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Further Studies On Nucleopeptides With DABA-based Backbone

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In continuing our studies on new ODN-like molecules suitable for a wide variety of biomedical and bioengineering applications, here we report further studies relative to a chiral nucleopeptide with a diaminobutyric acid (DABA) backbone. In particular, in this work we describe the synthesis of the nucleoaminoacid monomer with the D stereochemistry, performed in analogy to our previous reports on the L-DABA derivative, and the oligomerization using both enantiomers to form an alternate D,L-nucleopeptide. This oligomer was studied for its ability to bind complementary DNA by CD and UV spectroscopies. Furthermore, the new nucleopeptide showed binding evidence with a free nucleobase probably forming a three-dimensional network based on hydrogen bonding. This kind of structures is of particular interest for the development of new nanomaterials with many desirable properties as well as of new ODN-analogues for biotechnological applications.

In memory of Dr Silvano Fumero, an eminent scientist.

1. Introduction

Polymer gels, typically formed by molecular or macromolecular species cross-linked into network structures, have been intensively studied due to their interesting properties suitable for a wide variety of biomedical and bioengineering applications, including selective drug/gene delivery or sensing devices. Of particular interest are gels stabilized by noncovalent and relatively weak linkages allowing the cross-links to form in reversible fashion (Shay *et al.* 2001) and that respond to changes in the environment, such as temperature, pH or other external stimuli (Sato *et al.* 2001).
By taking advantage of the base recognition in nucleic acids, based on Watson-Crick (W-C)

hydrogen bonding, many researchers investigated gel systems based on DNA (Li *et al.* 2004) able to form extended oligonucleotide structures with well-defined sizes, shapes, branching density and surface functionality (Shchepinov *et al.* 1999) in order to develop new drug delivery systems (Luo *et al.* 2006) or new nanomaterials (Luo 2003) with many desirable properties.

Despite the remarkable advantages of DNA utilization in many research fields, some drawbacks, including a low cellular uptake, as well as enzimatic degradation or acidic depurination susceptivity, limit DNA use in biomedical or bioengineering applications (Uhlmann and Peyman 1990). Therefore, many efforts have been made to introduce new DNA-like molecules more resistant to enzymatic or chemical degradations but still able to form hydrogen bonding with complementary molecules (Matsukura *et al.* 1987, Nielsen *et al.* 1991, Kurreck *et al.* 2002).

In this context, recently we designed, synthesised and characterized a new ODN analogue containing a chiral γ-peptide backbone, based on the diaminobutyric acid (DABA), on which the nucleobase is anchored through a methylene carbonyl linker (Roviello *et al.* 2006 and 2007). Our interest for this non protein diamino carboxylic acid resides on the fact that DABA was found in many natural sources (Foster *et al.* 1987), was produced in simulated stellar conditions (Wolman *et al.* 1972) and also recovered in extraterrestrial meteoritic soil (Meierhenrich *et al.* 2004). Therefore, DABA-based molecules could be developed not only for biotechnological applications, as in the case of polyDABA for gene delivery systems (Iwashita *et al.* 2005), but also to throw light on their possible prebiotic role as nucleopeptides (Nielsen 1993, Meierhenrich *et al.* 2004, Strasdeit 2005). In our research the homothymine oligomer synthesized, containing a backbone entirely formed by L-DABA moieties, didn't show significant ability to bind complementary DNA (Roviello *et al.* 2006 and 2007).

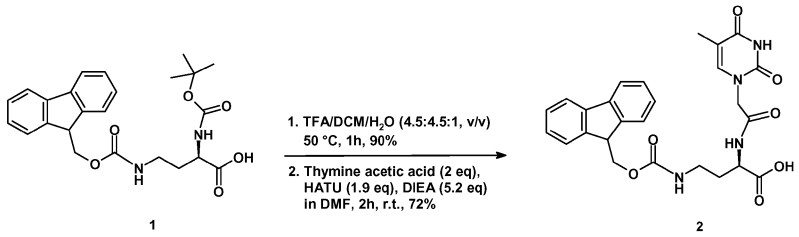
Here we report the synthesis of the homothymine oligomer containing an alternate D,L-DABA-based backbone. This oligomer was studied for its ability to bind complementary DNA or a free nucleobase in order to form a supramolecular structure based on hydrogen bonding and controlled in a thermoreversible fashion. This kind of structures is of particular interest for the development of new nanomaterials with many desirable properties or of new ODN-analogues in biotechnological applications.

