2′-O-[2-(Methylthio)ethyl]-modified Oligonucleotide: An Analog of 2′-O-[2-(Methoxy)ethyl]-modified Oligonucleotide with Improved Protein-binding Properties and a High Binding Affinity to Target RNA

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Introduction

A novel 2′-modification — 2′-*O*-[2-(methylthio) ethyl] or 2′-*O*-MTE æ has been incorporated into oligonucleotides and evaluated for its properties relevant to antisense activity. The results were compared with the previously characterized 2′-*O*-[2-(methoxy)ethyl] or 2′-*O*-MOE modification. As expected, the 2′-*O*-MTE-modified oligonucleotides bound better to human serum albumin than did the 2′-*O*-MOE-modified oligonucleotides. The 2′-*O*-MTE oligonucleotides maintained high binding affinity to target RNA. Nuclease digestion of 2′-*O*-MTE oligonucleotide showed that they have limited resistance to exonuclease degradation. We analyzed the crystal structure of a decamer palindrome sequence incorporating the 2′-*O*-MTE modification. An analysis of crystal structure explained the improved binding affinity, protein-binding affinity, and limited nuclease resistance of 2′-*O*-MTE to exonuclease degradation.

Methods and Materials

Optimal crystallization conditions for the modified decamer were screened by the sparse matrix crystallization technique; the Hampton Research (Laguna Niguel, CA) nucleic acid mini screen was used. Crystals for data collection were grown by the hanging-drop vapor-diffusion method. Equal volumes of a 2 mM oligonucleotide solution in water and a buffer solution, containing 40 mM sodium cacodylate (pH 7.0), 80 mM potassium chloride, 12 mM spermine tetrahydrochloride, and 10% (volume/volume or v/v) 2-methyl-2,4-pentanediol (MPD), were mixed and equilibrated against 1 mL of 35% (v/v) MPD. Diffraction data to a maximum resolution of 1.2 Å were collected on a single flash-frozen (100K) crystal at a wavelength of 1 Å at DND-CAT beamline 5-ID at the APS by using a MarCCD detector. Data were integrated and merged in the DENZO/SCALEPACK suite. The structure was solved by the molecular replacement method by using the program AMORE. Crystallographic refinements were performed with the programs CNS and SHELX-97.

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