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Effect of *trans*-diamminedichloroplatinum(II) on human serum albumin: conformational changes through partial disulfide bond cleavage

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Abstract

trans-Diamminedichloroplatinum(II) (*trans*-DDP), the *trans* isomer of *cis*-diamminedichloroplatinum(II) (*cis*-DDP), was bound to human serum albumin (HSA) in pH 7.4 buffer containing 0.1 M NaCl at 37°C. The amount of bound *trans*-DDP per mol of HSA was found to be 21.4 mol when the protein was incubated with a 40-fold excess of *trans*-DDP for 6 days. In *trans*-DDP-treated HSA, 3.4 disulfide (S-S) bonds were cleaved, where one HSA molecule contains 17 S-S bonds. The spectral characteristics of *trans*-DDP-treated HSA were examined in terms of the fluorescence spectrum of its lone tryptophan (Trp-214), and molar ellipticity. The relative fluorescence intensity of platinum-bound HSA decreased to 32.4% of that of the native state, suggesting that perturbation around the Trp-214 residue took place. This was confirmed by the destruction of the warfarin-binding site containing Trp-214 observed in the metal-bound HSA. Analysis of circular dichroism (CD) spectra showed a decreasing helix content from 50.5% in the native state to 30.6% in the metal-bound HSA. These conformational changes observed in HSA may be attributed to the S-S bond rupture induced by *trans*-DDP. Comparison with *cis*-DDP, which has already been shown to cleave S-S bonds in HSA, revealed that *trans*-DDP binds to HSA and cleaves S-S bonds more readily than the *cis* isomer.

Keywords: *trans*-Diamminedichloroplatinum(II); Human serum albumin; Protein binding; Conformational change; Disulfide bond; Secondary structure; Warfarin; Fluorescence; CD

1. Introduction

trans-Diamminedichloroplatinum(II) (*trans*-DDP), a stereo-isomer of *cis*-diamminedichloroplatinum(II) (*cis*-DDP), is known to bind DNA with a reactivity similar to that of *cis*-DDP in vitro but exerts less therapeutic effect (Lempers and Reedijk, 1991). Both *cis*- and *trans*-DDP are first hydrolyzed with the loss of one chloride to form the monoaquated species (Miller and House, 1990). This step is rate-determining for DNA binding to produce monofunctional adducts and there is little difference in reactivity between *cis*- and *trans*-DDP (Bancroft et al., 1990). A subsequent step for bifunctional adduct formation is

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also controlled by the second hydrolysis of another chloride. Similarity in the second hydrolysis rate constants for *cis*- and *trans*-DDP was also demonstrated (Bancroft et al., 1990).

However, Dedon and Borch (1987) reported that *trans*-DDP reacts with glutathione about 300-fold faster than *cis*-DDP. It suggests that the retarded biological activity of *trans*-DDP seems to partially result from the consumption of *trans*-DDP due to the rapid nucleophile displacement reaction with sulfur-containing nucleophiles such as glutathione before the drug reaches the DNA (Bancroft et al., 1990). Plasma contains many nucleophiles including proteins to which platinum complexes are exposed after administration. Proteins have been shown to react with the platinum complexes via a sulfhydryl group, methionine (Met) and histidine (His) residues (Howe-Grant and Lippard, 1980; Lempers and Reedijk, 1991). Platinum binding to serum albumin and inactivation of plasma proteinase inhibitors such as α_2-macroglobulin and α_1-antitrypsin have been reported (Gonias and Pizzo, 1983; Gonias et al., 1988; Roche et al., 1989).

We have reported that *cis*-DDP causes disulfide (S-S) bond cleavage of human serum albumin (HSA) and fibrinogen, and leads to changes in their secondary structure (Ohta et al., 1992b, 1993). At least four S-S bonds in HSA were cleaved among 17 S-S bonds when incubated with a 60-fold excess of *cis*-DDP for 2 weeks at 37°C. The result indicates that the S-S bond is also a target of *cis*-DDP in addition to the lone sulfhydryl group, cysteine (Cys)-34 in HSA. The present study is concerned with whether *trans*-DDP exerts S-S bond cleavage similar to *cis*-DDP. The effects of *trans*-DDP on the structural integrity of HSA were studied in terms of fluorescence and circular dichroism (CD) spectral measurements. Warfarin binding was examined as a measure of change in HSA structure.

