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A Designed β-Hairpin Peptide for Molecular Recognition of ATP in Water

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Binding of ATP by proteins is one of the most prominent molecular recognition events in nature and is crucial for many enzymatic processes. For these reasons, it has been a popular target for the design of molecular receptors.[1] The binding cavities or surfaces of designed receptors often contain functionality similar to those provided by nucleic acid binding sites on proteins, which primarily consist of regions of aromatic, cationic, and hydrogen-bonding residues.[2]

Although synthetic receptor systems have provided valuable insights into biological complexation phenomena, a more direct approach is the use of a structured peptide-based receptor to mimic protein recognition of ATP. Although single-stranded nucleic acid binding proteins typically interact via a β-sheet surface of a protein,[3] the use of β-sheet-based peptide receptors for nucleic acids is an undeveloped method in the field of molecular recognition. Monomeric β-hairpin peptides present novel mimics for protein recognition of nucleic acids through a β-sheet surface. In the present work, we demonstrate that an unconstrained 12-residue β-hairpin peptide with a diagonal tryptophan pair on one face of the β-hairpin binds nucleotides in water through a combination of aromatic and electrostatic interactions with an impressive affinity.

The general β-hairpin sequence is based on Gellman's peptide.[4a] The peptide **1** contains an Asn-Gly type I′ turn and incorporates a diagonal Trp-Trp pair to allow nucleobase intercalation. A diagonal placement of the Trp residues was selected on the basis of the NMR structure of the Trpzip peptide of Cochran et al., in which the diagonal Trp residues appear to form a cleft.[4b] Lysine residues were placed on the same face of the hairpin as the aromatic side chains to provide short-range electrostatic interactions with the nucleotide phosphates. The hairpin has a net +4 charge to increase solubility and prevent peptide aggregation.

1: Ac-Arg-**Trp**-Val-**Lys**-Val-Asn-Gly-Orn-**Trp**-Ile-**Lys**-Gln-NH_2

Alpha proton (H_α) chemical shift data and ROESY experiments demonstrate that peptide **1** exhibits a high β-hairpin population in water at 25 °C. Most H_α protons exhibit significant ($\Delta\delta H_\alpha > 0.1$ ppm) downfield shifting from random coil values, indicating a well-populated β-hairpin conformation (Figure 1).[5] Cross-strand NOEs were also observed, indicating a β-hairpin structure (see Supporting Information). In addition, the presence of a highly structured hairpin is indicated from the large splitting of the glycine diastereotopic protons ($\Delta\delta$Gly = 0.73 ppm), which corresponds to a 96% fold, using a cyclized disulfide reference peptide to represent the glycine splitting for the 100% fold (see Supporting Information). The magnitude of $\Delta\delta$Gly values have been directly correlated with hairpin folding in a number of structural studies.[6] Concentration dependence studies by CD indicate that peptide **1** is monomeric.

The ability of peptide **1** to bind ATP was investigated by fluorescence spectroscopy. ATP titration into a solution of peptide **1** produced pronounced quenching of the Trp side chain fluorescence at 348 nm. Fitting of the tryptophan fluorescence quenching as a function of ATP concentration gave an association constant of 5800 M^{-1} ($\Delta G = -5.1$ kcal/mol) (Table 1). The designed hairpin thus shows a remarkable affinity for ATP in aqueous solvent.

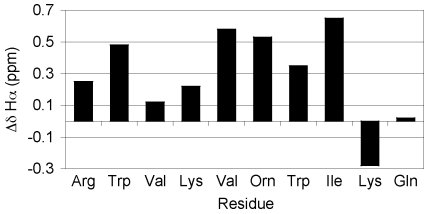

Figure 1. $\Delta\delta H_\alpha$ values for peptide **1** relative to random coil shifts determined from control peptides (see Supporting Information) with turn residues omitted.

Table 1. Affinity Constants for Nucleotide Recognition by Peptide **1**[a]

nucleotide	[NaCl] mM	K_{assoc} M^{-1}	K_d mM	$\Delta G_{binding}$ (error)[c] kcal/mol
ATP	0	5815	0.17	−5.1 (0.2)
ATP	200	70	14	−2.5 (0.2)
ATP	500	21[b]	48	−1.8 (0.2)
ADP	0	490	2.0	−3.7 (0.1)
AMP	0	109	9.2	−2.8 (0.1)
AMP	500	20[b]	50	−1.8 (0.2)

[a] Determined in 10 mM acetate buffer, pH 5.0 at 25 °C. Affinity constants were determined by fluorescence titrations unless otherwise stated. [b] Binding constants were determined by NMR titrations due to extensive inner filter effects by fluorescence at the concentration range required for the experiment. [c] Errors in binding strengths were determined by the average deviation between 2 and 4 separate titration experiments.

The mode of interaction between peptide **1** and ATP was investigated by NMR. Titration of ATP into peptide **1** produced significant upfield shifting of aromatic protons on both Trp residues as well as the adenine protons of ATP, indicating that the adenine base is interacting with both Trp residues of peptide **1**. The magnitude of upfield shifting was from 0.1 to 0.7 ppm. Interestingly, ATP titration produced no upfield shifting in peptide receptors containing a lateral Trp-Trp pair,[7] suggesting that the diagonal relationship is required to form a binding cleft for aromatic recognition in this system. A 1:1 binding ratio was confirmed with a Job plot by NMR.

