

Electronic Supporting Information

for

Tris-(2-carboxyethyl) Phosphine Significantly Promotes the Reaction of Cisplatin with Sp1 Zinc Finger Protein

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Experimental Details

Materials: Cisplatin was purchased from Sigma-Aldrich. TCEP-HCl was obtained from Amresco. 4-(2-pyridylazo) resorcinol (PAR) dye was obtained from Sangon (Shanghai). All other chemical reagents were of the highest purity available.

Protein expression and purification: The plasmid pGEX-Sp1(83-778) coding for the C-terminal 696 amino acids of Sp1 was a generous gift from Dr. Dimitris Kardassis at University of Crete Medical School, Greece. The gene sequence for the expression of the second zinc finger domain of Sp1 (Sp1-zf2, amino acids 565-595) (Scheme S1) was amplified by PCR from pGEX-Sp1(83-778) plasmid and was subcloned into the Pet-28a-His-SUMO expression vector. The Pet-28a-His-SUMO-Sp1-zf2 expression vector was transformed into *E. coli* BL21 (DE3) cells. Transformed cells were grown in LB containing 10 μ M ZnCl₂ and induced with 0.4 mM isopropyl-1-thio- β -D-galactopyranoside (IPTG) at an OD₆₀₀ of 0.8 and then incubated for 16 h at 16°C. The cells were harvested by centrifugation and lysed by sonication in the lysis buffer containing 5 mM imidazole, 200 mM NaCl, 10 μ M ZnCl₂, 5mM β -ME and 20 mM Tris-HCl, pH 8.0. The soluble extract, obtained after centrifugation at 16000 rpm for 30 min at 4°C, was incubated with pre equilibrated Ni-NTA resin (Qiagen) for 30 min at 4°C. The His-SUMO-tagged fusion protein bound to the Ni-NTA column was eluted in the elution buffer (250 mM imidazole, 200 mM NaCl, 10 μ M ZnCl₂, 5mM β -ME and 20 mM Tris-HCl, pH 8.0). The His-SUMO-tag was cleaved from the precursor fusion protein via SUMO-specific protease and the Sp1-zf2 was further purified by size-exclusion chromatography (HiLoad Superdex 75 pg 16/60, GE Healthcare). The protein further concentration and buffer change were achieved via ultrafiltration.

Fluorescence spectroscopy: The fluorescence measurements were carried out on a Perkin Elmer LS55 Fluorescence spectrometer equipped with temperature controller which was thermostatted at 25°C. Samples were in a capped 1 cm path length quartz cell and the excitation wavelength was set at 284 nm by scanning the emission spectra between 300 nm and 500 nm. The bandwidth for both excitation and emission spectra was 5 nm. Different concentration of cisplatin were added into a 10 μ M Sp1-zf2 protein in the absence or presence of TCEP reductant respectively in 10 mM HEPES buffer (pH 6.8) and the samples were incubated for 30 hour at 37°C prior to fluorescence measurements. All reactions were kept the ratios of [TCEP]/[cisplatin]=2 when reactions were performed in the presence of TCEP. Baseline-correction and volume-correction were used in the data processing.

Zinc release assay: Zn²⁺ ion released from the zinc finger domain of Sp1 protein was detected by UV-vis spectra by using a 4-(2-pyridylazo) resorcinol (PAR) dye which the formation of Zn-PAR complex has a new absorption maximum at 500 nm. Different concentration of cisplatin were added into a 30 μ M Sp1-zf2 protein solution containing 50 μ M PAR in 50 mM HEPES buffer (pH 6.8) in the absence or presence of TCEP reductant and were incubated for 30 h at 37°C prior to UV-vis measurements.

