

A short C-rich PNA fragment capable to form novel G-quadruplex-PNA complexes

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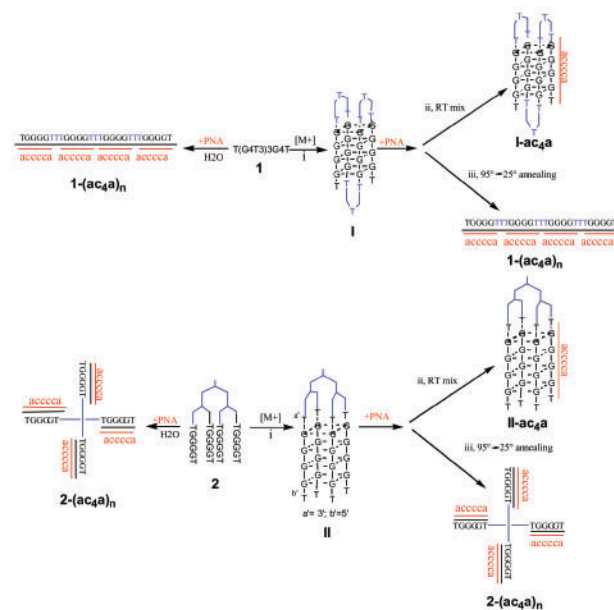
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ABSTRACT

In this work we investigated the interaction between the short ac₄a C-rich peptide nucleic acid (PNA) probe and two intramolecular G-quadruplex targets having the same G-tetrad core, but different folding topologies. The T(G₄T)₃G₄T and the recently reported tetra-end-linked-(TG₄T)₄ G-rich oligonucleotides (GROs) were chosen and synthesized for this study. UV, CD, and MS experiments revealed the formation of novel 1:1 G-quadruplex-PNA complexes besides the expected DNA-PNA heteroduplexes.

INTRODUCTION

Guanine rich oligonucleotides (GROs) are known to fold into orderly secondary structures stabilized by guanine tetrads known as G-quadruplexes. Due to the growing awareness of the biological value of quadruplex structures great efforts are dedicated in seeking for novel ligands capable of specifically binding to these structures or, more generally, to DNA putative quadruplex sequences (PQSs) to be used as therapeutics agents, diagnostic tools, and molecular probes or as DNA-fuel in nanotechnology applications. The different strategies aimed at the targeting of DNA quadruplexes so far proposed make use of: (1) small molecules with large aromatic surfaces that bind their DNA target via π -stacking to the guanine tetrads;^{1,2} (2) molecules that selectively bind to the grooves of the quadruplex structures;^{2,3} (3) C-rich complementary oligonucleotides;^{4,5} (4) C-rich homologous PNA oligomers that are able to form hybrid PNA-DNA quadruplexes.^{6,7} PNAs are a class of nuclease-resistant DNA mimics that selectively bind to their complementary DNA targets with higher affinity than the corresponding DNA analogues.⁸ In this study we investigate the interaction between GROs and the C-rich PNA hexanucleotide ac₄a. Two different GROs containing four TG₄T repeated units separated either by T loops, or by an alkylic tetra-end-linker⁹ were chosen for this study (**1** and **2**, respectively, Scheme 1). These DNA sequences lead to compact antiparallel (**I**, scheme 1) or parallel (**II**, Scheme 1) monomolecular quadruplexes when annealed in presence of NH₄⁺, Na⁺ or K⁺ cations ((i), Scheme 1).



Scheme 1 Schematic structural representation of GROs and GRO-PNA complexes (see text for descriptions).

RESULTS AND DISCUSSION

Three CD experiments were performed to characterize the interaction between the PNA and the quadruplexes **I** and **II**.^{10,11} First, CD spectra were recorded for **I** and **II** alone (red lines, Fig. 1), PNA alone (green lines, Fig. 1) and 1:4 DNA-PNA mixtures (solid black lines, Fig. 1) in 150 mM NH₄Cl and 10 mM NH₄Cl (data not shown) buffers (pH 7.0). The 1:4 DNA-PNA mixtures were prepared either by mixing at room temperature (RT) and storing at RT for one night ((ii), Scheme 1) or by the fast annealing procedure (heating at 95°C followed by fast cooling and storage at RT for one night, (iii), Scheme 1), prior to recording the spectra. In both cases the CD profiles of the DNA-PNA mixtures indicated that some interaction between the quadruplexes and the PNA occurred, because the spectra of the mixtures differ from the sum of the individual CD spectra. The CD data indicate the formation of quadruplex-PNA complexes (**I-ac₄a** and **II-ac₄a**,

