Why natural DNA is based on 2'-deoxyribose, with 3',5' phosphodiester links

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Abstract : Ribonucleosides are converted to deoxyribonucleosides by a biochemical process that removes the 2'-hydroxyl group, and that could have removed the 3'-hydroxyl group instead with a different enzymatic preference. This raises the question of whether there is some intrinsic chemical preference for the natural structure of DNA, with its phosphate links joining the 3' and 5' positions of adjacent bases.

We have prepared DNA isomers in which we use 3'-deoxynucleosides, and which have the unnatural 2',5' links. We find that they form duplexes using the normal genetic code pairings, but more weakly than does normal DNA. Computer modelling indicates some of the problems that the unnatural structure introduces.

Introduction.

In the course of our studies on the cleavage of RNA by enzyme models, we saw that the cleavage was accompanied by simultaneous isomerization of the natural 3',5'-linked RNA to its 2',5'-linked isomer (1). This and other considerations prompted us to consider the question of why genetic information is carried in DNA with a 3',5' link (2, 3). Of course there is now no option, since in DNA the nucleosides are 2'-deoxy, as the result of enzymatic removal of the 2'-hydroxyl group from ribonucleosides. However, the biochemical sequence by which the 2'-hydroxyl is removed would also work for the removal of the 3'-hydroxyl group, except for the current enzymatic preferences that direct the regiochemistry (4). Thus a question can be formulated: is the current preference simply an accident of evolution, or is there an intrinsic disadvantage to the use of a 3'-deoxy 2',5'-linked analog of natural DNA (5)?
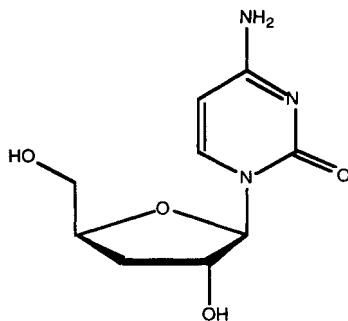

3'-Deoxycytidine
One of the 3'-deoxynucleosides that are linked as 2',5'-phosphodiesters
in our isomer of normal DNA.

We took up this question some years ago (2, 3), and at first used only the adenosine (<u>A</u>) and thymidine (<u>T</u>) analogs of normal A and T (we will use the underline to signify

isomers in which the 3'-hydroxyl is missing, so that all links are 2',5'). We concluded that there was indeed duplex formation with our complementary DNA oligomers constructed from mixed sequences of A and T, but that the strands were weakly bound together compared with analogous normal DNA sequences. This was in contrast with the conclusions of Switzer, who asserted that such abnormal DNA was even more strongly associated than was normal DNA (6). However, some of his melting data seemed to involve hairpin formation within a single strand, not duplex formation.

While mixed sequences of A and T hybridized poorly with complementary 2',5'-linked strands, we saw that there was strong association between a strand formed exclusively from A and one made up exclusively of T. It seemed likely that this reflected the formation of <u>triple</u> helices with the homopolymers, and this was confirmed in physical studies (7). Such triple helices—using both Watson-Crick and Hoogsteen base pairing—are seen with normal DNA and involve pyrimidine-purine-pyrimidine base triplets. Thus they are possible with the homopolymers, which use two strands of the T polymer along with one strand of the A polymer, but not possible with mixed sequences (except for segments containing significant runs of purines paired with runs of pyrimidines).

T•AT   **C⁺•GC**
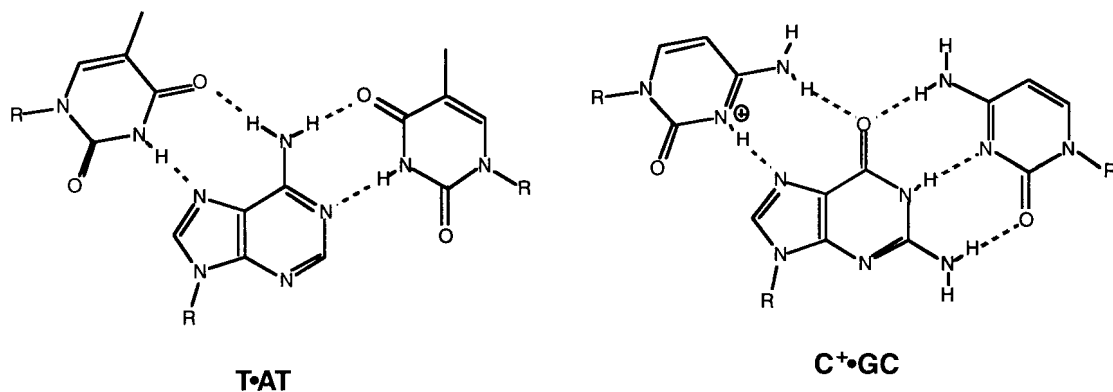
The base associations in triple helices, involving both Watson-Crick and Hoogsteen pairings. For C, Hoogsteen pairing with G requires protonation, and occurs at low pH.

