

# Sugar Pucker and Nucleic Acid Structure

Martin Egli\*

*“The world is made of sugar and dirt” (Döblin, 1929)*

DNA and RNA are acids, contain bases, form salts and are held together by sugars. In DNA, the sugar is 2'-deoxyribose and in RNA it is ribose. By the time I joined Alex's lab in 1989, the important role of the sugar in determining the shape and function of the nucleic acids had long been recognized. As recounted in a short paper titled “The double helix: a tale of two puckers” (Rich, 2003), Alex made a string of discoveries that paved the way to a deeper understanding of how the sugar influences the conformation of DNA and RNA.

Early fiber diffraction images had revealed two forms of the DNA duplex and it was later found that these were based on different conformations of the sugar moiety. In the B-form, the C2'-atom is out of the plane on the same side as the nucleobase (C2'-endo, Fig. 1A). In the A-form, the C3'-atom is out of the plane and the sugar conformation is C3'-endo (Fig. 1B). The change in sugar pucker has many consequences, including the duplex shape, groove widths and depths, intra-strand phosphate-phosphate distances and backbone hydration. Although DNA models based on fiber diffraction patterns led to an understanding of how sugar conformation affects the architecture of the double helix, single crystal diffraction studies of mini-helices [RNA (Rosenberg *et al.*, 1973)] and oligonucleotides at high resolution later routinely allowed accurate visualization of the sugar pucker.

The discoveries that poly(A) and poly(U) (Rich & Davies, 1956) and poly(A) and poly(dT) (Rich, 1960) could be hybridized had a tremendous impact, considering past and present applications as well as structure and stability of the nucleic acids. Thus, it became clear that the canonical RNA duplex resembled the DNA A-form and that DNA could adapt to RNA — but not the other way around — in a DNA:RNA hybrid duplex. We found later that a single ribonucleotide in an oligo-2'-deoxyribonucleotide could drive the entire duplex into the A-form (Egli *et al.*, 1993). As well, an Okazaki fragment, a chimeric RNA-DNA strand paired to DNA, was found to adopt a regular A-form in the crystal (Egli *et al.*, 1992). However, the conclusion that every DNA:RNA hybrid is of the A-form is wrong and in reality, the

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\*Department of Biochemistry, Vanderbilt University, Nashville, TN 37232

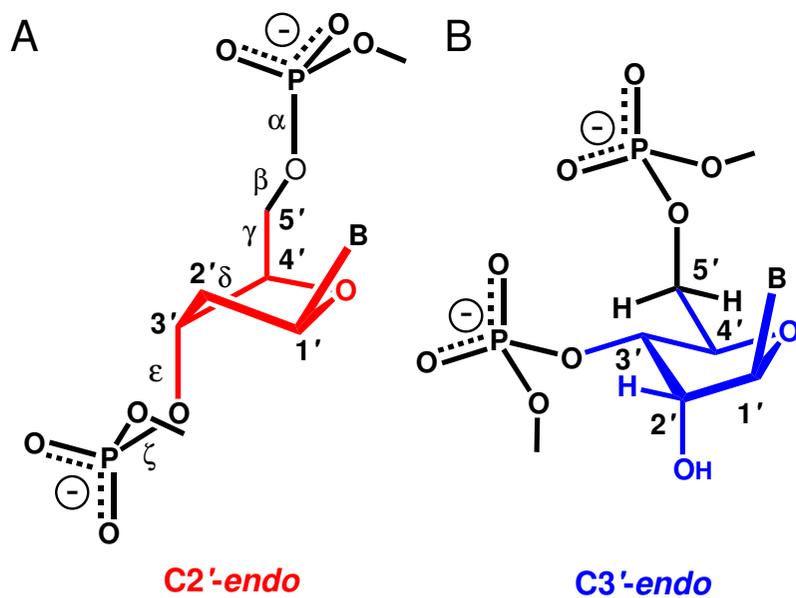


Fig. 1. Idealized diamond-lattice models of (A) DNA B-form, and (B) RNA A-form backbones.