2. Materials and methods

2.1. Materials

trans-DDP was purchased from Aldrich Chemical Co., Milwaukee and used as received. HSA (fraction V, lot no. 116F-9363, Sigma, St. Louis) was used after being defatted. Disodium 2-nitro-5-thiosulfobenzoate (NTSB) was synthesized according to the method of Thannhauser et al. (1984). Potassium warfarin (racemate) was a gift from the Eisai Co., Tokyo and used as received. A Coomassie Brilliant Blue G kit (Bio-Rad, Richmond, CA) was used for the protein assay. All other chemicals were of reagent grade.

2.2. Binding of trans-DDP to HSA

trans-DDP and HSA were incubated at various molar ratios up to 40 (*trans*-DDP/HSA) at 37°C in a phosphate buffer solution (0.05 M, pH 7.4) containing 0.1 M NaCl where the protein concentration was always maintained at 10 mM. Each mixed solution was filtered through a 0.2 μm sterilized filter (Toyo Roshi, Tokyo) and placed in a sterilized test tube with a screw cap. This operation was always conducted in a glove box to avoid contamination. The mixed solutions were incubated over a 6 day period under light protective conditions, during which one of the tubes was separately removed as a function of time for various analyses.

2.3. Separation of unbound trans-DDP and HSA

At appropriate time intervals, 0.5 ml of the reaction solution was removed and applied on a gel filtration column (Sephadex G-25, 1.0 cm i.d. $\times$ 18 cm, Pharmacia, Uppsala) equilibrated with a pH 7.4 phosphate buffer containing 0.1 M NaCl. The fraction of *trans*-DDP was assayed in each 2.0 ml fraction and the sum of the amount in each fraction was assumed to be the total amount of unbound *trans*-DDP in the mixture.

2.4. Determination of platinum complexes and HSA

trans-DDP was assayed using a flameless atomic absorption spectrophotometer (AA-670G, Shimadzu, Kyoto) equipped with a graphite furnace atomizer (GFA-4A, Shimadzu, Kyoto). The three-stage heating program used was as follows: 100°C for drying, 1000–1350°C for ashing for 30 s and 2700°C for atomizing for 8 s. The injection volume was 10 μl. The native HSA concentration

was assayed at 278 nm, and the protein treated with *trans*-DDP was assayed using the Coomassie Brilliant Blue G kit (Bio-Rad). The percentage of mercaptalbumin, in which Cys-34 is reduced, was 19.3%. All other chemicals were of reagent grade.

2.5. Determination of number of cleaved S-S bonds

The number of cleaved S-S bonds of HSA was estimated using the NTSB method (Thannhauser et al., 1984; Kella and Kinsella, 1985). HSA solution (0.5 ml) was mixed with an NTSB assay solution (3 ml) with a freshly prepared Na_2SO_3 solution (0.4 M). After 10 min incubation, the absorbance at 412 nm was monitored against an appropriate blank. The concentration of cleaved S-S bonds was calculated using the extinction coefficient of 2-nitro-5-thiobenzoic acid (1.36×10^4 M^{-1} cm^{-1}).

2.6. Fluorescence and CD spectral measurements

The fluorescence spectra of the Trp in HSA were recorded on a spectrofluorophotometer (RF-520, Shimadzu, Kyoto) at an excitation wavelength of 300 nm as described in a previous report (Ohta et al., 1992b). Relative fluorescence intensity changes at the emission maximum wavelength of 350 nm were followed for different incubation times at various molar ratios of the components (HSA, 10 mM; *trans*-DDP, 0.1–0.4 mM). The medium was the same as that used in the binding study. Using these samples, CD spectral measurement and estimation of secondary structure were performed as described previously (Ohta et al., 1992b). Calculations of the secondary structure combinations which provide the best fit to the CD spectra were carried out. The analysis utilizes the CD spectra of proteins containing known amounts of each secondary structure. The standard spectra of the following proteins were utilized for these estimates: myoglobin, parvalbumin, insulin, lactate dehydrogenase, lysozyme, cytochrome *c*, carboxypeptidase A, thermolysin, subtilisin BPN′, papain, trypsin inhibitor, ribonuclease S, nuclease, ribonuclease A, and concanavalin A, according to the method of Yang et al. (1986).

