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Minimalist Protein Design: A β-Hairpin Peptide That Binds ssDNA

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The interaction between proteins and single-stranded DNA (ssDNA) plays an important role in the regulation of critical biological processes such as telomeric-end protection[1] and DNA replication and repair.[2] The recognition of ssDNA by protein receptors occurs predominantly through aromatic and electrostatic interactions involving protein side chains extending from a solvent-exposed β-sheet surface, known as the OB-fold (oligonucleotide/oligosaccharide-binding fold).[3] Aromatic stacking interactions are believed to play a pivotal role in the ability of OB-fold proteins to distinguish ssDNA from duplex DNA (dsDNA) since the aromatic bases are buried within the π-stack of the duplex and are less accessible to stack with protein aromatic side chains.[4]

Although many proteins bind ssDNA on a β-sheet surface, there have been no examples of β-hairpin peptides as molecular receptors for ssDNA or RNA.[5] Such systems present a direct approach for elucidating the factors controlling protein−nucleic acid recognition while providing a potential scaffold for disrupting protein−ssDNA interactions. In a previous report, we have introduced a designed 12-residue β-hairpin, **WKWK**, which binds ATP with an affinity of 170 μM in water.[6] One face of the β-hairpin displays two Trp and two Lys residues which interact with ATP through aromatic and electrostatic interactions. In this Communication we describe the extension of this system to a β-hairpin receptor for ssDNA with a binding affinity that rivals that of natural protein receptors, providing a minimalist mimic of the OB-fold.

Peptide **(WKWK)$_2$** is a dimer of our ATP receptor,[6] linked by a disulfide bond between N-terminal Cys side chains (Figure 1). The structure of **(WKWK)$_2$** creates two nucleotide binding sites which were expected to be well-structured due to the highly populated β-hairpin conformation of **WKWK**.[6a] Indeed, NMR characterization of **(WKWK)$_2$** using standard methods including H$_\alpha$ and Gly chemical shifts[7] and cross-strand NOEs indicates that it is as folded as the parent peptide, **WKWK** (see Supporting Information).

The recognition of single-stranded pentanucleotide sequence 5′-d(AAAAA)-3′ (dA$_5$) by **(WKWK)$_2$** was investigated by fluorescence titrations following the Trp quenching with increasing oligonucleotide concentration. In pH 5.0 buffer, **(WKWK)$_2$** was shown to bind dA$_5$ with an affinity in the submicromolar range and was beyond the detection limit of Trp fluorescence.[8] A 1:1 binding stoichiometry for the **(WKWK)$_2$** interaction with dA$_5$ was demonstrated using the molar ratio variation method (see Supporting Information).[9]

The interaction of dA$_5$ with the peptide was probed by measuring the change in chemical shifts of the side-chain protons of **(WKWK)$_2$** in the presence of 1 equiv of the oligonucleotide. The magnitude of the upfield shifting of side-chain protons was generally larger at the Lys and Trp residues of the peptide, suggesting a direct interaction between the oligonucleotide and these side chains of the peptide (Figure 2). Significant chemical shift deviations were also observed at the Arg residues of the peptide, suggesting that it may also contribute to binding. Upfield shifting of the Trp aromatic

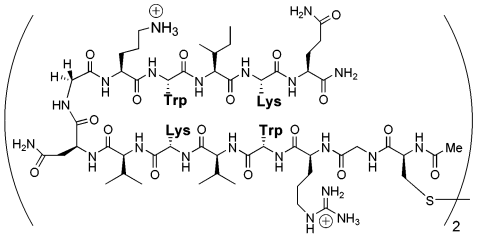

Figure 1. Structure of peptide **(WKWK)$_2$**. The residues in bold create the nucleic acid binding face.

