TNA as a Potential Alternative to Natural Nucleic Acids

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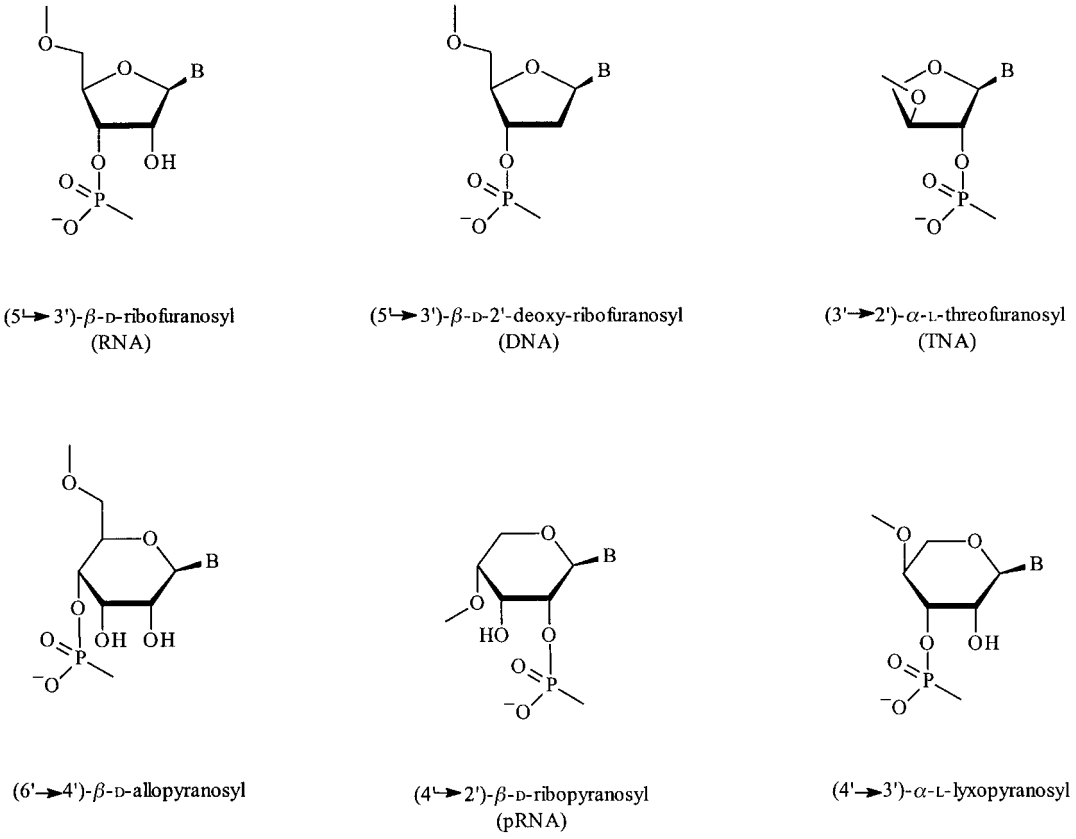
One of the intriguing questions related to the origin of life concerns the understanding of the chemical etiology of the present-day nucleic acids: RNA and DNA. This problem has been systematically approached by Eschenmoser and coworkers by analyzing several nucleic acid alternatives that might have been obtained under prebiotic conditions. Their analysis focused on the chemical properties that are fundamental to the biological functions of RNA and DNA, and has now led to the synthesis and the exploration of the properties of (3′→2′)-α-L-threofuranosyl oligonucleotides (TNA; Scheme 1).[1] The selection of TNA as a model system was based on previous investigations on the diastereoselective aldol condensation of glycolaldehyde phosphate, which has a kinetic preference for the formation of allose 2,4,6-triphosphate in the absence of formaldehyde and for ribose 2,4-diphosphate in the presence of formaldehyde. Significant amounts of racemic erythrose 2,4-diphosphate and threose 2,4-diphosphate are formed as intermediates of the hexoaldose synthesis, particularly in the absence of formaldehyde.[2] It is, therefore, not unrealistic to consider threofuranosyl nucleic acids as an ancestor to RNA or as an RNA alternative. The potential of TNA for base pairing was deduced from its structural analogy with (4′→3′)-α-L-lyxopyranosyl oligonucleotide that was known to show cross-pairing with DNA and RNA.[3] Previously, the β-aldohexose nucleic acids were ruled out as nucleic acid alternatives based on their functional inferiority to RNA. This conclusion was based on their lower base-pairing strength and base-pairing selectivity compared to RNA. The (4′→2′)-pentopyranosyl oligonucleotides, with pRNA as the prototype, have a stronger and more selective pairing system than RNA and have the potential for nonenzymatic replication,[4] which makes them potential RNA alternatives. The study with pRNA led to the conclusion that nature did not select RNA according to the criterion of maximal strength of base pairing. The quasi-linear structure of the thermodynamically more stable double-stranded pRNA shows only a weak left-handed twist, which is very different from the helical structure of natural nucleic acids. Since pRNA does not cross-pair with



Scheme 1. Various oligonucleotide building blocks.

