

Circular dichroism as a means to follow DNA gymnastics: on the shoulders of giants

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This is the first report of DNA stem-loops self-assembled by 'foot-loop' interactions into either two-dimensional strings or three-dimensional spirals, distinguished by circular dichroism spectroscopy. All subunits are linked by cooperative Watson-Crick hydrogen bonds.

Key words: DNA, circular dichroism, nanostructures, stem-loop structures, self-assembly

Introduction

Many of the key goals of DNA-based nanotechnology entail the use of periodic arrays with tunable features such as binding patterns and cavities,¹ with important applications in medicine as drug-delivery systems.² Branched networks that result from ligating three-way junctions of DNA together have been reported.^{3,4} This study introduces a new method to build either linear or three-dimensional self-assembling structures. Pairs of DNA stem-loops (46 bases) were designed to self-assemble by foot-loop interactions to form networks of strings (A+B) or spirals (C+D) (Fig. 1). The inter-subunit interactions are restricted to cooperative Watson-Crick hydrogen bonds. No ligation is used—making the assembly completely reversible.

Methods

Oligonucleotide design and synthesis

The sequences were designed to form a double-stranded stem of alternating guanine/cytosine base pairs⁵ holding a single-stranded, asymmetrical loop sequence from the polypurine tract of HIV-1 (A₄GA₃G₆A).⁶ The 'feet' (5'- and 3' extensions of the stem) were added to make complementary base pairs to the loop, thus forming either strings or spirals. Heterodimers were required to make the foot-loop interaction constitute an anti-parallel double helix. Oligonucleotides were synthesised by standard phosphoramidate chemistry using a Beckman 1000 M DNA synthesiser, and purified in dimethoxytrityl-on mode by reverse-phase high performance liquid chromatography (HPLC) using an acetonitrile gradient. Concentrations were expressed in strand molarity, using nearest-neighbour approximation for the extinction coefficients of the unfolded species.⁷

Ultraviolet (UV) melting

Thermal melting curves were recorded using a heating rate of 1°C min⁻¹ in an Uvikon spectropolarimeter, with a custom-made heating block equipped with an Oasis analog-to-digital converter. The DNA strand concentration was 1.5 μM ml⁻¹ and oligonucleotides were dissolved in 100 mM l⁻¹ sodium chloride, 5 mM l⁻¹ magnesium chloride, and 10 mM l⁻¹ sodium cacodylate, at pH 7.0.⁸

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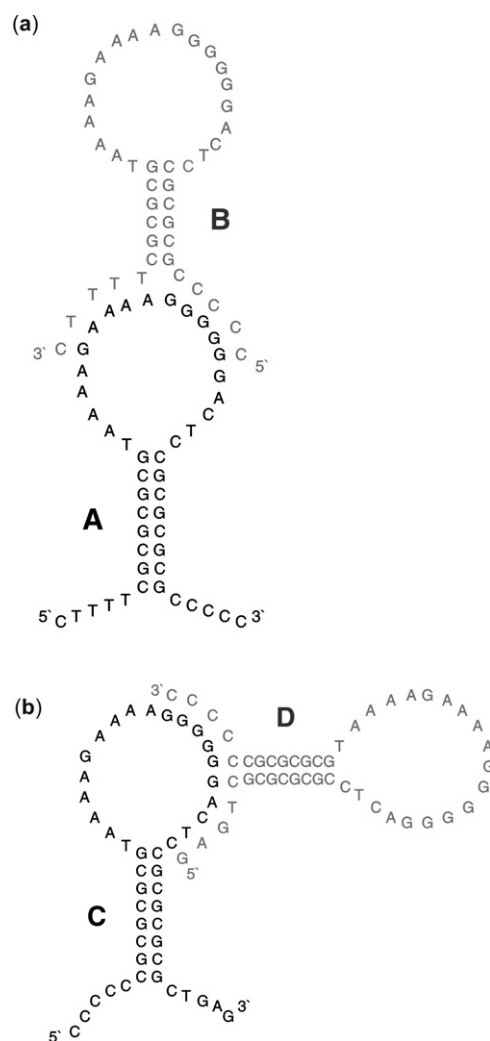


Fig. 1. Oligomers designed to self-assemble into (a) strings or (b) spirals.