The properties of G and C were clearly of interest. We reported the synthesis of the needed monomeric reagents (8), and Switzer reported some studies on the properties of oligomers containing only G and C (9). We now describe some of our studies with oligomers containing all four unnatural nucleosides—A, T, G, and C—and see that they confirm and expand our previous conclusions.

The preparation of the isomeric DNA oligomers.
We have described elsewhere the synthesis of phosphoramidites of protected nucleoside isomers in which the 3'-hydroxyl is missing, and phosphoramidation is on the 2'-hydroxyl group (2, 8). We find that these compounds are just as effectively used as are the normal isomers in automated solid phase oligonucleotide synthesis, as expected. We deprotected the resulting oligomers using standard procedures, to be described elsewhere. The oligonucleotides were then purified by denaturing polyacrylamide gel electrophoresis (PAGE), and the oligonucleotides were visualized using UV shadowing. They were cut from the gel and eluted from the gel slices using the crush and soak

method. They were finally purified on a Pharmacia NAP-10 column. The base compositions were confirmed by enzymatic digestions with snake venom phosphodiesterase and bacterial alkaline phosphatase, and the resulting 3'-deoxynucleosides were quantitatively analyzed by hplc.

The sequences relevant to the current paper are listed in Table 1. They were prepared in both the novel 2',5'-linked series and as the 3',5'-linked normal isomers.

TABLE 1. Oligonucleotides prepared in the 2',5' and 3',5' series.

NAME	SEQUENCE (5' to 2' or 3')
PUR-15	GGA GAG AGA GAG GGA
PYANTI-15	TCC CTC TCT CTC TCC
NORM-16	AGG CAT GCA AGC TTG T
ANTI-16	A CAA GCT TGC ATG CCT

Mixing experiments.

The composition of a complex can be determined by Job's method, in which some property of the complex reaches a maximum or minimum at the stoichiometric composition of the complex. This is shown in Figure 1 for normal 3,5PUR-15, containing G and A, mixed with normal 3,5PYANTI-15 that contains C and T in a sequence complementary to the purine sequence for antiparallel complexing. The Job's plot indicates a 1:1 complex, as is well known for these conditions (pH 7.0, 10 mM phosphate, 100 mM NaCl, 0.1 mM EDTA, total strand concentration 3.0 μM). Figure 2 shows the analogous plot for the abnormal 2,5PUR-15 with 2,5PYANTI-15; again there is a 1:1 complex formed under the above conditions. Thus both the normal 3',5' and the abnormal 2',5' isomers behave similarly under these conditions.
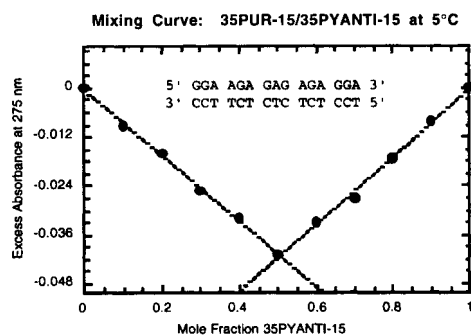

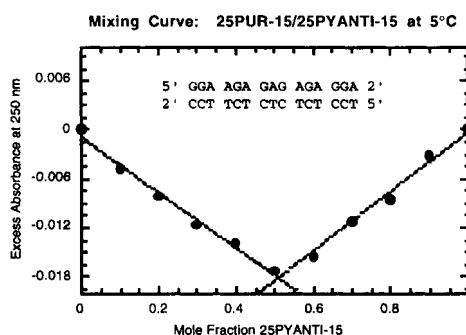Figure 1. Job plot for 3',5' antiparallel duplex,

Figure 2. Job plot for 2',5' antiparallel duplex,

Melting studies.

However, the strength of association is not the same. Determination of the UV intensity as a function of temperature, the standard "melting" studies, show that the duplex between 3,5PUR-15 and 3,5PYANTI-15 has a T_m of 50.6 ± 0.5 °C with 3 μM total strand concentration in buffer (10 mM phosphate, 100 mM NaCl, 0.1 mM EDTA, pH 7.0), while the duplex of 2,5PUR-15 with 2,5PYANTI-15 has a T_m of 34.2 ± 0.5 °C under the same conditions. Thus the normal duplex is more stable. An even more striking contrast is seen with the fully mixed sequences NORM-16 and ANTI-16.

These each contain all four bases, as one would see in genetic DNA, and they were examined at 4 μM total strand concentration and neutral pH (7.0), and with reasonable salt concentrations (10 mM phosphate buffer, 200 mM NaCl, 0.1 mM EDTA). As the melting curves in Figure 3 show, the normal 3,5 isomers have a T_m of 65.5 °C for their duplex, while that for the abnormal 2,5 isomers has a T_m of 20.5 °C. Thus complexing is much weaker in the 2,5 series.

We also examined the ability of the 2,5 series to hybridize with the 3,5 oligomers in a 1:1 ratio. The complex of 3,5NORM-16 with 2,5ANTI-16 had a T_m of 14.0 °C under the above conditions, as did the complex of 2,5NORM-16 with 3,5ANTI-16. Thus the mixed duplexes between the 3,5 and 2,5 series are the weakest of all.