Scheme 1) when the PNA is added to preformed **I** and **II** whereas almost exclusively DNA-PNA heteroduplexes (**1-(ac₄a)_n** and **2-(ac₄a)_n**, Scheme 1) were found in the samples obtained by (iii). These latter spectra were practically superimposable with those obtained when mixing **1** and **2** with the PNA in desalted H₂O, where only the heteroduplexes are likely to occur (data not shown). UV-melting curves were recorded to further assess the nature of PNA-DNA complexes (data not shown).¹¹ Monitoring the 295-nm UV absorbance of samples obtained according to (ii), we observed the quadruplex diagnostic hypochromic melting profiles, while hyperchromic curves were obtained from samples obtained by (iii) where heteroduplex denaturation is likely to occur. Better hyperchromic sigmoidal melting curves were obtained monitoring the UV absorbance at 272 nm for the samples annealed according to (iii). These data, in agreement with the CD data, confirmed that the quadruplex scaffold was still present in the complexes **I-ac₄a** and **II-ac₄a** obtained mixing **I** and **II** with the PNA at RT, while the corresponding heteroduplexes (**1-(ac₄a)_n** and **2-(ac₄a)_n**, respectively) were obtained when the UV melting experiments were performed according to (iii). The formation of 1:1 quadruplex-PNA complexes rather than 1:1 DNA-PNA heteroduplexes were further confirmed by electrospray mass spectrometry experiments that showed mass peaks relative to three-cations-containing 1:1 DNA-PNA species that can be unequivocally attributed to quadruplex species containing four G-tetrad planes.¹² On the other hand several mass peaks attributable to 1:n DNA:PNA heteroduplexes and even heterotriplexes ($2 < n < 8$) were detected from the samples prepared according to (iii) (see Table 1).

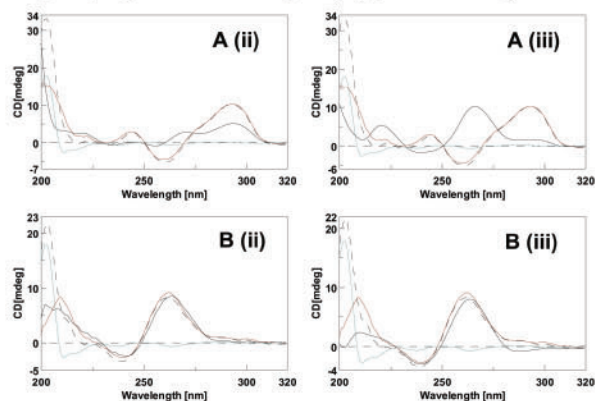


Fig. 1 CD spectra of **1** (A) and **2** (B) alone and in the presence of 4:1 PNA excess (150 mM NH₄Cl buffer, pH 7.0, 25 °C). The samples were prepared according to (ii) (left) and (iii) (right).

CONCLUSION

Herein we demonstrate that short C-rich PNA fragments are capable to bind their complementary quadruplex DNA target not only by strand invasion, but also by an external

Table 1 ESI-MS results (150 mM NH₄Cl buffer, pH 7.0).

Sample preparation	1	2
(ii)	[I]; [I+1PNA]	[II]; [II+1PNA]
(iii)	[I]; [1+2PNA]; [1+4PNA]	[II]; [2+1PNA]

binding mode that preserves the quadruplex scaffold. This finding is noteworthy in the light of PNA application as quadruplex binding agents.

REFERENCES

- Phan, A. T., Kuryavyi, V., Gaw, Y. H., Patel, D. J. (2005), *Nat. Chem. Biol.* **1**, 167-173.
- White, E. W., Tanius, F., Ismail, M. A., Reszka, A. P., Neidle, S., Boykin, D. W., Wilson, W. D. (2007) *Biophys. Chem.*, **126**, 140-153.
- Chen, Q., Kuntz, I. D., Shafer, R. H. (1996) *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 2635-2639.
- Datta, B., Armitage, B. A. (2001) *J. Am. Chem. Soc.*, **123**, 9612-9619.
- Green, J. J., Ying, L., Klenerman, D., Balasubramanian, S. (2003) *J. Am. Chem. Soc.*, **125**, 3763-3767.
- Datta, B., Schmitt, C., Armitage, B. A. (2003) *J. Am. Chem. Soc.*, **125**, 4111-4118.
- Roy, S., Tanius, F. A., Wilson, W. D., Ly, D. H., Armitage, B. A. (2007) *Biochemistry*, **46**, 10433-10443.
- Egholm, M.; Buchardt, O.; Christensen, L.; Behrens, C.; Freier, S. M.; Driver, D. A.; Berg, R. H.; Kim, S. K.; Norde, B.; Nielsen, P. E. *Nature* 1993, **365**, 566-568.
- Oliviero, G., Borbone, N., Galeone, A., Varra, M., Piccialli, G., Mayol, L. (2004) *Tetrahedron Letters*, **45**, 4869-4872.
- Mergny, J. L., Gros, J., De Cian, A., Bourdoncle, A., Rosu, F., Sacca, B., Guittat, L., Amrane, S., Mills, M., Alberti, P., Takasugi, M., Lacroix, L. (2006) *Quadruplex Nucleic Acids*, 31-80.
- Faccini, A., Tortori, A., Tedeschi, T., Sforza, S., R., Tonelli, R., Pession, A., Corradini, R., Marchelli, R. (2008) *Chirality*, **20**, 494-500.
- Rosu, F., Gabelica, V., Houssier, C., Colson, P., De Pauw, E. (2002) *Rapid Commun. Mass Spectrom.*, **16**, 1729-1736.

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