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# Decomposition Kinetics of Cisplatin in Human Biological Fluids

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## Abstract

Cisplatin is metabolized to high- and low-molecular mass complexes by exchanging of one or both chloride ligands with nucleophilic species. The decomposition of cisplatin and the formation of its metabolites in human biological fluids was investigated in-vitro.

In nucleophile-free medium, cisplatin was decomposed by a reversible chloride ligand exchange reaction, which was dependent upon both chloride ion concentration and medium pH. The effect of pH was observed in the second-order rate constant for cisplatin re-formation, which was far smaller in neutral and alkaline media than in acidic media. In the medium containing nucleophilic species (glutathione, human albumin and globulin), cisplatin was irreversibly decomposed according to apparent first-order kinetics. The linear relationship between apparent decomposition rate constant and concentration of nucleophilic species suggested that nucleophilic species in biological fluids might react with cisplatin directly. Both high- and low-molecular mass metabolites were formed in human plasma and ascites; however, only a low-molecular mass metabolite was produced in urine. The faster decomposition of cisplatin and the greater ratio of fixed and mobile metabolites formed in plasma were mainly attributed to higher albumin concentration in plasma than in ascites and urine.

The in-vitro decomposition of unchanged cisplatin and formation of high- and low-molecular mass metabolites in human biological fluids were simultaneously evaluated according to the kinetic model which combined both pathways via hydrolysis and by the direct reaction process with nucleophilic species.

The biotransformation of cisplatin is quite different from that of other drugs metabolized by enzymatic processes, losing its effect in biological fluids and tissues by the exchange of one or both chloride ligands with nucleophilic species to form high- and low-molecular mass complexes (Gullo et al 1980; Daley-Yates & McBrien 1984).

The chloride ligands are displaced by water molecules in a stepwise manner, forming more reactive hydrated species (Reishus & Martin 1961). In aqueous solutions, the first hydration process is considered to be the rate-determining step, and an equilibrium between cisplatin and the hydrated species can reasonably be assumed because of the reversible re-formation of cisplatin which occurs on addition of chloride ions. However, cisplatin has been shown to be unstable in biological fluids, despite the high extracellular chloride concentration (Repta & Long 1980). The addition of sodium chloride to plasma ultrafiltrate also had no effect on the stability of cisplatin (Long et al 1980).

In the present study, the effects of temperature, chloride ions and pH on the decomposition of cisplatin were examined in buffer solution.

## Materials and Methods

*Materials*

Cisplatin (Randa) was a gift from Nippon Kayaku Co., Ltd. (Tokyo, Japan). Glutathione was donated by Yamanouchi Pharmaceutical Co., Ltd (Tokyo, Japan). Human albumin (fraction V), human γ-globulin (Cohn fraction II, III) and human $\alpha_1$-acid glycoprotein (Cohn fraction VI) were purchased from Sigma Chemical Co., Ltd. (St Louis, MO, USA). All other chemicals and reagents were of analytical grade.

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*Decomposition of cisplatin in aqueous solution*

Separate experiments were performed to investigate the decomposition of cisplatin in 0·067 M phosphate buffer solution at various temperatures, chloride ion concentrations, pH values and in the presence of glutathione, albumin, globulin and $\alpha_1$-acid glycoprotein. A cisplatin stock solution (1 mg $mL^{-1}$) was prepared by dissolving cisplatin in 0·9% sodium chloride (saline), and a standard solution (100 μg $mL^{-1}$ was prepared by dilution with the same solvent. Phosphate buffer was added to the standard solution (1 : 10 v/v) to produce an initial test solution containing 30 μM cisplatin and 14 mM chloride ions.

A sample of each solution (200 μL) was immediately ultrafiltered (4000 *g*) at 4°C for 30 min using a 10 000-Da molecular mass cut-off membrane (UFC 3GC, Japan Millipore Ltd., Tokyo, Japan). A 50-μL portion of the ultrafiltrate was then used to assay unchanged cisplatin.

*Decomposition of cisplatin in biological fluids*

Blank plasma and urine samples were obtained from healthy subjects, and a blank ascites sample from a cancer patient. Cisplatin standard solution (100 μg $mL^{-1}$ cisplatin in saline) was added to 2 mL urine and 4 mL plasma or ascites (1 : 10 v/v) to an initial concentration of 30 μM cisplatin, and decomposition studies were performed at 37°C. Sample handling followed the procedures described above.

*Assay*

Unchanged cisplatin was analysed by high-performance liquid chromatography (HPLC) with post-column derivatization as reported previously (Kinoshita et al 1990).

