Speciation of Drugs in Blood Serum by Surfactant-Mediated HPLC/ICP-MS with Direct Sample Injection

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Speciation of cisplatin (CDDP; cis-diamminedichloroplatinum(II)), anti-cancer drug, in human blood serum has been carried out by surfactant- mediated HPLC/ICP-MS. In the surfactant-mediated HPLC, an ODS (octadesylsilica) column coated with CHAPS (3-[3-cholamidopropyl]-dimethylammonio) propanesulfonic acid), which is a zwitterionic surfactant obtained as a bile acid derivative, was employed as a stationary phase, and ICP-MS was used for detection of ^{195}Pt. Since the present surfactant-mediated stationary phase had the characteristics of rapid separation of large and small molecules with capability of direct sample injection even for blood serum without any pretreatment, CDDP and its complexes with cysteine and albumin in addition to some hydrolysis-type complexes were found in human blood serum.

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Introduction

Chemical speciation, which is hereafter referred to simply as "speciation", is defined as "analysis for identification and quantitation of the elements in the different chemical forms in various samples".[1,2] Although various elements provide some different biological, toxicological or physiological functions in the biological systems, such functions are more specific or characteristic, depending on their chemical forms even at the trace level. Presently, therefore, speciation analysis of various trace elements has received great attention in various scientific fields.[1,3]

In general, rather stable chemical species such as organometallic compounds of, Hg, As, Pb, Se, Sb and so on, have been subjected to speciation analysis because such organometallic compounds generally provide high toxicities to human bodies as well as to animals and plants. As is well known, CH_3HgCl caused Minamata disease as the result of environmental pollution in the Minamata Bay. In resent years, the present authors have reported the researches on speciation of trace elements in natural water[4,5] As a result, it was found that most of trace elements except for alkali and alkaline earth elements exist in the forms of large organic molecule-metal complexes (LOMMCs). In such a speciation study, a hyphenated analytical system combining a separation system with a detection system, for example, HPLC/ICP-MS, is generally employed for identification and quantitation of chemical species at the trace or ultratrace level.[1,3] Thus, high resolution of the separation system and high sensitivity of the detection system are required in efficient speciation analysis.

In recent years, the present authors have developed the CHAPS-coated ODS column[7-11] as a unique separation column, which allowed rapid separation of the large (molecular weight (MW) larger than 10000 Da) and small (MW smaller than 10000 Da) molecules. The CHAPS-coated ODS column was prepared by adsorbing CHAPS hydrophobically on the ODS column. Since proteins were not adsorbed on this column, it also allowed us to inject the blood serum samples directly without any pretreatment. Then, organic drugs and trace elements binding and non-binding with proteins in human blood serum could be successfully determined by using the CHAPS-coated ODS column with direct injection.[9-11]

Cysplatin is a well known anti-cancer drug in medicine.[12] However, it is also kown that cysplatin causes a serious kidney infection, when it is dosed into the cancer patients. Thus, it is still required to elucidate the chemical species in blood in relation with transport mechanisms to organs in body. In the present study, hence, a HPLC/ICP-MS system, in which the CHAPS-coated ODS column was employed as a separation column, was applied to speciation of cisplatin (CDDP; *cis*-diamminedichloroplatinum(II)) in blood serum to elucidate the chemical species or dissolved forms of CDDP in blood. As a result, it was found that CDDP existed in the complex forms bound with albumin and cysteine as well as in hydrolysis-type complexes.

Experimental

Apparatus

The HPCL system used in the present experiment consisted of a high-pressure pump (Model LC-10AD, Shimadzu, Kyoto, Japan), a sample injector (Model 7725, Rheodyne Cotani, CA, USA) with a 50 µl loop and a UV absorption detector (Model 870-UV, JASCO, Tokyo, Japan). An ODS column (L-column packed with 5 µm C_{18}-bonded silica, 250 mm x 4.6 mm i.d.) was purchased from the Chemical Inspection and Testing Institute, Tokyo, Japan). The ODS column coated with CHAPS was employed as the surfactant-mediated separation column in the present experiment. The CHAPS-coated ODS column was prepared by a dynamic coating method, in which CHAPS was adsorbed on the surface of the ODS column by the hydrophobic interactions, as described in the previous papers.[9,10] The 0.2 mM Tris-HCl buffer solution with 0.2 mM CHAPS (pH 7.4) was used as the mobile phase at the flow rate of 0.7 ml min^{-1}.

