Pseudo-complementary Nucleic Acids

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Pseudo-complementary (pc) nucleic acids contain base analogs that form weak base pairs with one another but form strong base pairs with standard bases(1,2). Consequently, pc-nucleic acids have diminished intramolecular and intermolecular secondary structures and can readily hybridize to unmodified nucleic-acids. The differential hybridization property of pc-nucleic acids is an attractive feature which can be utilized in microarray studies and in the targeting of duplex DNA.

One example of a pseudo-complementary base pair is the one between 2-aminoadenine (nA) and 2-thiothymine (sT) (Figure 1). This family of base analogs has been studied extensively in a number of polymer systems, which include DNA, RNA, and PNA(1,3,4,5). In comparison to the Watson-Crick base pair between adenine (A) and thymine (T), the pair between nA and sT is unstable because of the steric clash between the exocyclic amine of nA and the large size of the sulfur atom of sT. While the nA:sT base pair is unstable, the base pairing strength of the A:sT pair is similar to that of an A:T base pair, and the nA:T pair is more stable than A:T, presumably because three hydrogen bonds can now be formed between nA and T(6,7,8,9,10,11). This dynamic hybridization property of pc-nucleic acids is a desirable characteristic, which has found utility in a number of applications.

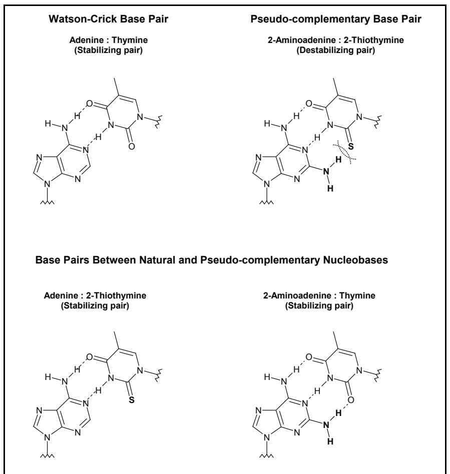


Figure 1: Proposed Base Pairs Between Natural and Pseudo-complementary Nucleobases. Differences between the pseudo-complementary and the corresponding natural nucleobases are represented in bold. Steric clashes within a base pair are represented by the dashed arcs.

Literature precedence has described how hybridization of a nucleic acid sequence to its corresponding complement can be hindered by significant secondary structure(12,13,14,15). Figure 2 depicts the advantage of utilizing pc-nucleic acids in hybridization studies, such as those which employ microarrays. As the degree of secondary structure should be significantly diminished for sequences composed of pc-nucleic acids, more representative hybridization to tiling microarrays is predicted. Advancements towards this ultimate goal have focused on incorporation of the corresponding pseudo-complementary nucleoside triphosphates during polymerase-mediated replication of the corresponding template. To accomplish this goal, the corresponding pseudo-complementary nucleoside triphosphate should be readily incorporated opposite its natural nucleobase with high fidelity. For example, in DNA polymerase replication, nA is selectively incorporated opposite thymine and sT is selectively incorporated opposite adenine, establishing the fidelity of recognition in DNA polymerase replication(16,17,18). Work with pc-nucleic acids has been recorded in a number of published manuscripts(3,16,17,18,19) as well as within a recent patent application filed by Sampson et al. from Agilent(20). Overall, encouraging results have been obtained, allowing for further advances towards more uniform nucleic acid hybridization.

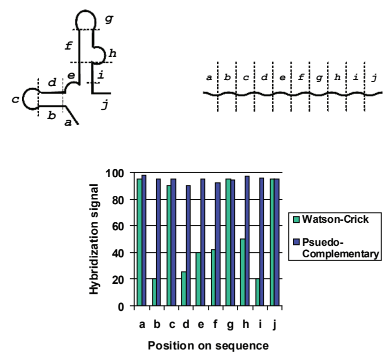
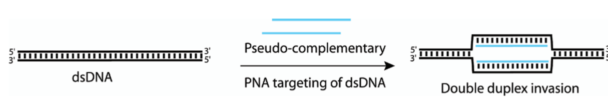


Figure 2. Effect of secondary structure on nucleic acid hybridization. a) Natural Watson-Crick sequence exhibiting a high degree of secondary structure. b) The same sequence, prepared with pc-nucleic acids displays reduced secondary structure. c) Expected hybridization performance of Watson-Crick and pc-nucleic acids to a tiling microarray reveals a much more uniform signal for sequences prepared with pc-nucleic acids

Peptide Nucleic Acids (PNAs) are noncharged polyamide sequences that form very stable duplexes with a complementary DNA strand(21). One interesting application of PNAs involves the use of pseudo-complementary PNAs to target duplex DNA (Figure 3)(4). The resultant complex is a stable double duplex invasion structure. If PNAs with the natural nucleobases attached were used, the duplex invasion would not be very efficient, as the dsDNA would remain intact and the two PNA strands would bind to one another with high affinity. The ability of pc-PNA to perform double duplex invasion has been utilized in a number of applications, which include inhibition of transcription initiation(4), modification of restriction endonuclease activity(22,23), and site-specific hydrolysis of duplex DNA(24).



Overall, the significant reduction of secondary structure in pc-nucleic acids is an attractive characteristic with a large number of potential applications ranging from nucleic acid detection and non-standard nucleic acid-based structure formation. As pseudo-complementary sequences form unstable sequences with one another and stronger complexes with their natural complement, there is a great potential for the development of novel applications that rely on the differential hybridization properties of nucleic acids.

TriLink is currently collaborating with Professor Howard Gamper of Thomas Jefferson University and was funded by a STTR grant to study pc-nucleic acids(25,26). Please contact us if you are interested in learning more about this exciting technology.

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