2. Results And Discussion

The synthesis of the new D-DABA-based thymine monomer (**2**), suitably protected for peptide solid phase synthesis based on Fmoc chemistry, is reported in Scheme 1 and is analogous to that recently reported in our previous works for the synthesis of the L-DABA monomer (Roviello *et al.* 2006 and 2007), but starting from the commercial Boc-(D)-DAB(Fmoc)-OH (**1**).

The DABA-based hexathymine with alternating chirality [$(t_{D\text{-}dab}t_{L\text{-}dab})_3$, **3**] was synthesized in solid phase by using $t_{D\text{-}dab}$ monomer **2** and its L-enantiomer, with a protocol which minimizes racemization during the coupling steps (Sforza *et al.* 2002, Roviello *et al.* 2006

and 2007). Oligomer **3** was cleaved from the solid support by acidic treatment (TFA/m-cresol, 4:1, v/v) and purified by RP-HPLC on a C-18 column with a linear gradient of CH_3CN (0.1% TFA) in H_2O (0.1% TFA) over 25 min. LC-ESI-MS characterization confirmed the identity of **3**: *m/z* 900.30 (found), 900.8 (expected for $[H\text{-}G\text{-}(t_{L\text{-}dab}\text{-}t_{D\text{-}dab})_3K\text{-}NH_2+2H]^{2+}$).



Scheme 1: Synthesis of the Fmoc-protected thymine D-DABA monomer.

CD binding experiments of the homothymine oligomer **3** with the complementary DNA (dA_6) were performed using a Tandem cell. This cuvette is constituted from two separated reservoir communicating just by the upper part of the cell in which two solutions, initially separated, come in contact only after mixing by turning upside down the cell. Initially, a solution of dA_6 in phosphate buffer was kept in one of the Tandem cell reservoirs (1 mL), while oligomer **3**, in the same buffer conditions, was in the other one. CD spectrum from 200 to 320 nm, corresponding to the sum spectrum of the separated strands, was recorded. Successively, the cell was turned upside down to allow the mixing of the two samples and again a CD spectrum was registered. Differences between sum and mix spectra are indicative of binding. In our case, $(t_{D\text{-}dab}t_{L\text{-}dab})_3$ didn't show evidence of binding with complementary DNA (Figure 1). No significant differences between the sum and mix spectra, and therefore no binding evidence, were revealed recording CD spectra of the "mix" sample at different times, performing a kinetic experiment. Furthermore, the binding was not detected even adding one more equivalent of oligomers **3**, in a 2:1 ratio of $(t_{dab})_6/dA_6$, in order to verify the possibility to form a triple helix complex.

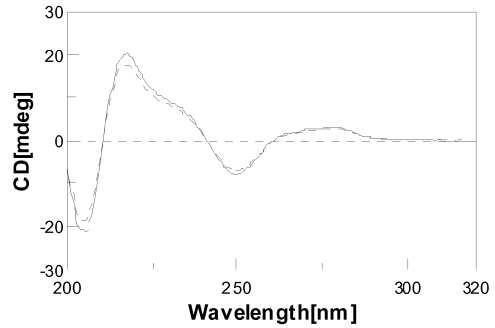


Figure 1: Sum (dashed line) and Mix (solid line) CD spectra of $(t_{D,L\text{-}dab})_6$ ***3*** *and DNA, 4μM in H_2O.*

Successively, the ability of the hexamer **3** to form molecular networks cross-linked with free adenine base was investigated.

Firstly, the CD profile of the hexathymine single strand in water (pH 7) was analysed. A weak CD signal was detected for our chiral oligomer and more exactly a maximum centred at 272 nm was observed (Figure 2, dashed line). This suggests a structural preorganization for our chiral nucleopeptide in aqueous medium that should be confirmed by NMR and RX studies.

