PEPTIDE NUCLEIC ACID (PNA): A MODEL STRUCTURE FOR THE PRIMORDIAL GENETIC MATERIAL?

PETER EGIL NIELSEN
Research Center for Medical Biotechnology, Department of Biochemistry B, The Panum Institute, Blegdamsvej 3c, 2200 Copenhagen N, Denmark

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Abstract. It is proposed that the primordial genetic material could have been peptide nucleic aicds, *i.e.*, DNA analogues having a peptide backbone. PNA momomers based on the amino acid, α, γ-diaminobutyric acid or ornithine are suggested as compounds that could have been formed in the prebiotic soup. Finally, the possibility of a PNA/RNA world is presented, in which PNA constitutes the stable genetic material, while RNA which may be polymerized using the PNA as template accounts for enzymatic activities including PNA replication.

1. Introduction

Ever since the identification of DNA as the genetic material and the elucidation of its unique information bearing structure (Watson and Crick, 1956) there has been speculations as to the evolutionary origin of this material and to life as we know it. The pionering experiments by Miller (Miller, 1953) and Oro (Oro, 1960) demonstrated a plausible way by which some of the fundamental building blocks of nucleic acids and proteins, the nucleobases and the amino acids, could have been formed in the prebiotic soup. It is, however, much less obvious how the sugars, such as ribose and deoxyribose, entered the scene (Joyce *et al.*, 1987; Eschenmoser and Loewenthal, 1992), and the introduction of chirality which determines the handedness of the DNA double helix is another enigma.

The prevailing theory at the moment is that our DNA/RNA/protein world was preceded by an RNA world. This theory was spurred by the discovery of catalytic RNA (Cech, 1987), which bridges the conceptual gap between information (DNA) and function (proteins) in biology by combining both properties in one molecule, the RNA (Cech, 1986). Due to the chemical fragility of RNA it is, however, highly unlikely that prebiotic life could have relied on RNA (Orgel, 1987, 1992; Eschenmoser and Loewenthal, 1992).

2. Peptide Nucleic Acids (PNA)

We recently described a novel DNA analog, PNA (peptide nucleic acid), which may be relevant for this discussion. PNA consists of a peptide (polyamide) backbone comprised of N-(2-aminoethyl)glycine units to which nucleobases are attached via carbonyl methylene linkers (Figure 1)(Nielsen *et al.*, 1991, Egholm *et al.*, 1992a, b; Cherny *et al.*, 1993) and we have found that PNA binds to oligo(deoxy)ribonucleotides obeying the Watson-Crick base pairing rules, i.e., A-T and G-C base pairs are

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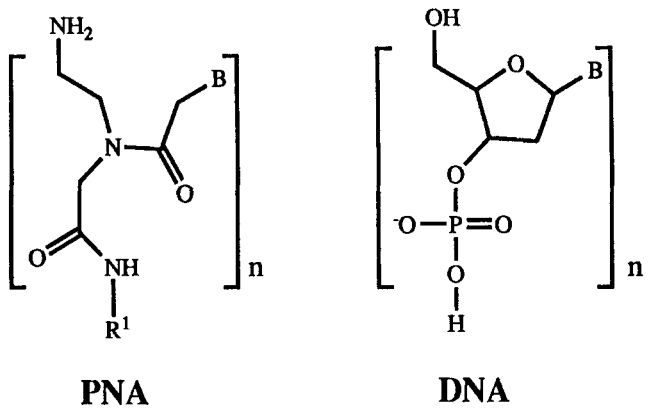

Fig. 1. Chemical structure of PNA and DNA. B is the nucleobase attached at N^1 (pyrimidines) or N^9 (purines) position, and R^1=H, or lysinyl amide for the PNAs discussed in this paper. The PNAs are written from the amino- to the carboxy-terminal using normal peptide conventions: "H-" signifies a free amino group, while "-NH_2" signifies a terminal carboxamide.

highly preferred (Egholm *et al.*, 1993a, b) (Figure 2). Thus in a chemical sense (but not in a functional sense) PNA bridges the gap between proteins and nucleic acids, and the results obtained with PNA clearly show that molecules with the potential of carrying genetic information are not required to contain either phosphates or sugars but could be 'peptides'.

