Electronic Supplementary Material (ESI) for Dalton Transactions.

Supporting Information

Dirhodium tetraacetate binding to a B-DNA double helical dodecamer probed by X-ray crystallography and mass spectrometry

Gabriella Tito,[a] Romualdo Troisi,[a] Giarita Ferraro,[a] Andrea Geri,[b] Lara Massai,[b] Luigi Messori,[b] Filomena Sica,[a] Antonello Merlino[a]*

[a] Department of Chemical Sciences, University of Naples Federico II, Complesso Universitario di Monte Sant'Angelo, via Cintia, I-80126, Naples, Italy. *E-mail: antonello.merlino@unina.it
[b] Department of Chemistry "U. Schiff", University of Florence, via della Lastruccia 3, 50019 Sesto Fiorentino, Italy

Experimental section

Materials

The C-G-C-G-A-A-T-T-C-G-C-G DNA sequence for crystallization experiments (HPLC purity grade) and for CD and UV-vis experiments (desalted purity grade) was purchased from Eurofins Genomics. Dirhodium tetraacetate [$Rh_2(\mu\text{-}O_2CCH_3)_4$] was purchased from Sigma-Aldrich (Merck).

Methods

Crystallization, data collection and refinement

To form the duplex and to prepare a homogeneous sample, annealing of DNA was carried out as follows: 1.9 mM DNA (single strand concentration) in 20 mM sodium cacodylate at pH 7.0 was kept to 90 °C for 5 min and then slowly cooled down in 50–60 min and stored at 20 °C overnight. Crystals of the duplex were grown using hanging drop vapor diffusion method at 20 °C mixing a 0.5 μL drop consisting of 0.95 mM DNA duplex and a 0.5 μL of 7% (v/v) 2-methyl-2,4-pentanediol, 20 mM $MgCl_2$, 80 mM spermine tetrahydrochloride, and 60 mM sodium cacodylate at pH 6.5 equilibrated against a reservoir containing 50% (v/v) MPD. The crystals were then soaked with dirhodium tetraacetate [$Rh_2(\mu\text{-}O_2CCH_3)_4$] for 7 days and then freezed with N_2.

X-ray diffraction data were collected on a Pilatus 6M detector at XRD2 beamline of Elettra synchrotron, Trieste, Italy, at 100 K, using a λ=1.00 Å. Data sets were processed and scaled using Autoproc pipeline.[1] Data collection statistics are reported in Table S1. The structure was solved by molecular replacement method using the program Phaser[2] and the coordinates of B-DNA from PDB code 5BNA,[3] without water molecules and ligands, as a search model. The refinement was carried out with Refmac5,[4] and model building and map inspections were performed using Coot.[5] The final structure converged to Rfactor of 0.197 and to Rfree of 0.223. Superpose program (CCP4 package) was used to calculate root-mean-square deviations (rmsd).[6] Analysis of DNA structure parameters was carried out using the Web 3DNA 2.0 server.[7] Refinement statistics are reported in Table S1. Coordinates and structure factors were deposited in the Protein Data Bank (PDB codes 8CE2). PyMOL (The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC) was used to generate all the figures.

UV-vis absorption and far UV-CD spectroscopy

UV–vis absorption spectra of 0.5 mM [$Rh_2(\mu\text{-}O_2CCH_3)_4$] in 60 mM sodium cacodylate at pH 6.5 were recorded at room temperature in the 370–700 nm range every 1 nm at a scan rate of 400 nm/min using a Jasco V-650 UV–vis spectrophotometer. Spectra of [$Rh_2(\mu\text{-}O_2CCH_3)_4$] were also recorded in the presence of DNA (DNA:dirhodium compound metal molar ratio 1:2). To perform the UV-vis experiments, 0.5 mM DNA solutions (single strand concentration) were annealed as described above.

Circular dichroism spectra of the adduct formed upon reaction of the DNA with the metal compound were collected in the far-UV region (from 200 to 350 nm) using a Jasco J-715 spectropolarimeter (JASCO Corp., Milan, Italy) at 25 °C. Each spectrum was obtained using a quartz cell with path length of 0.1 cm, averaging three scans, subtracting contributions from the corresponding references, and converting the signal to mean residue ellipticity in units of deg/mol $^{-1}cm^{-2}$. Spectra were collected upon

24 h incubation of the DNA with $[Rh_2(\mu\text{-}O_2CCH_3)_4]$ at a 1:2 and 1:10 molar ratio in 10 mM sodium cacodylate buffer at pH 6.5. 20 mM $MgCl_2$ was added to the solution since in the presence of this salt the thermal denaturation experiments produce a reproducible sigmoidal curve. To carry out these experiments, 43 µM (single strand concentration) DNA solutions were used. The DNA solutions were preventively annealed in 10 mM sodium cacodylate at pH 6.5, 20 mM $MgCl_2$. Other experimental settings were: 50 nm/min scan speed, 2.0 nm band width, 0.2 nm resolution, 100 mdeg sensitivity, and 2 s response.