2.7. Warfarin binding to HSA

Fluorometric titration was carried out as follows: HSA solution incubated with *trans*-DDP at various molar ratios up to 40 for 6 days was diluted to 2 mM and placed in a 1 cm quartz cell (25°C), followed by the addition of warfarin solution, giving a final concentration ranging from 0.5 to 15 mM using a microsyringe (Otagiri et al., 1979). The mixture was excited at 335 nm and the emission was measured at 378 nm using a spectrofluorophotometer.

3. Results and discussion

3.1. trans-DDP binding to HSA

Fig. 1 shows the binding of *trans*-DDP to HSA as a function of incubation time at various molar ratios of *trans*-DDP to HSA over a 6 day period. The characteristics of the time course were as follows by comparing the reaction of *cis*-DDP with HSA: (i) the binding reactions did not proceed to reach a common equilibrium, which were similar to those of *cis*-DDP; (ii) the binding of *trans*-DDP occurred in a relatively monotonic manner with time compared with that of *cis*-DDP which consisted of a two-stage reaction; and (iii) the magnitude of *trans*-DDP binding was greater

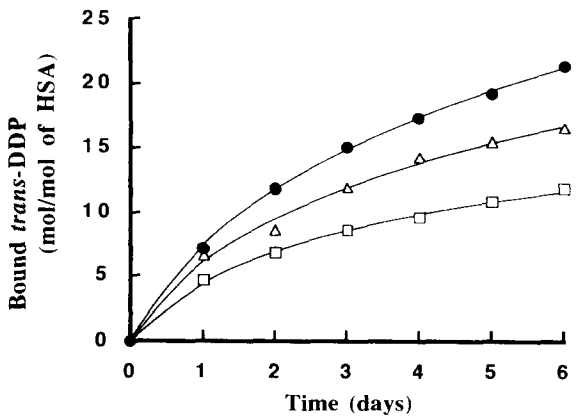

Fig. 1. Time course of *trans*-DDP binding to HSA incubated at various molar ratios at 37°C. Molar ratio (*trans*-DDP/HSA): 20 (□), 30 (△) and 40 (●). Concentration of HSA: 10 mM.

than that of *cis*-DDP binding except for early incubation periods (Yotsuyanagi et al., 1991). Binding of *trans*-DDP to HSA was about 2-fold greater in extent than that of *cis*-DDP. When a 20- or 40-fold excess of platinum complex to HSA was incubated, almost the same amounts of both platinums were bound with incubation for 1 day. However, after 6 days, the bound amount of *trans*-DDP was 11.9 or 21.4 mol per HSA molecule whereas bound *cis*-DDP amounted to 5.3 or 9.7 mol. It seems that further binding would take place by increasing the incubation time. This tendency was also demonstrated in *cis*-DDP binding. However, incubation with a drug concentration higher than 0.4 mM or for a period longer than 6 days in 0.4 mM *trans*-DDP solution made the reaction mixture slightly opaque. Binding sites in proteins likely to be available for platinum(II) complexes are Cys, Met and His residues (Ratilla et al., 1987; Lempers and Reedijk, 1991). The first binding of *cis*-DDP to HSA has been demonstrated to occur at the lone sulfhydryl group, Cys-34 (Gonias and Pizzo, 1983), whereas Pizzo et al. (1988) showed that Cys-34 is not involved in *trans*-DDP binding. This difference was not reflected in the different number of bound platinums observed between *trans*-DDP and *cis*-DDP. Since HSA contains six residues of Met and 16 residues of His (Dugaiczyk et al., 1982), these residues might be involved in the very large number of *trans*-DDP bindings (Fig. 1). In previous reports with *cis*-DDP, the S-S bond was found to serve as a platinum-binding site in plasma proteins, including HSA, fibrinogen and γ-globulin (Ohta et al., 1992a,b; Chen et al., 1994). Thus, the effect of *trans*-DDP on HSA S-S bonds was examined.

