Crystal Structure of an Oxaliplatin 1,2-d(GpG) Intrastrand Cross-Link in a DNA Dodecamer Duplex

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Introduction

((1*R*,2*R*)-Diaminocyclohexane)oxalatoplatinum(II) (**1**, "oxaliplatin") is clinically approved in Europe for the treatment of metastatic colorectal cancer. Like cisplatin (**3**), oxaliplatin acts by metalating the adjacent purines of DNA. So far, no structural study of a cisplatin analog-bound to double-stranded DNA has been published in the literature.

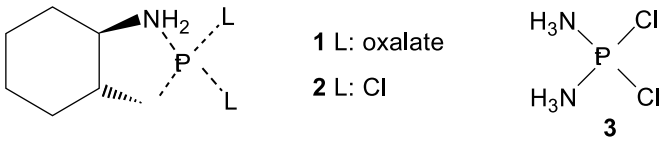

*FIG. 1. Oxaliplatin (*1*) and related structures.*

Materials and Methods

(1*R*,2*R*)-Diaminocyclohexanedichloroplatinum(II) (**2**) was prepared from (1*R*,2*R*)-diaminocyclohexane and potassium tetrachloroplatinate(II) in 82% yield as described.[1] The 12-mer with the sequence 5'-d(CCTCTGGTCTCC)-3' was modified at the G,G site with silver nitrate-activated **2** to give the oxaliplatin modified duplex DNA, as done previously with cisplatin.[2] The hanging drop vapor diffusion method was used for crystallization.[3] Crystals were grown at 4ºC from 3.0 μL drops containing 0.2 mM DNA, 10% MPD ((±)-2-methyl-2,4-pentanediol), 40 mM sodium cacodylate (pH 7.0), 12 mM spermine • 4HCl, 20 mM barium dichloride, and 5% ethyl acetate equilibrated against a reservoir of 30% MPD and 5% ethyl acetate. Crystals with maximum dimensions of 0.08 × 0.2 × 0.4 mm^3 appeared after 5 days.

Crystals were transferred to a solution of 30% MPD, 40 mM sodium cacodylate (pH 7.0), 12 mM spermine • 4HCl, 20 mM barium dichloride, and 5% ethyl acetate at 4°C, then mounted in loops and flash frozen. Multiwavelength anomalous dispersion (MAD) studies[4] using the oxaliplatin modified 12-mer were conducted at 100 K on beamline 19-ID of the Structural Biology Center-CAT at Argonne National Laboratory. The platinum absorption spectrum was measured as x-ray fluorescence using a Bicron scintillation counter. The values of f′ and f″ were calculated with the program CHOOCH.[5] Four wavelengths were selected for data collection, corresponding to the inflection point (1.0724 Å), the peak (1.0721 Å), and two remote energies (1.0277 Å, 1.1206 Å) with respect to the absorption edge of platinum. Complete data sets were collected successively for each wavelength on the SBC APS1 3 × 3 CCD detector. Each data set required approximately 12 min to collect (100 frames, 1º each, 4 s/frame). Intensities were integrated using DENZO and scaled with SCALEPACK.[6]

Results

MAD phases were calculated by using the program CNS (v1.0) for the space groups I222 and $I2_12_12_1$.[7] After solvent flattening, only the phase information for I222 produced a readily interpretable electron density map. An initial model was then subjected to rigid body refinement.7 Data to 2.8 Å resolution were used for cycles of positional, simulated-annealing, and B-factor refinements, interspersed with analysis of the geometry of the model. High electron density found at a distance of 2.6 Å to N7 of G31 was modeled as a fully occupied barium ion. This model was used as an input to refine 2.4 Å data collected at the SSRL.[8]

Discussion

The two DNA strands maintain their hydrogen bond base pairing throughout the entire helix despite the coordination of platinum. The two planes of the nucleobases G6 and G7 form a dihedral angle of 25º. The pseudo-equatorial hydrogen of the NH_2 group of the DACH ligand *cis* to the N7 atom of guanine G7 forms a hydrogen bond to oxygen O6 of G7 (N–O distance: 2.9 Å, angle of N–H–O: 136º). In the cisplatin analog, the corresponding N–O distance is 3.5 Å. This finding is in agreement with the recent findings of solution studies which also show that the biggest difference between cisplatin and oxaliplatin is found at the 3'- site of the GG lesion.[9] The refined model described here provides structural proof for such a hydrogen bonding interaction between a chiral ligand in a Pt(II) complex with the chiral DNA molecule to which it is coordinated. Binding of the DACH or DAB ligand to platinum both locks the conformation of the C–N bond, thereby fixing the position of the hydrogen atoms of the NH_2 groups, and directs the pseudo-equatorial hydrogen atom *cis* to the 3'-guanine to form a hydrogen bridge to the O6 of the very nucleobase.

Acknowledgments

This work was supported by a grant from the National Cancer Institute. B.S. was supported by the Swiss National Science Foundation and the Novartis Foundation. We thank Prof. C. Drennan, Drs. Uta-Maria Ohndorf and Tzanko Doukov, and Mr. Mike Sintchak for helpful discussions and Prof. George Sheldrick for providing us with a modified version of his SHELXL97 program. We thank Dr. Frank J. Rotella and the SBC team for assistance during our stay at Argonne. Use of the Argonne National Laboratory Structural Biology Center beamlines at the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Biological and Environmental Research, under Contract No. W-31-109-ENG-38. X-ray data were also collected at the Stanford Synchrotron Radiation Laboratory (SSRL), which is funded by the Department of Energy (BES, BER) and the National Institutes of Health (NCRR, NIGMS).

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