Circular dichroism spectroscopy

Circular dichroism (CD) spectroscopy of 1.5 μM ml⁻¹ DNA strand concentration in 100 mM l⁻¹ sodium chloride, 5 mM l⁻¹ magnesium chloride, and 10 mM l⁻¹ sodium cacodylate, at pH 7.0 in a 1 ml jacketed quartz cuvette, was performed in a Jasco J-810 spectropolarimeter with ten accumulations per scan. Circular dichroism, with units of millidegrees, reflects detailed helical

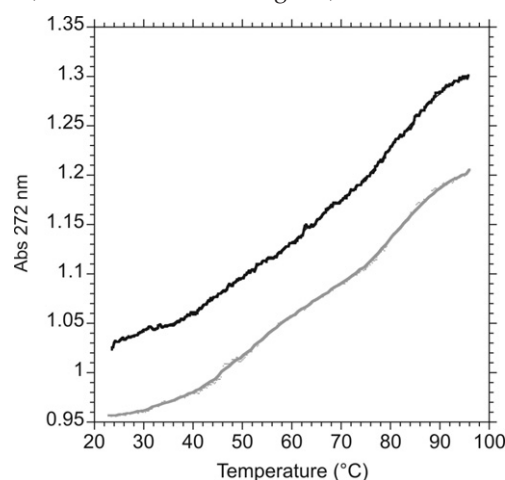


Fig. 2. Denaturation profiles obtained at 272 nm for mixtures of oligomers A + B (grey) and C + D (black) in 100 mM l⁻¹ sodium chloride, 5 mM l⁻¹ magnesium chloride and 10 mM l⁻¹ sodium cacodylate at pH 7.0, the DNA strand concentration was 1.5 μM ml⁻¹.