3.2. S-S bond cleavage

Fig. 2 shows that the number of S-S bonds decreased in HSA as a function of incubation time at various ratios of *trans*-DDP to HSA over a 6 day period. A decrease in the total number of 17 S-S bonds in HSA was observed, demonstrating that the S-S bond is susceptible to cleavage by *trans*-DDP as well as by *cis*-DDP. With the low molar ratios of *trans*-DDP to HSA, the cleavage

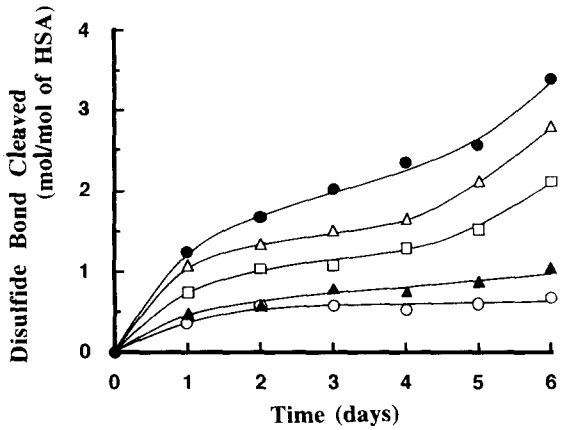

Fig. 2. Number of disulfide bonds of HSA cleaved with time at various molar ratios. Molar ratio (*trans*-DDP/HSA): 5 (○), 10 (▲), 20 (□), 30 (△) and 40 (●). Concentration of HSA: 10 mM.

proceeded at a moderate rate up to about one S-S bond and then reached a plateau. Meanwhile, with increased molar ratios and after the first or second S-S cleavage, the curves turned upward slightly indicating that further cleavage would occur at a relatively high rate. Since S-S bonds are essential for the maintenance of the native conformation of HSA (Lee and Hirose, 1992; Ohta et al., 1992a), the first S-S cleavage might induce a structural alteration such that the second or third S-S bond becomes more available for further cleavage. When a 20- or 40-fold excess of initial platinum complex concentration to HSA was incubated, the bonds cleaved by *trans*-DDP amounted to 2.1 or 3.4 mol whereas *cis*-DDP cleaved 1.2 or 1.6 mol after 6 day incubation. The number of S-S bonds cleaved by *trans*-DDP was about double that by *cis*-DDP. This is consistent with the difference in the bound platinum between *trans*- and *cis*-DDP previously described. It should be referred to the HSA fraction V used in this study, which contains about 15% of dimer as calculated by SDS-PAGE analysis. Since we used HSA without removal of polymer, the S-S bonds cleaved in HSA may contain intermolecular S-S bonds of dimer as well as intramolecular S-S bonds provided that the dimer is formed only by intermolecular S-S bridge and furthermore

this bridge is preferentially accessible to platinum attack.

Fig. 3 shows the relationship between the bound *trans*-DDP and the cleaved S-S bonds of HSA. A linear relationship having a slope of about 0.141 ($r = 0.947$) was obtained regardless of the initial *trans*-DDP to HSA molar ratios. This indicates that the S-S bonds were cleaved at a ratio of one S-S bond per 7.1 mol of *trans*-DDP binding in the range of the total bond cleavage up to about 3.4 bonds. The relatively low correlation coefficient of 0.947 compared with 0.991 for *cis*-DDP can be explained by the tendency to an upward increase in the cleaved number around 2 and 3. This result together with those in Fig. 2 suggests that the first or second S-S cleavage, and some resulting conformational changes might lead to further cleavage, rather than further binding, occurring more readily. Although the distribution of 17 S-S bonds shows homology in the whole albumin molecule and they link together several regions of equal size, the accessibility of *trans*-DDP to these S-S bonds appears to be different (Saber et al., 1977). In addition to the S-S bond, Met and His residues in HSA are conceivably responsible for the multiple binding of *trans*-DDP. Cys-34 has been shown not to involve *trans*-DDP binding to albumin (Pizzo et al., 1988).