Total platinum levels in the biological fluids and platinum levels in the ultrafiltrate were determined with an atomic absorption spectrometer (Hitachi model Z-8000, Hitachi

Co., Ltd., Tokyo, Japan). A 5- or 10-μL sample of ultrafiltrate, diluted plasma or ascites (1 : 2 v/v, with 0·25% Triton X-100) or diluted urine (1 : 10 v/v, with distilled water) was injected into the tube cuvette (Kinoshita et al 1990).

The concentrations of fixed and mobile metabolites were estimated by the following equations:

$$\text{Fixed metabolite} = \text{total platinum concn} - \text{platinum concn in the ultrafiltrate} \quad (1)$$

$$\text{Mobile metabolite} = \text{platinum concn in the ultrafiltrate} - \text{unchanged cisplatin concn (platinum equivalent)} \quad (2)$$

The concentration of chloride ions in the biological fluids was measured using an ion-selective electrode (CL-125B; TOA Electronics Ltd., Tokyo, Japan). The protein concentration of the biological fluids was determined by the method of Lowry et al (1951) using human serum albumin as a standard.

*Kinetic analysis for cisplatin decomposition in aqueous solution*

Cisplatin undergoes reversible hydration and deprotonation-protonation reactions, which are dependent on the initial concentrations of cisplatin and chloride ions in the medium. In chloride-free solution at a high cisplatin concentration (> 1 mM), monohydrated cisplatin is predominantly produced, although significant amounts of dihydrated cisplatin are produced at a low cisplatin concentration (< 1 mM) (Segal & Pecq 1985). However, in solution containing chloride ion concentration of more than 0·01 M over the pH range 5–7·5, cisplatin exists mainly (> 90 mol%) as unchanged cisplatin and monohydrated cisplatin in equilibrium, although the studies which showed this were performed at a low initial cisplatin concentration (1 μM) (LeRoy et al 1979; Lippard 1982). Thus, an equilibrium between cisplatin and monohydrated cisplatin could be assumed in the present experimental medium in which the initial concentration of cisplatin was 30 μM and the chloride ion concentration was in excess (14 mM).

The following model has been proposed to describe the decomposition of cisplatin in aqueous solution.

Model 1 (Reishus & Martin 1961; Greene et al 1979)

$$\underset{[A]}{\text{CDDP}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{[B]}{\text{Monoaqua CDDP}} + \underset{[Cl^-]}{Cl^-} \qquad \text{model 1}$$

where $k_1$ is the forward first-order rate constant for cisplatin decomposition and $k_{-1}$ is the second-order rate constant for cisplatin re-formation. [A], [B] and [$Cl^-$] are the concentrations of unchanged cisplatin, monohydrated cisplatin and chloride ions, respectively. A mathematical expression can be developed to describe the reaction as follows:

$$\frac{-d[A]}{dt} = k_1[A] - k_{-1}[Cl^-][B] \quad (3)$$

The percentages of unchanged cisplatin remaining at time t ($[A]/[A]_0(\%)$), and at equilibrium (Eq(%)), can be calculated by integrating equation 3 and using the relationships, $[A]_0 = [A] + [B]$, $[A] = [A]_0$ and $[B] = 0$ at $t = 0$:

$$\frac{[A]}{[A]_0}(\%) = \frac{100}{k_1 + k_{-1}[Cl^-]}(k_1 e^{-(k_1 + k_{-1}[Cl^-])t} + k_{-1}[Cl^-]) \quad (4)$$

$$\text{Eq}(\%) = \frac{k_{-1}[Cl^-]}{k_1 + k_{-1}[Cl^-]}100 \quad (5)$$

*Kinetic analysis for reaction of cisplatin with nucleophiles*

Two possible pathways have been proposed to explain the reaction between cisplatin and nucleophiles. One of these pathways involves the formation of intermediate (monohydrated cisplatin) as a rate-determining step, followed by a further rapid reaction between intermediate and various reactive nucleophiles. The fixed and mobile metabolites formed show good thermodynamic stability, therefore; the reverse reaction involving re-formation of the intermediate is negligible and model 2 can be invoked:

Model 2 (Repta & Long 1980; Segal & Pecq 1985; LeRoy & Thompson 1989):

$$\underset{[A]}{\text{CDDP}} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{[B]}{\text{Intermediates}} + \underset{[Cl^-]}{Cl^-}$$

$$+[Nu] \downarrow k_2$$

Fixed metabolites
Mobile metabolites

model 2

where $k_2$ is the rate constant for formation of fixed and mobile metabolites. The mathematical expressions can be developed from model 2 as follows:

$$\frac{-d[A]}{dt} = k_1[A] - k_{-1}[Cl^-][B]$$

$$\frac{d[B]}{dt} = k_1[A] - k_{-1}[Cl^-][B] - k_2[Nu][B] \quad (6)$$

If a steady-state approximation is introduced for [B], $d[B]/dt = 0$, the apparent rate of decomposition of cisplatin becomes:

$$\frac{-d[A]}{dt} = k_1[A] - k_{-1}[Cl^-]\frac{k_1[A]}{k_{-1}[Cl^-] + k_2[Nu]} \quad (7)$$

and the apparent rate constant for cisplatin decomposition ($k_{app}$) can be defined as:

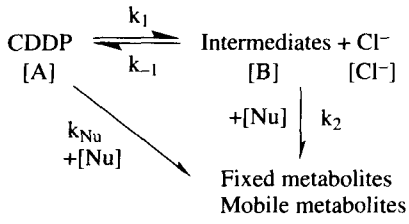
$$k_{app} = \frac{k_1 k_2[Nu]}{k_{-1}[Cl^-] + k_2[Nu]} \quad (8)$$

Inverting equation 8 yields equation 9 (Repta & Long 1980):

$$\frac{1}{k_{app}} = \frac{1}{k_1} + \frac{k_{-1}[Cl^-]}{k_1 k_2[Nu]} \quad (9)$$

In the second possible pathway, nucleophiles may react with both unchanged cisplatin and its intermediates. The model for this reaction (model 3) can be represented as follows:

Model 3 (Long et al 1980; Segal & Pecq 1985):




model 3

where $k_{Nu}$ is the second-order rate constant for the direct reaction between cisplatin and nucleophiles. Mathematical expressions for model 3 can be derived as follows:

$$\frac{-d[A]}{dt} = k_1[A] - k_{-1}[Cl^-][B] + k_{Nu}[Nu][A]$$

$$\frac{d[B]}{dt} = k_1[A] - k_{-1}[Cl^-][B] - k_2[Nu][B] \quad (10)$$

When [B] is at steady-state ($d[B]/dt = 0$), the apparent decomposition rate of cisplatin can be expressed as:

$$\frac{-d[A]}{dt} = k_1[A] - k_{-1}[Cl^-]\frac{k_1[A]}{k_{-1}[Cl^-] + k_2[Nu]} + k_{Nu}[Nu][A] \quad (11)$$

and the apparent rate constant for cisplatin decomposition ($k_{app}$) can be defined as:

$$k_{app} = \frac{k_1 k_2[Nu]}{k_{-1}[Cl^-] + k_2[Nu]} + k_{Nu}[Nu] \quad (12)$$

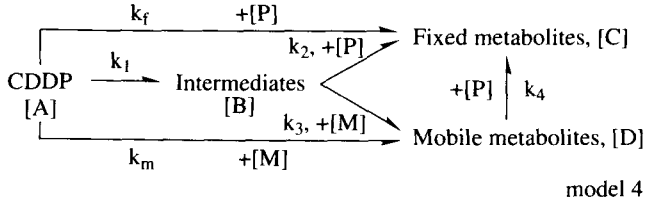
If cisplatin is decomposed according to model 2, a plot of $1/k_{app}$ vs $1/[Nu]$ will yield a straight line with an intercept at $1/k_1$ according to equation 9. If cisplatin is decomposed according to model 3 and $k_2[Nu]$ is far greater than $k_{-1}[Cl^-]$, $k_{app}$ can be simplified as equation 13:

$$k_{app} = k_1 + k_{Nu}[Nu] \quad (13)$$

A plot of $k_{app}$ versus [Nu] will yield a straight line with a slope of $k_{Nu}$ and an intercept at $k_1$.

*Decomposition of cisplatin in biological fluids*

If cisplatin reacts with nucleophilic species according to model 3, and fixed metabolites are also formed from mobile metabolites when $k_2[Nu]$ is much greater than $k_{-1}[Cl^-]$ the decomposition of cisplatin in biological fluids can be analysed according to model 4:




model 4

where $k_f$ and $k_m$ represent the second-order rate constants for direct formation of fixed and mobile metabolites from cisplatin, respectively, $k_2$ and $k_3$ represent the second-order rate constants for the formation of fixed and mobile metabolites from the intermediates (monohydrated cisplatin) and $k_4$ represents the second-order rate constant for the formation of fixed metabolites from mobile metabolites. [C], [D], [P] and [M] are the concentrations of fixed metabolites, mobile metabolites, proteins and low molecular mass nucleophiles, respectively. Mathematical expressions for unchanged cisplatin, fixed and mobile metabolites can be described by equation 14, and each rate constant can be simultaneously analysed.