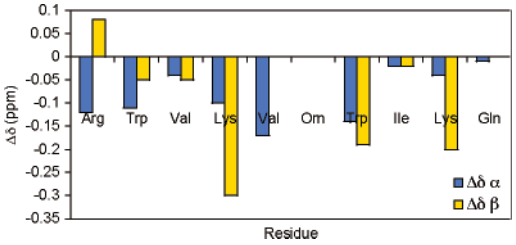

Figure 2. Change in Hα and Hβ chemical shifts of **(WKWK)$_2$** in the presence of 1 equiv of dA$_5$, 10 mM acetate-d_3 buffer, pH 5.0, at 298 K (the turn sequence, Asn-Gly, is omitted).

protons by 0.1−0.4 ppm and of the adenine aromatic protons by $\geq$0.3 ppm was observed, indicating that stacking interactions are involved in complex formation. Additionally, the $\Delta\delta$Gly value and cross-strand NOE correlations of the dimer in the presence of dA$_5$ indicate that the β-hairpin conformation is not disrupted upon binding to the pentanucleotide (see Supporting Information). These results demonstrate that **(WKWK)$_2$** binds the oligonucleotide while maintaining a β-hairpin conformation through interactions with cationic and aromatic residues on one face of the β-hairpin, similar to the binding motif of the OB-fold.

The dissociation constant for binding to dA$_5$ was found to be 12 μM in pH 7.0 buffer (Table 1), which is weaker than that in pH 5.0 buffer, possibly due to incomplete protonation of the Lys side chains at pH 7.0.[5a] Investigation of sequence selectivity indicates that the interaction with dA$_5$ is −0.3 to −0.6 kcal/mol stronger than the interaction with sequences dC$_5$ and dT$_5$ (Table 1). The modest selectivity pattern is not surprising, given that ssDNA-binding proteins interact with their oligonucleotide targets in a largely sequence-independent manner.[1c] However, it should be noted that the binding affinity for dA$_5$ may be attenuated due to a tendency for intramolecular stacking between the bases in the ssDNA sequence,[10] whereas intramolecular stacking in dT$_5$ and dC$_5$ is unlikely to occur under the solvent conditions in this study.[11]

We investigated the binding of **(WKWK)$_2$** to an 11-residue single-stranded oligonucleotide and its corresponding duplex to determine the selectivity for ssDNA relative to dsDNA (Table 1). We found that **(WKWK)$_2$** binds the 11-residue oligonucleotide with a K_d of 3 μM in 100 mM NaCl, pH 7.0 buffer (Table 1). This is comparable to the binding affinity of Cold Shock protein for

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Table 1. Affinity Constants for **(WKWK)$_2$** with DNA Sequences[a]

entry	sequence	[NaCl], mM	K_a, M^{-1}	K_d, μM	ΔG (error),[b] kcal/mol
1	5′-AAAAA-3′	0	8×10^4	12	−6.7 (0.1)
2	5′-TTTTT-3′	0	3×10^4	30	−6.1 (0.1)
3	5′-CCCCC-3′	0	5×10^5	20	−6.4 (0.1)
4	5′-CCATCGCTACC-3′	100	3×10^5	3	−7.5 (0.1)
5	5′-CCATCGCTACC-3′ 3′-GGTAGCGATGG-5′	100	2×10^5	5	−7.2 (0.2)

[a] In 10 mM sodium phosphate buffer, pH 7.0, at 298 K. [b] Errors determined from two to four separate titration experiments.

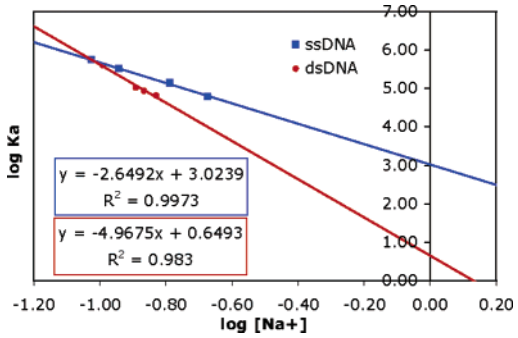

Figure 3. Dependence of log K values for **(WKWK)$_2$** binding to ssDNA sequence 5′-CCATCGCTACC-3′ on log [Na^+] in 10 mM sodium phosphate buffer, pH 7.5, at room temperature. Each point represents the average of 2−4 runs.

ssDNA, which contains a single OB-fold.[12] The stronger interaction with the 11-mer relative to the pentanucleotides can be attributed to an increased number of favorable contacts that are possible with the longer oligonucleotide.

