[Chem. Pharm. Bull. 33(6)2190—2194(1985)]

Kinetic Study on the Formation of Platinum-Blue Complexes with Oxygen

TETSUO OSA,* HIROAKI HINO, and SHIGEAKI FUJIEDA[1)]

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan

(Received September 27, 1984)

A kinetic study of Pt-blue complex formation was carried out in a solution containing $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and uracil under oxygen bubbling, with monitoring by means of an oxygen analyzer and visible spectrometer. The rate of oxygen consumption was first-order with respect to concentration of $[Pt(NH_3)_2(H_2O)_2]^{2+}$, uracil, or oxygen. A correlation was found between the decrease of oxygen consumption in the solution and the increase of the absorbance at 640 nm, namely formation of the blue complex. The formation mechanism of the blue complex is considered to be 1) first the equilibrium reaction of the coordination of uracil to Pt, 2) next the oxidation of the Pt–uracil complex with oxygen, and 3) finally a follow-up reaction presumably *via* formation of polynuclear complexes. Eight other blue complexes were formed by the use of N-aromatic base ligands possessing an amide linkage. The oxygen consumption rates of these complexes were found to be large in the case of the ligands which form many structurally isomeric complexes.

Keywords——platinum; platinum-blue complex; *cis*-dichlorodiammineplatinum(II); *cis*-diaquadiammineplatinum(II) nitrate; oxidation; platinum complex oxidation; reaction kinetics

It is well known that a combination of platinum and acetamide forms a blue complex, called "Platinblau," but the structure and the platinum valence number of the complex have not been clarified due to the difficulty of crystallization.[2)] In 1975, Rosenberg *et al.* reported that *cis*-diaquadiammineplatinum(II) {*cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, **1**} reacts slowly with pyrimidine bases, such as uracil and thymine, at pH 7 and 37 °C under air to yield a "platinum–pyrimidine blue" resembling Platinblau.[3)] The blue complexes have received a great deal of attention owing to their high antitumor activity, high water solubility, and low toxicity.

However, little structural information is available on the platinum–pyrimidine blue. Various attempts have been made to clarify the structure of Platinblau but with only limited success. Various types of blue complexes have also been synthesized with many pyrimidine derivatives, and it is believed nowadays that platinum–pyrimidine blue is not a square planar complex with Pt(II) but is a polynuclear complex with a higher valency, Pt(III) or Pt(IV).

We have investigated the kinetics of platinum-blue formation in aqueous solutions by monitoring the absorbance at 640 nm and the oxygen concentration. The results are presented here.

Experimental

Apparatus and Instruments——The vessel used for oxygen consumption measurement is shown in Fig. 1. The concentration of oxygen in the solution was determined with a Beckman model 0260 oxygen analyzer. Absorption spectroscopy was carried out with a Shimadzu UV-360 connected to a Haake water circulator (model F2-C) to maintain a constant temperature of the vessel. The pH of the solution was determined with a Hitachi-Horiba M-7 pH meter. The buffer solution consisted of CH_3COOH–CH_3COONa for the pH range 3—6 and HNO_3–CH_3COONa for pH 2.

Preparation of Reaction Solution and Oxidation of Prepared Solution——*cis*-Dichlorodiammineplatinum(II) {*cis*-

$[PtCl_2(NH_3)_2]$, **2**} was synthesized according to the literature.[4] **2** (0.150 g, 0.5 mmol) was first added to 2 ml of an aqueous solution containing 0.169 g $AgNO_3$, and the solution was stirred for 12 h in the dark. After the reaction, AgCl formed was filtered off and the filtrate was confirmed to be free from Ag^+ and Cl^-. To the filtrate (which contained **1**), uracil (0.056 g, 0.5 mmol) and 1 N acetate buffer (3 ml) were added, followed by the addition of water to give a total volume of 10 ml. The solution was then stirred on a water bath (70 °C) for 10 min to remove excess air. In the kinetic measurement, the oxygen concentration in the solution was adjusted by bubbling with oxygen and/or argon. Platinum–uracil blue complex was gradually formed by the oxidation of the solution. In order to determine the formation rate of the blue complex, the oxygen concentration remaining in the solution was monitored with an oxygen analyzer and the absorbance at 640 nm was measured. For the preparation of completely oxygen-free solution, the freeze-and-melt treatment with liquid nitrogen was repeated three times.

In order to obtain other Pt-blue complexes, hydantoin, 2-imidazolidinone, 2-pyrrolidone, succinimide, maleimide, pyrimidine, thymine, nicotinamide, or 2-pyridone instead of uracil was added to the **1** solution and the same oxidation procedure as described above was carried out.