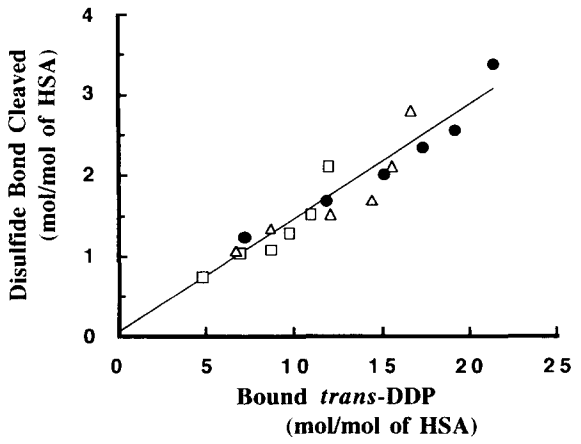

Fig. 3. Relationship between bound *trans*-DDP and cleaved disulfide bond. *trans*-DDP and HSA were incubated at pH 7.4 (phosphate buffer with 0.1 M NaCl) and 37°C for 6 days. Molar ratio (*trans*-DDP/HSA): 20 (□), 30 (△) and 40 (●). Concentration of HSA: 10 mM. A linear regression analysis gave $Y = 0.141X + 0.046$ ($r = 0.947$).

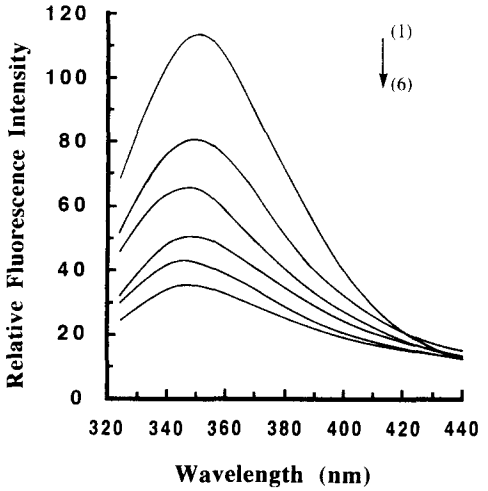

Fig. 4. Fluorescence spectral changes of HSA incubated with *trans*-DDP at various molar ratio for 6 days. Molar ratio (*trans*-DDP/HSA): (1) 0, (2) 5, (3) 10, (4) 20, (5) 30, (6) 40. Concentration of HSA: 10 mM. Excitation at 300 nm.

3.3. Fluorescence spectral change

The cleavage of S-S bonds by *trans*-DDP would give rise to a structural alteration, since the S-S bond is important for stabilizing the native structure of globular proteins. Trp fluorescence is the most frequently used among the three intrinsic aromatic fluors in HSA molecules as a measure of conformational perturbation at or near the residue. Excitation at 300 nm, a fluorescence efficiency around 350 nm reflects changes in the environment of the lone Trp residue (Trp-214). Fig. 4 shows typical changes in the fluorescence spectrum of the reaction mixture in which *trans*-DDP and HSA were incubated at various molar ratios for 6 days. The fluorescence spectra of HSA were gradually quenched with increasing amounts of *trans*-DDP. The maximum fluorescence wavelength showed a blue shift from 350 to 346 nm on excitation at 300 nm. The observation of a similar blue shift in the intrinsic Trp residue(s) has been reported in the reduced forms of HSA and BSA (Kella et al., 1988; Lee and Hirose, 1992). On excitation at 295 nm, the emis-

sion maximum at 340 nm for the native form shifted to 328 nm for the completely reduced HSA prepared under nondenaturing conditions (Lee and Hirose, 1992). According to the latter authors and Kuwajima (1989), the blue shift can be possibly explained by the 'molten globule'-like state, which is compact to some extent and assumes a native-like secondary structure. However, the differentiation from the native state is the absence of close packing throughout the molecule and the increase in fluctuations in the side chains. In the molten globule-like state of the reduced HSA, a hydrophobic cluster is likely to be formed from the remaining secondary structure segments by hydrophobic interactions. The interaction of the hydrophobic cluster and Trp residue probably accounts for the blue shift in Trp fluorescence.

