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Double zipper helical assembly of deoxyoligonucleotides: mutual templating and chiral imprinting to form hybrid DNA ensembles

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Herein, the conventional and unconventional hydrogen bonding potential of adenine in APA for double zipper helical assembly of deoxyoligonucleotides is demonstrated at ambient conditions. The quantum mechanical calculations supported the formation of hybrid DNA ensembles.

The magnificent structure-property correlations of biological systems is exemplified by the elegant molecular design and functioning of nucleic acids. In particular, deoxyribonucleic acid (DNA) has structurally evolved over billions of years to effectively store and communicate the genetic information in majority of all living organisms. In recent times, numerous efforts have been directed at utilizing DNA as a potential biomaterial, a biomolecular system capable of conducting electricity, single molecular wire and material building block in celebrated nanotechnological advances. Here, we report an adenine functionalized perylene bisimide (PBI) conjugate (APA) as a promising molecular template to construct hybrid DNA ensembles through double zipper helical assembly (Fig. 1). The intriguing property of adenine to form hydrogen bond with complementary (thymine) and non-complementary (adenine and guanine) nucleobases inspired us to design APA as a double zipper template to construct new hybrid DNA structures. PBI is one of the most promising aromatic π-conjugated systems with potential applications in organic electronics biology and supramolecular architecture. Recently, supramolecular architecture resulting from covalent functionalization of PBI with single-stranded (ss) DNA has been reported. In this context, APA operates through noncovalent interactions, thus, avoiding synthetic difficulties prevalent in the covalent approaches. To the best of our knowledge, this is the first report on the construction of ordered hybrid DNA ensembles through double zipper helical assembly of deoxyoligonucleotides employing versatile hydrogen bonding and π-stacking potential of adenine in APA.

Fig. 1  a) Molecular structure of APA and deoxyoligonucleotides (dBₙ) used in the present study and schematic of hybrid DNA ensemble formation. b-d) Hydrogen bonding in A-T, A-G and A-A base pairs. W = Watson-Crick hydrogen bonding site.

Herein, we present a new strategy for the construction of hybrid DNA ensembles of deoxyoligonucleotides (dBₙ) employing APA (Fig.1). APA conjugate was prepared following our earlier reported procedure, and various dBₙ (dAₙ/Tₙ/Gₙ/Cₙ) were employed to construct double helical assembly of APA and dBₙ (Fig. S1). First, the molecular interactions of APA were studied by evaluating its photophysical properties. The UV-vis absorption spectrum of APA (50 µM) in DMSO exhibited three strong characteristic bands at 530, 493 and 461 nm corresponding to characteristic 0-0, 0-1 and 0-2 electronic transitions of perylene chromophore, respectively. The absorption spectrum of APA (50 µM) in aqueous solution (Water/DMSO = 90:10, v/v%) exhibited hypsochromic shift in the 400-550 nm region and a new band at 561 nm, which is attributed to the hydrophobic force-induced aggregation of APA (Fig. S2a). The fluorescence spectrum of APA in DMSO displayed mirror image emission bands and these bands were completely quenched in aqueous solution as a result of aggregation (Fig. S2b).
The stoichiometric ratio of APA:dT₁₀ (10:2), we performed concentration dependent CD measurements by titrating increasing concentrations of APA (0 to 70 µM) to a fixed concentration of dT₁₀ (10 µM). The stoichiometry of APA:dT₁₀ in hybrid DNA ensemble is given in terms of base pairs ratio i.e. A:T (A of APA:T of dT₁₀). Plot of CD intensity at 561 nm (perylene) against the A:T ratio showed saturation at 1:1 suggesting stoichiometry of 10:2 for the formation of [dT₁₀(APA)₁₀:dT₁₀] type ensemble (Fig. 2c). We also recorded the CD spectra of fixed concentration of APA (50 µM) by adding increasing concentration of dT₁₀ (0 to 12 µM). The plot of CD intensity monitored at 561 nm against concentration of dT₁₀ showed saturation at 10 µM (Fig. S4). Thus, CD studies confirmed stoichiometric ratio of 10:2 for the complexation of APA and dT₁₀ to form [dT₁₀(APA)₁₀:dT₁₀] ensembles.