Electrospray Ionization Mass Spectrometry

For the mass spectrometry measurements, all samples were prepared in LC-MS grade solvents or solutions. To form the duplex and to prepare a homogeneous sample, annealing of DNA was carried out as follows: 1 mM DNA (single strand concentration) in 100 mM ammonium acetate at pH 6.8 was kept to 90 °C for 5 min and then slowly cooled down in 50–60 min and stored at 20 °C overnight. For the reaction with the selected metal complex, an aliquot of the B-DNA duplex solution was mixed with an aliquot of the 10^{-3} M metal complex solution and diluted with 100 mM ammonium acetate solution to a final concentration of 10^{-4} M. The DNA-to-complex ratio was 1 : 5. The obtained mix was incubated up to 24 h at 37 °C. All samples were diluted to a final protein concentration of 5 x 10^{-6} M using 100 mM of ammonium acetate solution. 20% methanol was added just before injection to acquire a stable electrospray signal. It has been verified that under these conditions $[Rh_2(\mu\text{-}O_2CCH_3)_4]$ remains stable for 24 h.

The ESI-MS investigations were performed using a TripleTOF® 5600+ high-resolution mass spectrometer (Sciex, Framingham, MA, U.S.A.), equipped with a DuoSpray® interface operating with an ESI probe. All the ESI mass spectra were acquired through direct infusion at 7 µL/min flow rate. The ESI source parameters optimized for the B-DNA are the follows: negative polarity, ion-spray voltage floating (ISFV) -4000 V, temperature (TEM) 0 °C, ion source gas 1 (GS1) 40 L/min; ion source gas 2 (GS2) 0 L/min; curtain gas (CUR) 30 L/min, collision energy (CE) -10 V; declustering potential (DP) -10 V, acquisition range 1000-2400 m/z.

For acquisition, Analyst TF software 1.7.1 (Sciex) was used and deconvoluted spectra were obtained by using the Bio Tool Kit micro-application v.2.2 embedded in PeakView™ software v.2.2 (Sciex).

References

1 C. Vonrhein, C. Flensburg, P. Keller, A. Sharff, O. Smart, W. Paciorek, T. Womack and G. Bricogne, *Acta Crystallogr D Biol Crystallogr*, 2011, **67**, 293–302.

2 A. J. McCoy, R. W. Grosse-Kunstleve, P. D. Adams, M. D. Winn, L. C. Storoni and R. J. Read, *J Appl Crystallogr*, 2007, **40**, 658–674.

3 R. M. Wing, P. Pjura, H. R. Drew and R. E. Dickerson, *The EMBO Journal*, 1984, **3**, 1201–1206.

4 G. N. Murshudov, P. Skubák, A. A. Lebedev, N. S. Pannu, R. A. Steiner, R. A. Nicholls, M. D. Winn, F. Long and A. A. Vagin, *Acta Crystallogr D Biol Crystallogr*, 2011, **67**, 355–367.

5 P. Emsley, B. Lohkamp, W. G. Scott and K. Cowtan, *Acta Crystallogr D Biol Crystallogr*, 2010, **66**, 486–501.

6 L. Potterton, S. McNicholas, E. Krissinel, J. Gruber, K. Cowtan, P. Emsley, G. N. Murshudov, S. Cohen, A. Perrakis and M. Noble, *Acta Crystallogr D Biol Crystallogr*, 2004, **60**, 2288–2294.