Comparison of the 11-mer to its corresponding duplex at 100 mM NaCl demonstrates modest energetic preference for ssDNA (Table 1). We performed a salt study to quantify the effect of aromatic and electrostatic interactions on the binding affinity (Figure 3). Extrapolation to 1 M salt demonstrates a binding free energy of approximately -4.2 kcal/mol for the 11-mer (Figure 3). As the electrostatic interactions are screened at 1 M salt,[8] we estimate that −4.2 kcal/mol represents the contribution of aromatic interactions to binding. This is much greater than has been previously observed in unstructured $(Lys)_n(Trp)_m$ peptides with ssDNA[13] or found here for **(WKWK)$_2$** with the duplex (Figure 3), where the non-ionic contributions for binding were found to be less than −1 kcal/mol.[5a,13] Previously, we have shown that stacking of adenine between the diagonal Trp residues of **WKWK** is worth approximately −1.8 kcal/mol.[6a] Therefore, it appears that, for the 11-mer, a base is intercalating within both aromatic binding pockets of the dimer, with each interaction providing about −2 kcal/mol.

Although stacking interactions provide selectivity for ssDNA, the affinity for the dsDNA sequence at 100 mM NaCl results from the greater negative charge density of duplex DNA.[8] A linear decrease in the magnitude of log K_a with increasing log [Na^+] was found for both the 11-mer and the duplex due to electrostatic screening of the phosphates, but the duplex exhibited a greater dependence on salt concentration than the single-stranded oligonucleotide (Figure 3). Equation 1 correlates the salt sensitivity of

$$\partial \log K_a / \partial \log [Na^+] = -z\psi \qquad (1)$$

peptide-nucleic acid interactions with the charge of the peptide, z, and a proportionality constant, ψ, which has been correlated with the fraction of cation released.[8] The net charge for **(WKWK)$_2$** is +8, such that binding of **(WKWK)$_2$** to the 11-mer gives $\psi = 0.33$, whereas binding to the duplex gives $\psi = 0.62$, indicating significant differences in the contribution of electrostatic interactions to binding. Importantly, the difference in salt dependence of the binding oligoLys peptides to ss- or ds-*poly*nucleotides is less than that observed here for *oligo*nucleotides due to the polyelectrolyte effect: $\psi = 0.74$ for ssDNA and $\psi = 0.88$ for dsDNA.[8] Hence, greater selectivity for binding of **(WKWK)$_2$** to ssDNA is expected in *poly*nucleotides relative to *oligo*nucleotides at physiological salt concentrations.

In summary, the designed β-hairpin receptor **(WKWK)$_2$** binds ssDNA in aqueous solution with an affinity that is comparable to that of naturally occurring protein receptors for ssDNA. The results are consistent with a stacking interaction of a base with each Trp binding pocket of the dimeric β-hairpin. The aromatic and electrostatic interactions that regulate recognition in our system are similar to those that regulate ssDNA binding by protein receptors. Moreover, the contribution of aromatic interactions is much more significant than observed with unstructured $(Lys)_n(Trp)_m$ peptides. Thus, by incorporating Trp side chains into a structured peptide that is preorganized for binding, we have gained specificity that is not observed for unstructured peptides. Peptide **(WKWK)$_2$** introduces an excellent scaffold for investigating protein interactions with ssDNA and has implications for drug design pertaining to antigene and antisense therapeutics.[14]

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Supporting Information Available: Experimental procedures, NMR data, and binding curve fits. This material is available free of charge via the Internet at http://pubs.acs.org.

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