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

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Abstract

The two main factors that stabilize pairing between nucleic acid strands are stacking interactions and hydrogen bonding, and these properties form the basis for biomolecular recognition. Our understanding of this mechanism of recognition 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, as it is necessary to maintain the specific hydrogen bonding motifs required for base pair specificity. Here, we report structural details of a crystal structure at 1.0 Å resolution of a modified DNA decamer containing a novel G-clamp analogue 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 16ºC relative to the wild-type DNA.

Mehods and Materials

A crystal was picked up from a droplet with a nylon loop and transferred into a cold N_2 stream (120 K). High- and low-resolution data sets were collected on the 5-ID beam line (λ = 0.978 Å) of the DND-CAT at the APS 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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Reference

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