The blue shift of Trp by *trans*-DDP observed in Fig. 4 could be similarly explained. This suggests that *trans*-DDP might induce S-S cleavage and thus the formation of the molten globule-like state of HSA and the hydrophobic cluster which is capable of interacting with Trp-214. The fact that no blue shift was detected in *cis*-DDP-treated HSA requires the consideration of an additional factor which induces a red shift, such as an unfolded state. Fig. 5 demonstrates the time courses of fluorescence quenching at 350 nm. The relative fluorescence intensity decreased with incubation time at each molar ratio. In a preliminary experiment, the fluorescence spectrum of HSA was not immediately changed after the addition of *trans*-DDP. Furthermore, no changes in the fluorescence intensity were observed for the control HSA solution without *trans*-DDP over an incubation period of 6 days. Therefore, the fluorescence quenching was exclusively induced by some change in HSA which was caused by platinum binding to the region including Trp-214. In 3.4 S-S cleaved HSA, a decrease in intensity to 32.4% was observed. Some changes around Trp-214 were also confirmed by a warfarin-binding study which is discussed in section 3.5.

3.4. CD spectral change

To obtain further information about the structurally perturbed HSA, CD measurement and

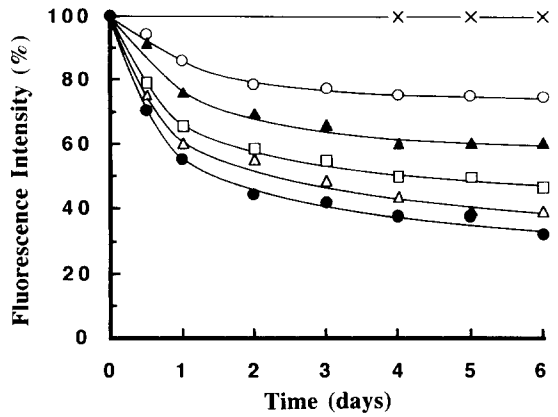

Fig. 5. Time courses of relative fluorescence changes of HSA incubated with *trans*-DDP at various molar ratios for 6 days. Molar ratio (*trans*-DDP/HSA): 0 (×), 5 (○), 10 (▲), 20 (□), 30 (△) and 40 (●). Concentration of HSA: 10 mM. Excitation at 300 nm and emission at 350 nm. The relative fluorescence intensity of native HSA was set as 100%.

secondary element analysis were performed. The CD spectra for *trans*-DDP-treated and untreated HSA are presented in Fig. 6. CD spectra in the ultraviolet region exhibit two negative bands at 208 and 222 nm which are characteristic of an α-helix. These bands gradually increased with increasing amounts of the initial *trans*-DDP, although they are similar in shape. For secondary structural analysis, 45 data points at intervals of 1 nm between 196 and 240 nm were obtained, and the CD spectra were analyzed according to the

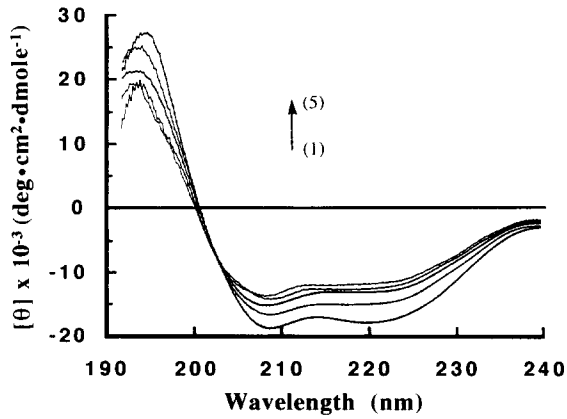

Fig. 6. CD spectra of HSA incubated with *trans*-DDP at various ratios. Molar ratio (*trans*-DDP/HSA): (1) 0, (2) 10, (3) 20, (4) 30, (5) 40. Concentration of HSA: 10 mM.

