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DNA Binding and Recognition of a CC Mismatch in a DNA Duplex by Water-Soluble Peptidocalix[4]arenes: Synthesis and Applications

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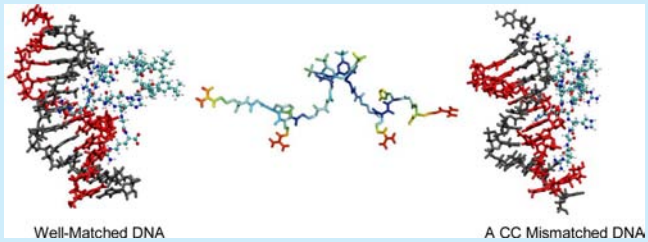
Supporting Information

ABSTRACT: Water-soluble peptidocalix[4]arenes were synthesized by the introduction of arginine-rich narrow groove-binding residues at lower rims through solid-phase synthesis. The study of binding of these water-soluble bidentate ligands to well-matched and mismatched DNA duplexes by fluorescent titrations, ethidium bromide (EB) displacement assays, DNA-melting experiments, and circular dichroism (CD) analysis revealed a sequence-dependent groove-binding mechanism.



Sequence-specific DNA-binding proteins play a central role in the regulation of transcription.[1] Two well-known protein−DNA recognition mechanisms are hydrogen bond formation between amino acid side chains and nucleic acid bases primarily in the major groove (direct readout) and sequence-dependent DNA shape recognition (indirect readout).[2] A newly described readout mechanism is the recognition of the local sequence-dependent minor groove shape based on the sequence dependence of the minor groove width and the resulting electrostatic potential variations.[3] This mechanism implies that the interaction of the well-positioned positively charged residues, primarily arginine residues, with the specific binding sites within the narrow minor grooves (width <5.0 Å) tends to play a prominent role in the sequence-specific protein−DNA recognition.[4] Despite specific interactions between proteins and DNA, the use of DNA-binding proteins for DNA recognition has limitations like the loss of native structure and biological activity of target proteins during purification.[5]

Subsequently, the design of artificial binders possessing a cliplike structure able to bind to DNA duplexes is of great interest, especially in light of the broad range of possible diagnostic and therapeutic applications.[6−8] During the past decades, various synthetic classes of compounds, demonstrated to interact with DNA noncovalently, have been developed.[7] Although calixarenes, the cyclic oligomers obtained by condensation of phenols or resorcinols with aldehydes, are ideal synthetic multivalent ligands, only limited studies have been made on the calixarene scaffolds for the design of DNA-binding agents to date.[9] Calixarenes are easily accessible versatile templates which allow the introduction of various functionalities with preorganized shape and programmed multivalent pattern of binding sites and, therefore, can be promising candidates for the study of noncovalent DNA interactions.[10]

Our contribution to research in this field has been focused on calixarene dimers.[11,12] We have demonstrated that major groove binding dimeric aminocalixarenes bind tightly to double-stranded DNAs (dsDNAs) irrespective of their number of base pairs. However, all of the synthesized calixarenes were insoluble in water and made no distinction between matched and mismatched DNA duplexes.[12] Thus, synthesis of a new class of water-soluble artificial DNA binder-based calixarenes able to distinguish mismatched from matched DNA represents an important and long-standing goal in chemistry. In this regard, considering changes in groove dimension, particularly the minor one, related to the presence of mismatches may have consequences in design of novel DNA-mismatch-binding ligands. Here, we report the synthesis of novel water-soluble bidentate peptidocalix[4]arenes[13] possessing short arginine-rich narrow groove binding residues (Arg-Gly-Gly-Arg in the human orphan estrogen related receptor-2,[14] Arg-Arg-Gly-Arg in the monomeric orphan nuclear receptor NGFI-B,[15] Arg-Gly-His-Arg in MATα1-MATα2,[16] Arg-Lys-Lys-Arg in POU homeo domains,[17] and Arg-Gln-Arg in the Hox protein SCR[3]) at their lower rims that can contribute to the specificity of local DNA shape recognition.

The lower rim 1,3-dihydrazidecalix[4]arene precursor in the cone conformation has been synthesized as previously described.[18] Water-soluble peptidocalix[4]arenes (**PCs 1−5**, Figure 1) have been produced starting from the standard Fmoc solid-phase synthesis of short peptide fragments attached to the