Results and Discussion

Formation of Platinum Blue Complex

No consumption of oxygen, change of absorbance, or appearance of blue complex was observed in pH 4 solution containing 5×10^{-2} M uracil or **1** at 60 °C under oxygen. Furthermore, no change was detected in the completely degassed solution containing both uracil and **1**. Therefore, all of uracil, **1** and oxygen are necessary for the complex formation.

Dependence on Oxygen Concentration

Figure 2 depicts the time courses of the oxygen concentration and the absorbance at 640 nm in the pH 4 buffer solution containing 1.00×10^{-1} M **1** and 1.00×10^{-1} M uracil. The solution became blue with a decrease of oxygen concentration, but the absorbance still increased after complete consumption of oxygen. This phenomenon indicates that there are some further reaction steps after the coordination reaction of uracil to Pt followed by oxidation.

The initial concentration of oxygen was set at 7.96×10^{-4}, 1.60×10^{-4}, or 8.10×10^{-5} M by bubbling the reaction solution with O_2, air, and/or Ar in order to investigate the influence of oxygen concentration. The concentrations of **1** and uracil used were both 5.00×10^{-2} M. The reaction was carried out in the pH 4 buffer solution at 60 °C. Figure 3 shows the relationship between the initial oxygen concentration and the consumption rate of oxygen. The consumption rate of oxygen was obtained from the slope at the initial stage of the log $([O_2]_0/[O_2])$ *vs.* reaction time plot, where $[O_2]_0$ is the initial concentration of oxygen, and $[O_2]$ is the oxygen concentration during the reaction. The reaction depends on the oxygen

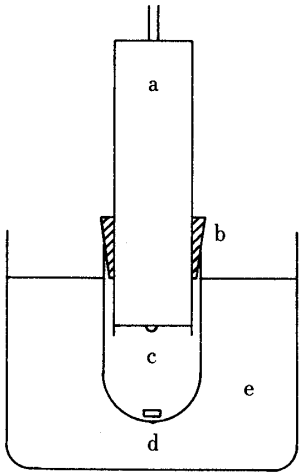

Fig. 1. Vessel Used for Oxygen Consumption Measurement

a, oxygen sensor; b, silicon rubber stopper; c, reaction solution; d, magnetic stirring bar; e, thermostat.

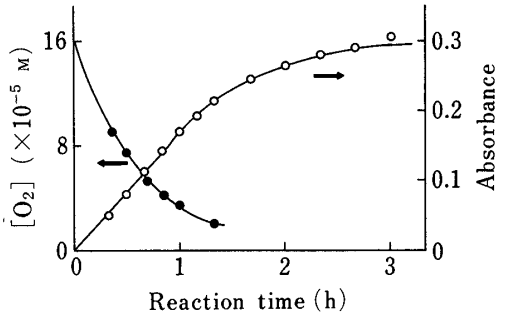

Fig. 2. Time Courses of O_2 Concentration and Absorbance at 640 nm in the Solution

●, oxygen concentration; ○, absorbance at 640 nm.
Concentration of $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and uracil were each 100 mM; 60 °C; pH 4 acetate buffer.

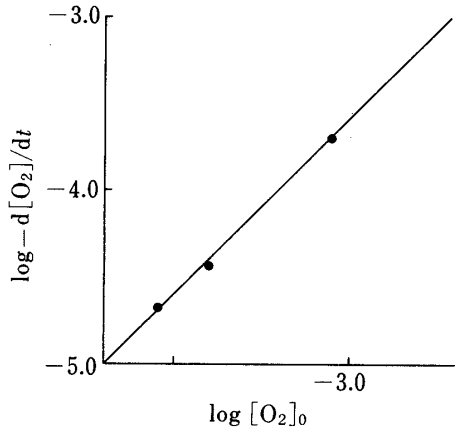

Fig. 3. Dependence of Oxygen Consumption Rate on the Concentration of O_2

Concentrations of $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and uracil were each 50 mM; 60 °C; pH 4 acetate buffer.

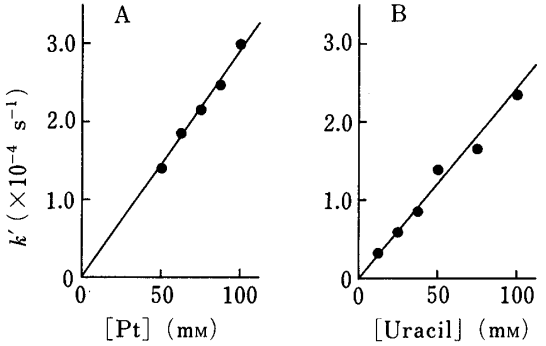

Fig. 4. Dependence of k' on the Concentrations of Uracil and $[Pt(NH_3)_2(H_2O)_2]^{2+}$

A; concentration of uracil, 50 mM; initial concentration of oxygen, 1.6×10^{-4} M; 60 °C; pH 4 acetate buffer.
B; concentration of $[Pt(NH_3)_2(H_2O)_2]^{2+}$, 50 mM; initial concentration of oxygen, 1.6×10^{-4} M; 60 °C; pH 4 acetate buffer.

