Synthesis, Characterization and Interaction with DNA of Dichlorobiscyclo-(glycyl-L-methionyl)platinum(II)

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Pt(II) complexes such as cisplatin [*cis*-dichlorodiammineplatinum(II)] constitute a new class of potent anticancer drugs for general use in cancer chemotherapy. A variety of studies (see for example [1]) points towards DNA as the primary target molecule for platinum interaction. However, one possibility [2–4] is a DNA–protein crosslink. The high affinity of platinum for sulfur suggests the possibility of metalation at cysteine or methionine residues of a polymerase.

Models for such interaction should therefore be platinum(II) derivatives containing S(methionyl)–Pt coordination bond(s). Given the well known [5] usefulness of cyclic dipeptides (diketopiperazines) as model compounds in peptide and protein research, we decided to employ as Pt(II) ligands methionyl-containing diketopiperazines. We describe in this communication the coordination compound by reaction between Pt(II) and cyclo-(glycyl-L-methionyl) (CGM) and its interaction with salmon sperm DNA.

The ^{1}H NMR data for the free ligand in $(CD_3)_2SO$ show the following features. two broad signals at δ = 8.18 and 7.94 (NH); a pseudotriplet centered at δ = 3.84 (CH); a doublet at δ = 3.74 (CH_2 glycyl), ^{3}J (NHCH) = 1.8 Hz; a sharp singlet at δ = 2.02 (CH_3); a complex pattern in the δ range 1.94–2.50 (two CH_2 of methionyl side chain). A similar pattern is obtained in D_2O solution (apart from the obvious disappearance of the NH signals): a pseudotriplet centered at δ = 4.15 (CH); a singlet at δ = 3.99 (CH_2 glycyl); a complex pattern at δ = 2.75–2.45 (CH_2); a sharp singlet at δ = 2.04 (CH_3), a complex pattern at δ = 1.81–2.2 (γCH_2).

From the ^{3}J(NHCH) of glycyl protons it can be argued that the diketopiperazine ring is essentially planar in both solvents [5]. This is corroborated by circular dichroism studies [6] and is in agreement with the X-ray structure [7]. In the case of the Pt(II)-complex in $(CD_3)_2SO_2$ in addition to minor shifts concerning the resonance of CH (δ = 3.90) and NH (broad signals in the region at δ = 8.23–7.90), the major change involves the CH_3 signal (δ = 2.48). This clearly points out that the metal is coordinated through the S atom, analogously to that found for platinum(II) interacting with cyclo-(L-methionyl-L-methionyl) [8] and linear L-methionyl-L-methionine [9]. However, it is to be noted that the NMR spectrum also shows the pattern typical of the uncoordinated CGM, thus indicating that some coordination equilibria with the solvent are occuring.

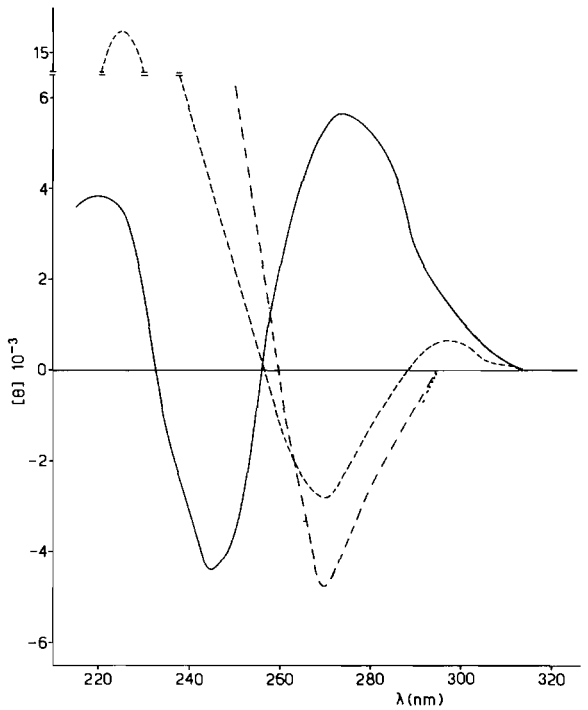

Fig. 1. Circular dichroism spectra of salmon sperm DNA (——) (0.82 10^{-3} *M*) and of DNA in the presence of Pt(CGM)Cl after subtraction of the Pt(CGM)Cl optical activity contribution. Pt/P input ratios were: 0.1 (– – –), 0.3 (–·–·–) and 0.5 (·····). Solvent 5 m*M* NaCl.

In D_2O solution a similar shift is observed for the CH_3 resonance (complex pattern centred at $\delta \sim$ 2.55, including also the two CH_2 groups of methionyl) while CH_2 (glycyl) and CH signals are observed at δ = 4.05 and $\delta \sim$ 4.3, respectively. In any event, from the NMR data, it seems clear that in the complex (for which the formula $Pt(CGM)_2Cl_2$ is deduced from the elemental analysis) the platinum(II) is bonded to the two diketopiperazine moieties through the S atoms. Furthermore, from the IR doublet found for the Pt–Cl stretching (see Experimental), a *cis* configuration (at least in part) for the coordination compound can be suggested.

