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Binding ability of a thymine-functionalized oligolysine towards nucleic acids

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ABSTRACT

In this Letter, we investigated the binding properties towards nucleic acids of a thymine-functionalized oligolysine, composed of nucleobase-bearing amino acid moieties and underivatized L-lysine residues alternate in the backbone. The basic nucleopeptide proved to be well soluble in water and able to interact with both DNA and RNA, as suggested by circular dichroism, UV and surface plasmon resonance studies performed on the thymine-containing oligomer with both adenine-containing DNA (dA_{12}) and RNA (rA_{12} and poly rA) molecules. In both cases the thymine-functionalized oligolysine was proven to form complexes characterized by a 1:1 T/A stoichiometric ratio, as evidenced by CD titration. UV melting experiments revealed that the complex formed between the homothymine oligolysine and rA_{12} RNA was more stable than the complex with dA_{12} DNA probably due to the additional H-bonding of the 2′-OH groups in RNA, that reinforces the overall interaction with the nucleopeptide. Finally, human serum stability assays were conducted on the thymine-bearing nucleopeptide which showed a half-life of 45 min.

1. Introduction

In the last decades, several scientific studies were conducted on DNA analogues in which the sugar-phosphodiester backbone of natural nucleic acids underwent various modifications leading to artificial oligonucleotides with interesting properties.[1–5] In particular, peptide-based DNA analogues were able to interact with natural nucleic acids[6–8] by forming complexes characterized by high thermal stability and a significant intrinsic cell-membrane permeability. Furthermore, the possibility to obtain supramolecular assemblies, as well as the formation of complexes with heteroaromatic molecules based on such nucleobase-containing peptides was proven in different studies.[9–13] Interestingly, positively-charged DNA analogues with pseudopeptide backbones showed useful properties such as good cell uptake and specific DNA recognition via stable duplex formation.[14–17] However, various attempts to use real peptide backbones as alternative oligonucleotide linkages have also been reported, leading to chiral nucleobase-containing peptides with interesting features.[18,19] Peptide-like DNA analogues with long side chains were also described in the literature to form stable complexes of high sequence specificity with natural oligonucleotides.[20,21]

Diamino acids represent a class of natural building blocks frequently used for the realization of peptide-like analogues of nucleic acids. Indeed, such molecules offer the possibility to introduce the nucleobases, by amidation to one of the two amino groups, into the main peptide backbone, which is constructed by oligomerization of the diamino acid moieties also in combination with other amino acids.[17,22–24]

Taking into account all these considerations, and particularly focusing on the importance of diamino acid-containing nucleopeptides, recently we realized an oligonucleotide analogue,[25] characterized by a peptide backbone comprising both nucleobase-functionalized amino acids and underivatized L-lysine moieties in an alternate sequence. The diamino acid moieties allowed for the linkage to the DNA nucleobase by means of an amide bond to the epsilon amino group, whereas the underivatized L-lysine residues conferred a positive charge to the oligonucleotide analogue, especially valuable for their potential in improving the water-solubility of the nucleopeptide, as well as its ability to recognize the negatively-charged natural nucleic acids.

Some biological properties of this sequential nucleopeptide, such as the ability to interact with DNA and RNA, as well as the human serum stability, are described for the first time in the present work.

2. Results and discussion

2.1. Chemistry

Recently, we described the synthesis of a new thymine-based nucleoamino acid, suitably protected for solid phase peptide

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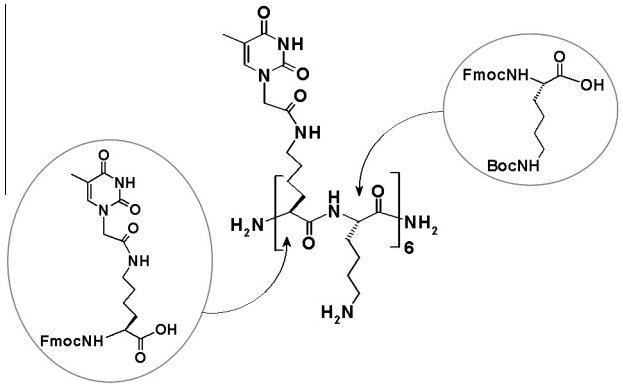

Figure 1. The hexathymine nucleopeptide and its building blocks.

synthesis (Fmoc chemistry), and its oligomerization to an hexathymine nucleopeptide (Fig. 1), obtained by alternating thyminyl lysine monomers and unfunctionalized L-lysine units in the sequence.[25]