This is particularly interesting when considering the types of compounds identified in the Miller experiments designed to mimic the prebiotic conditions (Miller, 1953). In these experiments organic acids including many of the natural amino acids were produced (Miller, 1953), and other experiments have been divised which produce the nucleobases (Oro, 1960). Thus it is conceivable that prebiotic mimicing conditions which produces nucleobase-amino acid conjugates can be found (Wong, 1991).

The compounds 1–4 (Figure 3) are suggestions for such conjugates. Formally, these PNA-monomers are built from α, γ-diaminobutyric acid or ornithine, carbonic acid or glycolic acid and the nucleobases, all of which, except for ornithine, have already been identified in the Miller-Oro experiments (Miller, 1987; Ferris, 1987).

It should be emphasized that although we have shown that PNA with the (2-aminoethyl)glycine backbone is an amazingly good mimic of DNA, we do not yet know the structural constraints within which this property exists. Recent results have shown, however, that extending the backbone by using β-alanine in place of glycine is deleterious for PNA/DNA complex stability (Hyrup *et al.*, 1993). The monomers 1 and 2 (Figure 3) results in a shortened backbone which at least in the case of the acetic acid linker (structure 2) should have increased flexibility and thus may be able to adopt the proper backbone conformation, whereas 3 and 4 should

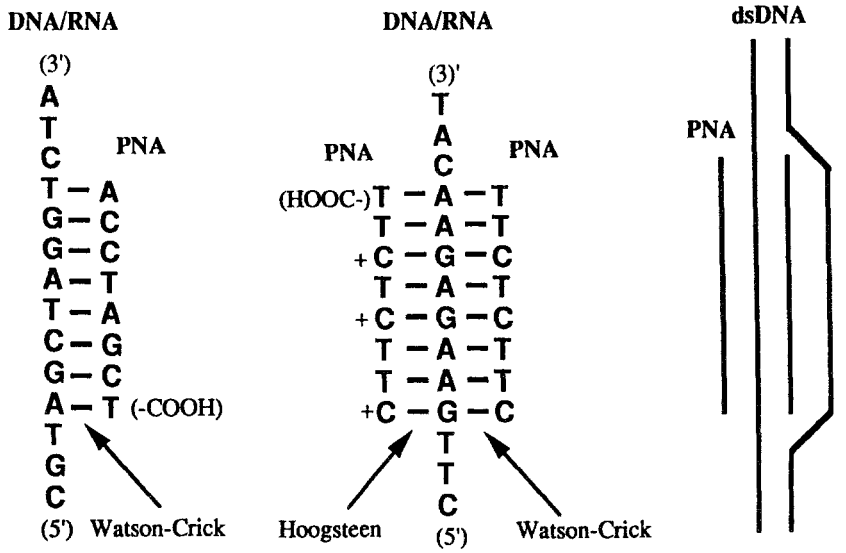

Fig. 2. Binding modes of PNA to single stranded DNA/RNA and double stranded DNA. (a) Purine/pyrimidine mixed sequence PNA binding to single stranded DNA (or RNA) forming PNA/DNA (or RNA) duplexes by Watson-Crick base pairing. (b) Homopyrimidine PNA bind to single stranded DNA (or RNA) in 2:1 complexes forming $(PNA)_2$/DNA (or RNA) triplexes employing Watson-Crick/Hoogsteen base pairing. (c) Homopyrimidine PNA binds to double stranded DNA by strand displacement (purine/pyrimidine mixed sequence PNA does not form strand displacement complexes). In PNA/DNA (or RNA) duplexes PNA can bind in both orientations, but the anti-parallel as shown on the Figure is preferred (the conventional nucleic acid and peptide conventions are used, i.e., 5'-3' and amino-carboxy terminal directions). In $(PNA)_2$/DNA triplexes the parallel orientation seems to be preferred.

result in a PNA backbone which is equivalent to our original (2-aminoethyl)-glycine PNA backbone. It can also be argued that the optimal backbone for a genetic material is not necessarily the backbone that gives the most stable duplex. Indeed, if the duplex is too stable, the genetic information is not available for transcription and replication processes.