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RNA, a direct exchange of information between these oligonucleotides seems difficult. However, the absence of cross-pairing between pRNA and RNA does not preclude the possibility of pRNA being an ancestor of RNA, but only suggests that it is very unlikely. A nucleic acid that can adopt several conformations and that in this way can hybridize equally with pRNA and RNA, might be considered as an evolutionary intermediate between them.[5]

Analysis of the properties of TNA might answer the question why TNA could have, but did not, become nature's genetic system, and it may add to our understanding of the functional superiority of RNA over its potential alternatives. Eschenmoser and co-workers demonstrated that this tetrose-based oligonucleotide shows base pairing which is similar to that of pentose-based RNA in regard to specificity, strand orientation, and pairing strength. Moreover, TNA hybridizes with the natural nucleic acids RNA and DNA, and informational transfer based on complementary (presumed) Watson–Crick base-pair recognition between these furanosyl oligonucleotides has been demonstrated. This finding is unique because the previously studied nucleic acid alternatives (excluding pentofuranosyl nucleic acid analogues such as arabinofuranosyl nucleic acids[6]) do not demonstrate this characteristic. It is also innovative because it demonstrates that one of the bonds responsible for nucleic acid flexibility (the repeating unit of the TNA backbone has five bonds instead of the usual six) can be removed without abolishing the capacity of furanosyl nucleic acids for functional selection by base pairing. This observation has its precedent in the field of artificial nucleic acids. Carbocyclic 5′-*nor* oligonucleotides (five bonds)[7] and carbocyclic oxetanocin oligonucleotides (seven bonds)[8] both hybridize with RNA.

Cross-pairing between TNA and RNA is possible because of the stretched conformation of TNA, in which all the substituents on the furanose ring are quasi-axially oriented (3′-*endo* conformation). This is an interesting example of nucleoside conformation mainly being determined by stereoelectronic effects (gauche and anomeric effects). β-D-Xylofuranosyl nucleosides have a similar conformation, which makes (3′ →2′)-β-D-xylofuranosyl nucleic acid another candidate for cross-pairing with RNA (assuming that the 5′-CH_2OH group does not cause steric hindrance).[9] An important observation is that TNA is able to form hairpin structures and, thus, does not restrict its conformational diversity to classical duplex formation.

The criteria which have been put forward for selecting potentially natural (transient) nucleic acid alternatives as genetic material for a primitive life are:[10]

1) a potential natural type of molecular structure,
2) a capacity for informational base pairing,
3) a capability for self-replication,
4) a capacity to express a chemical phenotype (reactivity and catalytic properties), and
5) a potential for evolution.

Which of these criteria are met by TNA? TNA could be considered as a structural alternative for RNA as it can be potentially formed by self-organization of organic material (first requirement). The question of whether RNA or TNA is most easily available under prebiotic conditions (simplicity of synthesis) can only be answered when nitrogenous systems are included in the experiments for investigating constitutional self-assembly.[1] It has also been demonstrated that TNA survives the second requirement, that is, a thermodynamic functional selection procedure based on base pairing. If TNA is to be regarded as either a precursor or an alternative to RNA then it must be demonstrated that TNA is inferior to RNA on the basis of functional criteria, since TNA was not selected by nature as its genetic system. It may be doubted that non-enzymatic replication of TNA will work as well as with pRNA because of the lower base-pairing strength, but (based on this single criteria) its potential for replication should be similar to (or even exceed) that of RNA. Both RNA and TNA have pairing capabilities which seem to be optimal for nature's purposes as a functional nucleic acid.