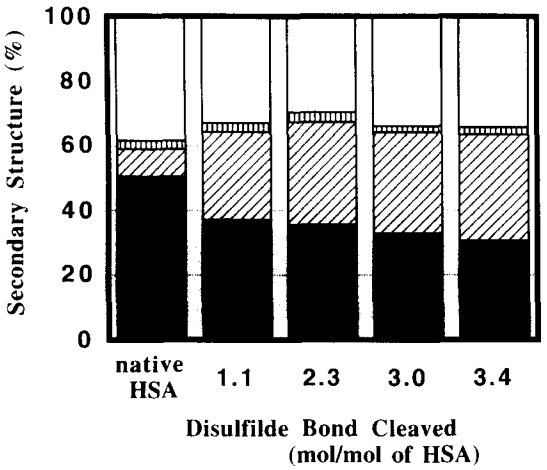

Fig. 7. Secondary structural changes of HSA incubated with *trans*-DDP for 6 days. Relative contents of α-helix (cross-hatched bars), β-sheet (diagonally hatched bars), β-turn (vertically hatched bars) and random coil (empty bars) are expressed as a function of disulfide bonds cleaved by *trans*-DDP.

method of Yang et al. (1986). These data points were fitted by a superposition of the spectra of 15 reference proteins of known three-dimensional structure. Secondary structure compositions were calculated by the non-linear curve-fitting program written in BASIC (Ohta, unpublished data). The quality of the fit was evaluated by calculating a normalized standard deviation (NRMSD) (Brahms and Brahms, 1980). An NRMSD value of less than 0.1 was assumed to provide a good fit of a CD spectrum obtained. Fig. 7 shows the relative proportions of α-helix, β-sheet, β-turn and random coil. The content of α-helix, 50.5% in native HSA, was decreased to 30.6% when HSA was incubated with a 40-fold excess of *trans*-DDP for 6 days. This represents the loss of helical conformation corresponding to 116 residues out of 295 residues in the native form, according to our calculation. This conformational change seems to be significantly large despite small changes in the CD spectra (Fig. 6). Such large conformational changes have not been reported in the drug-HSA binding studies being carried out widely. The helix content gradually decreased with increasing cleavage of the S-S bond, and the decrease approximately corresponds with an increase in the β-sheet.

This result suggests that *trans*-DDP binding to HSA through S-S cleavage causes a secondary structural alteration destabilizing the α-helix as well as the local structural change around Trp-214. A similar tendency was demonstrated in CD studies of *cis*-DDP-treated HSA or reduced HSA by sulfitolysis (Ohta et al., 1992b). The degree of helix destabilization was not dependent on the S-S cleaving reagent used but on the number of cleaved S-S bonds.

3.5. Binding of warfarin to trans-DDP-treated HSA

The high-affinity binding site for warfarin has been shown to locate in a region including Trp-214 and shares a common binding site with phenylbutazone, aspirin and some sulfonamides (Kragh-Hansen, 1990). The above fluorescence study reflects the environment around Trp-214 and leads to the expectation of an unfavorable effect of *trans*-DDP on the warfarin-binding site of HSA. Thus, the binding of warfarin to *trans*-DDP-treated HSA was investigated using fluorescence spectroscopy. When excited at 335 nm, warfarin shows a weak fluorescence which is increased by

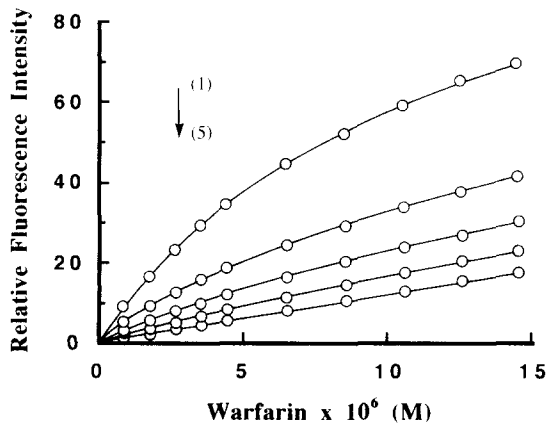

Fig. 8. Relative fluorescence changes of warfarin with *trans*-DDP-treated HSA. *trans*-DDP-treated HSA (2 mM throughout) was used after the protein was incubated with *trans*-DDP at different molar ratios for 6 days. Molar ratio (*trans*-DDP/HSA): (1) 0, (2) 10, (3) 20, (4) 30, (5) 40 at the concentration of 10 mM HSA. Excitation at 335 nm and emission at 378 nm.