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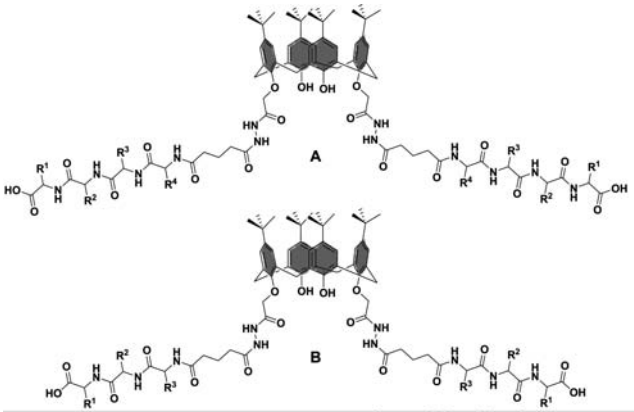

name	structure	amino acids side chains R^1	R^2	R^3	R^4
PC 1	A	Arg	Gly	Gly	Arg
PC 2	A	Arg	Arg	Gly	Arg
PC 3	A	Arg	Gly	His	Arg
PC 4	A	Arg	Lys	Lys	Arg
PC 5	B	Arg	Gln	Arg	-

Figure 1. Structures of novel water-soluble peptidocalix[4]arenes.

lower rim 1,3-dihydrazidecalix[4]arene precursor using glutaric anhydride and purified by HPLC. The products have been characterized using mass spectroscopy, ^{1}H NMR, and ^{13}C NMR.

To investigate ds-DNA recognition, fluorescence titrations of all compounds were carried out with a 5′-fluorescein-labeled 12 bp DNA (12 bp, WM) in Hepes-buffered solutions (2 mM HEPES and 150 mM NaCl in water, pH = 7.4). The DNA sequences used in this study are shown in Table 1.

Table 1. DNA Sequences Used in This Study

name	sequence
ds Fl-DNA (12 bp, WM)	5′-[Fl]GTGACGAACCTC-3′ 3′-CACTGCTTGGAG-5′
ds Fl-DNA (12 bp, MM)	5′-[Fl]GTGACGAACCTC-3′ 3′-CACTGCTTCGAG-5′
ds Fl-DNA (12 bp, AT)	5′-[Fl]AAAAAAAAAAAA-3′ 3′-TTTTTTTTTTTT-5′

A marked increase in fluorescence intensity indicated a tight interaction with all of the water-soluble peptidocalix[4]arenes producing a sharply kinked titration curve. Fluorescence enhancement upon binding may result from the hydrophobic microenvironment. The stoichiometric ratio decreases with an increase in positive charge of the nucleus. The most positively charged peptidocalix[4]arene **PC 4** has the highest binding affinity toward well-matched DNA. Nevertheless, the constants are slightly different. In addition, we examined the emission responses of all of the compounds toward the analogous DNA duplex containing a single CC mismatch, which narrows the minor groove. Interestingly, **PC3** exhibits a higher emission response in the presence of a DNA mismatch relative to the well-matched DNA (Figure 2).

Given a 27-fold higher binding affinity toward the mismatched DNA indicates that **PC 3** is quite selective for binding to the single base mismatch (Table 2). This selectivity might stem from the structural impact of CC mismatch, which clearly alters the groove dimensions and the capability to bind cations through the simultaneously very low DNA breathing

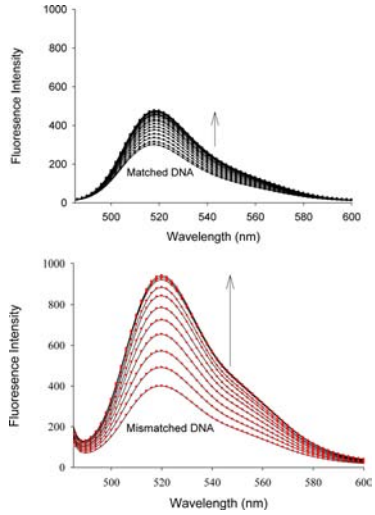

Figure 2. Fluorescence spectra of matched and mismatched DNA in response to the presence of **PC 3**.

Table 2. Association Constants of Water-Soluble Bidentate Peptidocalix[4]arenes with Various DNA Strands

receptor	ds Fl-DNA (12 bp)	Ka [M^{-1}][a]	stoichiometry[b]
PC 1	WM	$2.699\times 10^{+7}$	17:1
PC 1	MM	$1.750\times 10^{+7}$	12:1
PC 2	WM	$6.691\times 10^{+6}$	9:1
PC 2	MM	$4.355\times 10^{+7}$	8:1
PC 3	WM	$7.867\times 10^{+6}$	15:1
PC 3	MM	$2.105\times 10^{+8}$	13:1
PC 4	WM	$3.614\times 10^{+7}$	6:1
PC 4	MM	$2.085\times 10^{+7}$	3:1
PC 5	WM	$2.654\times 10^{+7}$	15:1
PC 5	MM	$1.602\times 10^{+7}$	14:1

[a]Calculated by nonlinear least-squares curve fitting (SigmaPlot Version 10.0). [b]From Job plots: ratios are given in receptor: DNA.

