribution functions of Pt blue in concentrations of 9.8–140 mg/ml. The functions were calculated by extrapolating the data to $k = 0$ with Guinier's law and to large k with the classical Porod's law [19] by means of a Fourier transform procedure.

TABLE 1. The number and duration of the measurements

Concentration (mg/ml)	No of scans	Durations of scans (h)
1 4	2	3 2, 8.6
8 9	2	0 5, 1.4
14.0	3	0 5, 0 6, 1.1
140.0	4	1 1, 2.1, 0.8, 0.8

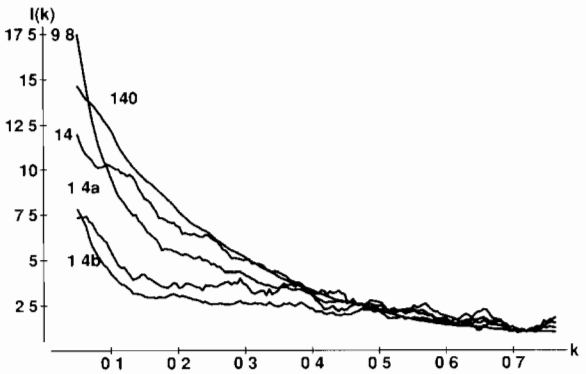

Fig. 1. Experimental intensity curves (in arbitrary units) as a function of k ($Å^{-1}$).

TABLE 2. The radii of gyration (Å) and k ranges of determination as a function of Pt blue concentration of the solution[a]

Concentration (mg/ml)	Range of k ($Å^{-1}$)	R_{g1} (Å)	Range of k ($Å^{-1}$)	R_{g2} (Å)
1 4 (a)	0.05–0.10	11	0.10–0 30	3.3
1 4 (b)	0.05–0.10	15	0.10–0 30	3 8
9.8	0.05–0.10	15	0 15–0 30	4.6
14.0	0.05–0 15	6	0 15–0 30	4 7
140.0	0 05–0 15	8	0 15–0.30	5 2

[a]Data (a) and (b) represent two consecutive scans of the same sample

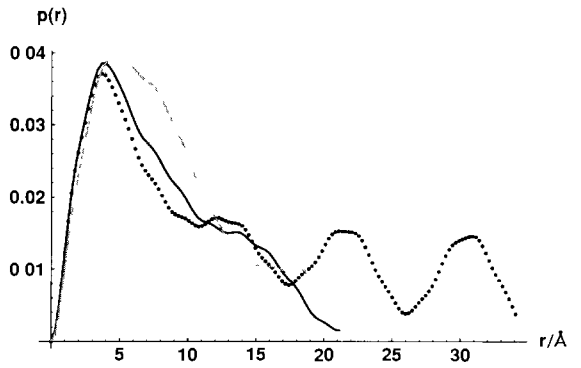

Fig. 2 Distance distribution functions in concentrations of 9.8 (dotted), 14 (solid black) and 140 (solid gray) mg/ml.

4. Discussion

Two radii of gyration may be obtained if the scattering arises from aggregates or from a mixture of independently scattering particles of two different sizes. We conclude (Fig. 1) that the dependence of the shape of the intensity curve on concentration arises rather from aggregation than from interparticle interference effects in a solution of discrete particles of two sizes. The time dependence of the intensity curve at 1.4 mg/ml also supports this conclusion. The larger of the radii of gyration, R_{g1}, gives the average size of the aggregate and the smaller of them, R_{g2}, gives the approximative size of the primary particle. It is proposed that the average primary Pt unit in the solution is dinuclear, since R_{g2} values agree with the radius of gyration [13] of a dinuclear Pt unit of crystalline 1-methyluracil blue [22]. The first quite well resolved maximum of the distance distribution function $p(r)$ agrees also quite well with the calculated $p(r)$ of the dinuclear Pt unit.

