Conformational Preorganization, Hydration, and Nucleic Acid Duplex Stability

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Chemical modification of DNA and RNA and the generation of artificial oligonucleotide pairing systems have been spurred by two central objectives. Although initially unlinked, both promise new insights into the nature of the double helical conformations assumed by DNA and RNA. The first concerns issues of molecular evolution and, among many others (Brosilow, 1998), seeks a rationalization for nature’s selection of pentoses over other potential candidates as the carbohydrate building blocks of the genetic material (Eschenmoser and Loewenthal, 1992). Chemical synthesis of alternative nucleic acid pairing systems and an exploration of their physical-chemical properties can potentially yield insights into such questions (Böhlinger et al., 1992; Eschenmoser and Kisliuk, 1996).

For example, comparison of the conformational and pairing properties of hexose-DNA and DNA has led to the view that the helicity of DNA is a consequence of the inherent geometric constraints of the deoxynucleosides in its backbone (the situation in RNA is similar) (Eschenmoser and Dobler, 1992). Furthermore, the Watson-Crick base pairing principles in DNA duplexes (C-G > A-T) are no longer valid in duplexes formed by (3′,2′-dideoxy-β-D-glucopyranose) based oligonucleotides (G-C > A-T - G-G > A-T) (Hunner et al., 1993). Thus, the standard base pairs in DNA are not simply a consequence of the chemical nature of the four bases, but their formation is also related to the nature of the backbone sugar moiety.

The search for oligonucleotides with sufficient metabolic stability for in vivo applications has provided the second strong incentive for chemically modifying DNA and RNA. DNA and RNA oligonucleotides are readily degraded biologically, and in the case of RNA, chemical stability is also insufficient. Chemical modification may render them more resistant to degradation by various nucleases. However, chemical modification should not impair RNA affinity and pairing selectivity. 200 modifications were reviewed recently, and their hybridization affinities for complementary RNA were analyzed in the context of the expected structural changes (Frieser and Altermann, 1997).

This structure-activity correlation has led to the identification of several stabilizing features. Thus, modifications that provide higher RNA affinity either (1) preorganize the backbone in conformations favorable for hybridization to RNA, (2) improve stacking through addition of a polarizable group to the base heterocycle, (3) increase the number of hydrogen bonds, for example, replacement of A by 2,5-diaminothymine, (4) neutralize the negative charge of the phosphate group, or (5) favor conformations determined by the separation of the associated DNA-RNA duplexes (Bläsielin et al., 1996) despite the preference of the ss DNA backbone for an A-type C3′-endo conformation and the fact that the negatively charged phosphodiester moiety is replaced with a neutral linker (Roughan et al., 1996; Eglı, 1996). This example demonstrates that subtle geometric changes caused by a chemical modification (S-C vs. P-O bond lengths, S-C-C vs. P-O-C bond angles) can offset features that in principle should enhance RNA affinity (Northern-type packer, neutral backbone).

N3′ → P5′ phosphoramidate DNA (3′-NP DNA), a second-generation nucleic acid analog in which O3′ is replaced with an amino group, displays drastically enhanced RNA affinity compared with DNA and RNA (Fig. 1A). Gryaznov and Chen, 1994; Chen et al., 1995; Gryaznov et al., 1995). Moreover, 3′-NP DNA self-pairing is considerably more stable than duplex formation in the case of either DNA or RNA. It is noteworthy that stable formation of Py3-Py-type triplexes with both duplex DNA and RNA and 3′-NP DNA as the third strand was observed at neutral pH, where triplex formation with a phosphodiester third strand does not occur (Escudero et al., 1996). Circular dichroism (CD) experiments suggested that 3′-NP DNA duplexes resemble the RNA A-form (Gryaznov et al., 1995), and 2-dimensional NMR data showed that the 3′-aminoaruranoses in 3′-NP DNA duplexes adopt predominantly A-type puckerings (Ding et al., 1996). 3′-NP DNA oligonucleotides display improved nuclease resistance relative to DNA and RNA, and the accumulated in vitro and in vivo data suggest that phosphoramidates can serve as potent and specific antitumor (Heidelbach et al., 1997; Skrzek et al., 1997) and antigenic agents (Gleaves et al., 1996; 1997) and potentially as detergents (Eglı et al., 1997).

We have recently determined the crystal structure of the 3′-NP DNA duplex with sequence 5′-d(CpGpGpCpGpAmpAmpTApTApCpGpGpCpGpCpGp) at 2.4 Å resolution (Tereshko et al., 1998). This is the first crystal structure of an all-chemically modified DNA double helix. Consistent with the earlier CD and