dynamics and a narrow minor groove. Such a narrow groove gives rise to tight bindings and displays enhanced negative electrostatic potential ideally suited for the insertion of arginines that, when spaced appropriately as part of short sequence motifs, provide a complementary set of positive charges that can contribute to the specificity of local DNA shape recognition.[19] In addition, histidine probably reinforces the interactions through further π stacking, and T-shaped interactions with the DNA nucleobases.[20]

The effect of **PC 3**−DNA complex formation on the stability of the double helix was assayed by recording the dsDNA melting profiles before and after addition of **PC 3**. Groove binding interactions by a combination of electrostatic, van der Waals, and hydrogen-bonding interactions exert stabilizing effects on dsDNA structure, which leads to a rise in its melting temperature.[21] The more and stronger the interactions are, the higher the melting temperature of dsDNA is. The difference in binding affinity agrees quite well with the selectivity observed in the thermal denaturation experiments. The melting temperature (T_m) of DNA in the presence of excess **PC 3** was increased by 21 °C for mismatched DNA, whereas only a 14 °C increase was found for matched DNA (Figure 3).

Moreover, DNA structural changes upon binding of **PC 3** to CC mismatched dsDNA were investigated in the absence and presence of **PC 3**−DNA complex using circular dichroism

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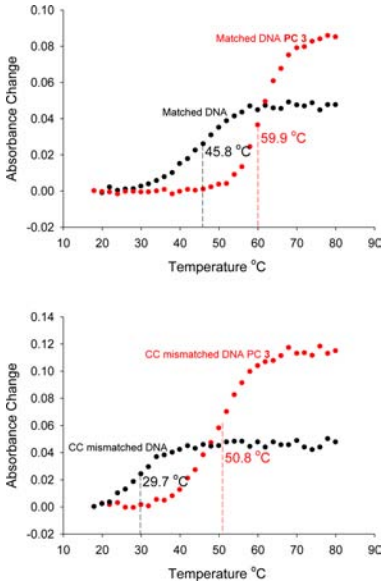


Figure 3. Melting curves of dsDNAs before and after addition of excess **PC 3**.

(CD) spectroscopy (Figure 4). The differential CD spectra were calculated by subtracting **PC 3** spectra from the corresponding **PC 3**–DNA spectra to minimize the interference from **PC 3**.

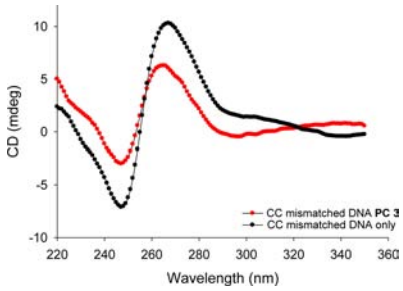
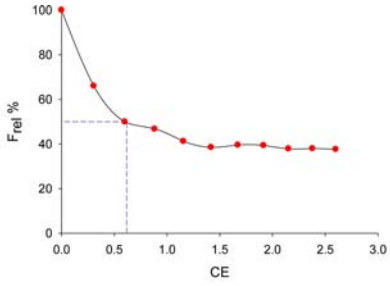


Figure 4. CD spectra of the free CC mismatched dsDNA and its differential CD spectra of **PC 3** complex.

While the intensities of positive and negative bands are reduced in the presence of excess **PC 3**, the CD pattern is reminiscent of B-form DNA composed of a positive band at about 260–280 nm and a negative band around 245 nm.

To elucidate the binding mechanism, similar spectroscopic titrations were also carried out using fluorescein-labeled $(AT)_{12}$ model DNA. The nearly 25-fold difference in the K_D value $(1.952 \times 10^{+8})$ compared with the well-matched DNA demonstrates a clear preference for the binding of **PC 3** to $(AT)_{12}$. This sequence selectivity is not unexpected given the fact that A-tracts often referred to as the B* form are known to create narrow minor grooves associated with more negative electrostatic potential, optimal for electrostatic interaction of the guanidinium groups of arginine.[22]

On the other hand, the efficient binding affinity of the **PC 3**–DNA complex was experimentally assessed by using the ethidium bromide (EthBr) displacement assay. Addition of **PC 3** resulted in complex formation and significant displacement of intercalated EthBr from the double helix of unlabeled calf thymus DNA monitored by a decrease in fluorescence emission (Figure 5). For comparative analysis, the data was



receptor	charge	C_{50} [μM]	CE_{50}
PC 3	+4	3	0.6
spermine	+4	390	1560

Figure 5. EthBr displacement assay for nonfluorescent calf thymus DNA by **PC 3** in Hepes-buffered solutions (2 mM HEPES and 150 mM NaCl in water, pH = 7.4). F_{rel} = relative fluorescence emission intensity, CE = charge excess ratio.