Computer simulations were made in order to test how well the model of aggregated dinuclear primary Pt units and the counter ions fits to the data. Refinements were also made with a more flexible monomeric model, since one cannot rule out that alternative. Because of the large scattering power of Pt, both the ligands and the counter-ions have only a small effect on the intensity curve. Pt atoms were modelled as spheres with a radius of 1 Å and ligands as spheres with a radius of 3 Å. Thus a dinuclear Pt unit consisted of four spheres. The distance between the centers of two Pt spheres was 3 Å and the distance between the centers of the ligand spheres was 10 Å.

R_{g2} does not give the precise size of the primary Pt unit because of the interparticle interference effects. According to computer simulations, an intensity curve of an aggregate of two dinuclear Pt units yields two well-resolved radii of gyration, if the distance of the Pt units is larger than about 20 Å. For a dense aggregate only one radius of gyration is obtained.

In further simulations, the following refinement procedure was used. Pt units were placed at random in a simulation cell and, with a random diffusion process, fit to the experimental intensity curve. The best fitting configuration is found by means of the simulated annealing method [22, 23]. The details of the fitting are given in the Appendix. To prevent overlapping the centers of two Pt spheres belonging to different units were not allowed to be closer to each other than 3 Å. The minimum distance between the centers of the ligand spheres was 6 Å.

The results of the refinement of (8–12) dinuclear Pt units in a simulation cell are presented in Table 3. The experimental intensity curve together with the model (140 mg/ml) is shown in Fig. 3. If the distance between the dinuclear units was smaller than 25 Å the units were considered to be aggregated. This distance is short enough to allow hydrogen bonds between the ribose parts of two Pt units. The number of isolated Pt units increased in scans (a) and (b) at the concentration of 1.4 mg/ml. The average number of Pt in an aggregate remained the same but the average distance of the dinuclear Pt units belonging to aggregates decreased from 22 to 18 Å in this concentration. The

TABLE 3. Results of the refinement with 8 dimers The number of Pt atoms is denoted by $\langle n \rangle$ in an average aggregate, the share of discrete dinuclear units (%) in the solution by n_{free}, and the Pt–Pt coordination number at 3 Å by CN

Concentration (mg/ml)	$\langle n \rangle$	n_{free} (%)	CN	χ
1.4 (a)	3	38	1.0	2.4
1.4 (b)	3	50	1.0	1.9
9.8	4	38	1.0	1.0
14.0	4	38	1.2	2.1
140.0	8	12	1.5	1.3

The goodness of fit was calculated as

$$\chi^2 = \frac{1}{N} \Sigma_{i=1,\ N} (I^{exp}(k_i) - I^{calc}(k_i))^2 / I^{exp}(k_i),$$

where I^{exp} is the experimental, I^{calc} the calculated intensity and N the number of points.

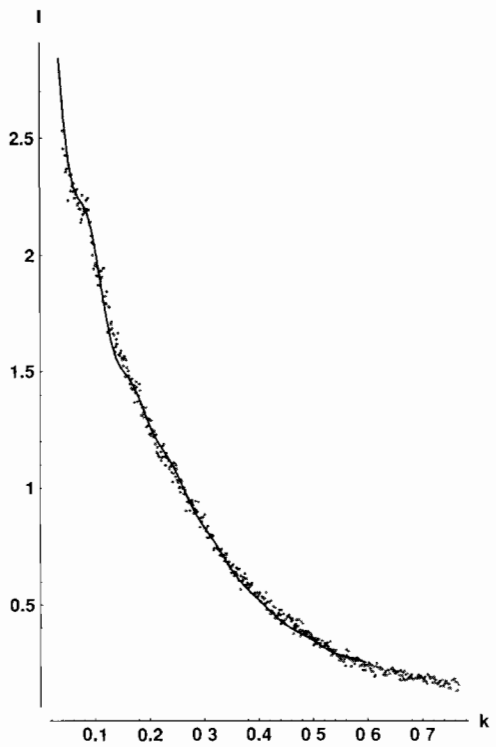

Fig 3 Experimental intensity curve (dotted) (in arbitrary units) at 140 mg/ml compared to dinuclear model (solid) as a function of k ($Å^{-1}$).