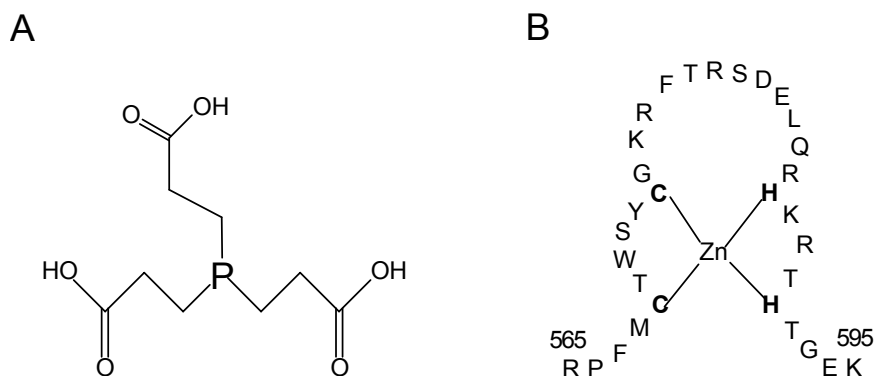
All reactions were kept the ratios of [TCEP]/[cisplatin]=2 when the reactions were carried out in the presence of TCEP. Each sample was corrected against a blank containing 50 μ M PAR in 50 mM HEPES but no containing Sp1-zf2 protein. The amount of zinc released from the Sp1-zf2 protein was quantitatively determined by the absorption at 500 nm.

HPLC: High pressure liquid chromatography (HPLC) was carried out on an Agilent 1200 system equipped with a Kromasil C18 column (250 $\times$ 4.6 mm, 5 μ m). HPLC profiles were recorded on UV detection at 230 nm. Linear gradient of 10-52% B in 7 minutes was used. (eluent: A: 0.1% TFA in H₂O, B: CH₃OH).

UV-Vis spectrum: The absorbance at 230 nm (which reflects the presence of Pt-P coordination) was measured on an Agilent 8453 spectrophotometer equipped with Peltier temperature controller. Reactions were kept in a capped 1-cm path length quartz cell, which was thermostatted at 37°C. The reaction of 100 μ M cisplatin with 200 μ M TCEP was carried out in H₂O in different pH conditions (pH 2.0 and pH 6.0) at 37°C. The reaction was monitored immediately after cisplatin adding. The control experiments were also carried out for measuring the absorbance of UV230 contained correspond concentration of TCEP in similar buffer, but no cisplatin adding.

NMR Spectroscopy: ³¹P NMR was performed on a Bruker 500 MHz spectrometer. ³¹P NMR spectra of 2 mM TCEP samples in D₂O were recorded at 298 K. To monitor the reaction, 1 mM cisplatin was added to the 2 mM TCEP in D₂O in similar reaction condition.

Electrospray Ionization Mass Spectrometry (ESI-MS): Electrospray ionization mass spectra (ESI-MS) was performed on a Thermo Xcalibur 2.0 instrument using a Thermo LTQ linear ion trap mass spectrometry (Thermo Fisher, San Jose, CA, USA) equipped with a nano-ESI source in positive mode. The drying gas was heated to 200°C and employed voltage by application of 1.8 kV. Samples were diluted by 50%/50% water/methanol containing 0.1% formic acid before injection in order to obtain a good volatilization and were injected into the Nano-ESI source at the flow rate of 3 μ L/min. Tandem mass and zoom scan were used to analyze the structures of the products. Data were evaluated using XCalibur software (version 2.0, Thermo Finnigan).



Scheme S1. The structures of TCEP molecule (A) and the sequence of Sp1-zf2 zinc finger protein (B)