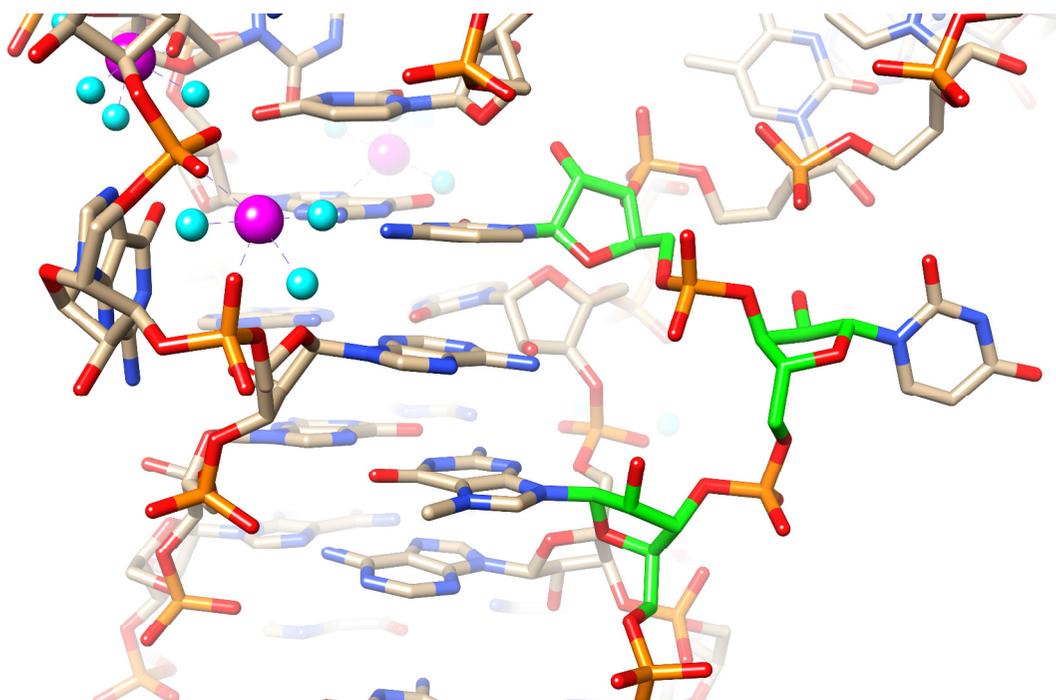


Fig. 2. Ribonucleotides with C2'-endo pucker (carbon atoms of sugar moieties highlighted in green) in the anticodon arm of tRNA<sup>Phe</sup> (PDB ID 1ehz; [www.rcsb.org](http://www.rcsb.org)).

DNA strand displays a range of conformations in structures of such duplexes, either alone or in complex with proteins. For example, in the hybrid bound to RNase H, all RNA riboses exhibit C3'-endo pucker, but the pucker of DNA 2'-deoxyriboses is predominantly of the C2'-endo type (Nowotny *et al.*, 2005).

That the ribose pucker in RNA is not limited to C3'-endo becomes clear when one inspects the structures of more complex RNA molecules such as transfer RNA (Kim *et al.*, 1974; Robertus *et al.*, 1974). Outside canonical stems, nucleotides in single-stranded regions can be found in the C2'-endo pucker (Fig. 2). The 2'-hydroxyl group then assumes a pseudo-equatorial orientation instead of the axial one associated with the C3'-endo pucker (Figs. 1B, 2).

Another momentous discovery in the areas of DNA structure and function in Alex's lab was the unusual conformation of Z-DNA (Wang *et al.*, 1979). It was the first crystal structure of an oligonucleotide and did not reveal the expected right-handed B-form geometry. Half the sugars were not of the C2'-endo type; in fact, Gs adopt C3'-endo pucker and their nucleobase is swung around and in a syn orientation. Combined with the standard C2'-endo/anti conformation of Cs, this gives rise to the characteristic zig-zag shape of the backbones and alternating short and long separations between adjacent intra-strand phosphate groups. Z-DNA offered a rich source for insights into DNA structure, stability, hydration and stereoelectronic effects (Gessner *et al.*, 1994; Egli & Gessner, 1995).

That the ribose 2'-hydroxyl group will have an important impact on the structure of the double helix (Watson & Crick, 1953; Rich & Davies, 1956) and the energetic barrier between the C3'-endo and C2'-endo sugar conformations is not surprising. However, it is less obvious that the different sugar puckers in DNA and RNA also affect the inclination between the backbone and base pair axes (Egli *et al.*, 2007). In DNA, the two are virtually perpendicular and in RNA they are negatively inclined. Thus, the sugar pucker is at the origin of the relative strand polarity in double helices. Besides the normal antiparallel arrangement, DNA strands can pair in a parallel orientation (van de Sande *et al.*, 1988). Conversely, RNA strands in duplexes are limited to the antiparallel orientation.