2.2. Binding studies with nucleic acids

The ability of the thymine-functionalized oligolysine to bind nucleic acids was investigated and, more particularly, a CD binding assay was first performed by using a dA_{12} DNA. This binding experiment was performed on the homothymine nucleopeptide with the complementary DNA in a tandem cell recording the sum CD spectrum of the separated components and the complex spectrum, obtained after mixing the two solutions. In more detail, 24 nmol in nucleobase (T) of nucleopeptide and 12 nmol in nucleobase (A) of dA_{12} DNA, both dissolved in 10 mM phosphate buffer (pH = 7.5), were introduced separately in the two *tandem* cell reservoirs, and the sum CD spectrum was recorded at 5 °C (Fig. 2a). The CD contribution of nucleopeptide to the sum spectrum was weak compared to the signal due to the homoadenine DNA single strand. Upon mixing the two solutions, a difference in the shape and intensity of the CD profiles was observed between the sum and complex CD spectra, revealing an interaction between the nucleopeptide and DNA (Fig. 2a). In order to evaluate the contribution to the CD spectrum of the DNA/nucleopeptide complex due to the electrostatic interactions, we performed an analogous experiment by using a DNA strand (dT_{12}) non-complementary to the homothymine nucleopeptide. In this case a minor variation of the CD spectrum, mainly due to the electrostatic interaction between the negatively-charged DNA and the cationic nucleopeptide, was revealed upon mixing the solutions of the oligomer and dT_{12} (Fig. 2b). The difference between the *mix* and *sum* spectra in the experiment involving the hexathymine oligomer and dA_{12} accounts for the sum of both electrostatic and nucleobase-recognition contributions (pink line, Fig. 2c). On the other hand, pure electrostatic contribution to the CD spectrum can be evidenced by subtracting complex and sum spectra obtained in the binding assay of the nucleopeptide with dT_{12} (blue line, Fig. 2c). The component due to the pure nucleobase recognition was, thus, obtained by subtracting the spectra of Figure 2c (green line, Fig. 2d) and proved to be significantly stronger than the electrostatic contribution. The above-reported observations suggest that the interaction between the artificial nucleopeptide and the homoadenine nucleic acid is mainly due to the interaction of nucleobases, even if ionic interaction is also detectable as expected.

Subsequently, a CD titration of dA_{12} DNA with the nucleopeptide was performed by adding increasing amounts of the nucleopeptide to the DNA solution and a stabilization of the CD signal at ca. 260 nm in correspondence of a 2/1 $[T_6]/[dA_{12}]$ ratio ($T/A = 1$) was revealed. Indeed, no significant changes in the spectrum were observed by adding a further aliquot of nucleopeptide in correspondence of 2.5/1 $[T_6]/[dA_{12}]$ nucleopeptide/DNA ratio (Fig. 3).

Furthermore, the ability of the nucleopeptide to bind homoadenine RNA was investigated by CD in analogy to the procedure employed in case of DNA. A strong variation of the CD profile was

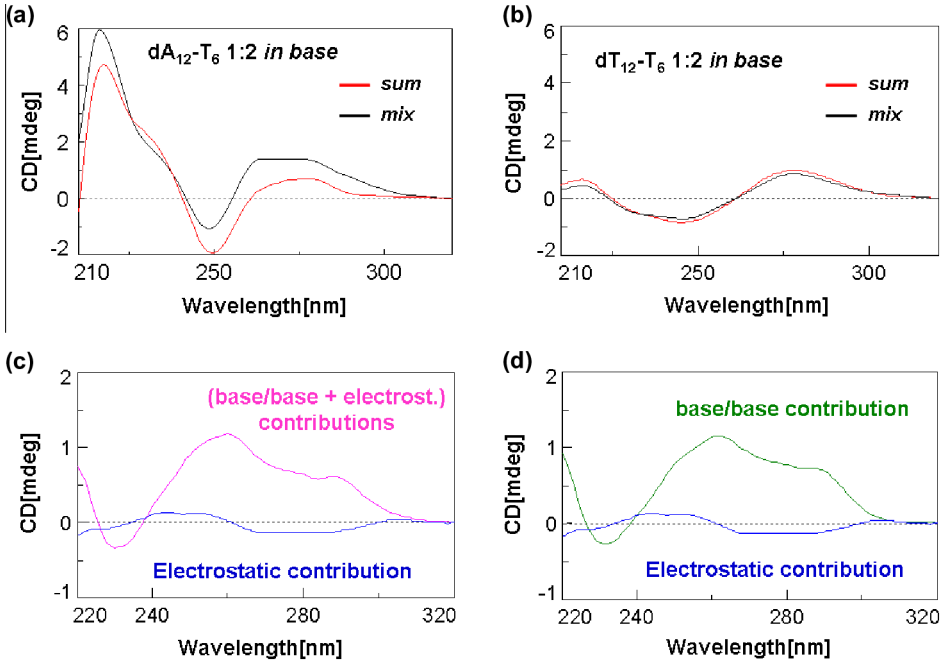

Figure 2. CD binding study of the nucleopeptide with DNA (a): t_6 oligomer (24 nmol in T) + dA_{12} (12 nmol in A) in 10 mM phosphate buffer (pH = 7.5, 2 ml) before (*sum*) and after mixing (*mix*). (b) t_6 oligomer (24 nmol in T) + dT_{12} (12 nmol in T) in 10 mM phosphate buffer (pH = 7.5, 2 ml) before (*sum*) and after mixing (*mix*). (c) Contribution to CD spectrum of electrostatic (blue line) and electrostatic + base/base (pink) interactions. (d) Comparison between the contributions due to the nucleobase recognition and electrostatic interactions.