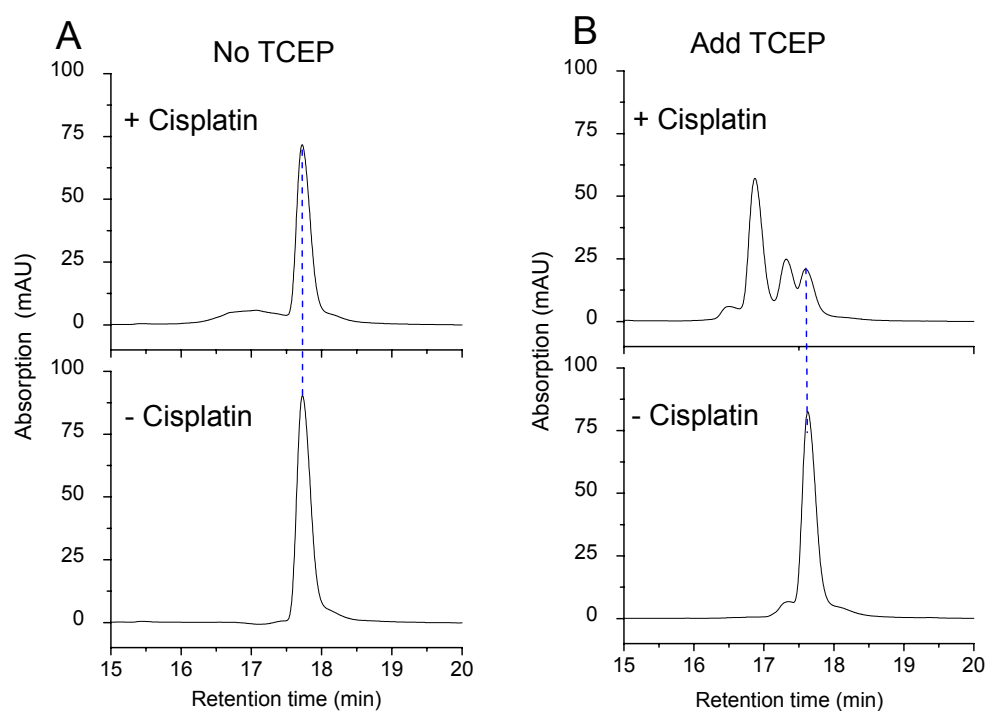


Figure S1. HPLC profiles of Zn-Sp1-zf2 in the reaction with cisplatin. (A) in the absence of TCEP. (B) in the presence of TCEP at a ratio of $[TCEP]/[cisplatin] = 2$. The reactions were performed on 100 μM Zn-Sp1-zf2 and 150 μM cisplatin in 50 mM HEPES (pH 6.8) at 37°C for 30 hour incubation prior to HPLC analysis. HPLC profiles were recorded with UV detection at 280 nm. Linear gradient of 20-80% B in 20 minutes was used (eluent: A: 0.1% TFA in H_2O , B: CH_3OH).

Table S1. Analyses of the MS peaks from the platination adducts Sp1-zf2 in Figure 2.

peak	Composition	Molecular Formula	m/z (charge)	MW: obsd./cald.
(a)	$[\text{ZFP}+\text{Pt}+\text{TCEP}-2\text{H}]^+$	$\text{C}_{174}\text{H}_{269}\text{N}_{55}\text{O}_{52}\text{S}_3\text{PPt}$	1072.48(+4) 858.18(+5) 715.32(+6) 613.28(+7)	4285.92/4285.64
(b)	$[\text{ZFP}+\text{Pt}+2\text{TCEP}-2\text{H}]^+$	$\text{C}_{183}\text{H}_{284}\text{N}_{55}\text{O}_{58}\text{S}_3\text{P}_2\text{Pt}$	1135.00(+4) 908.20(+5) 757.00(+6) 649.00(+7) 568.00(+8)	4536.00/4535.83
(c)	$[\text{ZFP}+2\text{Pt}+2\text{TCEP}-4\text{H}]^+$	$\text{C}_{183}\text{H}_{282}\text{N}_{55}\text{O}_{58}\text{S}_3\text{P}_2\text{Pt}_2$	1183.23(+4) 946.79(+5) 789.16(+6)	4728.95/4728.89
(d)	$[\text{ZFP}-2\text{H}]^+$	$\text{C}_{165}\text{H}_{256}\text{N}_{55}\text{O}_{46}\text{S}_3$	641.49(+6) 549.99(+7) 481.36(+8)	3842.91/3842.4

Note: Some additional minor product peaks corresponding to Sp1-zf2 were also presented on the MS spectra due to the product overlaps during HPLC separations.