As well, it is not unexpected that the 2'-hydroxyl group in RNA changes the hydration of the RNA duplex compared to that of DNA. The presence of the C2'-substituent that can act as a H-bond donor and acceptor results in a stable and regular water structure in the minor groove (Egli *et al.*, 1996). However, the steric and electronic influence of the 2'-oxygen on sugar pucker and RNA structure may have overshadowed the fact that the 2'-substituent consists of oxygen *and* hydrogen. X-ray crystal structures even at high resolution do not reveal the positions of hydrogen atoms. Moreover, the hydration patterns around the ribose do not allow a definitive answer as to the whereabouts of the hydrogen atom, i.e. whether it lies between O2' and base, or O2' and backbone (Egli *et al.*, 1996). MacKerell Jr. proposed that this very issue is at the origin of the conformational versatility of RNA (Denning & MacKerell Jr., 2012). In canonical duplexes, the hydrogen sits between O2' and base (water-mediated interaction). A rotation of the 2'-hydroxyl

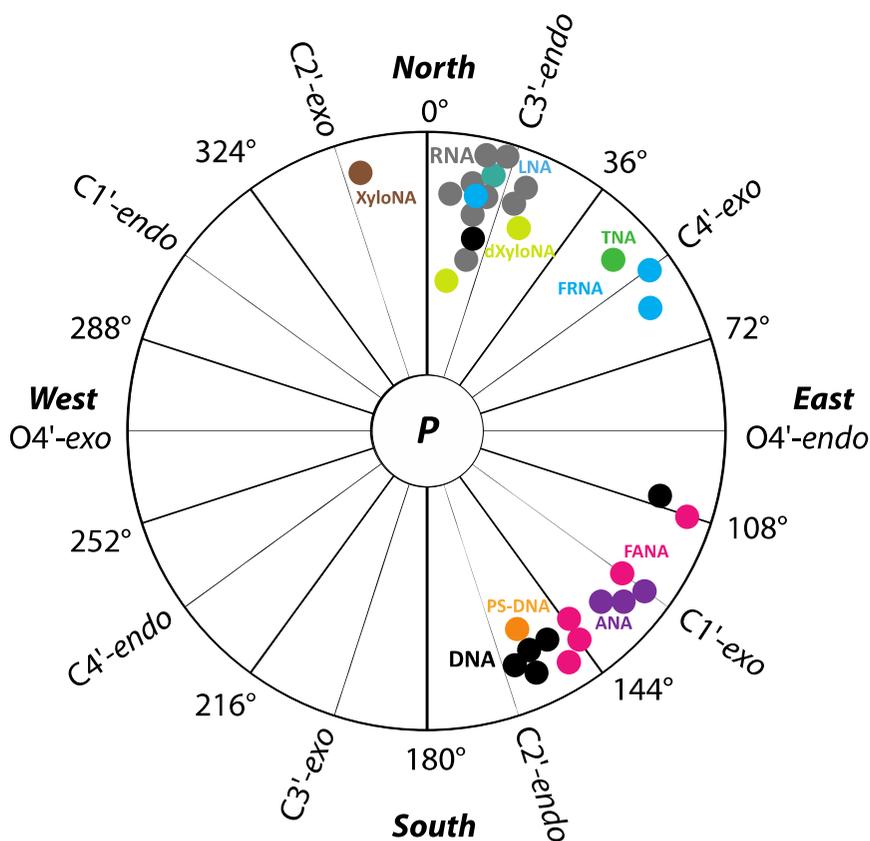


Fig. 3. Average pseudorotation phase angles of sugars in DNA and RNA and five-membered sugar rings of a selection of synthetic nucleic acid analogs (xeno-nucleic acids, XNA) (Anosova *et al.*, 2016). Different filled circles for DNA, RNA, FRNA, ANA, FANA, and deoxy-xylosenucleic acid, dXyNA, represent various duplex types (e.g. DNA:RNA, DNA:RNA, DNA:PNA, ANA:FANA, ANA-FANA-ANA:ANA-FANA-ANA, etc.). PS-DNA is phosphorothioate DNA.

group around the C2'-O2' bond swings the hydrogen over to the backbone, thus potentially favoring non-canonical conformations of the backbone and contributing to the rich tertiary structural repertoire of RNA. More insight into this topic has to await the determination of neutron crystal structures of RNAs. This demonstrates that RNA and ribose, 60 years after the pioneering fiber diffraction studies by Alex, still harbors secrets.