Next, CD spectra of APA in the presence of non-complementary dA₁₀, dG₁₀ and dC₁₀ were recorded. Notably, APA:dC₁₀ (10:2) combination did not display any characteristic CD signals indicating the absence of ordered assembly between APA and dC₁₀ (Fig. 2a). Surprisingly, APA:dA₁₀ (10:2) and APA:dG₁₀ (10:2) showed unprecedented P-(right-handed) and M-helical arrangements with respect to both perylene chromophore (400-600 nm) and dBₙ (B = A or G) (250-300 nm) (Fig. 2a). The stoichiometry study performed with APA and dBₙ (dA₁₀ and dG₁₀) also suggested the formation of [dBₙ(APA)₁₀:dBₙ] in the ratio of 10:2 (APA:dBₙ) (Fig. 2d and Fig. S5). This indicated mutual templating between APA and dBₙ/dGₙ through unconventional hydrogen bonding leading to P- and M-helical imprinting in the hybrid DNA ensembles. In the control study, spectra of individual dA₁₀ showed positive and negative signal at 270 nm and 250 nm respectively (Fig. S6). Similarly, dT₁₀ showed positive and negative signals at 280 nm and 250 nm respectively. These CD signals correspond to their respective random coil structures. Therefore, the characteristic features in the CD spectra of dT₁₀, dA₁₀ and dG₁₀ with APA in the absence of complementary dAₙ and dGₙ in PBS buffer. APA (50 µM) showed hyperchromicity in the presence and absorption region of DNA complex (250-300 nm) region corresponding to the absorption of APA and dT₁₀, respectively (Fig. 2a). The intense negative CD signal in APA absorption region originated from the orientation of transition moments of perylene chromophores in the counter-clockwise direction (M-helix). The negative CD signal in 250-300 nm region revealed M-helical arrangement of dT₁₀ strands around the molecular template APA. To validate our proposed
electrostatic (sugar phosphate backbone) interactions of dBn. In the pH range of 2-11, a flat CD signal was observed for APA in the absence of dBn. APA:dT10 (10:2) and APA:dT20 (20:2) showed very weak CD features corresponding to perylene chromophore (400-600 nm) and dT10/dT20 (250-300 nm) absorption regions in the acidic pH range of 2-4 (Fig. 2e). However, strong CD signals were observed for perylene chromophore (400-600 nm) and dT10/dT20 (250-300 nm) in the pH range of 7-11. CD spectra of APA:dA10/dA20 displayed a flat signal in the 400-600 nm region under acidic conditions (pH = 2-4). Remarkably, at neutral pH APA:dA10/dA20 exhibited strong positive CD signals in the 400-600 nm and 250-300 nm regions. The intensities of these signals were further enhanced by increasing the pH from 7 to 11 (Fig. 2f). Interestingly, APA:dA20 showed a positive CD signal while APA:dA10 did not show any appreciable CD signal in the adenine absorption region (250-300 nm) under acidic conditions (pH = 2-4). These pH-dependent transformations in the CD signatures of APA:dA10/dA20 are mainly attributed to protonation of adenine. In acidic media (pH = 2-4), adenine (N7) undergoes protonation with the protonated N7-H, triggering self-complementary base pairing [d(AH+):d(AH+)]. This facilitates the formation of homoduplexes (A-motifs) of dA10/dA20, which are further stabilized by electrostatic interactions with the phosphate backbone. It should be noted that well-defined and characteristic CD signature for self-complementary A-motif is observed with dA where n ≥ 12. However, under neutral and basic conditions the unconventional A:A hydrogen bonding interaction driven by APA templating of dA10/dA20 dominates to form hybrid DNA ensembles (Fig. 1).

Next, we recorded CD spectra of APA in the presence of dG10 and dC10 over a pH range of 2-11. Surprisingly, APA:dG10 (10:2) showed CD features similar to APA:dT10 (10:2), albeit only under neutral pH conditions (Fig. S9). Stable unconventional hydrogen bonding-driven G-A pairing between APA and dG10 at neutral conditions led to the formation of [dG10:APA]dG10, and [dC10:APA]dC10, in their stable form.

The CD spectra of APA and dC10 mixture (10:2) displayed a flat signal in the pH range of 7-11, as these conditions do not favor the formation of A:C base pairing. However, under acidic conditions (pH = 4) an intense positive CD signal at 294 nm and a negative CD signal at 265 nm were observed. These are the characteristic CD features well-documented in the literature for i-motifs of dC10 under acidic conditions (Fig. S9a). Overall, pH ≥ 7 favored the mutual templating and chiral imprinting of APA and dBn (B = T/A/G) through conventional and unconventional hydrogen bonding to form hybrid DNA ensembles of the type dBn(APA)n dBn.