$$\frac{-d[A]}{dt} = (k_1 + k_f[P] + k_m[M])[A]$$

$$\frac{d[C]}{dt} = k_f[P][A] + k_2[P][B] + k_4[P][D] \quad (14)$$

$$\frac{d[D]}{dt} = k_m[M][A] + k_3[M][B] - k_4[P][D]$$

*Data analysis*

The data are expressed as percentage of the initial cisplatin concentration ± s.d. The apparent rate constant for cisplatin decomposition ($k_{app}$) was calculated by linear least-square regression analysis, and the rate constants for cisplatin decomposition according to models 1 and 4 were calculated by non-linear least-square regression analysis (MULTI program, Yamaoka et al 1981) using a personal computer.

## Results and Discussion

*Effects of chloride ion and pH on cisplatin decomposition*

The activation energy for cisplatin decomposition in phosphate buffer containing 14 mM chloride ions (pH 7) was estimated as 17·8 kcal $mol^{-1}$ by the Arrhenius plot. This is in agreement with values obtained in water (19–20 kcal $mol^{-1}$, Repta & Long 1980) and in phosphate solution (19 kcal $mol^{-1}$, Bose et al 1984). Fig. 1 shows the influence of chloride ion concentration and pH on the time-course of cisplatin decomposition in phosphate buffer. Cisplatin was eliminated according to apparent first-order kinetics, and completely decomposed in the chloride-free solution. However, the equilibrium between cisplatin and monohydrated cisplatin could be obtained within 24 h in the solution containing excessive chloride ions (7 and 14 mM). Although $k_{app}$ was almost the same in the chloride-free solution within the pH range 3–7 (data not shown), cisplatin was more stable in acidic solution than in the neutral or alkaline solution containing 14 mM chloride ions.

The simulation curves using equation 4 fitted well with the observed values (Fig. 1). Furthermore, the percentage of cisplatin remaining at 24 h was in good agreement with the values calculated at equilibrium by equation 5 (pH 3, 84·4% mean observed value compared with 80·9% mean predicted value; pH 5, 72·2 and 72·8%; pH 6, 30·6 and 30·7%; pH 7, 4·25 and 4·36%; pH 9, 1·61 and 1·65%; pH 7 ($Cl^- = 7$ mM), 2·30 and 2·40%). These results support model 1 as being suitable for describing the decomposition behaviour of cisplatin in the present medium.

Fig. 2 shows the pH profiles for the decomposition rate constants of cisplatin estimated using equation 4. The first-order decomposition rate constant ($k_1$) was almost constant over the pH range 3–9. However, the effect of pH on cisplatin decomposition was observed in the second-order re-formation rate constant ($k_{-1}$) which varied between 99·5 $M^{-1}$ $h^{-1}$ at pH 3 and 0·392 $M^{-1}$ $h^{-1}$ at pH 9.

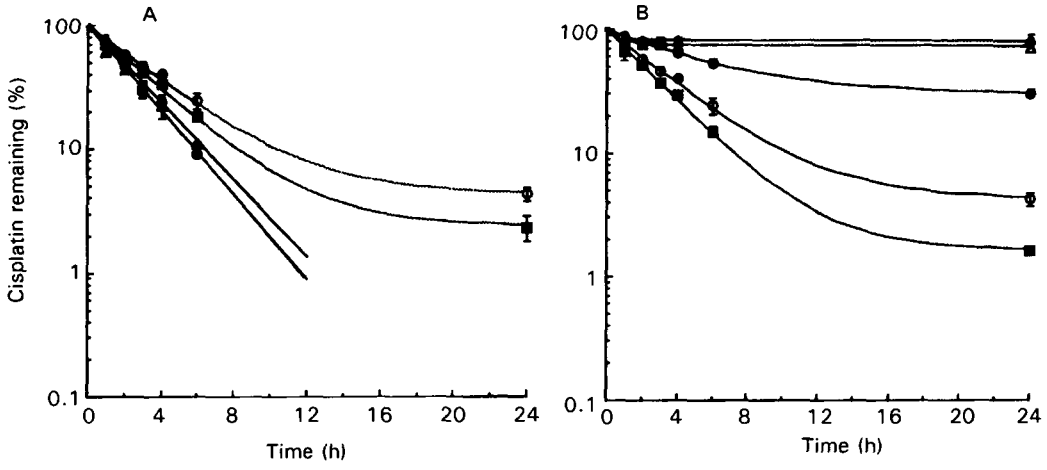



FIG. 1. Effects of chloride ions (A) and pH (B) on the decomposition of cisplatin (30 $\mu$M) in phosphate buffer (pH 7) at 37°C. [$Cl^-$]; ● 0, ▲ 1·4, ■ 7, ○ 14 mM at pH 7. pH; ▲ 3, △ 5, ● 6, ○ 7, ■ 9, in the presence of 14 mM chloride ions. The solid lines are simulation curves predicted using equation 4 in the text.

