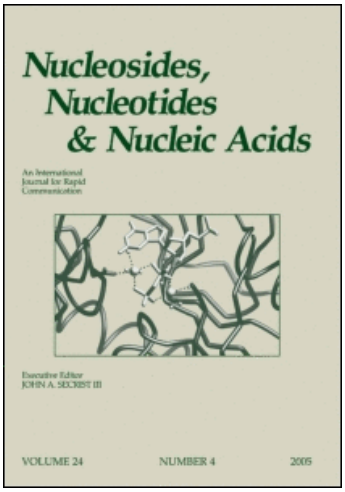

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THERMODYNAMIC STUDIES ON PNA AND PNA/DNA DENDRIMER FORMATION

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□ *In this work we report a kinetic and thermodynamic study relative to the formation of gel systems based on PNA and PNA/DNA dendrimers, useful for drug delivery or diagnostic applications. We realized two kinds of systems: a PNA-based monomolecular system formed by an autoassembling PNA tridendron (***A***) and a PNA/DNA bimolecular system based on a PNA tridendron with a mixed sequence and a DNA crosslinker (***B***). Both systems have the ability to form a three-dimensional network by means of specific W-C base pairing.*

Keywords PNA; dendrimers; gel systems

INTRODUCTION

The possibility to form extended oligonucleotide (ODN) systems with well-defined sizes and shapes[1] could be of eminent interest in developing new materials with innovative characteristics for nanotechnologies,[2] or in medical applications, such as drug delivery, gene transfection, and imaging. To form extensive ODN-based three-dimensional network, branched oligonucleotides (dendrimers) are of particular interest, as

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already reported in the literature.[3] The high level of control possible over the architectural design of oligonucleotide dendrimers, their size, shape, branching length/density, and their surface functionality, clearly distinguishes these structures as unique and optimal candidates for many biotechnological applications.

In order to develop ODN dendrimers, with enhanced thermal stability and good nuclease resistance, to be used for drug delivery or diagnostic applications, we realized systems based on PNA or PNA/ DNA building blocks.

RESULTS AND DISCUSSION

With the aim to obtain oligonucleotide-based gels in drug delivery or diagnostic applications, we realized two kinds of systems: a monomolecular PNA system (**A**, Figure 1) and a PNA and DNA bimolecular one (**B**, Figure 1). For the synthesis of such systems we used linear and branched oligonucleotide molecules as building blocks.

The system A is based on a three-branched PNA, constituted by an L-Lysine residue at the C-terminus, useful to improve the final product solubility, a polyadenine branch (a_5), a L-lysine core and two polythymine branches (t_5) assembled simultaneously on both amino groups of the central Lysine.

The PNA tridendron sequence of the system **A** was: (Ac t- t- t -t- t)$_2$- K-a-a-a-a-a-KNH$_2$ (**1**). The melting temperature (T_m) of the autoassembling PNA system **A**, determined by UV melting experiments, was 58°C at 4 μM concentration. By recording UV melting curves at different PNA oligomer concentrations, we observed a logarithmic dependence of T_m from concentration, with an increasing of the T_m value from 56°C to 60°C going from 1 to 17 μM. A linear PNA with the self-complementary sequence Ac-t_5-K-a_5-KNH$_2$ was synthesized as control. A 5 μM solution of this PNA oligomer showed a T_m of 54°C and no significant changes in the T_m by varying its

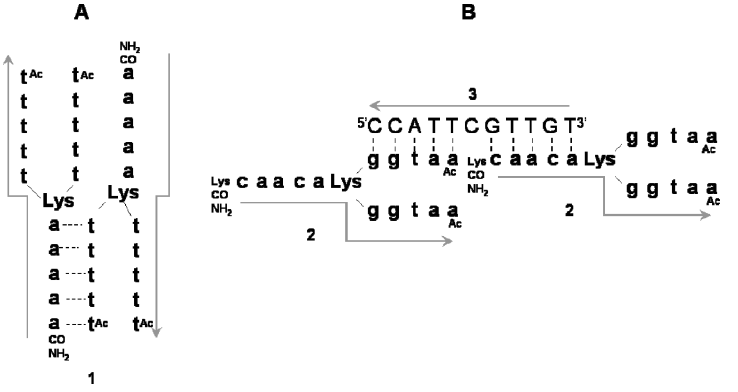

FIGURE 1 Monomolecular system **A** formed by PNA tridendron **1**; bimolecular system **B** formed by PNA tridendron **2** and DNA **3** (gray arrows indicate the synthesis directions).