3. Chirality

The origin of chirality is a central enigma for the understanding of the origin of life. It is difficult to envisage how only one isomer of the building blocks, e.g., for RNA polymers, could be used unless a chiral template is employed. Thus it has been suggested that chiral minerals could have served this purpose as well as being the catalyst (Lahav and White, 1980; Gedulin and Arrhenius, 1992). It is noteworthy that the (2-aminoethyl)glycine PNA (Figure 1) is achiral, and thus in principle could be the basis of an achiral genetic material. Taking this argument a little further, such a PNA could act as a chiral template, e.g., for the synthesis of RNA, if adsorbed on a chiral mineral surface that would favour for instance a

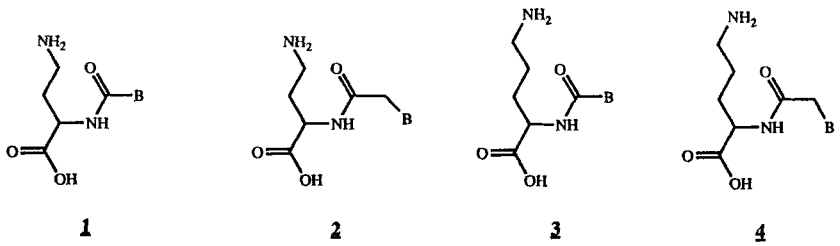

Fig. 3. Possible structures of prebiotic PNA building blocks based on α, γ-diaminobutyric acid (1 and 2) or ornithin (3 and 4). B signifies the nucleobase attached at N^1 (pyrimidines) or N^9 (purines) position.

righthanded helical structure of the single stranded PNA.

4. PNA/RNA World

A combined PNA/RNA world is also an interesting possibility. In this scenario, PNA would be the stable genetic material, while the RNA could carry out enzymatic functions, such as transcription of RNA from PNA and also PNA replication. Since PNA is assembled by amide (peptide) linkages, it may be highly relevant in this connection that a large body of evidence indicate that RNA is the essential component of the peptidyl transferase activity of ribosomes (Noller *et al.*, 1992). Thus RNA alone may be capable of catalyzing peptide bond formation.

5. Experimental Approach

Admittedly, all of the above discussion is pure speculation until a few basic questions have been answered. It must be shown that PNA/PNA duplexes can be formed and that a simple base pair recognition code (presumably the Watson-Crick) is obeyed.* Furthermore, experiments must be devised to show that one PNA molecule can act as a genetic template for the synthesis of a complementary one in a model molecular replication system as the ones devised for 'chemical replication' of RNA (Orgel, 1992). Finally 'PNA monomers' that give functional PNA polymers of the above criteria should be identified from prebiotic soup experiments.

It could also be worthwhile to search for amino acid-nucleobase conjugates within modern day living organisms as relics of the ancient genetic material, keeping in mind that novel functions for such material may have evolved.

* Preliminary results have shown that Watson-Crick complementary PNA molecules do interact with each other in a specific way, identified by a monophasic thermal transition.

6. Closing remarks

Although this presentation has been highly speculative, it is my hope that it may inspire others to think along new lines, and to design experiments that could support or disprove the ideas. However, it is my belief that the finding that a sugar-phosphate backbone is not a prerequisite for a material that could carry genetic information is important for our understanding of DNA as the genetic material of the life we know, and hopefully we will someday understand why nature chose a (deoxy)ribose-phosphate backbone and how it evolved.

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