In order to ascertain the thermal stability of helical ensembles of dBn and APA, we carried out variable-temperature absorption and CD studies (Fig. S10 & S11). Hybrid ensembles of purine-containing dBn and APA exhibited high thermal stability as indicated by the melting temperatures ($T_m$) (Table 1). The thermal denaturation data revealed that all helical DNA ensembles of APA and dBn were highly stable under ambient conditions. To visualize the structural morphology of hybrid helical DNA ensemble, we carried out atomic force microscopy (AFM) measurements on [dT20(APA)20] (A/T = 1:1) assembly structures. AFM micrograph clearly showed formation of ordered left-handed helical assembly of [dT20(APA)20] (Fig. 3a and Fig. S12a). The left-handed helical assembly structure is well-corroborated with the observed negative cotton effect in the CD spectrum of [dT20(APA)20] ensemble (Fig. S7). The AFM section profiles of [dT20(APA)20]structures revealed typical helical pitch of ~ 22 nm along A-axis (Fig. 3b) and height (thickness) of 5 ± 0.5 nm along B-axis (Fig. 3c). The observed thickness (5 ± 0.5 nm) is in agreement with the theoretically calculated value of 4.5 nm across (B-axis) hydrogen bonded APA and dT20 in [dT20(APA)20] (Fig. S12b). Further, the length of [dT20(APA)20] structures are in the range of 100-400 nm which is more than individual ensemble (Fig. S12). The observed longer helical assembly structures allowed us to consider end-to-end extension of individual ensembles through weak interactions which is very much anticipated on the surface. Overall, these studies proved the versatility of conventional and weighted averages of heat of formation values (in kcal/mol) of the left (M) and right (P) handed [dBn(APA)]dBn (B = A/T/G) helices. Models of hybrid DNA ensembles [dT20(APA)20] (A/T = 1:1), [dC20(APA)20] (B =ApA), dA30, and [dG20(APA)20]dG30, in their stable form.
unconventional hydrogen bonding potential of adenine as the key factor for constructing stable helical hybrid DNA ensembles.

To understand the structure and energetics of the double zipper assembly, quantum mechanical (QM) calculations were performed on carefully chosen model systems, details of which are given in the ESI. High level QM calculations at the RI-MP2 level of theory indicated that both A:A and A:G prefer W:W type base pairing over other possibilities (Fig. 1c & d). The relative energies of four model systems with two base pair steps for each of the M- and P-helical forms were calculated at the semi-empirical PM7 and the Boltzmann weighted differences are presented in Fig. 4c. The energies suggest that dA8 and dG8 prefer M-helical forms, whereas dT8 prefers to form the P-helix, which is in excellent agreement with the experimental observation discussed above. Based on these structures, three-dimensional model for the most stable helical ensemble in each of the three cases were built and are given in Fig. 4d-f. A complex combination of several factors including unique conformational preferences of the backbone increased pitch, and solvent effects are proposed to yield such structures of the assemblies.

In conclusion, we demonstrated the versatility of conventional and unconventional hydrogen bonding ability of adenine in APA as a robust double zipper molecular template to construct hybrid DNA ensembles of random coiled deoxyoligonucleotides. The formation of ordered M- and P-helical DNA ensembles was achieved by distinct base pairing (A-T, A-A and A-G) in the processes of mutual templating and chiral imprinting of APA and deoxyoligonucleotides at pH ≥ 7. These experimental results were further supported by AFM analysis and computational calculations. DNA-templated studies that have been reported so far probe the helical assembly of chromophores based on their characteristic CD signatures. In the present work, we showed mutual helical assembly of functional chromophore and oligonucleotides with corresponding characteristic CD signatures in their respective absorption regions for the formation of M- and P-helical DNA ensembles. The results reported here are likely to inspire the development of new hybrid DNA ensembles of functional molecules (organic chromophores with interesting optical, electronic and biological properties) and oligonucleotides for diverse applications. The properties and applications range from electronics to nanotechnology to biomedicine. The pH dependent hydrogen bonding ability of nucleobases in the DNA ensembles can be used as tool for the development of stimuli responsive (pH-triggered) delivery systems for therapeutic small molecules and oligonucleotides.

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Notes and references
Graphical Abstract

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