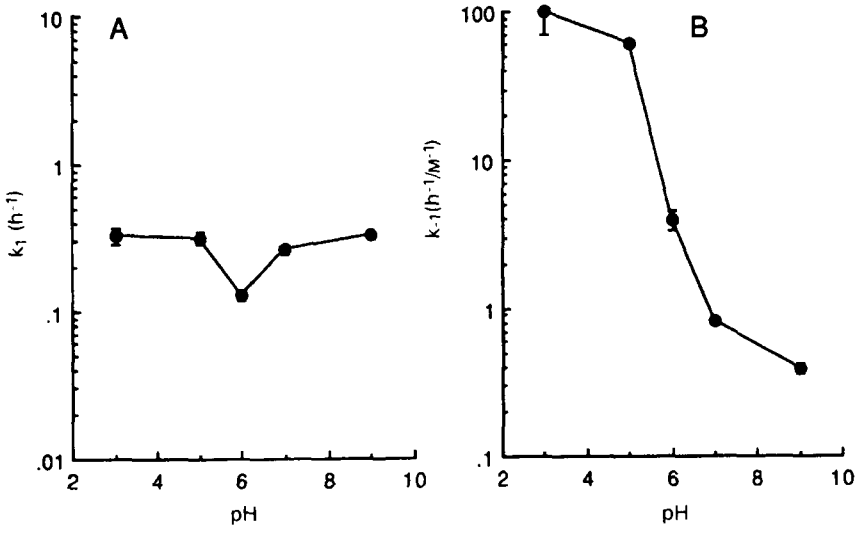



FIG. 2. Rate constant-pH profiles of the decomposition of cisplatin (30 $\mu$M) in phosphate buffer containing 14 mM chloride ions at 37°C. The rate constants were calculated using equation 4 in the text.

Phosphate ion is capable of reacting with cisplatin; this reaction may possibly occur in the present medium. However, the decomposition behaviour of cisplatin in the present medium was predicted by the same model as proposed previously in which reversible cisplatin decomposition was dependent upon chloride ion concentration. Therefore, the decomposition behaviour of cisplatin in the present phosphate buffer is probably best described by model 1.

*Mechanism for reaction of cisplatin with nucleophilic species*

Fig. 3 represents two plots relating the concentration of glutathione as a low molecular mass nucleophile with $k_{app}$ for cisplatin decomposition at 37°C in pH 7 phosphate buffer containing 14 mM chloride ions, either of which is derived from model 2 or model 3. Since $k_{-1}$ was 0·822 $M^{-1}$ $h^{-1}$ at pH 7 (Fig. 2) and the chloride ion concentration was 14 mM, $k_{-1}[Cl^-]$ can be calculated to be 0·0115 $h^{-1}$. On the other hand, $k_2$ has been suggested to be far greater than $k_{-1}$ at pH > 5 (Segal & Pecq 1984), and the concentration of glutathione was in the same concentration range as the chloride ions. In equation 12, it can be hypothesized that $k_2[Nu]$ is far greater than $k_{-1}[Cl^-]$ at pH 7. Therefore, $k_{app}$ could be approximately expressed as the sum of $k_1$ and $k_{Nu}[Nu]$ in a pH 7 solution (eqn 13). The plot of $k_{app}$ versus glutathione concentration showed a straight line, expressed as $k_{app} = 0{\cdot}0441 \cdot [\text{glutathione}] + 0{\cdot}270$, ($r = 0{\cdot}990$, $P < 0{\cdot}01$). The intercept was almost the same value as $k_1$ (0·261 $h^{-1}$) at pH 7 (Table 1). These results suggest the reaction between cisplatin and low molecular mass nucleophilic species probably occurs according to model 3. The second-order rate constant for the direct reaction between cisplatin and glutathione was estimated to be 0·0441 $M^{-1}$ $h^{-1}$.