The C2'-endo and C3'-endo puckers map to opposite poles of the pseudorotation phase angle ( $P$ ) cycle: South and North, respectively (Fig. 3). They represent the most common conformations adopted by pentose sugars in the native nucleic acids. Other puckers also occur and the five 36° angle segments of the Eastern hemisphere can be populated by 2'-deoxynucleotides. In addition to DNA and RNA, a large number of nucleic acid analogs have been explored in regard to pairing stability and conformational properties in the last 30 years. Many of them were synthesized in the context of medicinal chemistry and to test them as antisense, siRNA or aptamer

oligonucleotides against a variety of targets (Egli & Herdewijn, 2012). Mimics of RNA with  $P$  angles that lie in the Northern region are much more numerous than those that mimic the conformational features of DNA. The reason for this imbalance is that antisense and siRNA oligonucleotides are mostly targeting mRNAs, and RNA analogs can be expected to pair more strongly with the target. Locked nucleic acid (LNA) and 2'-deoxy-2'-fluoro-ribonucleic acid (FRNA) constitute two prominent members of this class of molecules (Fig. 3). Another analog modified with fluorine is an excellent mimic of DNA: 2'-deoxy-2'-fluoro-arabinonucleic acid (FANA). In fact, FANA is one of only a handful of analogs that opposite RNA elicit cleavage by RNase H and therefore afford perfect mimicry of DNA conformation.

Investigations as part of an etiology of nucleic acid structure showed that the chemistry of the sugar affects the base-pairing rule (G:C > A:T). (Eschenmoser, 2011). Thus, purine-purine pairs are of similar stability as G:C pairs for ( $6' \rightarrow 4'$ ) 2',3'-dideoxyglucopyranose nucleic acid (homo-DNA), and homo-DNA displays a propensity for reverse-Hoogsteen pairing. This observation demonstrates that ribose and 2'-deoxyribose not only share a pucker type, but also support the preference for the Watson-Crick pairing mode and the relative stability of the two fundamental base pairs. XNAs exhibit a wide range of pairing properties (Anosova *et al.*, 2016). Some are capable of self-pairing and cross-pairing with both DNA and RNA, for example  $3' \rightarrow 2'$  L- $\alpha$ -threofuranose nucleic acid (TNA) and peptide nucleic acid (PNA). Others such as (*S*)-glycol nucleic acid (GNA) only pair with RNA but not with DNA. Yet others, such as homo-DNA and pyranosyl-RNA (pRNA) form autonomous pairing systems that exceed in stability by far those of DNA, RNA and their hybrids.

An important determinant of pairing by the various XNAs (hexose, pentose, tetrose or acyclic sugar moieties) that is influenced by the sugar pucker (cyclic sugars) is the particular combination of base-backbone inclination and helical twist angles. As it turns out, the right-handed twists of DNA and RNA strands and the ca.  $30^\circ$  difference between their inclination angles ( $0^\circ$  vs. ca.  $-30^\circ$ , respectively), allow stable cross-pairing. Opposite twist angles and/or differences in inclinations that are larger, e.g.  $45^\circ$ , will however prevent cross-pairing among XNAs and between XNAs and DNA or RNA. XNAs are now being studied in synthetic biology and synthetic genetics, and analyses of their 3D structures are beginning to reveal how significantly they deviate from the well-known structural motifs formed by DNA and RNA (Anosova *et al.*, 2016).

A question of particular interest is whether such artificial polymers populate an expanded fold space compared with DNA and RNA. Just like in the days of Alex's early fiber and single crystal X-ray diffraction analyses of DNA and RNA, the conformation of the sugar is bound to play an important role in XNA structure and function. The example of TNA with a tetrose in its backbone that appears to be limited to a C4'-exo pucker (Pallan *et al.*, 2003), but is nevertheless capable of pairing with DNA and RNA, may support the notion that chemical simplicity does not preclude complexity in folding and function.

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