presented in terms of C_{50} and CE_{50} values, which represent the concentration of **PC 3** causing a 50% decrease in fluorescence intensity and the necessary "charge excess" required to cause 50% EthBr displacement, respectively. According to the obtained very low C_{50} and CE_{50} values which are among the lowest ever reported for DNA binders, **PC 3** proved to be an overall strong DNA binder. Moreover, as phosphate backbone binders possess high C_{50} and CE_{50} values this can be strong evidence for groove binding.[12]

Although the experimental scanning time scale is too far from the accessible time scale for all-atom MD simulations even on today's fastest computers, these simulations can provide insights into the early stages of the complex formation process. These simulations reported here reveal that guanidinium cations, the most mobile parts of the molecule, play crucial roles in DNA interactions (Figure 6) and suggest significantly different interaction patterns between matched and mismatched complexes.

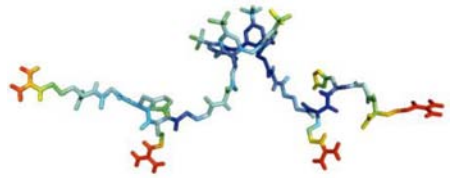


Figure 6. Molecular modeling of **PC 3**: The molecule is colored according to the B factors, or temperature factors. Blue regions represent cool areas, whereas the most mobile parts of the molecule are colored red. Only polar hydrogens are shown.

These data illustrate that mismatched DNA recognition occurs by extensive minor groove interactions, while matched DNA is recognized by major groove interactions in the early stages (Figure 7).

Intermolecular interaction energies, electrostatic and nonelectrostatic interactions represented by Coulomb and Lennard-Jones potentials, between **PC 3** and different parts of well-matched and mismatched DNA duplexes have been calculated to shed more light on the interactions. The diagrams below depict the interaction of **PC 3** with the CC mismatched base pair and the CG base pair of the well-matched DNA at the same position (Figure 8). Negative values indicate attractive interactions, while positive values indicate repulsive interactions. The presented results show that the interaction with

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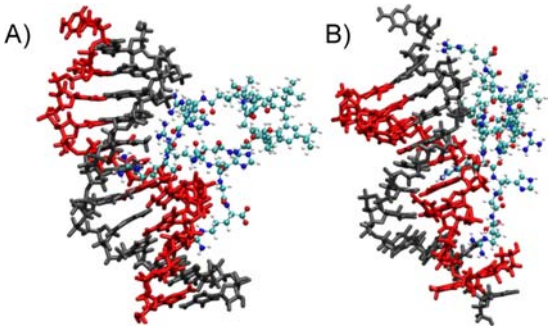

Figure 7. Molecular modeling of **PC 3**–DNA complex with matched (A) and mismatched DNA (B).

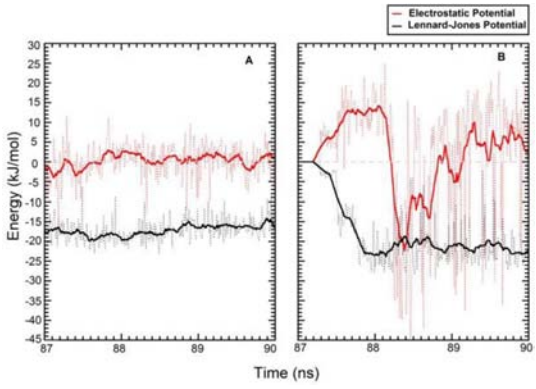

Figure 8. Intermolecular interaction energies of **PC 3** with the CC mismatched base pair (B) and the CG (A) base pair of matched DNA at the same position.

CC base pair, which leads to some noticeable decreases in energies, is somehow stronger even in the early stages of complex formation.

In summary, water-soluble peptidocalix[4]arenes have been designed by incorporating arginine-rich short narrow groove binding residues on the lower rim of the calix[4]arene scaffold with the goal of recognizing well-matched and mismatched DNA duplexes. Our fluorescence measurements revealed that the synthesized compounds have high affinity for DNA. Most importantly, comparison between the fluorescence titrations of the matched and mismatched DNA proved that **PC 3** is quite selective in recognition of a CC mismatch in a DNA duplex. This selectivity is reflected in the 27-fold higher binding affinity toward the mismatched DNA and 21 °C temperature increase in the T_m of mismatched DNA without causing any major conformational changes of the B-DNA. This study opens new doors for DNA binding and base pair mismatch detection in DNA duplexes through consideration of significant alterations in groove dimension, particularly the minor one, related to the presence of mismatches irrespective of the sequence environment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01995.

Experimental procedures and characterizations, fluorescent titrations, EB displacement assays, DNA melting experiments, CD analysis, and computational methods (PDF)

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Notes

The authors declare no competing financial interest.

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