The decomposition of cisplatin was dependent on the concentrations of human albumin and globulin, but not on the $\alpha_1$-acid glycoprotein concentration as shown in Fig. 4. The $k_{app}$ value was linearly related to the concentration of albumin or

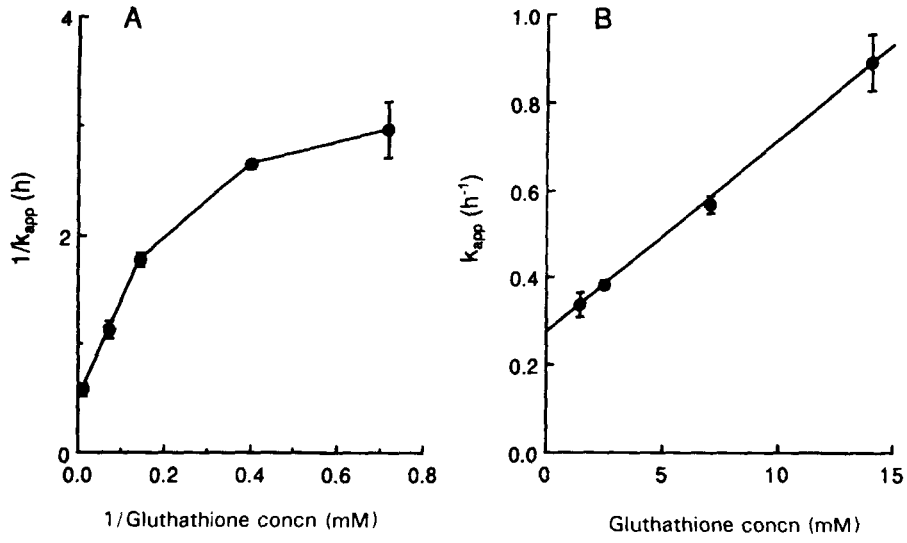



FIG. 3. Plots of $1/k_{app}$ versus 1/[glutathione] according to model 2 (A) and $k_{app}$ versus [glutathione] according to model 3 (B).

Table 1. Decomposition rate constants of cisplatin in aqueous solution, human plasma, ascites and urine.

| | pH | $[Cl^-]$ (mM) | Protein (g $dL^{-1}$) | $k_{app}$ ($h^{-1}$) | $k_1$ ($h^{-1}$) | $k_f[P]$ ($h^{-1}$) | $k_2[P]$ ($h^{-1}$) | $k_m[M]$ ($h^{-1}$) | $k_3[M]$ ($h^{-1}$) | $k_4[P]$ ($h^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 7·0 | 14 | 0 | 0·216 ±0·02 | 0·261 ±0·015 | | | | | |
| A + albumin | 7·0 | 14 | 1 | 0·317 ±0·005 | 0·263 | 0·405[Albumin] | | | | |
| | | | 2 | 0·509 ±0·008 | | | | | | |
| | | | 5 | 0·579 ±0·021 | | | | | | |
| | | | 10 | 0·817 ±0·166 | | | | | | |
| Plasma | 7·4 | 140 | 6·3 | 0·531 ±0·049 | 0·203 ±0·007 | 0·327 ±0·058 | 0·087 ±0·038 | 0·029 ±0·014 | 0·027 ±0·016 | 0·047 ±0·006 |
| Ascites | 8·0 | 110 | 1·5 | 0·437 ±0·004 | 0·269 ±0·018 | 0·118 ±0·037 | 0·077 ±0·055 | 0·079 ±0·044 | 0·059 ±0·039 | 0·041 ±0·017 |
| Urine | 5·5 | 420 | 0·24 | 0·235 ±0·044 | | | | | | |

A = phosphate buffer, ionic strength 0·148 M.

globulin ($k_{app} = 0{\cdot}405 \cdot [albumin] + 0{\cdot}263$; $k_{app} = 0{\cdot}717 \cdot [globulin] + 0{\cdot}236$ (Fig. 5)). The intercepts were very close to $k_1$ at pH 7 (0·261 $h^{-1}$). Therefore, the reaction between cisplatin and biological macromolecules also probably occurs according to model 3.

The sulphydryl groups act as nucleophiles to react with cisplatin. Of the sulphydryl groups, amino acids containing the SH group (cysteine and methionine) possibly form low molecular mass complexes with cisplatin (Riley et al 1983; Daley-Yates & McBrien 1984; Mistry et al 1989). In addition to low molecular mass nucleophilic species, the lone sulphydryl group (Cys-34) in human serum albumin is also responsible for the reaction with cisplatin (Momburg et al 1987). Multiple sites in human serum albumin which react with cisplatin have also been suggested (Momburg et al 1987). The rate constants for the direct reaction between cisplatin and proteins ($k_{Nu}$ in equation 13) were estimated to be about 9 (albumin) or 16 (globulin) times that of glutathione, suggesting that there are multiple reactive sites on proteins.