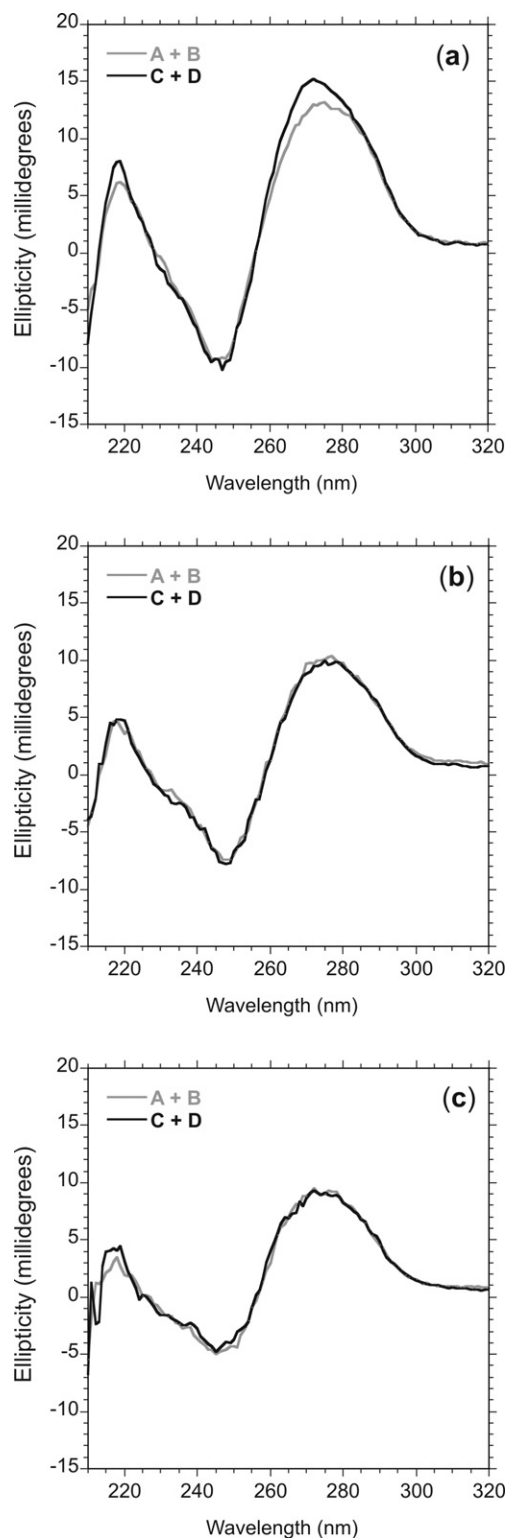


Fig. 3. Circular dichroism spectra of mixtures A + B (grey) and C + D (black) at (a) 25°C, (b) 70°C and (c) 90°C, respectively.

geometry and is defined as the difference between the absorption of left- and right-handed, circularly-polarised light measured as a function of wavelength (nm). The sample was kept at a constant temperature by a Haake D8 programmable water bath during each set of scans.

Polyacrylamide gel electrophoresis (PAGE)

Native PAGE was performed with samples at a DNA strand concentration of $1 \mu\text{M ml}^{-1}$, pre-heated to 80°C, and incubated at

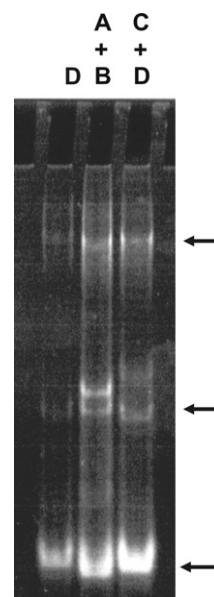


Fig. 4. Native (10%) polyacrylamide gel of oligomer D and mixtures A+B and C+D incubated in 100 mM l^{-1} sodium chloride, 5 mM l^{-1} magnesium chloride and 10 mM l^{-1} sodium cacodylate at pH 7.0.

room temperature in 100 mM l^{-1} sodium chloride, 5 mM l^{-1} magnesium chloride, and 10 mM l^{-1} sodium cacodylate, pH 7.0, before loading on a 10% gel run at 70 volts with tris-borate running buffer (pH 8.3), and visualised under UV light by ethidium bromide intercalation.

Results

Melting behaviour of the networks

The melting of both assemblies (Fig. 1.) was biphasic at a neutral pH (Fig. 2.) corresponding to the denaturation of the intermolecular 'foot-loop' double helix (melting temperature (T_m) = 52°C), followed by the unfolding of the intramolecular stem double helix (T_m = 82°C) in 1 ml of 100 mM l^{-1} sodium chloride, 5 mM l^{-1} magnesium chloride, and 10 mM l^{-1} sodium cacodylate.

Circular dichroism

Circular dichroism spectra revealed that the network made up of oligomers C+D showed more helicity (15 m°) than oligomers A+B (13 m°) at 272 nm (Fig. 3a), as expected. Upon raising the temperature above the melting temperature (T_m , the temperature at which half of the observed absorbance change has been recorded) of the 'foot-loop' interactions, the spectra of the individual stem-loops were similar at 70°C (Fig. 3b). Further heating to 90°C resulted in similar spectra for the random coil species (Fig. 3c).

Polyacrylamide gel electrophoresis

Lanes 1, 2 and 3 of Fig. 4 show the monomer stem-loop D and the dimers and higher networks formed by A+B and C+D, respectively. The migration patterns of lanes 2 and 3 were different, as expected, supporting the CD evidence that networks A+B and C+D had different secondary structures.

Conclusion

This is the first time that 'foot-loop' interactions have been shown to create either linear strings or spirals that were distinguished by CD. Future work will include electron and atomic-force microscopy of the networks, as well as incorporate a transcription factor-binding site into the stem sequence. Hetero-multimers, whether they are formed by single strands or

represent i-motif, parallel or anti-parallel guanine-duplexes, Hoogsteen or anti-Hoogsteen triplexes, can self-assemble in this way under the controlling influence of ionic strength and/or pH.

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