the addition of HSA. Fig. 8 shows the developments in warfarin fluorescence on the addition of an increasing amount of warfarin into the *trans*-DDP-treated HSAs. In these measurements, the incubation mixtures of *trans*-DDP and HSA were used without removing unbound *trans*-DDP, since a preliminary test ascertained that the fluorescence of warfarin was independent of the presence of free *trans*-DDP. Thus, the intensity changes can be considered as a measure of the magnitude of warfarin binding. The development of warfarin fluorescence by HSA was increasingly diminished when the preparation of *trans*-DDP-treated HSA was carried out with increasing amounts of *trans*-DDP. The conditions used in this study (2 μM HSA and 1–15 μM warfarin) seem to mainly reflect the primary binding for warfarin. The number of primary and secondary binding sites and corresponding dissociation constants, n_1, n_2, K_1 and K_2 are 1, 1, 5.4×10^5 M^{-1} and 1.1×10^4 M^{-1}, respectively (Otagiri et al., 1979). From these values, the occupancy of the primary and secondary binding sites by 14 μM warfarin is calculated to be 86.9 and 12.5%, respectively. Thus, the result shown in Fig. 8 suggests a reduction in the warfarin-binding capacity at the primary site of HSA due to *trans*-DDP. The result indicates that *trans*-DDP binding and the rupture of S-S bridges causes an impairment of the warfarin-binding site located near Trp-214, consistent with the result obtained by the fluorescence quenching study.

When comparing the activity of *trans*-DDP and *cis*-DDP, the *cis* complex required a longer incubation time than the *trans* isomer to bind an equal number of platinum atoms or to cleave an equal number of S-S bridges in HSA. For example, in order to bind 15 mol of platinum per mol of HSA, 3 days are required for *trans*-DDP, while 14 days are needed for *cis*-DDP, when the initial molar ratio of the platinum complex to HSA is 40. In order to cleave three S-S bonds of HSA, 5 or 6 days are required for *trans*-DDP, while 14 days are necessary for *cis*-DDP. Thus, the overall reaction rate of *trans*-DDP is about 2-fold greater than that of *cis*-DDP. In contrast, the extent to which alterations in the HSA structure, reflected in the quenching of Trp-214 fluorescence, helix destabilization and decreased binding of warfarin, induced probably by bound platinums through the S-S bond were almost the same except that the blue shift of Trp-214 was induced only by the *trans* isomer, where an equal number of S-S bonds was cleaved by both DDPs. This observed higher reactivity of *trans*-DDP than *cis*-DDP is consistent with a previous report in which *trans*-DDP was observed to be more readily incorporated than *cis*-DDP into proteins, including α_2-macroglobulin, α_1-proteinase inhibitor, and human and bovine serum albumin (Gonias et al., 1988), although the authors suggested a Cys or Met residue as platinum-binding site.

From these results, it is apparent that the interaction of *trans*-DDP with HSA causes: (i) the binding of *trans*-DDP at multiple sites and the cleavage of S-S bonds in HSA; (ii) a local perturbation of the warfarin-binding site on the HSA molecule including Trp-214; and (iii) a conformational change with the loss of helix stability in HSA. Several factors which influence the stability of the helix structure in proteins have been discussed (Sheridan et al., 1982). Among them, the antiparallel alignment of helices is likely to stabilize the helical structure in the case of HSA (He and Carter, 1992). The HSA molecule consists of three domains, each with a domain formed by two subdomains. The three helices, which are constructed to make a subdomain, are fixed by two double Cys bridges. Since 16 S-S bonds out of 17 bonds on the HSA molecule form eight pairs of double Cys bridges, the observed helix destabilization induced by *trans*-DDP may arise from the cleavage of some of these S-S bridges. Thus, a possible explanation is that the S-S bonds play a critical role in the stability of the α-helix in the HSA molecule.

The previous work suggests that the biological inactivity of *trans*-DDP may be the result of selective trapping by sulfur-containing nucleophiles such as reduced glutathione in cell or body fluid before it binds to DNA (Bancroft et al., 1990), although they were not referring to the S-S bond but to a Cys or Met residue as the binding site. The present study shows the possible contribution of the S-S bond in proteins to the inactivity of *trans*-DDP on administration.

Acknowledgements

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