Covalent Incorporation of Selenium into Oligonucleotides for X-ray Crystal Structure Determination via MAD: Proof of Principle

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Introduction

Selenium was incorporated into an oligodeoxynucleotide in the form of 2′-methyl-seleno-uridine (U_{Se}). The x-ray crystal structure of the duplex $[d(GCGTA)U_{Se}d(ACGC)]_2$ was determined by the multiwavelength anomalous dispersion (MAD) technique and refined to a resolution of 1.3 Å, demonstrating that selenium can selectively substitute oxygen in DNA and that the resulting compounds are chemically stable. Since derivatization at the 2′-α-position with selenium does not affect the preference of the sugar for the C3′-endo conformation, this strategy is suitable for incorporating selenium into RNA. The availability of selenium-containing nucleic acids for crystallographic phasing offers an attractive alternative to the commonly used halogenated pyrimidines.

Methods and Materials

Oligonucleotide Synthesis and Purification

The synthesis of the 2′-$SeCH_3$ ribo U phosphoramidite was described previously. All other oligonucleotide reagents and 3′-CE deoxyphosphoramidites were purchased from Glen Research (Sterling, VA). The DNA decamer $GCGTAU_{Se}ACGC$ was synthesized on a 2-μmol scale on an Applied Biosystems Inc. 381A DNA synthesizer. Coupling times of 90 seconds and 10 minutes were used for 3′-CE deoxyphosphoramidites and the 2′-$SeCH_3$ phosphoramidite, respectively, and the 5′-trityl group was retained following synthesis. All couplings were greater than 75% as judged by the trityl assay. Cleavage of the oligonucleotide from the solid support and deprotection was achieved by using 28% NH_4OH at 55°C for 8 hours. Reverse phase (RP) high-pressure liquid chromatography (HPLC) analysis and purification were carried out on an Applied Biosystems Inc. chromatograph with a Hewlett-Packard Hypersil ODS-5 column (4.6 × 200). A 1% gradient of acetonitrile in 0.03 M triethylammonium acetate buffer (pH 7.0) was used with a flow rate of 1.0 mL/min. The crude "trityl-on" oligonucleotides were purified by RP HPLC, detritylated in 80% acetic acid for 30 minutes, and then purified again by RP HPLC.

Crystallizations

Crystals of the selenium-modified decamer duplex were grown by the hanging-drop vapor-diffusion method; a commercially available sparse matrix screen (Hampton Research Inc., Laguna Niguel, CA) was used. Crystals for data collection were obtained by mixing equal volumes of an aqueous DNA solution (2 mM) and a solution containing 40 mM sodium cacodylate (pH 7.0), 12 mM sodium chloride, 80 mM potassium chloride, 12 mM spermine tetrahydrochloride, and 10% (volume/volume or v/v) 2-methyl-2,4-pentanediol (solution 19, Hampton Research's nucleic acid mini screen), then equilibrating the droplets over 1 mL of a 35% (volume/volume or v/v) 2-methyl-2,4-pentanediol reservoir solution. Several other conditions also resulted in crystal growth. Crystals typically appeared within 1 week and belonged to the orthorhombic space group $P2_12_12_1$ with unit cell constants of a = 24.56 Å, b = 43.97 Å, and c = 45.34 Å, and one duplex per asymmetric unit. The crystals were shock-frozen directly in the mother liquor for data collection.

MAD Data Collection and Crystal Structure Determination

X-ray diffraction data to a maximum resolution of 1.3 Å were collected at 100K on DND-CAT beamline 5-ID at the APS. MAD data from the K edge of selenium were collected on a single 2′-methylseleno-modified crystal. High- and low-resolution data sets were collected separately. Before data collection, a fluorescence scan centered around the selenium K edge was recorded in order to accurately tune the x-ray beam to the three wavelengths: those at the inflection point (edge), the absorption maximum (peak), and about 460 eV above the peak wavelength (remote). The three wavelengths were alternated after each sweep of 45° in phi with a 1° oscillation. All data were integrated and merged by using DENZO and SCALEPACK, respectively. The positions of the two selenium atoms per duplex were determined with CNS-solve. Following refinement of the heavy atom parameters, experimental MAD phases were calculated with the same program. Solvent flattening and density modification significantly

improved the initial map, revealing excellent density for all 20 nucleotides of the duplex. The structural model of an A-form DNA duplex was used to fit atom positions into the modified experimental electron density. Initial positional and isotropic B-factor refinements of the duplex were carried out with CNS, using inflection point data and with 5% of the reflections set aside as a test set to monitor the R_{free}. Anisotropic B-factor refinement for all DNA atoms and selected solvent molecules was performed with SHELX-97. After an R_{free} of 20.0% was reached, all reflections to 1.3 Å resolution were used in the final rounds of refinement. Fixed positions for hydrogen atoms that were based on the positions of heavier atoms were included in the refinement, resulting in an R_{work} of 14.9%.

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