

Internal Derivatization of Oligonucleotides with Selenium for X-ray Crystallography Using MAD

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Introduction

Determination of the 3-D structures of DNA oligonucleotides, DNA-drug complexes, ribozymes, and viral RNAs with high resolution is invaluable for gaining insights into their functions and mechanisms. Several approaches, including heavy-atom soaking, cocrystallization, and halogen derivatization, have been used to label DNA and RNA for nucleic acid x-ray crystallography. Heavy atom soaking and cocrystallization have not often been very successful for nucleic acid x-ray crystallography, and halogen derivatization is usually limited to short nucleotides. In protein x-ray crystallography, selenium replaces sulfur in order to mimic methionine, and this selenomethionine derivatization method is widely used in determining the phase and structure of proteins by multiwavelength anomalous dispersion (MAD). Indirect derivatization of RNAs by using the selenomethionine-labeled RNA-binding protein U1A for phase and structure determination has been successfully demonstrated, even though the RNA-binding protein U1A has to be prepared and even though appropriate positions for inserting the U1A binding site have to be identified by building numerous ribozyme constructs and screening their complexes with the protein. Therefore, direct labeling of nucleotides with selenium will greatly simplify the derivatization effort and will facilitate nucleic acid x-ray crystallography.

We have developed a route for the synthesis of 2'-selenium uridine analogues and oligonucleotides containing selenium labels, and we have demonstrated, for the first time, a new strategy to covalently derivatize nucleotides with selenium for phase and structure determination in x-ray crystallography. The 2'-position selenium derivatization retains the native C3'-endo conformation of A-form DNA and RNA molecules. Because the solid-phase synthesis allows Se-RNA and Se-DNA to be prepared at large scales, this approach (unlike the phosphoroselenonate-mediated autoligation of DNA strands), is suitable for RNA and A-form DNA derivatization for x-ray crystallography. Selenium labels can also be incorporated into a large RNA molecule via ligation of a transcribed fragment and a

synthetic fragment containing selenium labels. This derivatization method may serve as an alternative approach in phase and structure determination of RNA-protein and DNA-protein complexes by derivatizing RNA and DNA instead of proteins.

Methods and Materials

DNA and RNA analogues containing selenium at the 2'-positions [DNA-octamer 5'-GU_{Se}GTACAC, DNA-decamer 5'-GCGTAU_{Se}ACGC-3', and RNA-hexamer 5'-r(CGU_{Se}AC)dG-3'] were synthesized after standard solid-phase synthesis. The potential for scale-up was demonstrated by 10- μ mol syntheses. As expected, the protected selenide functionality was found to be stable in mild I₂ treatment (20 mM, 20 seconds) for the phosphite oxidation. The Se-oligonucleotides with methyl protection were purified by high-pressure liquid chromatography (HPLC), and selenium functionality was confirmed by electrospray mass spectrometry (MS). Crystallization conditions were screened, and diffraction-quality crystals were identified. X-ray fluorescence spectra recorded at DND-CAT beamline 5-ID at the APS confirmed the presence of selenium in crystals. MAD data of the Se-decamer to 1.2 Å resolution were collected at the APS and the diffraction data were successfully phased on the basis of the selenium anomalous signal. Likewise, diffraction data of the octamer to 1.8 Å resolution were collected, and the structure of the octamer was determined by the molecular replacement technique. These x-ray structures confirmed the presence of the 2'-methylseleno group at the 2'-position of uridine.

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