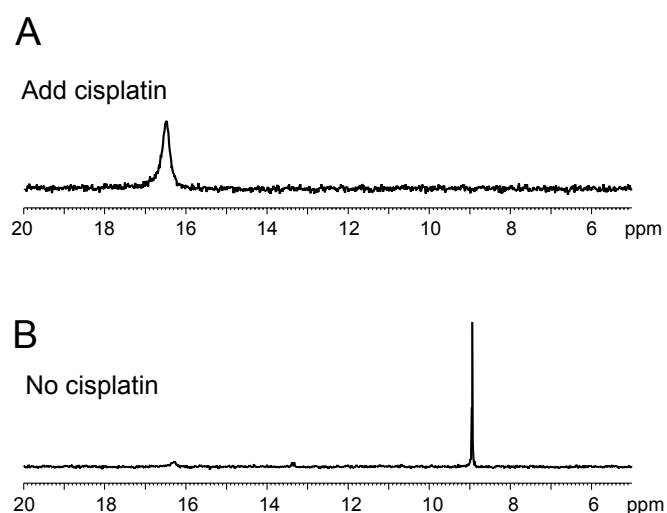


Figure S2. ^{31}P NMR spectra of TCEP in the reaction with cisplatin. (A) 2 mM TCEP; (B) 2 mM TCEP reacted with 1 mM cisplatin for 3 hours at 25°C. Spectra were recorded at 25°C in D_2O at pH 2.0.

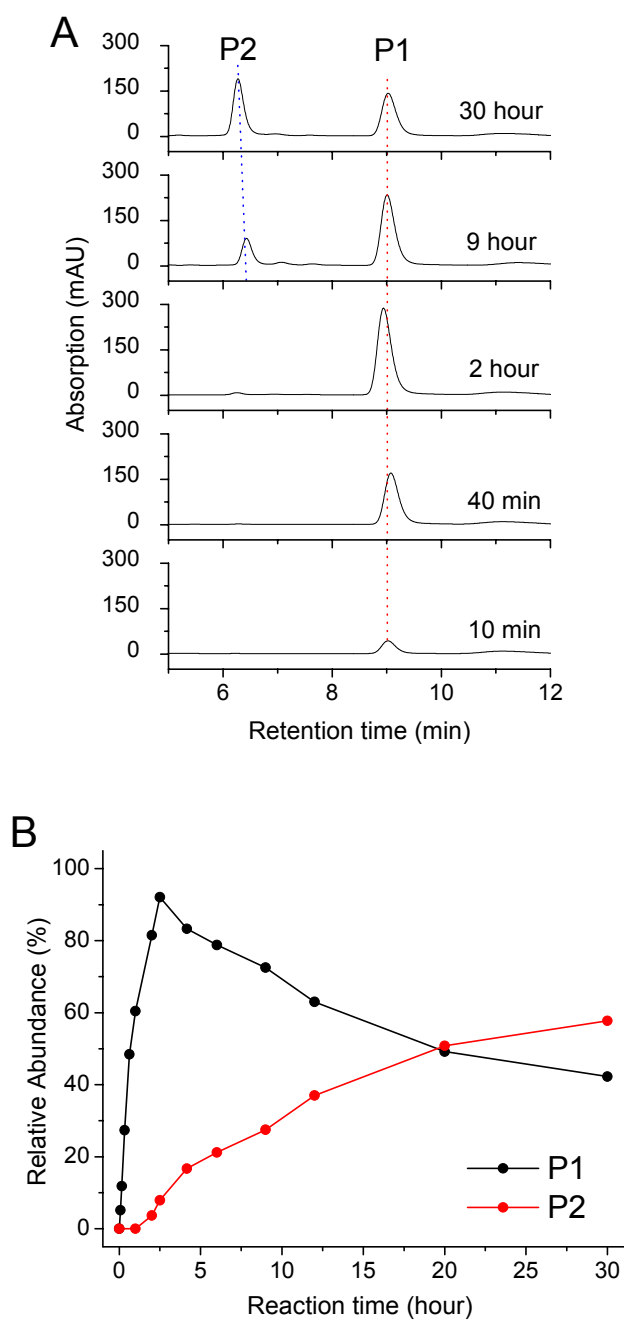


Figure S3. HPLC monitors the reaction of cisplatin with TCEP at pH 6.0. (A) HPLC profiles at different reaction time. (B) the relative ratio of P1 and P2 as a function of reaction time (B). The reaction was performed on 100 μ M cisplatin with 200 μ M TCEP at pH 6.0 in H₂O at 37°C.