Shown in Fig. 1 are the circular dichroism spectra of salmon sperm DNA in the absence and in the pre-

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sence of $Pt(CGM)_2Cl_2$. To the experimental curve of the mixture the spectrum of $Pt(CGM)_2Cl_2$ (at the same pH and concentration) was subracted. Different Pt/P input ratios were used. In the presence of the Pt(II) derivative the DNA spectrum is dramatically changed, the main differences being an apparent inversion of the sign of the dichroic bands and a wavelength shift of the extrema in the long-wavelength part of the spectrum, and a very noticeable increase of the band at 220–225 nm. Because of the acidic character of $Pt(CGM)_2Cl_2$ ($\sim 10^{-3}$ *M* solutions gave a pH 3), a protonation of DNA surely occurs; however the differences between the two curves need a different explanation, such as a direct interaction of the metal with DNA bases at acidic pH. Studies to clarify this point and to check the biological activity of the complex are in progress in our laboratory.

Experimental

Tert-butyloxycarbonylglycylmethionine ethyl ester (I)

Isobutyl chloroformate (5.13 ml, 39.9 mmol) was added at −15 °C to a solution of tert-butyloxycarbonylglycine (7.0 g, 39.9 mmol) and N-methylmorpholine (4.0 ml, 39.9 mmol) in chloroform (170 ml). The temperature was kept at −15 °C for 1 h, then methionine ethyl ester hydrochloride (8.5 g, 39.9 mmol) and N-methylmorpholine (4.0 ml, 39.9 mmol) were added. The mixture was kept at room temperature for 24 h and washed with 5% sodium bicarbonate solution, water, 5% citric acid solution, water and dried over sodium sulphate. The solution was evaporated to dryness and the residue was crystallized from ethanol–diethyl ether: yield 11.0 g (82%), m.p. 97–98 °C, $[\alpha]_D^{20} = +18.0$ (c, 1.0, AcOEt). TLC on silica gel using n-butanol–glacial acetic acid–water (3.1:1) showed a single spot, ninhydrin negative, iodine positive. *Anal.* calcd. for $C_{14}H_{26}N_2O_5S$: C, 50.28; H, 7.85; N, 8.37; S, 9.59. Found: C, 50.37; H, 8.00; N, 8.20; S, 9.70.

Cyclo-(glycyl-L-methionyl) (CGM) (II)

9.5 g (28.4 mmol) of I were dissolved in 98% formic acid (500 ml) and the solution was kept at room temperature for 24 h. The solvent was removed under reduced pressure and the residue, dissolved in sec-butanol (590 ml) and toluene (295 ml), was refluxed for 3 h. After evaporation to dryness, the residue was crystallized from sec-butanol and washed with diethyl ether. yield 4 g (75%), m.p. 208–209 °C, $[\alpha]_D^{20} = -17.0$ (c, 1.0, DMF).

TLC on silica gel using n-butanol–glacial acetic acid–water (3:1:1) showed a single spot, ninhydrin negative, iodine positive, IR (nujol). ν(NH) 3200 cm^{-1}; ν(CO) 1690 cm^{-1}.

Anal. calcd. for $C_7H_{12}N_2O_2S$. C, 44.66; H, 6.44; N, 14.87; S, 17.03. Found. C, 44.86; H, 6.47, N, 14.93; S, 17.23.

$Pt(CGM)_2Cl_2$

0.85 g (4.5 mmol) of II and 0.6 g (1.5 mmol) of potassium chloroplatinite, dissolved in water (25 ml), were stirred at room temperature for 4 h. The formed light yellow precipitate was filtered and washed with water, ethanol and diethyl ether: yield 0.6 g (66%). IR (nujol): ν(NH) ~3190 cm^{-1}, ν(CO) 1675 cm^{-1}, ν(Pt–Cl) 330 and 320 cm^{-1}.

Anal. calcd. for $C_{14}H_{24}N_4O_4S_2Cl_2Pt$: C, 26.17; H, 3.77; N, 8 72; S, 9.98. Found: C, 26.00; H, 3.75, N, 8.78; S, 9.85. This compound has been independently prepared by a different procedure by Bressan *et al.* [7]. The two products display identical physicochemical properties.

NMR spectra were recorded on a Varian EM 360 A spectrometer. Tetramethylsilane and acetone were used as internal standards. In each case chemical shifts were referred to tetramethylsilane.

Circular dichroism spectra were obtained with a Cary model 61 dichrograph. Cylindrical quartz cells were used with 0.1 and 0.5 cm optical paths. The data are expressed in terms of $[\theta]_\lambda$, the mean residue molecular ellipticity in units of degrees·cm^2 $dmol^{-1}$.

The concentration of salmon sperm DNA (Sigma Lot No. D-I626) solution was determined spectrophotometrically on a Perkin Elmer S 550 instrument.

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