An ICP-MS instrument (Model SPQ 8000A, Seiko Instruments, Chiba, Japan) was used as an element-selective detector for HPLC. The capillary tubing of the nebulizer in the ICP-MS instrument was connected to the outlet of the UV absorption detector with a Teflon tubing. The operating conditions of the ICP-MS instrument were as usual, described in the previous paper.[6,7]

Blood serum samples

The human blood samples were collected from healthy volunteers through a silica-coated staineless-steel needle equipped on a 20 ml polypropylene syringe. These blood samples were stored in a silica-coated glass tube, and centrifuged at 3000 rpm for 20 min. The supernatant was used as the blood serum sample in the following experiment.

Chemicals

CHAPS was perchased from Aldrich Japan (Tokyo, Japan). Albumin and other proteins (blue detran, β-amylase, cytochrome C) used for the present experiment as well as for the column calibration were purchased from Sigma (St. Louis, USA). Inorganic salts and amino acids also used for the column calibration were of analytical reagent grade, obtained from Wako Pure Chemicals (Osaka, Japan). Pure water used throughout the present experiment was prepared with a Milli-Q pureification system (Nihon Millipore Kogyo, Tokyo, Japan).

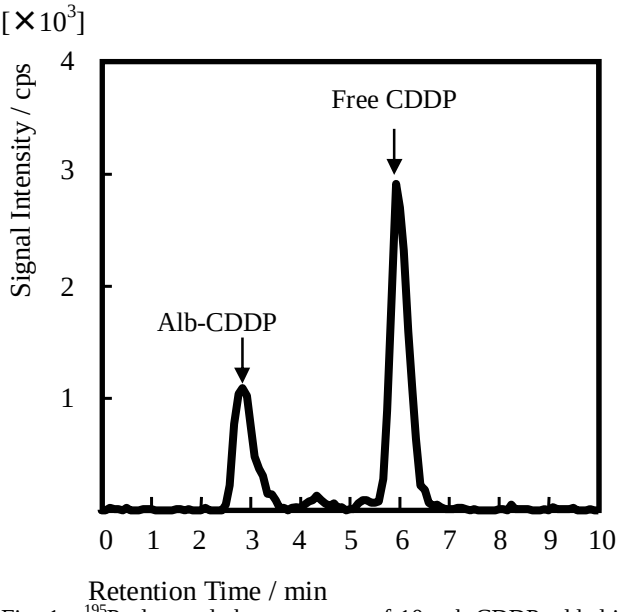

Fig. 1 ^{195}Pt-detected chromatogram of 10 ppb CDDP added in 2% albumin.
Column, CHAPS-coated ODS; mobile phase, 0.2 mM Tris-HCl and 0.2mM CHAPS (pH 7.4); flow rate, 0.7 ml/min; injection volume, 50 μl; detector, ICP-MS (m/z = 195).

Results and Discussion

Separation characteristics of CHAPS-coated ODS column

As reported previously,[10,11] the CHAPS-coated ODS column had an excellent separation capability for large and small molecules in a short retention time. However, when the CHAPS-coated ODS column was used only with 0.2 mM Tris-HCl buffer solution (pH 7.4) as the mobile phase, some deterioration in the long-term stability as well as in separation capability of the column was noticed in the previous experiment,[11] because CHAPSs adsorbed on the ODS column were gradually desorbed during the long-time use. In the present experiment, therefore, 0.2 mM CHAPS was added into 0.2 mM Tris-HCl buffer solution as a mobile phase to maintain the long-term stability of the CHAPS-coated ODS separation column. Since a new mobile phase, i.e., Tris-HCl buffer added with 0.2 mM CHAPS, was used in the present experiment, the retention behaviors of large and small molecules, such asproteins, inorganic anions, amino acids and caffeine, were examined again in a similar manner to the previous experiment.[10] The results for the retention times of analytes are summarized in Table 1 together with their molecular weights, where all the chromatographic signals were observed by a UV detector at 210 nm.