*Decomposition of cisplatin in biological fluids*

It has already been suggested that $k_{app}$ could be expressed approximately as the sum of $k_1$ and $k_{Nu}[Nu]$ (eqn 13). Furthermore, since previous studies and our unpublished data have suggested that fixed metabolites are also formed from mobile metabolites (Elferink et al 1986), the second-order formation rate constant of fixed metabolites from mobile metabolites ($k_4$) was considered. To describe the decomposition of cisplatin in human plasma and ascites, model 4 was proposed in the present study.

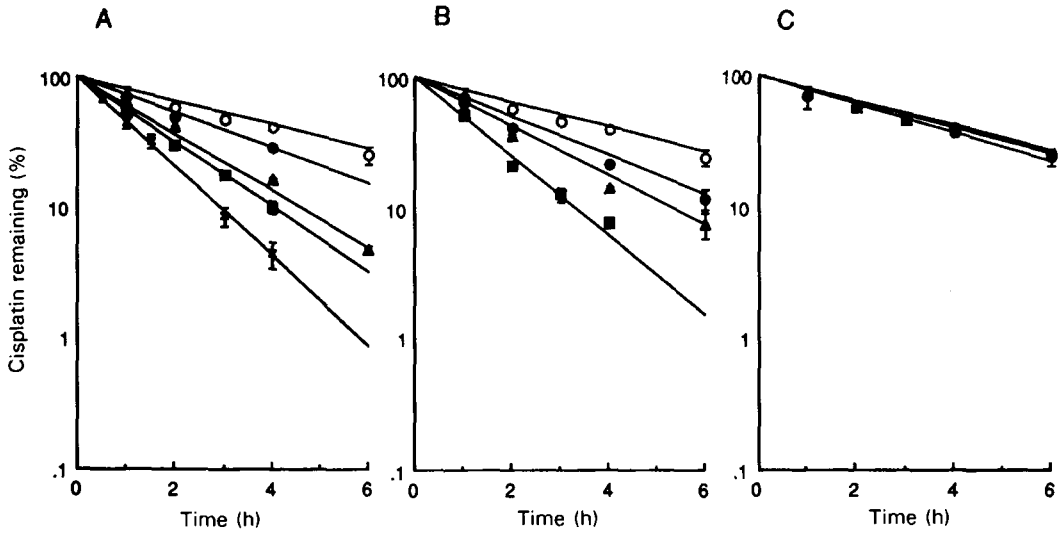



FIG. 4. Effects of albumin (A), globulin (B) and $\alpha_1$-acid glycoprotein (C) on the decomposition of cisplatin (30 $\mu$M) in phosphate buffer (pH 7) containing 14 mM chloride ions at 37°C. Albumin; ○ 0, ● 0·151 ▲ 0·302 ■ 0·754 × 1·508 mM; globulin; ○ 0, ● 0·125 ▲ 0·313 ■ 0·625 mM; $\alpha_1$-acid glycoprotein; ○ 0, ● 0·0123 ▲ 0·05 mM.

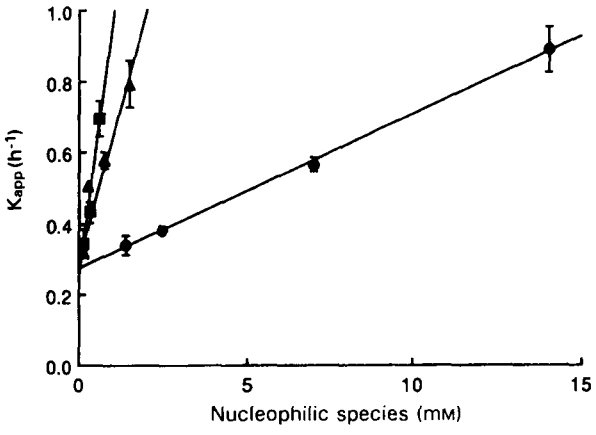



FIG. 5. Relationship between the concentration of nucleophilic species and $k_{app}$ in phosphate buffer (pH 7) containing 14 mM chloride ions at 37°C. ● Glutathione; ▲ albumin; ■ globulin.

