Direct Observation of a Cytosine Analogue That Forms Five Hydrogen Bonds to Guanosine: Guanyl G-clamp

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Introduction

The two main factors that stabilize pairing between nucleic acid strands are stacking interactions and hydrogen bonding. These properties form the basis for biomolecular recognition. Our understanding of the recognition mechanism has spurred the development of a family of antisense molecules as potential therapeutics. Although modified oligonucleotides offer unparalleled potential in terms of binding selectivity, there are still numerous obstacles to overcome in terms of nuclease resistance, optimum induction of RNase H activity, uptake into cells, and tissue distribution. While the phosphodiester linkage and the sugar moiety have been modified extensively, modifications to the heterocyclic base have been relatively limited, since it is necessary to maintain the specific hydrogen bonding motifs required for base pair specificity. Here, we provide details at 1.0-Å resolution on the crystal structure of a modified DNA decamer containing a novel G-clamp analog that features a guanidinium group tethered to a phenoxazine ring system capable of forming five hydrogen bonds to guanosine. Binding studies of oligomers containing a single unit to an RNA target revealed an increase in the melting temperature of 16°C relative to the wild-type DNA.

Methods and Materials

A crystal was picked up from a droplet with a nylon loop and transferred into a cold N_2 stream (120K). High- and low-resolution data sets were collected on DND-CAT beamline 5-ID (λ = 0.978 Å) at the APS by using a MarCCD detector. Data were integrated and merged with DENZO/SCALEPACK. The overall R_{merge} for all reflections between 20 and 1 Å was 4.7%. Refinement was performed with the programs CNS and SHELX-97.

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