X-ray Diffraction Analysis of M-DNA

S. L. Labiuk, J. S. Lee, L. T. J. Delbaere
Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Introduction

Metal ions interact extensively with DNA, altering its stability and conformation. M-DNA is a novel conformation where, at alkaline pH, the divalent cations Co^{2+}, Ni^{2+} or Zn^{2+} intercalate between the bases, inhibiting the binding of ethidium [1]. Evidence suggests that the metal ions reversibly replace the imino protons of every thymine and guanine, resulting in a DNA conformation that can act as an efficient conductor [2]. Though the details of the structure are largely unknown, A still pairs with T and G with C. Various lines of evidence indicate that M-DNA maintains a double helical conformation [1-3].

In order to better understand structural details of the M-DNA conformation, we have undertaken an x-ray analysis of crystals of the DNA sequence d(GGCGCC) grown in the presence of cobalt, nickel, or zinc ions at alkaline pH. Multiwavelength anomalous dispersion (MAD) phasing experiments have been performed on the cobalt conformation at the Bio Consortium for Advanced Radiation Sources (BioCARS) beamline 14-BM-D.

Methods and Materials

Crystals were grown by using the hanging drop vapor diffusion method with 2-methyl-2,4-pentanediol (MPD) as the precipitant. Data were collected at beamline 14-BM-D on a crystal ($0.2 \times 0.2 \times 0.2$ mm^3) at approximately 110K. A fluorescence spectrum was measured across the Co K-absorption edge. Reflections were measured at the inflection point (λ = 1.6066 Å), the absorption peak (λ = 1.6052 Å), and a high-energy remote point (λ = 1.5088 Å) by using an ADSC Quantum-4 detector. Maximum resolution was 2.8 Å, and data were processed by using DENZO and SCALEPACK (R_{sym}= 0.092, completeness = 90% at a resolution of 3.2 Å for the high-energy remote point).

The crystallography and nuclear magnetic resonance (NMR0 program suite was used for locating heavy atom positions and solving the MAD phases.

Results

The Co(II)-d(GGCGCC) crystals exhibit a tetragonal unit cell with a = b = 69.0 Å and c = 55.3 Å. The space group is $P4_12_12$, with five single strands of DNA per asymmetric unit.

The MAD-phasing experiment allowed the positions of the DNA helical axes within the asymmetric unit to be determined. Solution and refinement of the structure are ongoing.

Discussion

Although the quality of the data collected at beamline 14-BM-D allowed the determination of the locations of the helical axes within the asymmetric unit, higher-resolution data are desirable so that greater detail can be observed. A collection strategy aimed at this goal is being prepared. We are also planning to collect data for the nickel and zinc complexes as well as newly acquired crystals with other DNA sequences.

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References

[1] J. S. Lee, L. J. P. Latimer, and R. S. Reid, Biochem. Cell Biol. **71**, 162-168 (1993).
[2] P. Aich, S. L. Labiuk, L. W. Tari, L. T. J. Delbaere, W. J. Roesler, K. J. Falk, R. P. Steer and J. S. Lee, J. Mol. Biol. **294**, 477-485 (1999).
[3] S. L. Labiuk, L. T. J. Delbaere and J. S. Lee, Photochem. and Photobiol. **73**, 579-584 (2001).