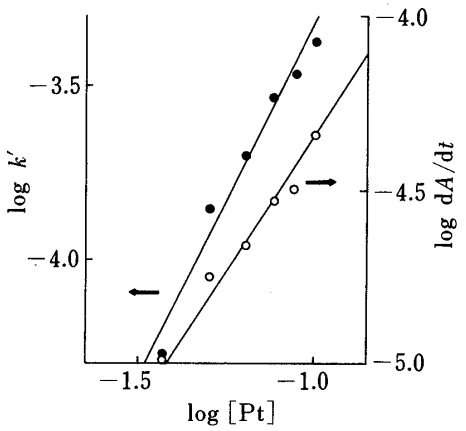

Fig. 5. Dependence of k' and dA/dt on the Concentrations of Uracil and $[Pt(NH_3)_2(H_2O)_2]^{2+}$

The concentrations of $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and uracil were equal; initial concentration of oxygen, 1.6×10^{-4} M; 60 °C; pH 4 acetate buffer.

concentration in the solution and the slope of the line indicates that the reaction is 1st-order as given by Eq. 1.

$$-d[O_2]/dt = k'[O_2] \quad (1)$$

Dependence on 1 and Uracil Concentrations

Figure 4 shows the relationship between the observed 1st-order rate constant (k') for oxygen consumption and the concentration of **1**. This linear plot (Fig. 4A) indicates that the reaction is 1st-order with respect to **1** (Eq. 2).

$$k' = k_1[Pt] \quad (2)$$

where [Pt] represents the concentration of **1**. The dependence of k' on the concentration of uracil (Fig. 4B) also suggests 1st-order kinetics with respect to uracil (Eq. 3).

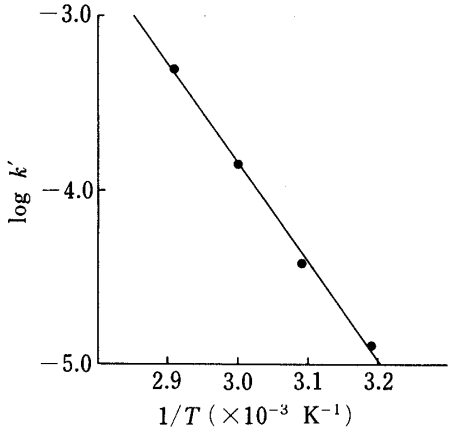

Fig. 6. Arrhenius Plot for the Oxidation of Pt–Uracil

Concentrations of $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and uracil were each 50 mM; pH 4 acetate buffer.

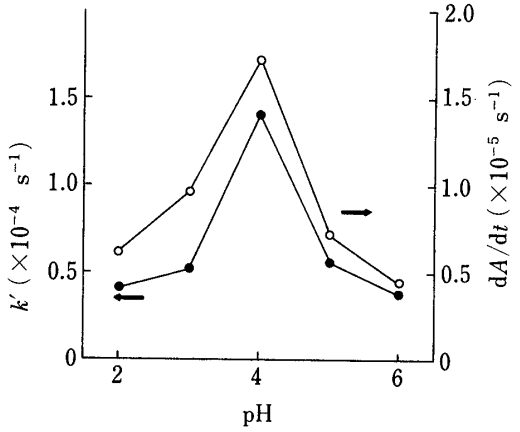

Fig. 7. Dependence of k' and dA/dt on pH

Concentrations of $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and uracil were each 50 mM; 60 °C; HNO_3–CH_3COONa buffer for pH 2, CH_3COOH–CH_3COONa buffer for pH 3—6.

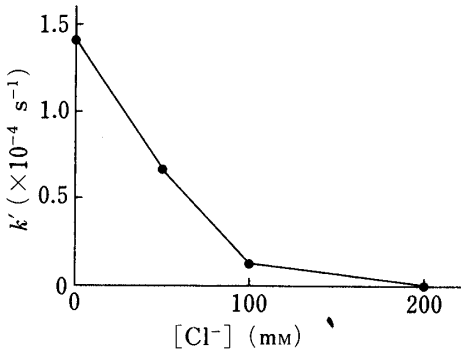

Fig. 8. Effect of Cl^- Ion

Concentrations of $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and uracil were each 50 mM; 60 °C; pH 4 acetate buffer.

$$k' = k_2[U] \quad (3)$$

where [U] represents the concentration of uracil.