Further NMR investigations have demonstrated that ATP is preferentially bound on one face of the β-hairpin. Assignment of the alpha and beta protons of peptide **1** in the presence of 10 equiv of ATP showed some upfield shifting at nearly all residues, yet the magnitude of upfield shifting was generally 2−3 times greater at Lys-4, Lys-11, and the Trp side chains (Figure 2a), indicating close proximity of these residues to the adenine ring. In addition, cross-strand NOEs in the presence of 10 equiv of ATP are observed (Figure 2b), and the spitting of the glycine diastereotopic protons remains unaffected ($\Delta\delta$Gly = 0.74 ppm). These results support a model in which ATP binding by peptide **1** occurs in the folded

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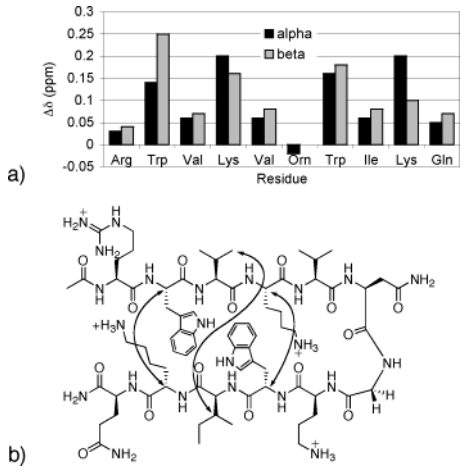

Figure 2. (a) Change in alpha (H_α) and beta (H_β) protons chemical shifts of peptide **1** in the presence of 10 equiv of ATP, with $\Delta\delta H_\alpha = \delta H_{\alpha,0ATP} - \delta H_{\alpha,10ATP}$. A positive change in chemical shift indicates upfield shifting. (b) Cross-strand NOEs in the presence of 10 equiv of ATP.

conformation through interactions with the Trp and Lys residues on one face of the hairpin.

The strength of ATP binding to peptide **1** was shown to drop off with electrostatic screening with added salt, demonstrating the contribution of electrostatic interactions between the nucleotide phosphates and the peptide receptor (Table 1). In addition, peptide **1** showed reduced recognition affinity for ADP ($\Delta G = -3.7$ kcal/mol) and AMP ($\Delta G = -2.8$ kcal/mol) relative to ATP in the absence of salt (Table 1). These results indicate that electrostatic interactions involving each phosphate of the nucleotide contributes approximately -1 kcal/mol to the binding event, consistent with previous estimates for the strength of individual salt bridges between nucleotide phosphates and polyammonium bearing synthetic hosts in water.[1e,f]

The binding constant for ATP recognition by peptide **1** in 500 mM NaCl was identical to the binding observed for AMP recognition in 500 mM NaCl (Table 1), suggesting that electrostatic interactions involving the phosphates are effectively screened at this salt concentration. From this result, we estimate that intercalation of adenine into the aromatic binding pocket of the β-hairpin contributes -1.8 kcal/mol to ATP recognition. This agrees well with the value of -2 kcal/mol determined for the Tyr-Adenine stacking interaction in aminoglycoside phosphotransferase.[2e]

Few synthetic receptors have achieved strong nucleotide recognition in aqueous media, which is due to the fact that hydrogen bonding and electrostatic interactions are intrinsically weak in this solvent system. Effective recognition in water in designed systems has been accomplished by coupling hydrophobic or stacking interactions with electrostatic or hydrogen-bonding interactions. For example, Schneider and co-workers have constructed polyammonium bearing calixarene-based receptors[1f] as well as azoniacylclophane hydrophobic cavities[1e] having binding constants for nucleotides up to 10^4-10^5 M^{-1} in water. Anslyn and co-workers have recently described a synthetic benzene scaffold with positively charged guanidinium arms to which was attached a library of tripeptides.[1g] "Hits" in this library showed binding constants for ATP on the order of 3400 M^{-1} in water. Recognition of ATP by our designed peptide receptor **1** is comparable to these synthetic systems, showing strong nucleotide recognition in aqueous solvent, but unique in that is composed of entirely naturally occurring amino acids and binds ATP via a secondary structure that is directly relevant for modeling nucleic acid recognition by proteins.

In conclusion, we have presented the first example of a β-hairpin peptide which functions as a molecular receptor for nucleotides in water. We have shown that a diagonal Trp-Trp pair in the non-hydrogen-bonding sites of the β-hairpin provides a binding cleft for aromatic intercalation. Designed electrostatic complementarity between the peptide side chains and a nucleotide guest enhances association, leading to appreciable binding affinities in aqueous media, which has been a challenge for many synthetic receptors systems. Furthermore, this host−guest system presents a novel minimalist model for nucleic acid recognition through a β-sheet surface, as is common in proteins, and suggests an initial design for the construction of single-stranded DNA binding peptides. The de novo design of β-hairpin receptors for single-stranded oligonucleotides is currently under investigation.

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Supporting Information Available: Experimental data and procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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