7 S. Li, W. K. Olson, X.-J. Lu, N*ucleic Acids Research*, 2019, **47**, W1, W26–W34.

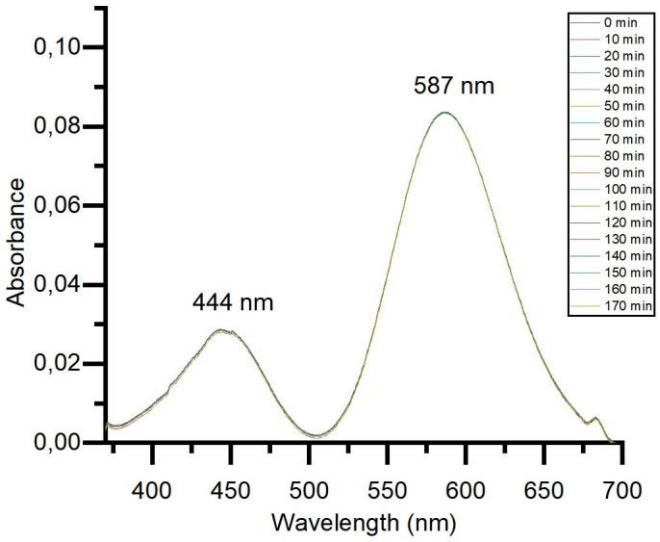

Figure S1. Time course UV-vis spectra of 0.5 mM $[Rh_2(\mu\text{-}O_2CCH_3)_4]$ in 60 mM sodium cacodylate buffer at pH 6.5.

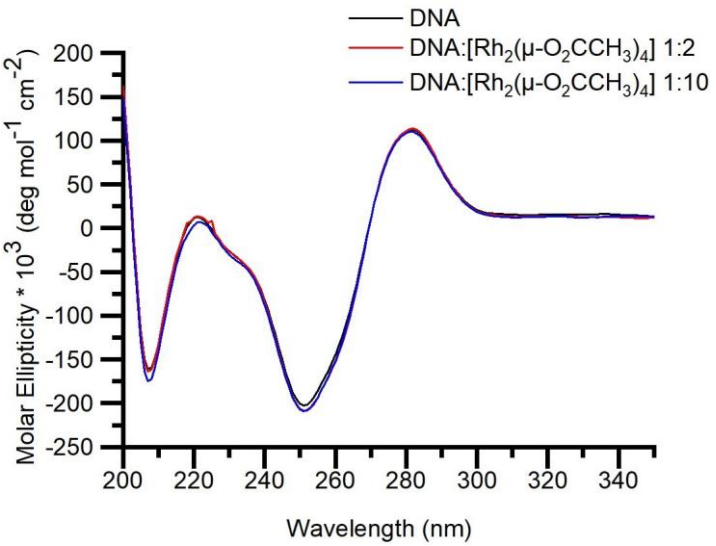

Figure S2. Circular dichroism spectra of 43 μM DNA in 10 mM sodium cacodylate buffer at pH 6.5 and 20 mM $MgCl_2$, incubated for 24 h with $[Rh_2(\mu\text{-}O_2CCH_3)_4]$ at 1:2 and 1:10 DNA:dirhodium compound molar ratio.

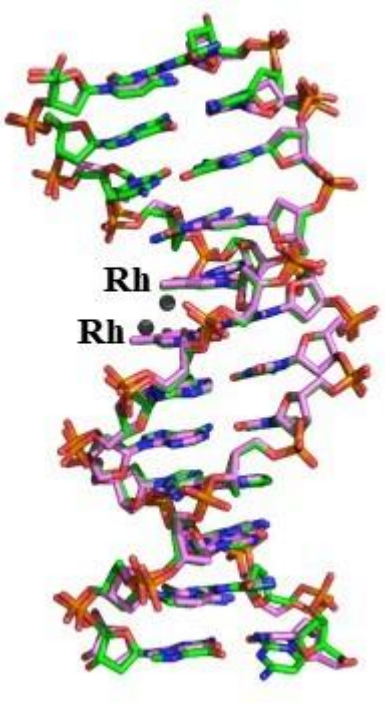

Figure S3. Superposition of the structures of metallodrugs-free DNA duplex (carbon atoms in green) and of the DNA adduct with dirhodium tetraacetate (carbon atoms in violet), corresponding to the relative orientation with the smallest rmsd (0.722 Å). Oxygens are in red, phosphorous in orange, nitrogens in blue.

