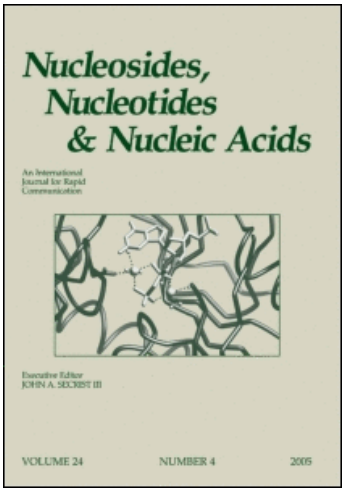

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Crystal and Solution Structure of the Bi-Loop Motif in Cyclic Octanucleotides

N. Escaja[a]; E. Pedroso[a]; S. A. Salisbury[b]; C. Gonzàlez[c]
[a] Departament de Química Orgànica, Facultat de Química, Universitat de Barcelona, Barcelona, Spain [b] Cambridge Crystallographic Data Centre, Cambridge, UK [c] Instituto de Estructura de la Materia, CSIC, Madrid, Spain

To cite this Article Escaja, N. , Pedroso, E. , Salisbury, S. A. and Gonzàlez, C.(1999) 'Crystal and Solution Structure of the Bi-Loop Motif in Cyclic Octanucleotides', Nucleosides, Nucleotides and Nucleic Acids, 18: 6, 1601 — 1602
To link to this Article: DOI: 10.1080/07328319908044796
URL: http://dx.doi.org/10.1080/07328319908044796

NUCLEOSIDES & NUCLEOTIDES, 18(6&7), 1601–1602 (1999)

CRYSTAL AND SOLUTION STRUCTURE OF THE BI-LOOP MOTIF IN CYCLIC OCTANUCLEOTIDES

N. Escaja[1], E. Pedroso*[1], S.A. Salisbury[2] and C. González[3]

[1]Departament de Química Orgànica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain
[2]Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK
[3]Instituto de Estructura de la Materia, CSIC, Serrano 119, 28006 Madrid, Spain

ABSTRACT: The refined NMR structures of the cyclic octamers d<pATTCATTC> **1** and d<pGCTCGCTT> **2** are consistent with the reported crystallographic data of **1** and the linear heptamer d(GCATGCT), respectively. The NMR study of four new cyclic octamers shows that in all cases a dimeric bi-loop structure is observed in equilibrium with a monomeric form.

The recent development of efficient synthesis procedures for obtaining milligram amounts of cyclic oligonucleotides[1] has opened the possibility of studying these compounds as models for a variety of DNA motifs.

We have recently described that the crystal structure of the cyclic octamer d<pATTCATTC> **1** contains two independent molecules which form a novel quadruplex by means of intermolecular Watson-Crick pairing and base stacking[2].

The NMR structure of two cyclic octamers, d<pATTCATTC> **1** and d<pGCTCGCTT> **2**, has also shown that the dimeric bi-loop motif exists in solution in slow exchanging equilibrium with a monomeric form[3]. At low oligonucleotide concentration, both molecules adopt a dumbbell form, with four of the bases involved in two intramolecular A·T or G·C base pairs, and the other four forming two mini-hairpin loops of two nucleotides each. At higher concentration, dimeric bi-loop structures are observed with four intermolecular Watson-Crick base pairs. Cross-peaks due to chemical exchange between the two forms can be identified in ROESY spectra, facilitating a complete assignment of all the proton resonances in the two species. More than 400 distance constraints obtained from NOE cross-peak intensities using a complete relaxation matrix analysis have now been used in a restrained molecular dynamics refinement to

calculate a set of 10 structures for each molecule. In both cases, calculations converge to a well defined four-stranded symmetric structure, with a RMSD around 1 Å (excluding the cytidines in the loop). The average structure of **1** is fully consistent with the reported crystallographic data. The average NMR structure of octamer **2** closely resembles the crystal structure of the linear heptamer d(GCATGCT)[4].

In order to get a deeper insight into the bi-loop motif we have also undertaken a systematic study to determine the sequence requirements that favour its formation. We report on our preliminary results of the NMR studies of four new cyclic octamers: d<pGCATGCAT> **3**, d<pGCTAGCTA> **4**, d<pCGTCCGTC> **5** and d<pGCTCATTC> **6**. In all cases a dimeric bi-loop structure is observed in equilibrium with a monomer, although the concentrations of **1** and **6** have to be much higher (ca. 20mM and 10mM in water, respectively, 25°C) than for the rest of the series (about 1mM) to observe equally intense dimer and monomer signals. Estimated melting temperatures from NMR data for the dimer to random coil transition are in the range of 40 to 60°C for the octamers **2** to **5**, and below 30°C for **1** and **6**. As expected, bi-loops with four intermolecular G·C base-pairs are more stable than those with two G·C and two A·T (octamer **6**) or four A·T pairs (octamer **1**). This is confirmed by the fact that only the dimer with G·C base-pairs is formed in the case of **3** and **4**. Interestingly, the order of the quadruplex forming complementary bases in the sequence of the octamer can be reversed ($^{5'}$CG$^{3'}$ in **5** instead of $^{5'}$GC$^{3'}$ in **2**, **3** and **4**) without a dramatic change in the stability of the dimer. Bi-loop formation from **3** and **4** indicates that either of the two bases in the loop can be a purine.

Finally, it should be mentioned that the monomeric form in equilibrium with the bi-loop does not always adopt a well defined structure. This is obviously the case in **6** (no dumbbell can be formed), but also in **5**, most probably because the $^{5'}$GTCC$^{3'}$ sequence does not allow the formation of stable mini-hairpin loops such as $^{5'}$CTTG$^{3'}$ and $^{5'}$CTCG$^{3'}$ of the dumbbell form of **2**.

The existence of this variety of four-stranded structures, both in the solid state and in solution, highlights the relevance of the bi-loop motif and strongly supports the hypothesis that we may be observing a generally occurring motif in natural DNA that could provide a molecular mechanism for recombination of double-stranded DNA in vivo.

REFERENCES

1 Alazzouzi, E.; Escaja, N.; Grandas, A.; Pedroso, E. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1506-1508.
2 Salisbury, S.A.; Wilson, S.E.; Powell, H.; Kennard, O.; Lubini, P.; Sheldrick, G.M.; Escaja, N.; Alazzouzi, E.; Grandas, A.; Pedroso, E. *Proc. Nat. Acad. Sci. USA* **1997**, *94*, 5515-5518.
3 González, C.; Escaja, N.; Rico, M.; Pedroso, E. *J. Am. Chem. Soc.* **1998**, *120*, 2176-2177.
4 Leonard, G.A.; Zhang, S.; Peterson, M.R.; Harrop, S.J.; Helliwell, J.R.; Cruse, W.B.T.; d'Estaintot, B.L.; Kennard, O.; Brown, T.; Hunter, W.N. *Structure* **1995**, *3*, 335-340.