On the other hand, as expected, no CD signal was detected for solutions at different concentration of free adenine in water, as reported in Figure 2 for the 0.1 mM case (solid line).

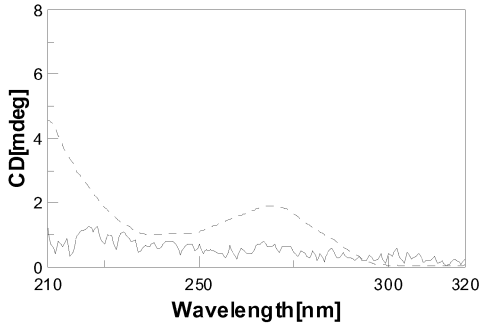


Figure 2: CD spectra of 15 μM $(t_{D\text{-}dab}t_{L\text{-}dab})_3$ ***3*** *(dashed line) and 0.1 mM adenine in* H_2O *(solid line, noise).*

CD titration of the $(t_{D\text{-}dab}t_{L\text{-}dab})_3$ oligomer with increasing amounts of adenine furnished many interesting evidences of interaction that could be at the basis of extensive molecular network formation.

Increasing amounts (30 nmol each) of a concentrated solution of adenine in water were added to 2 mL of 15 μM oligomer **3** solution (180 nmol in thymine). The CD band centred at 272 nm underwent an increase in intensity and a shift of the maximum to lower wavelengths, as adenine was added. Since adenine solutions don't give rise to CD signal, the differences revealed between the CD spectrum of **3** alone and of a mixture of **3**/adenine, is a clear evidence of interaction, as reported in Figure 3 for the case of **3**/adenine in a 2/1 ratio in bases (solid line).

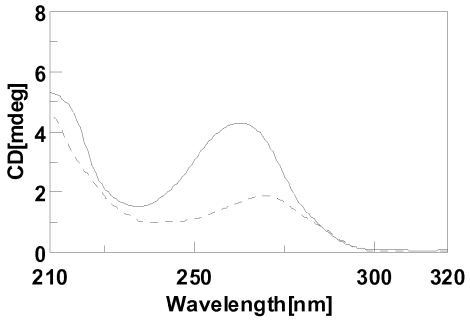


Figure 3. CD spectra of 15 μM $(t_{D\text{-}dab}t_{L\text{-}dab})_3$ ***3*** *(dashed line) and a 2:1 ratio in bases of* ***3*** *and adenine in* H_2O *(solid line).*

In our opinion, when thymines in **3** are in excess respect to the adenines, it could be hypothized the formation of a three-dimensional network in which DABA-based hexathymine strands are crosslinked by adenine molecules, by means of both W-C and Hoogsteen hydrogen bonds. In analogy to the polyT_2/polyA triplexes, our system could be schematically represented as in Figure 4. Obviously, adenines, not anchored to sugar moieties, have two more sites for hydrogen bonding formation (indicated in Figure 4 by the arrows) that are other probable cross-linking points.

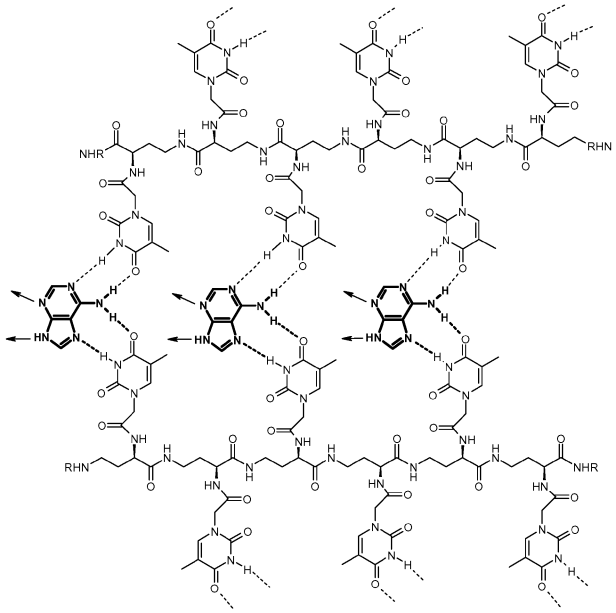


Figure 4: Schematic representation (for simplicity in 2D) of a T_2*/A nucleopetide/adenine network.*

Nevertheless, the comparison of literature CD data and the completely different CD profile obtained in our case, suggests a substantial structural difference between our chiral system, involving free adenine molecules, and the classical polyT_2 /polyA one, in which adenine moieties are anchored to the sugar-phosphate backbone.

