Crystal Structure of an Acridine-4-Carboxamide Complex of $d(CG^{5Br}UACG)_2$: A Novel Form of Intercalation Involving Four DNA Duplexes

A. Adams,[1] J. Mitchell Guss,[1] C. A. Collyer,[1] W. A. Denny,[2] L. P.G. Wakelin[3]

[1]*Department of Biochemistry, University of Sydney, Australia*

[2]*Auckland Cancer Society Research Centre, Faculty of Medicine and Health Science, The University of Auckland, Auckland, New Zealand*

[3]*School of Physiology and Pharmacology, University of New South Wales, Australia*

Introduction

DNA intercalating agents that poison the enzyme topoisomerase II are important in the treatment of cancer. 9-Aminoacridine-4-carboxamides are potent DNA-binding poisons of topoisomerase II with antitumour activity against experimental leukemias. The related des-9-amino compound, DACA (NSC 601316), is active in mouse solid tumour models and is currently in clinical trial. There are tight correlations between ligand structure, cytotoxicity, and antitumor efficacy for the 9-aminoacridine-4-carboxamides. To probe the molecular determinants of these structure/activity relationships, so that we may begin to understand what structural features distinguish the DNA complexes of active from inactive 9-aminoacridine-4-carboxamides, we have initiated x-ray crystallographic studies of their complexes with oligonucleotides.

Methods and Materials

Crystals of the complex of 9-amino-[N-(2-dimethylamino)butyl]acridine-4-carboxamide (9-aminobutyl-DACA) with $d(CG^{5Br}UACG)^2$ grew at 285 K by vapor diffusion in 10-mL sitting drops. Initially these drops contained 20 mM sodium cacodylate buffer (pH 6.5), 0.5 mM DNA, 3 mM magnesium acetate, 0.5 mM Co(II) chloride, 4.5 mM spermine, 1 mM 9-aminobutyl-DACA, and 7% 2-methyl-2,4-pentanediol (MPD). The drops were equilibrated against reservoirs of 1 mL of 35% MPD. Yellow crystals, with approximate dimensions 0.2 x 0.2 x 0.2 mm, appeared after several months. The crystals belong to space group C222 with cell dimensions a = 29.01 Å, b = 52.70 Å, and c = 40.53 Å.

The absorption edge was located with a fluorescence scan around 13,500 eV, the standard bromine edge. X-ray diffraction data were recorded at four different energies around the absorption edge using a single crystal frozen at 110 K in a N_2 cryostream. At each energy level, a data set of longer exposure (10 or 15 s) and shorter exposure (2 s) were measured to account for the very large dynamic range that occurs in the diffraction patterns of DNA due to the strong base-stacking reflections. All data were recorded on a Quantum 1 image plate positioned 105 mm from the crystal for all the data sets except for the long exposure at the highest energy, for which it was moved to a distance of 75 mm. Two data sets were collected at each energy and exposure time, starting with their f values separated by 180° to ensure the Bijvoet pairs were optimally measured.

The two Br positions were located by the heavy atom search procedure of the program suite CNS using the sum of the anomalous Patterson functions and the dispersive Patterson functions. The sites and phases were then refined to a resolution of 2.2 Å. The overall figure of merit for the 2992 reflections was 0.86. The structure of the $d(CG^{5Br}UACG)_2$/9-aminobutyl-DACA complex was immediately apparent from the experimental multiple anaomalous diffraction (MAD)-phased map, and the two strands of DNA and the 9-aminoacridine portion of the drug were built into the density with the program O. The structure was refined to a resolution of 1.6 Å with SHELX 97-2 using the highest energy data set.

Results

An asymmetric unit comprises 2 strands of DNA, one disordered drug molecule, 2 cobalt(II) ions, and 19 water molecules. The structure exhibits novel features not previously observed in crystals of DNA/drug complexes. The DNA helices stack in continuous columns with their central four base pairs adopting a B-like motif. However, despite being a palindromic sequence, the terminal GC base pairs engage in quite different interactions. At one end of the duplex, there is a CpG dinucleotide overlap modified by ligand intercalation and terminal cytosine exchange between symmetry-related duplexes. A novel intercalation complex is formed involving four DNA duplexes, four ligand molecules, and two pairs of base tetrads. The other end of the DNA is frayed, with the terminal guanine lying in the minor groove of the next duplex in the column. The structure is stabilized by guanine N7/cobalt(II) coordination.

Discussion

The most significant finding of our study is the discovery of a novel intercalation complex involving four DNA duplexes, four ligand molecules, and two sets of paired (un)stacked base tetrads. It appears that the geometrical requirements to pack the hexanucleotide into a crystal lattice in the presence of Co(II) and the ligand are the major determinants of the drug/DNA complex we see. The unique intercalation geometry we observe here, may provide new insights into understanding the biological properties of intercalating agents. This work has been published[1]: NDB accession number DD0032, PDB accession number 1FN1.

Acknowledgments

We wish to thank Barry Fields for help with collection of the MAD data set, the Association for International Cancer Research (grant to authors CAC and LPGW), and the National Health and Medical Research Council of Australia (grant to authors JMG and WAD) for financial support. Access to the BioCARS Collabora-

tive Access Team Sector 14 at the Advanced Photon Source at Argonne National Laboratory, Illinois, was provided by the Australian Synchrotron Research Programme, which is funded by the Commonwealth of Australia as a Major National Research Facility. BioCARS Sector 14 is supported by grant RR07707 from the U.S. National Institutes of Health, National Center for Research Resources. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

References

[1] A. Adams, J.M. Guss, C.A. Collyer, W.A. Denny, L.P.G. Wakelin, Nucleic Acids Res. 28, 4244-4253 (2000).