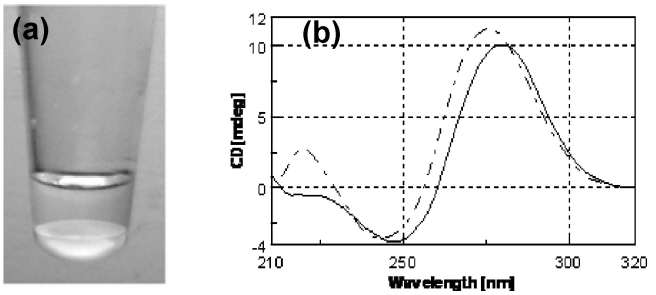

FIGURE 2 A preliminary gelation test on system **A**. The gel is visible on the bottom of the glass vial as a whitish substance (a); CD experiments on system **B** with the Tandem mix cell: sum (solid line) and complex (dashed line) CD spectra of **2** and **3** (b).

concentration from 1 to 30 μM. All these findings support the formation of a three-dimensional network for system **A**.

A preliminary gelation test relative to system **A** was performed on oligomer **1** and led to the formation of a gel layer as showed in Figure 2a.

Since the size of a monomolecular self-assembling gel system is not easily controllable, we also realized a bimolecular system (**B**, Figure 1) constituted of PNA and DNA moieties in which – one DNA oligomer was complementary to two branches of two PNA tridendron units. The PNA was made up of two arms of the same sequence at the N-termini, a central L-Lysine followed by the third arm and a further L-Lysine at C-terminus, analogously to PNA tridendron **1**.

The sequences of the system **B** were: $(Ac\text{-}a\text{-}a\text{-}t\text{-}g\text{-}g)_2$- K-a-c-a-a-c-$KNH_2$ (**2**, PNA tridendron); $^{5'}$C C A T T C G T T G T$^{3'}$ (**3**, DNA crosslinker).

We verified the formation of a PNA/DNA duplex by circular dichroism (CD) experiments performed at 1:1 nucleobase ratio of the two oligomers with a Tandem mix cell (Figure 2b).

In order to find the optimal crosslinker/tridendron ratio to obtain the most extensive network, we performed UV melting studies holding the tridendron concentration fixed at 3 μM and increasing the crosslinker amount from 3 to 9 μM. Table contained in Figure 3 summarizes T_m values at the corresponding crosslinker/tridendron ratios. By reporting

Crosslinker (3)/ Tridendron (2)	T_m ±0.5 (°C)
1	38.8
1.5	41.6
2	42.8
2.5	40.3
3	37.0

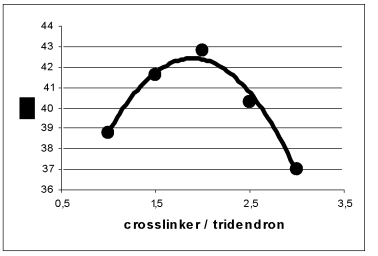

FIGURE 3 UV T_m values at different crosslinker/tridendron ratios.

crosslinker/tridendron ratios *vs* T_m values, we obtained a bell curve showing a maximum at 2:1 ratio (Figure 3), corresponding to the most extensive formation of the three-dimensional net.

On the basis of these findings, we decided to use a crosslinker/tridendron ratio of 2:1 for our future gelation tests on the bimolecular system **B**.

Further attempts to embed in the gel systems small molecules, like dyes or organic molecule-based drugs, will be object of our future investigations in continuing our research in this field.

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