Continuing the CD titration of **3**, further adding of adenine beyond the 2:1 T/A ratio caused an increase in the signal intensity and a substantial change in the CD spectrum shape. At 1:1 A/T ratio, the CD spectrum appeared as reported in Figure 5a (solid line). In this case we observed the formation of complexes that are probably based mainly on W-C hydrogen bonding.

The effect of further adding of adenine was the increase of the CD signals and the appearance of three bands centred at about 215, 247 and 269 nm (Figure 5b). It's very interesting to note that when adenine amounts exceeded the 1:1 ratio in A/T bases we didn't observe the stabilization of the CD spectrum, but a continuing increase of the band intensities was revealed, even though adenine alone, also in high concentrations, does not show any CD signal (Figure 2, solid line).

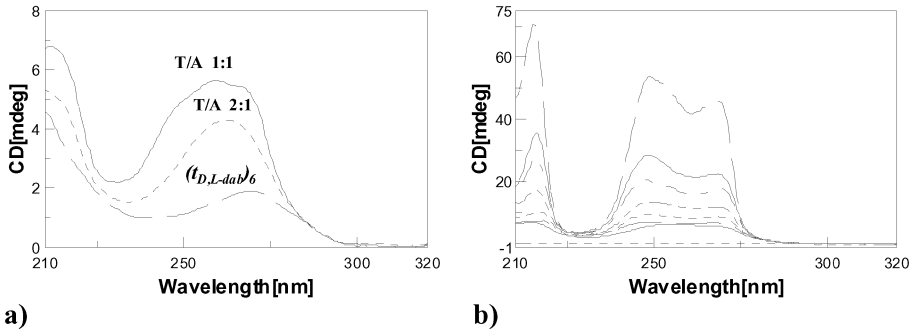


*Figure 5 a) comparison between the CD spectra of $(t_{D,L\text{-}dab})_6$ **3** and of the complex **3**/adenine (2:1 and 1:1 ratio in bases); b) CD spectra recorded after adenine adding to the solution containing 30 nmol of **3**: from 180 to 480 nmol adenine, corresponding, respectively, to 1:1 and 2.7:1 ratio in T/A bases.*

Indeed, when the chiral solution of **3**, also in traces, was added to the adenine solution (1 mM), achiral and lacking of CD signal, we observed a strong CD signal (Figure 6) not justifiable by the presence of so little amounts of the chiral compound **3**, but explainable by admitting the induction of a chiral molecular disposition of adenines in solution.

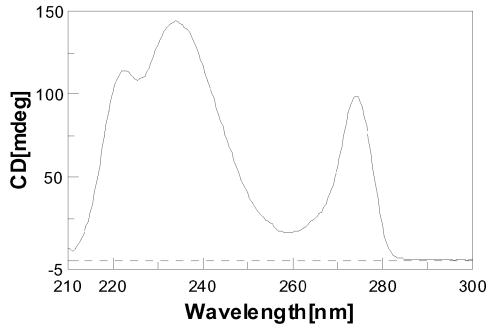


*Figure 6: CD spectrum of 1 mM adenine solution in presence of oligomer **3** (traces).*

The stability of the system composed of $(t_{D\text{-}dab}t_{L\text{-}dab})_3$ **3** and free adenine in water was studied by UV denaturation experiment. The solution of 1:18 ratio **3**/adenine (1:3 in bases) afforded a melting temperature (T_m) of 37 °C, as reported in Figure 7.

