Detection of Alkali Metal Ions in DNA Crystals Using State-of-the-Art X-ray Diffraction Experiments

V. Tereshko,[1] C. J. Wilds,[1] G. Minasov,[2] M. A. Maier,[3] T. Prakash,[3]
A. Howard,[4,5] Z. Wawrzak,[6] M. Manoharan,[3] M. Egli[1,*]

[1] *Department of Biological Sciences, Vanderbilt University, Nashville, TN, U.S.A.*

[2] *Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL, U.S.A.*

[3] *Department of Medicinal Chemistry, Isis Pharmaceuticals Inc., Carlsbad, CA, U.S.A.*

[4] *Department of Biological, Chemical and Physical Sciences, Illinois Institute of Technology, Chicago, IL, U.S.A.*

[5] *IMCA-CAT, Sector 17, Advanced Photon Source, Argonne National Laboratory, Argonne, IL, U.S.A.*

[6] *DND-CAT Synchrotron Research Center, Sector 5, Advanced Photon Source, Argonne, IL, U.S.A.*

**To whom correspondence should be addressed (phone 615-343-8070, e-mail martin.egli@ vanderbilt.edu)*

Abstract

The observation of light metal ions in nucleic acids crystals is generally a fortuitous event. Sodium ions in particular are notoriously difficult to detect because their x-ray scattering contributions are virtually identical to those of water, and $Na^+\cdots O$ distances are only slightly shorter than strong hydrogen bonds between well-ordered water molecules. We demonstrate here that replacement of Na^+ by K^+, Rb^+, or Cs^+ and precise measurements of anomalous differences in intensities provide a particularly sensitive method for detecting alkali metal ion binding sites in nucleic acid crystals. Not only can alkali metal ions be readily located in such structures, but the presence of Rb^+ or Cs^+ also allows structure determination by the single-wavelength anomalous diffraction (SAD) technique. Besides allowing identification of high-occupancy binding sites, the combination of high resolution and anomalous diffraction data established here can also pinpoint binding sites that feature only partial occupancy. Conversely, high resolution of the data alone does not necessarily allow differentiation between water and partially ordered metal ions, as demonstrated with the crystal structure of a DNA duplex determined to a resolution of 0.6 Å.

Methods and Materials

Crystals suitable for data collection were mounted in nylon loops and frozen and stored in liquid nitrogen. Data sets with crystals containing Ba^{2+} were collected on an in-house rotating anode generator/image plate setup. All other data collections were conducted at the APS, using the insertion device beamlines (ID-B) of the DND and IMCA collaborative access teams, located at Sectors 5 and 17, respectively. Both are equipped with 165- mm MARCCD detectors. Data to the maximum resolution limits of the individual crystals were collected at wavelengths below 1- Å. To improve completeness and to avoid overloads in the lower resolution bins, separate data sets were measured for the low- and high-resolution ranges in each case. Anomalous data were collected at a wavelength of 0.8151 Å for Rb^+-containing crystals (3rd harmonic range) and in the low-energy range (≥ 1.54 Å) for all other crystals. All data were integrated and scaled either in the DENZO/SCALEPACK or HKL2000 suites. Heavy atom searches, Patterson map and electron density map calculations, and SAD phasing were performed with the program CNS. Maps were displayed with the program TURBO FRODO. For all structures, initial refinements were performed with CNS. To calculate the R-free values, 10% of the data were set aside prior to the refinements. All anisotropic refinements with high-resolution data were conducted with the program SHELX-97.

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Reference

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