share of discrete dinuclear Pt units remained constant between 1.4 and 14.0 mg/ml. At higher concentrations the aggregates contained more Pt atoms, but the distance of the dinuclear Pt units was only 10 Å, thus the aggregates are more compact than in 1.4 mg/ml. The largest aggregate contained 7 dimers in a concentration of 140 mg/ml. The determined Pt–Pt coordination numbers (Table 3) imply that, when mixed with water, the tetranuclear primary particles decompose into dinuclear Pt units. According to the simulations, dilute solutions (1.4–9.8 mg/ml) contain only discrete or aggregated dinuclear Pt units and concentrated solutions (14–140 mg/ml) contain both di- and tetranuclear Pt units.

In the mononuclear simulation 12 monomers were placed in a simulation cell and the Pt spheres and the ligand spheres were allowed to move separately. The refinement was not sensitive to the positions of the ligands. An important result of the calculation was that no isolated monomers existed in the concentration range 9.8–140.0 mg/ml. Both the di- and mononuclear models gave the same average number of Pt atoms in an aggregate and the same *CN* at 3 Å. However, the dinuclear model gave a better goodness of fit in the concentration range 9.8–140 mg/ml. In the concentration 1.4 mg/ml the mononuclear model agreed slightly better with the experimental data. The numbers of free monomers in the concentration 1.4 mg/ml were 17 and 33% for scans (a) and (b), respectively. The existence of monomers is, however, controversial because of experimental difficulties in the case of the most dilute concentrations.

The formation of Pt–uridine blue has been followed by Raman and NMR spectroscopy [9], which indicated that the reaction gives a mixture. Our samples may not be equal to the reaction mixture, since they were obtained by dissolving the solid product, which was precipitated from the reaction mixture, into water. Our Pt–uridine blue and the reported products [10, 8] are synthesized under only slightly different conditions. The elemental analyses, and the Vis and IR spectra are very similar, and we believe that we have the same major complex. The C/Pt ratios fit with a structure analogous to crystalline Pt-methyluracil blue [24], a *cis*-diammine Pt_2 cation complex, which is double bridged by uridine It was concluded on the basis of elemental analyses that the structure of Pt blue is either that of a tetranuclear complex [10] or that of a chain with 28 platinum atoms [8]. Our WAXS studies on the solid product indicated that the Pt complex, which dominates the scattering, is tetranuclear [13]. In solution we find aggregates of up to 14 Pt, but these aggregates consist of dinuclear or tetranuclear Pt units. The existence of polymeric structures [3, 25] is generally accepted, but on the basis of the SAXS data we conclude that the Pt structures in water solutions are aggregates of di- or tetranuclear Pt units. Finally, these supramolecular level interactions may be crucial factors in determining the biological activity of platinum blues.

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Appendix

Refinement of mononuclear model for Pt blue (1.4 mg/ml (a))

The function to be minimized is $E=\sum_i^N(I^{calc}(k_i)-I^{exp}(k_i))^2$, where I^{exp} is the experimental, I^{calc} the calculated intensity, and N the number of points. In the simulated annealing procedure the parameter T ('temperature') controls the probability of accepting displacements. A linear cooling scheme, $T_{new}=\alpha T$, where α is the cooling factor, was applied.

The size of the problem [23]	
Size of the simulation cell (Å^3)	70^3
Maximum displacement parameter (Å)	1.0
No. configurations [23]	4.3×10^{16}
Minimum no. trials per T [23]	3675
Refinement	
Cooling factor α	0.9
Metropolis rejection rate [22]	0.75
Maximum number of configuration changes at one T	6000
Stopping cycle	130
No. accepted configurations	671 000
No. rejected (Metropolis criteria)	267 000
No. rejected unphysical configurations	60 000
Total no. trials	998 000