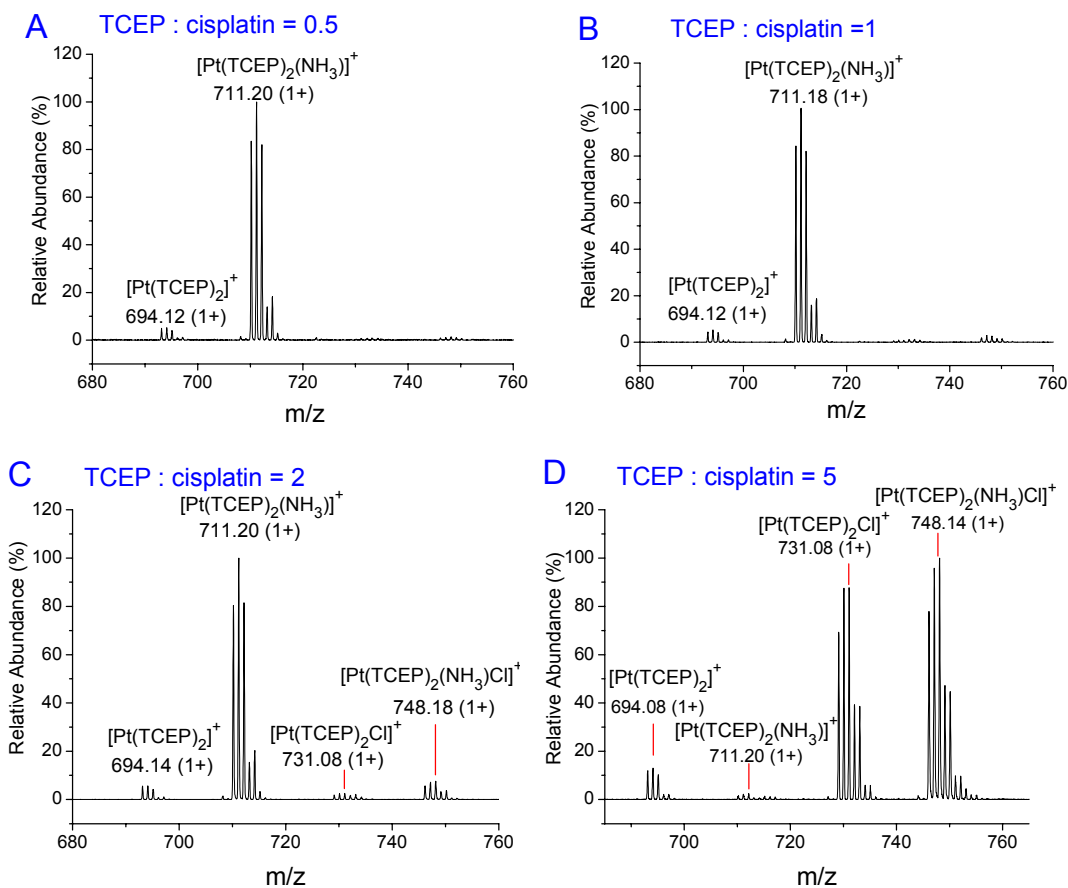


Figure S4. ESI-MS spectra of the products from the reaction of cisplatin with TCEP at different reaction ratios (TCEP/cisplatin). The reactions were carried out on 100 μ M cisplatin and different concentrations of TCEP at 37°C for 10 hour. The ratio of TCEP/cisplatin varies from 0.5 to 5 (labeled on each ESI-MS spectrum). The relative abundance of chloro coordination species is higher with the increase of TCEP concentration since TCEP·HCl was used in the reaction. The compositions of products at m/z 748.14 and 731.08 was further analyzed by MS/MS (Figure S5).