As can be seen in Table 1, large molecules such as blue dextran and proteins were eluted before 3 min, and other small

Table 1. Retention times of various analytes obtained by HPLC using the CHAPS-coated ODS column with UV absorption detection[a)]

Analyte	MW[b)]/ Da	Retention time/ min
Blue dextran	200000	2.29
β-Amylase	200000	2.35
Albumin	66000	2.55
Cytochrome C	12800	2.80
Cl^-	35.5	4.38
Br^-	60	4.78
I^-	79.9	5.80
L-glycine	75	3.87
L-leucine	131	4.00
L-phenylalanine	165	4.25
L-tryptophan	204	6.57
Caffeine	194	6.33

a) 0.2 mM Tris-HCl buffer solution containing 0.2 mM CHAPS (pH 7.4) was used.as the mobile phase
b) Molecular weight.

molecules were eluted after 3 min, but within 10 min. As has been reported previously,[7,8] halide ions (Cl^-, Br^-, I^-) can be separated in the present CHAPS-coated ODS column due to the electrostaic interactions between analytes and CHAPS, which is zwitterionic surfactant with cation and anion in one molecule. It is noted here that the retention times of the analytes shown in Table 1 were generally shorter than those observed by using the mobile phase without 0.2 mM CHAPS. However, the resolution in the chromatographic separation was not deteriorated even in the use of the mobile phase with 0.2 mM CHAPS. In addition, proteins were not adsorbed on the column, which allowed direct sample injection of the blood serum samples. Therefore, the CHAPS-coated ODS column was used as a surfactant-mediated stationary phase with the mobile phase with 0.2 mM CHAPS (pH 7.4) in the folllowing speciation study on cysplatin in blood serum.

Complex formation of cisplatin with albumin

The present surfactant-mediated HPLC/ICP-MS system was applied to speciation of cysplatin in the serum-model samples and real human blood serum samples. The chromatogram for 10 ng/ml (= ppb) CDDP added in the aqueous solution of 2% albumin is shown in Fig. 1, where 0.2 mM Tris-HCl with 0.2 mM CHSPA was used as the mobile phase and ^{195}Pt was detected by ICP-MS. The chromatogram in Fig. 1 was obtained 2 h after adding CDDP in albumin. It should be noted here that the peak at *ca.* 3 min as the retention time was not detected at 5 min after sample preparation, and its peak intensities increased with time, as will be seen in the time-sequential chromatograms shown in Fig. 2. It was confirmed by UV-absorption detection that albumin provided the chromatographic peak at *ca*. 3 min under the same conditions as those employed in the present experiment. Thus, the peak at *ca*. 3 min was assigned to albumin-CDDP complex. The peak at *ca*. 6 min is considered to correspond to free CDDP.

Formation of hydrolysis-type complexes of cisplatin

In Fig. 1, the ^{195}Pt-detected chromatograms for (i) 60 ppb CDDP and (ii) 60 ppb with 20 ppm (= μg/ml) $AgNO_3$ ([CDDP] : [$AgNO_3$] = 1 : 1000) are shown. In the chromatogram for CDDP without $AgNO_3$, a large peak corresponding to free CDDP was observed together with some other small peaks. In CDDP, 2 chloride ions are labile ligands, while 2 ammonia molecules are rigid ligands. Thus, chloride ions in CDDP are easily exchanged with water molecules in aqueous solution to form $[Pt(NH_3)_2(H_2O)Cl]$ and/or $[Pt(NH_3)_2(H_2O)_2]$.[14] It should be noticed here that the Tris-HCl buffer solution whose pH was 7.4 was used in the present experiment. Therefore, water molecules in platinum complexes are rapidly hydrolyzed at pH 7.4, and thus the above complexes including H_2O may be transformed to hydrolysis-type complexes such as $[Pt(NH_3)_2(OH)Cl]$ and/or $[Pt(NH_3)_2(OH)_2]$. In the experiment shown in Fig. 2, $AgNO_3$ was added to eliminate Cl^- as the precipitate of AgCl from the solution. Then, the peak at *ca*. 5.1 min could be assigned to $[Pt(NH_3)_2(OH)_2]$.