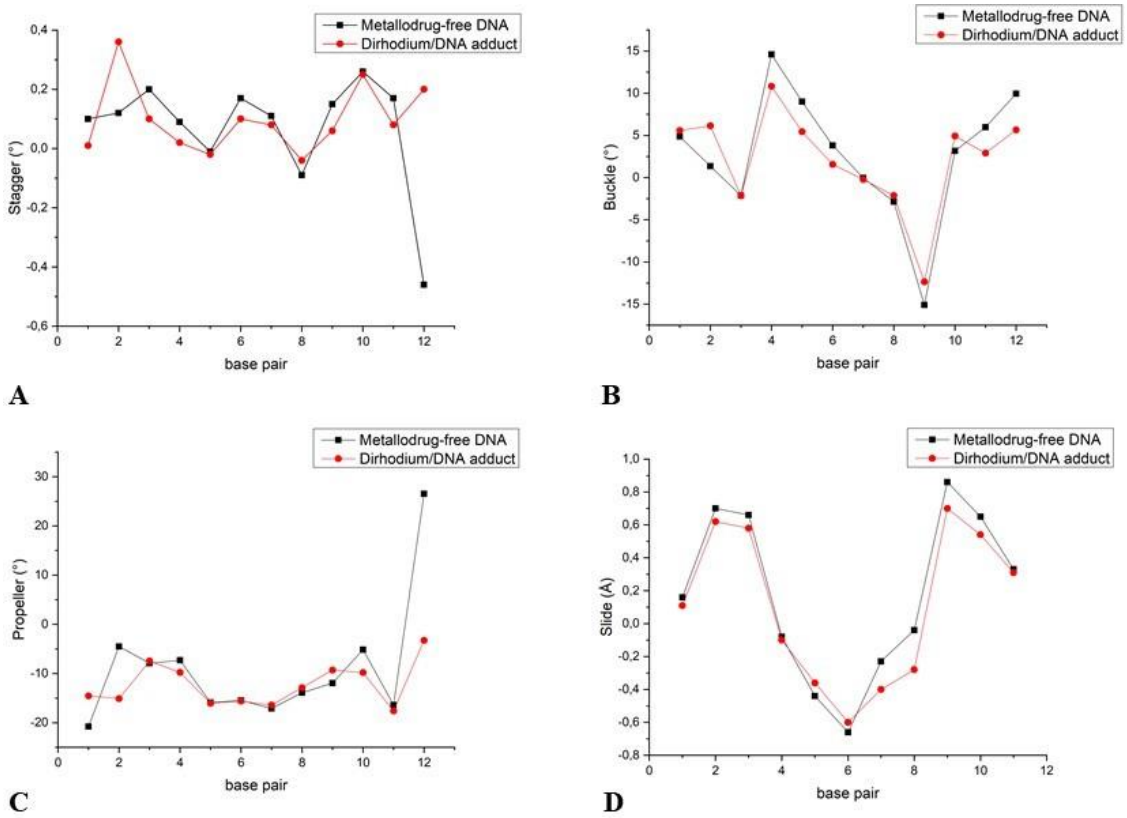

Figure S4. Position-specific analysis of local base pair parameters. Stagger angle (A), Buckle angle (B), propeller angle (C), and slide (in Å) are plotted for each position of the duplex in the metallodrug-free (black squares) and in the adduct with dirhodium tetraacetate (red circles).

Table S1. Data collection and refinement statistics for $[Rh_2(\mu\text{-}O_2CCH_3)_4]$/DNA adduct. Values in brackets refer to the highest resolution shell.

	$[Rh_2(\mu\text{-}O_2CCH_3)_4]$/DNA adduct
Crystal data	
Space group	$P2_12_12_1$
Unit-cell parameters	
a, b, c (Å)	25.53, 40.31, 65.71
α, β, γ (°)	90.00, 90.00, 90.00
No. of molecules in the asymmetric unit	1 (duplex)
Data collection	
Resolution limits (Å)	34.36 – 1.24 (1.26– 1.24)
No. of observations	215433 (8369)
No. of unique reflections	19898 (996)
Completeness (%)	99.9 (100.0)
<I/σ(I)>	23.2 (2.2)
Average multiplicity	10.8 (8.4)
$CC_{1/2}$	0.999 (0.785)
Refinement	
Resolution limits (Å)	34.36 – 1.24
No. of reflections	18926
R_{factor}/R_{free}	0.197/0.223
No. of atoms	658
Mean B value (Å^2)	16.35
Rmsd from ideal values	
Bond lengths (Å)	0.012
Bond angles (°)	3.073
Rh occupancy	0.20/0.20
Rh B-factors (Å^2)	19.94/24.30
PDB code	8CE2

Table S2. Melting temperature of DNA and its adducts with dirhodium tetraacetate in 1:2 and 1:10 DNA to metal compound molar ratio in 10 mM sodium cacodylate buffer at pH 6.5 and 20 mM $MgCl_2$

	Melting Temperature in ˚C (±1˚C)
DNA	70 ˚C
DNA:$[Rh_2(\mu\text{-}O_2CCH_3)_4]$ adduct in 1:2 molar ratio	70 ˚C
DNA:$[Rh_2(\mu\text{-}O_2CCH_3)_4]$ adduct in 1:10 molar ratio	70 ˚C