Table S2. Analyses of the MS peaks from the reaction of cisplatin with TCEP at different reaction ratios (TCEP/cisplatin) in Figure S4.

m/z	Composition	Molecular Formula	MW: obsd./cald.
711.20(1+)	Pt(TCEP) ₂ (NH) ₃	C ₁₈ H ₃₂ NO ₁₂ P ₂ Pt	711.20/711.1047
694.12(1+)	Pt(TCEP) ₂	C ₁₈ H ₂₉ O ₁₂ P ₂ Pt	694.12/694.07836
731.08(1+)	Pt(TCEP) ₂ Cl	C ₁₈ H ₃₀ O ₁₂ P ₂ Pt Cl	731.08/731.062
748.14(1+)	Pt(TCEP) ₂ (NH) ₃ Cl	C ₁₈ H ₃₃ N ₁ O ₁₂ P ₂ ClPt	748.18/748.08895

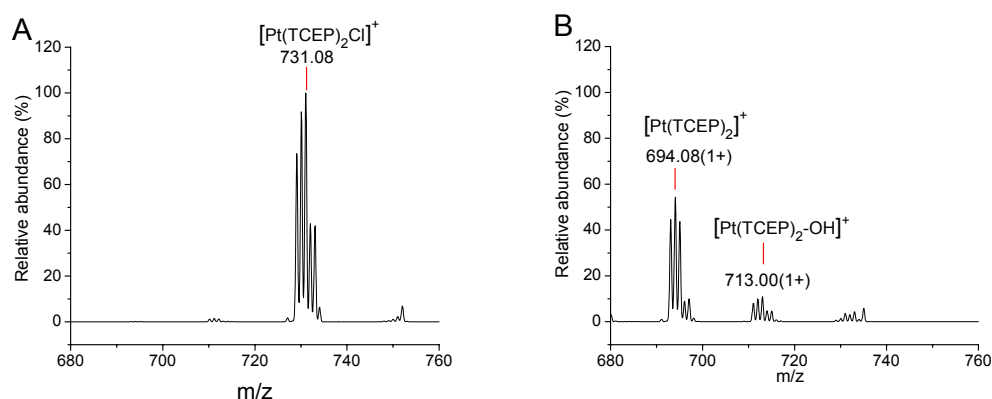


Figure S5. MS/MS spectra of the product from the reaction of cisplatin with TCEP. (A) Collision of the precursor peak of 748.14 (m/z) in Figure S4D. The release of NH_3 ligand results in the m/z 731.08 (1+). (B) Collision of the precursor peak of 731.08 (m/z) in Figure S4D. The release of Cl^- ligand generates product at m/z 694.08 (1+).

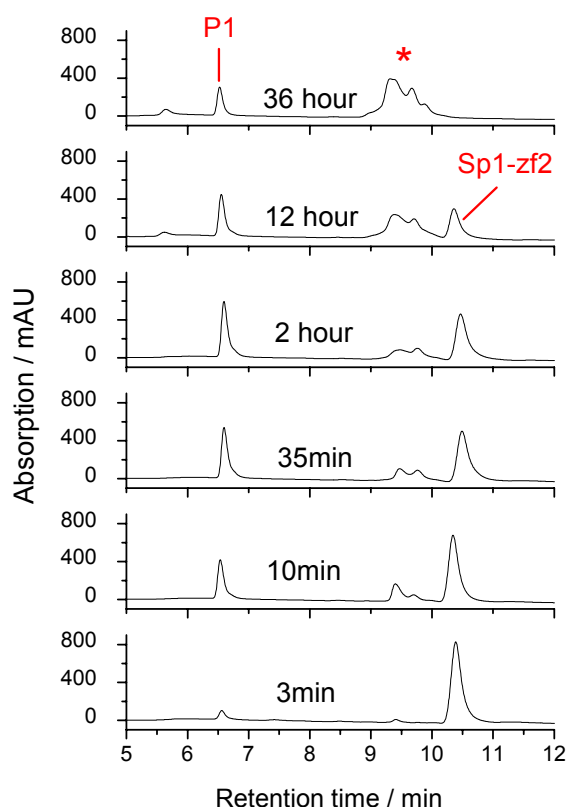


Figure S6. HPLC monitors the reaction of cisplatin with Sp1-zf2 protein in the presence of TCEP. The reaction was performed on 100 μM Sp1-zf2 and 300 μM cisplatin in 50 mM HEPES buffer containing 600 μM TCEP at pH 6.8 at 37°C. Reaction time is labeled on each profile, and the symbol (*) denotes the multiple platination adduct of Sp1-zf2. The retention time of P1 is different from that on Figure S3A since different elution gradient was used.