Development of Ligands That Selectively Target T-T Mismatches in CTG Trinucleotide Repeats

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Myotonic dystrophy type 1 (DM1) is one of >30 inheritable diseases whose origin can be traced to unstable repeating sequences in genomic DNA. DM1 is caused in part by the dysregulation of alternative pre-mRNA splicing that arises from the sequestration of proteins in the muscleblind-like (MBNL) family by expanded, non-coding r(CUG) trinucleotide repeats (TNR). Most therapeutic approaches have focused on developing agents that strongly and selectively bind the toxic $r(CUG)^{exp}$, thereby inhibiting its sequestration of MBNL proteins. However, some drawbacks include the continuous production of progressively longer $r(CUG)^{exp}$ transcripts and, more importantly, not stopping disease progression and transmission between generations. A more powerful approach is to target the expanded CTG repeats $(CTG)_n$ in DNA. Most models propose that TNR expansion occurs in DNA metabolic processes including replication, transcription, and mismatch repair, often involving unusual secondary structures during these processes, such as intra-strand hairpins and loop-outs. We hypothesize ligands which selectively bind hairpin CTG, loop-out CAG, or CTG•CAG duplex repeats in DNA will allow for an alternative approach to treat DM1 by possibly contracting $(CTG)_n$. Furthermore, these ligands may serve as useful probes for $(CTG)_n$ detection. We report a pyrroloquinazoline ligand that selectively binds T-T mismatch-containing DNA. Herein, derivatization, binding mode studies of the ligand, and future work are described.

Characterization of DhpH-C, a tRNA-Dependent Enzyme in Dehydrophos Biosynthesis

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Understanding the biochemical reactions employed by bacteria to produce antibiotics will aid in our ability to study and manipulate the selectivity and potency of these antibacterial compounds. Dehydrophos is an antibiotic that belongs to the phosphonates class of natural products. Phosphonates have a hydrolytically stable C-P bond, which allows them to mimic carboxylic acids and phosphate esters to inhibit a number of cellular processes. As a result, phosphonates are pharmaceutically relevant compounds with biosynthetic pathways that contain unusual enzymology. Dehydrophos is one of a number of phosphonates classified as phosphonopeptides. In particular, dehydrophos is a tripeptide antibiotic produced by *Streptomyces luridus* that includes glycine and leucine attached to a phosphonate analog of dehydroalanine. The gene cluster contains two genes predicted to encode FemX-like peptidyltransferases. FemX from *Weissella viridescens* interacts with $Ala\text{-}tRNA^{Ala}$ to add the first branched amino acid to the pentapeptide cell wall precursor. The C-terminal domain of the enzyme DhpH (DhpH-C) encoded within the dehydrophos gene cluster depends on $Leu\text{-}tRNA^{Leu}$ to form the first amide bond of the tripeptide. The goal of this study was to investigate the substrate specificity of DhpH-C, as well as the tRNA-enzyme interaction by determining residues of DhpH-C and elements of tRNA important for activity. Examining how tRNA elements affect reaction efficiency will enhance our understanding of this new class of enzymes involved in secondary metabolism.