Triple helix formation.
As we have described earlier, the poly$\underline{T}$ and poly$\underline{A}$ strands tend to form triple helices readily (3, 7). However, for triple helix formation involving G's and C's, low pH is required. The C needs an extra proton in order to participate in Hoogsteen base-pairing with G. We have not seen any evidence of triple helix formation with our new oligomers, containing $\underline{G}$ and $\underline{C}$, at pH 7.0. However, at lower pH triple helices are formed; the full story on this will be described elsewhere. The bottom line is that at pH 5.0 we see an antiparallel triple helix formed with PUR-15 and PYANTI-15 in both the 3,5 and the 2,5 series. As we saw with poly$\underline{A}$ and poly$\underline{T}$ (7), this triplex is somewhat more stable in the 2,5 series than in the 3,5 series, in contrast to the lower stability of the double helices in the abnormal series.

NORM-16/ANTI-16 Hybridization

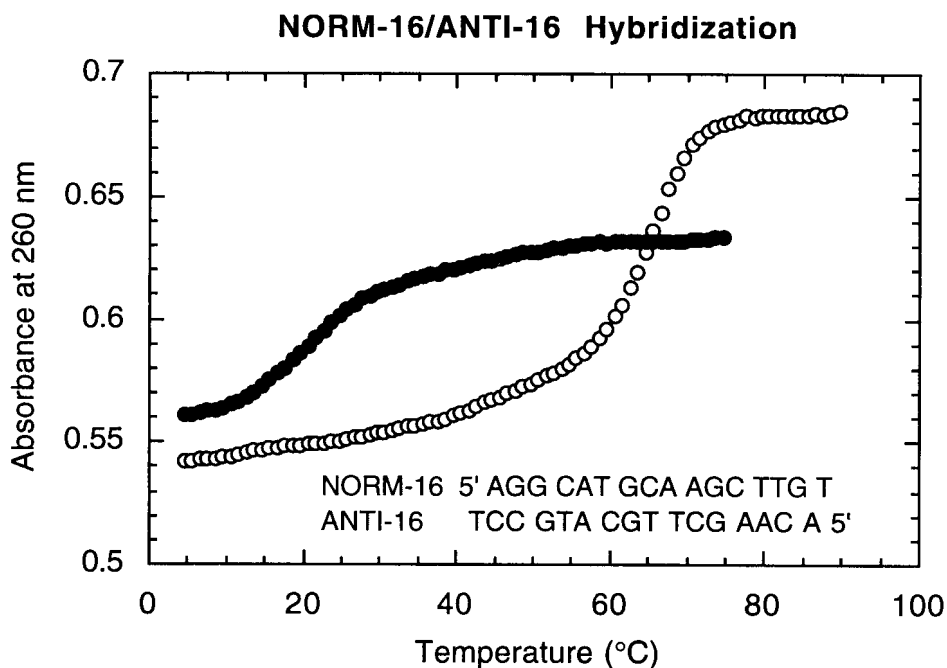NORM-16 5' AGG CAT GCA AGC TTG T
ANTI-16   TCC GTA CGT TCG AAC A 5'

Figure 3. Melting curves for duplexes of complementary mixed sequences at pH 7.0 with 10 mM phosphate buffer and 200 mM NaCl. The open circles are for normal 3',5'-linked DNA, while the closed circles are for the 2',5'-linked isomer.

Why do the 2',5'-linked DNA's form weaker duplexes?

Molecular modelling studies show that double helices consisting of our 2',5'-linked oligonucleotides differ geometrically from those of normal DNA (3, 7). The base stacking is imperfect, in the sense that not all the hydrophobic surfaces of the bases are covered by their stacking neighbors. Base stacking is the principal driving force for duplex formation, driven by hydrophobic energy and pi electronic stacking interactions. Furthermore, in the helix constructed from the 2',5'-linked nucleosides, there is a central hole.

In triple helices, models show (7) that the third strand helps cover some of the exposed surface of the bases in a 2',5'-linked duplex. This factor makes the triple helix more stable than that of a normal DNA.

Could 2',5'-linked DNA perform the genetic function of normal DNA?

For the transfer of genetic information it is critical that duplexes form under physiological conditions. Thus the weaker association in the 2',5' series means that it would not function as well. In the primitive earth there may well have been spontaneous random formation of both 2'-deoxy- and 3'-deoxynucleosides, but if so the selection process would favor the normal nucleosides that are now part of natural DNA. We think it quite unlikely that any organisms will be found that use our abnormal isomer.

However, abnormal DNA may still have useful properties. It does bind both to its abnormal complement and to its normal complement, albeit more weakly than does normal DNA. As we will describe elsewhere, it also binds well—essentially as well as does normal DNA—to normal RNA. It may have applications in research and in medicine, especially since it is not a substrates for some nucleases. In any case, its properties give us a fuller appreciation of the biological importance of one detail of DNA structure, the use of a 3',5'-link.

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