Speciation of cisplatin in human blood serum

The dissolved states of cisplatin in human blood serum were investigated by observing the ^{195}Pt-detected chromatograms for the analyte solution of cisplatin added in human blood serum. The time-sequential chromatograms obtained are shown in Fig. 3, where the chromatograms were observed at 5 min, 2 h, 5 h, and 25 h after adding CDDP in blood serum. It is clearly seen in Fig. 3 that the peak intensities of free CDDP at *ca*. 6 min decreased with passage of time, and the peak for free CDDP almost disappeared after 25 h. On the other hand, the peak

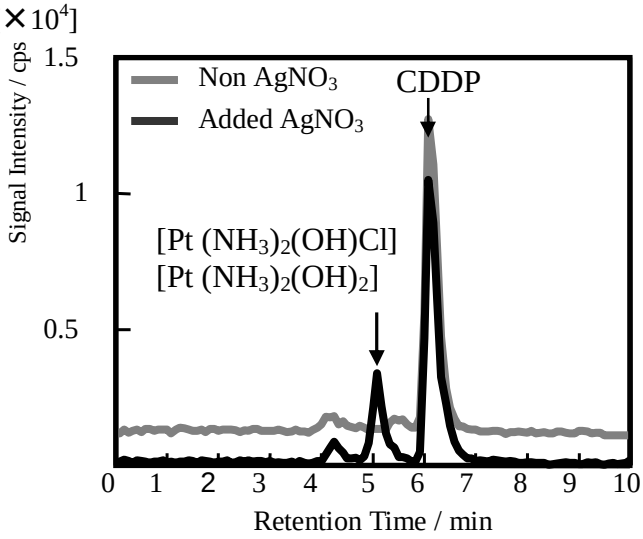

Fig. 2 ^{195}Pt-detected chromatograms of 60 ppb CDDP with and without 20 ppm $AgNO_3$.
Column, CHAPS -oated ODS; mobile phase, 0.2 mM Tris-HCl and 0.2mM CHAPS (pH 7.4); flow rate, 0.7 ml/min; injection volume, 50 μl; detector, ICP-MS (m/z = 195).

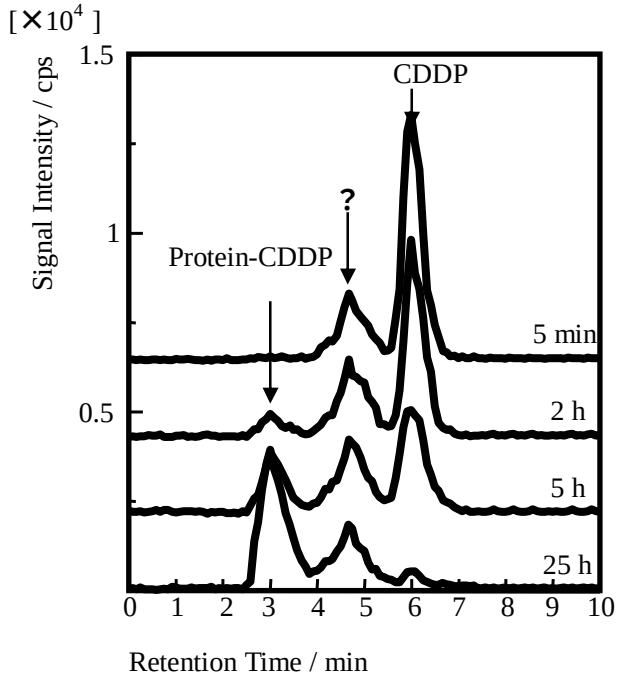

Fig. 3 ^{195}Pt-detected chromatogram of 30 ppb CDDP added in human serum;.
Column, CHAPS-coated ODS; mobile phase, 0.2 mM Tris-HCl and 0.2mM CHAPS (pH 7.4); flow rate, 0.7 ml/min; injection volume, 50 μl; detector, ICP-MS (m/z = 195).

intensities at *ca*. 3 min significantly increased with time. These results indicate that CDDP gradually combines with albumin, which is main blood serum protein.

In addition, a new peak was observed near 4.8 min immediately after adding CDDP into blood serum, as is seen on the chromatograms shown in Fig. 3. According to the recent work by El-Khateeb *et al*.,[14] it is known that CDDP forms the complexes with cysteine in blood serum. The mono-nuclear and bi-nuclear complexes of CDDP with cysteine (Cys), such

as $[Pt(NH_3)_2(OH)Cys]$ and/or $[Pt_2(NH_3)_4(Cys)_2]$ have been suggested by their NMR study. Although we cannot identify the species corresponding to the peak at *ca.* 5 min in Fig. 3 only from the ^{195}Pt-detected chromatograms, it is quite reasonable that the peak at *ca.* 5 min corresponds to one of the mixed complexes suggested above.

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