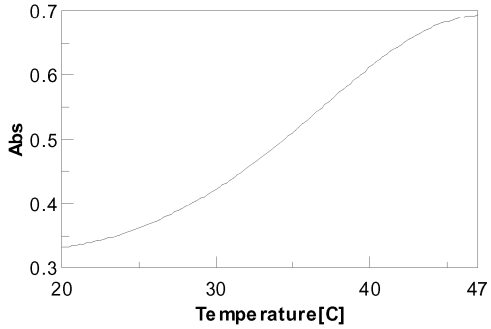


*Figure 7: UV melting of the complex **3**/adenine (1:18 ratio) in H_2O, pH 6.5*

3. Conclusions

In conclusion, our experiments showed that chiral nucleopeptides like our hexathymine oligomer with alternating D,L-chirality, are able to interact with adenine molecules in aqueous solution as clearly evidenced by CD spectroscopy. This finding also supports the use of chiral nucleopeptide systems for the realization of molecular networks, like hydrogels, to be used in drug delivery applications that we are going to study as a prosecution of the present research. Indeed, gelation and microrheology experiments on complexes $(t_{D,L\text{-}dab})_6$/adenine are in progress in various conditions of concentration, temperature and pH.

Another interesting point resides in the influence of DABA-based nucleopeptide chirality on the formation of a chiral environment in optically inactive solutions of adenine. Our results, together with the isolation of D,L-DABA and nucleobases in the extraterrestrial soil of the Murchison meteorite, reinforce the hypothesis that chiral nucleopeptides constituted a

primordial genetic material that introduced chirality in the actual nucleic acids/proteins world.

4. References

Foster J.G., W.D. Cress, S.F. Wright, J.L. Hess, 1987, Plant Physiol. 83(4), 900-904.
Iwashita Y., S. Goto, M. Tominaga, A. Sasaki, N. Ohmori, T. Goto, S. Sato, M. Ohta, S. Kitano, 2005, Cancer Sci. 96, 303-307.
Kurreck J., E. Wyszko, C. Gillen, V.A. Erdmann, 2002, Nucleic acid Res. 30, 1911-1918.
Li Y., Y.D. Tseng, S.Y. Kwon, L. D'Espaux, J.S. Bunch, P.L. Mceuen, D. Luo, 2004, Nat. Mater. 3, 38-42.
Luo D., 2003, Materials today 6 (11), 38-43.
Luo D., Y. Li, S. Ho Um, Y. Cu, 2006, Methods Mol Med. 127, 115-26.
Matsukura M., K. Shinozuka, G. Zon, H. Mitsuya, M. Reitz, J.S. Cohen, S. Broder, 1987, Proc. Natl Acad Sci. 84, 7706-7719.
Meierhenrich U.J., G.M. Muñoz Caro, J.H. Bredehöft, E.K. Jessberger, W.H.P. Thiemann, 2004, Proc. Natl. Acad. Sci. 101(25), 9182-9186.
Nielsen P.E, M. Egholm, R.H. Berg, O. Buchardt, 1991, Science 254, 1497-1500.
Nielsen P. E., 1993 Origins Life Evol. Biosphere 23, 323-327.
Roviello G.N., M. Moccia, R. Sapio, M. Valente, E.M. Bucci, M. Castiglione, C. Pedone, G. Perretta, E. Benedetti and D. Musumeci, 2006, J. Pept. Sci. 12, 829-835.
Roviello G.N., D. Musumeci, M. Moccia, M. Castiglione, R. Sapio, M. Valente, E.M. Bucci, G. Perretta, C. Pedone, 2007, Nucleos. Nucleot. Nucleic Acids 26, 1307-1310.
Sato Y., A. Hashidzume, Y. Morishima, 2001, Macromolecules 34, 6121-6130.
Sforza S., G. Galaverna, A. Dossena, R. Corradini, R. Marchelli, 2002, Chirality 14, 591-8.
Shay J.S., S.R. Raghavan, S.A. Khan, 2001, J. Rheol. 45, 913-927.
Shchepinov M.S., K.U. Mir, J.K. Elder, M. D. Frank-Kamenetskii, E.M. Southern, 1999, Nucleic Acids Res. 27(15), 3035–3041.
Strasdeit H., 2005, *ChemBioChem.* 6 (5), 801-3.
Uhlmann E., A. Peyman, 1990, Chem. Rev. 90, 543-584.
Wolman Y., W.J. Haverland, S.L. Miller, 1972, Proc. Nat. Acad. Sci. 69(4), 809-811.

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