The absence of a free hydroxyl function (the equivalent of the 2′-OH group in RNA) restricts the catalytic potential of TNA (for example, for self-splicing), and from the point of view of catalysis, TNA can be considered as an analogue of DNA. It is clear, however, that the increasing efficiency and diversity of catalytic activities is an important evolutionary driving force towards RNA, and therefore that ancestral RNA might have had less catalytic activity combined with a higher chemical stability. In this respect, TNA and pRNA are both candidates for a precursor RNA, although it is not clear how the catalytic potential of TNA and pRNA can be translated into RNA catalysis. Although TNA could be an excellent candidate for the storage of genetic information, nature preferred instead to use complicated chemistry for the conversion of RNA into DNA. However, as creation of life most probably precedes storage of information, this does not exclude TNA from being a precursor of RNA or an RNA alternative. Indeed, in a primitive life the same molecular structure might be required to play both roles: information storage and structure-specific reactivity and catalysis.[10] Although TNA might have evolved its own characteristic functionalities as a genetic model, evolution is conservative in such a way that it might re-use substances from one primitive life form for different purposes. From this point of view, it is interesting to note that pentopyranose nucleosides have been isolated from nature (that is, out of culture medium of *Streptomyces griseochromogenes*), but the extraction of threofuranosyl nucleosides from natural sources is not known.

As previously discussed, "life" implies the potential for information storage, for self-replication, and for exponential growth, and it has been speculated that threose, furanose, and (pento)pyranose nucleic acids may all have this potential. Evolution (and survival) implies the potential for the transfer of information and for making selection (maybe in a combinatorial way), first by evolution of one system into another and thereafter by recombination and mutation to explore new functions. In evolution, conformational diversity might be a substitute for structural diversity since extensive structural diversity might not be available in a primitive world, and also not sufficiently available in more complex systems. Conformational diversity is, likewise, required for catalysis—the essential factor in every living organism. One possible conclusion might be that the selection of furanose nucleic acids rather than pyranose nucleic acids is more a

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question of evolution than a question of the creation of life. As an example, replication and transcription (information transfer) assumes that the double-stranded nucleic acid opens and recloses. The strength of the duplex is defined by the degree of interstrand and intrastrand stacking interactions. In the thermodynamically more stable A form of double-stranded nucleic acids (3′-*endo*-furanose conformation) interstrand stacking is more pronounced than in the less stable B form (2′-*endo*-furanose conformation). Conformational transformation from A type to B type might facilitate the opening of double-stranded nucleic acids and, hence, replication and transcription; this transformation implies conformational flexibility. Such considerations together with the increased chemical stability of DNA might make DNA a better candidate than RNA for information storage (in the way information storage was concepted by nature) and exclude the use of pyranosyl nucleic acids. Similar considerations concerning the catalytic reactivity of RNA restricts considerably the choice of potential structural alternatives for nature's nucleic acid. The reduced conformational flexibility (absence of the 5′-CH_2OH group) might make TNA both an inferior candidate to DNA for storage of information and an inferior candidate to RNA for catalysis.

There is at this moment no indication (or suggestion) that either TNA or pRNA were precursors or former evolutionary competitors of RNA (the opposite cannot be demonstrated either). The investigation of Eschenmoser and co-workers on TNA and its properties provides an additional piece of information to be added to the puzzle which might eventually lead to a general understanding of the genesis of present day life and to the proposition of alternative models of life. It has also become clear that the power of natural selection cannot be understood fully solely on the basis of information generated by the synthesis and analysis of the properties of RNA alternatives.

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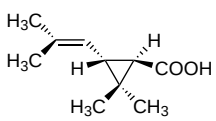
Fascinating Natural and Artificial Cyclopropane Architectures

Rüdiger Faust*

The various facets of the chemistry of cyclopropane derivatives are amazingly diverse and continue to fascinate scientists from a broad range of backgrounds, among them theoreticians, synthetic or structural chemists, and researchers with interests in natural product and/or medicinal chemistry. The challenges posed by the intriguing cyclic arrangement of only three tetravalent carbons are multitudinous, ranging from fundamental aspects of bonding, over the synthesis of highly strained molecules to an understanding of the mode of action of biologically active cyclopropyl derivatives. Selected examples of cyclopropane architectures encountered in compounds either derived from natural sources or prepared for the first time in the laboratory are highlighted below.

That nature has chosen to use a cyclopropane skeleton to design a defense mechanism for certain pyrethrum flowers against insect attack has been known since 1924, when Staudinger and Ruzicka isolated and characterized (+)-*trans*-chrysanthemic acid **1** from the petals of these plants.[1] The active insecticidal ingredients in these plants are in fact



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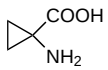

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esters of **1**, which can be easily modified and have been commercially exploited to give birth to one of the most successful classes of biomimetic insecticides, the pyrethroids. In 1997, the market value of this class of insecticides amounted to a staggering 1.5 billion US dollars.[2]

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