The time dependence of the oxygen concentration at various concentrations of uracil and **1** (in this case, the concentrations of uracil and **1** are equal) is shown in Fig. 5. It is clear that the oxygen consumption becomes faster with each increase of uracil and **1** concentrations, and k' is expressed by Eq. 4.

$$k' = k_3[Pt][U] = k_3[Pt]^2 \quad ([Pt] = [U]) \quad (4)$$

Figure 5 also shows that the rate of increase of the absorbance (dA/dt) at 640 nm became faster with increase of the concentrations of uracil and **1**. The values of dA/dt were obtained from the slope at the initial stage of the $\log(A_0/A)$ *vs.* reaction time plot as well as the values of $\log d[O_2]/dt$; A_0 is the initial absorbance at 640 nm of the solution. If oxygen consumption and blue formation occur simultaneously, both lines in Fig. 5 would be parallel. However, the slope of the rate of blue complex formation is slightly lower than that of k'. This means that the blue complex formation requires some reaction time after the process of oxygen uptake in the mixed solution of **1** and uracil.

Dependence on Temperature and pH

The k' value increased with temperature. The activation energy was found to be 25.9 kcal/mol from the slope of the Arrhenius plot (Fig. 6). The rate of increase in the absorbance also became larger with temperature.

Figure 7 shows the pH dependence of k'. From this figure it can be seen that the highest

TABLE I. Dependence of k' on the Kind of Ligands in Pt-Blue Complexes

Amine	k' ($\times 10^{-4}$ s^{-1})	Amine	k' ($\times 10^{-4}$ s^{-1})
Hydantoin	0.056	Pyrimidine	0.000
2-Imidazolidinone	0.313	Thymine	0.020
Succinimide	0.062	Uracil	1.410
2-Pyrrolidone	0.070	Nicotinamide	0.058
Maleimide	1.937	2-Pyridone	0.526

rate appears at *ca.* pH 4. The increase in the absorbance was also fast around this pH region.

Dependence of Halide Concentration

The presence of halide ion retarded the rate of oxygen consumption (Fig. 8). This may be caused by a reaction of chloride ion with **1** to form **2**, to which uracil cannot coordinate. A yellow precipitate, presumably *cis*-$[PtCl_2(NH_3)_2]$, was formed in the presence of relatively high concentrations (above 0.1 M) of NaCl.

Influence of Coordination Compounds

The reaction was also investigated using hydantoin, 2-imidazolidinone, 2-pyrrolidone, succinimide, maleimide, pyrimidine, thymine, nicotinamide, or 2-pyridone as a coordination compound (Table I). No oxygen consumption was observed with pyrimidine, which has no amide structure. The oxygen consumption was fast with coordination compounds which can form many structurally isomeric complexes.

From the above results, it became clear that the blue complex is formed by the oxidation of Pt–uracil complex with oxygen. The first step of this reaction is the equilibrium reaction of the coordination of uracil to Pt, and the next is the oxidation of the Pt–uracil complex with oxygen. The first step is favored at high pHs, while the next is favored at low pHs. The reaction shows 1st-order kinetics with respect to Pt (**1**), uracil or oxygen, being a 3rd-order reaction overall (Eq. 5).

$$-d[O_2]/dt = k[O_2][Pt][U] \quad (5)$$

The blue complex may be formed through a follow-up reaction, presumably *via* formation of a polynuclear complex, after the oxidation of the Pt–uracil complex by oxygen.

In conclusion, it has been clarified that oxygen dissolved in a solution is essential for formation of the Pt-blue complex. Formation of the blue complex through the reaction of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with pyrimidine bases under air has been considered to require a long time. However, there was no evidence that oxygen was the actual oxidant. We have here demonstrated that the blue complex cannot be formed after deaeration, and the rate of oxygen consumption is 1st-order in terms of the oxygen concentration dissolved in the solution. In addition, a correlation was found between the decrease of oxygen concentration in the solution and the increase of the absorbance at 640 nm, namely formation of the blue complex. From the above results, we conclude that the oxidation step with oxygen is essential for formation of the blue complex.

References and Notes

1) Present address: *Technical Research Center, Nippon Mining Co., Ltd., 3 Niizo-minami, Toda-shi, Saitama 335.*
2) N. Kurnakow, *J. Prakt. Chem.*, **51**, 234 (1895).
3) J. P. Davidson, P. J. Faber, R. G. Fisher, Jr., S. Mansy, H. J. Peresie, B. Rosenberg, and L. VanCamp, *Cancer Chemother. Rep., Part 1,* **59**, 287 (1975).
4) "Inorganic Syntheses," Vol. VII, McGraw-Hill Book Company, Inc., New York, 1963, p. 241.