Fig. 6 shows the decomposition of cisplatin (30 $\mu$M) in human biological fluids (plasma, ascites and urine) at 37°C. Cisplatin was eliminated mono-exponentially in all biological fluids. Fixed metabolites were a main product in plasma. Both fixed and mobile metabolites were formed in ascites. However, fixed metabolites were not detected in urine. The mobile metabolites tended to decrease over 4 and 8 h in both plasma and ascites. The simulation curves derived from equation 14 fitted well with the observed values. Table 1 summarizes the decomposition rate constants of cisplatin. The values of $k_{app}$ in plasma, ascites and urine were 0·531, 0·437 and 0·235 $h^{-1}$, respectively. The measured protein concentrations were 6·3 g $dL^{-1}$ (plasma), 1·5 g $dL^{-1}$ (ascites) and 0·24 g $dL^{-1}$ (urine), respectively. As about 70% of the total protein in plasma is albumin, the rate constant for the direct reaction between cisplatin and albumin can be calculated by substituting the albumin contribution (70%) of the plasma protein

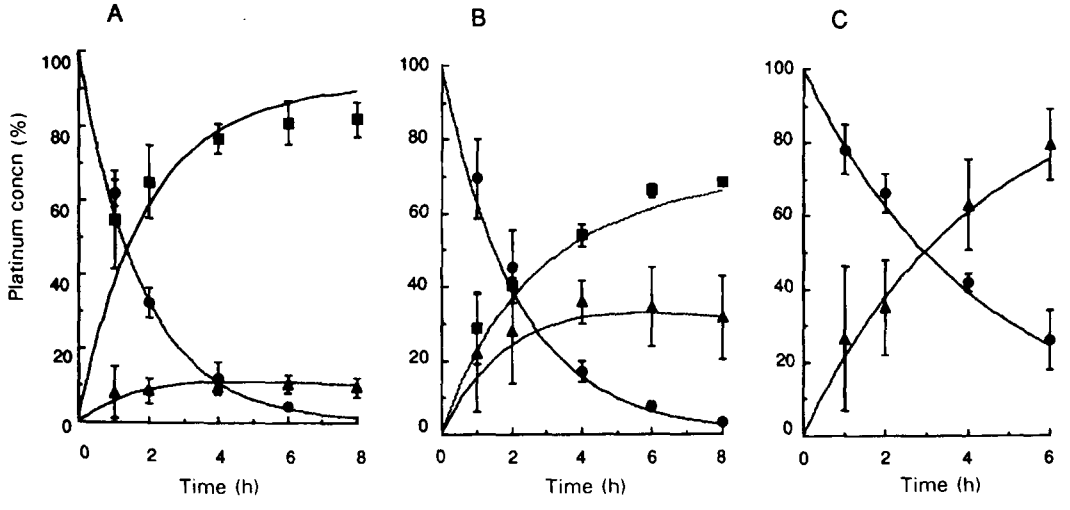



FIG. 6. Decomposition of cisplatin (30 $\mu$M) and formation of fixed and mobile metabolites in human plasma (A), ascites (B) and urine (C) at 37°C. ● Cisplatin; ■ fixed metabolites; ▲ mobile metabolites. The solid lines are simulation curves predicted according to model 4.

concentration (6·3 g $dL^{-1}$) in the obtained relationship (0·405 · [albumin], Table 1). The estimated albumin contribution was close to the computed value of $k_f[P]$ (0·327 $h^{-1}$, Table 1). Therefore, we suggest that a direct reaction between cisplatin and albumin might be the main route for cisplatin decomposition in plasma. Although the rate constant for the direct reaction between cisplatin and globulin was greater (0·717 $M^{-1}$ $h^{-1}$) than that of albumin (0·405 $M^{-1}$ $h^{-1}$), plasma globulin may not contribute to the decomposition of cisplatin because of the lower plasma level of globulin ( < 1 g $dL^{-1}$).

The protein concentration in urine was far less than that in plasma and ascites (Table 1). This may be the main reason why only mobile metabolites were detected in urine. Since the $k_{-1}$ value was more than 50 $M^{-1}$ $h^{-1}$ at pH < 5 (Fig. 2) and a higher chloride ion concentration was observed in urine, the same approximation (eqn 13) as used in the analysis of plasma and ascites can not be applied. The $k_{app}$ in acidic urine should thus be calculated as $k_1 \cdot k_2[Nu]/(k_{-1}[Cl^-] + k_2[Nu]) + k_{Nu}[Nu]$ (eqn 12).

We conclude that cisplatin is capable of reacting with numerous species in biological fluids to form fixed and mobile metabolites by the chloride ligand exchange reaction (Gullo et al 1980; Daley-Yates & McBrien 1984). The chloride ion concentration is a major determinant of cisplatin decomposition. The effect of pH was kinetically observed in the second-order rate constant for cisplatin re-formation. The decomposition behaviour of cisplatin in biological fluids could be described by a combination of hydration and direct reaction with nucleophiles leading to the formation of both fixed and mobile metabolites. The present results also show that albumin could play a significant role in the decomposition of cisplatin in plasma.

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