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Selective Binding of TAR RNA by a Tat-Derived β-Peptide

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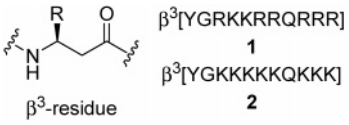
ABSTRACT



The interaction between the HIV-1 Tat protein and the TAR RNA element in the nascent viral genomic transcript is required for viral replication. An 11-residue β-peptide (1), an all-β homologue of the Arg-rich region Tat 47–57, binds TAR RNA with $K_d = 29 \pm 4$ nM. A control β-peptide (2) in which all Arg side chains are replaced by Lys side chains shows increased affinity but decreased specificity for wild-type vs bulge-deleted TAR RNA, as do the α-peptide analogues of 1 and 2.

The search for new therapies for AIDS has focused on events in the life cycle of HIV other than reverse transcription and proteolysis. Forward transcription of viral genomic RNA from proviral DNA is an attractive target, particularly because inhibition of transcription might prevent reactivation of latent or suppressed HIV infection.[1] Transcription of HIV RNA requires the interaction of the virally encoded Tat protein with the transcriptional activator-responsive element (TAR), a bulged RNA hairpin structure formed by the nascent transcript.[2] The key determinants of the Tat−TAR interaction have been localized to a trinucleotide bulge in TAR RNA[3] and the 11-amino acid basic region of Tat (residues 47−57).[4] This interaction can be disrupted by a variety of backbone-modified Tat 47−57 analogues, including a D-peptide,[5] an oligocarbamate,[6] an oligourea,[7] and various peptoid-based structures.[8] Here, we demonstrate that a β-amino acid oligomer ("β-peptide") analogue of Tat 47−57 (**1**) binds TAR RNA with nanomolar affinity.

The affinity of the Tat basic sequence for TAR RNA has been proposed to depend on two key features: a single arginine side chain, which may specifically bridge two phosphates in the TAR bulge, and a cluster of cationic residues, which appears to provide a polyelectrolyte-like affinity for RNA.[9] Backbone-modified Tat analogues preserve the side chains of the Tat basic region but vary (in

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spacing or chirality) the relative positions of the functional groups. The success of these analogues in disrupting the Tat−TAR interaction provides further evidence that the presentation of an arginine side chain in a cationic context is a primary determinant of affinity and also suggests that other peptidomimetics might have similar potency.

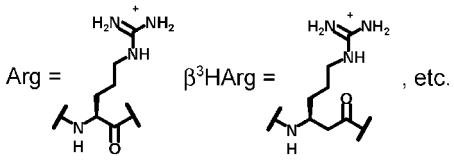
The study of β-peptides has accelerated over the past decade, propelled by demonstrations that they can be programmed to adopt protein-like secondary structures.[10] These structures have given rise to a variety of biological activities,[11] and the protease resistance of β-peptides makes them potentially attractive from a pharmaceutical standpoint.[12] During our previous work on membrane translocation of analogues of the Tat 47−57 sequence,[13] we noted that β-peptide **1** is unstructured in aqueous solution. Because this region of the native Tat protein adopts an extended conformation,[14] we hypothesized that flexible β-peptide **1** would itself be competent to bind TAR.

1 = β³HTyr-β³HGly-β³HArg-β³HLys-β³HLys-β³HArg-β³HArg-β³HArg-β³HGln-β³HArg-β³HArg-β³HArg

2 = β³HTyr-β³HGly-β³HLys-β³HLys-β³HLys-β³HLys-β³HLys-β³HLys-β³HGln-β³HLys-β³HLys-β³HLys

3 = Tyr-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg

4 = Tyr-Gly-Lys-Lys-Lys-Lys-Lys-Gln-Lys-Lys-Lys



The syntheses of β-peptide **1** (as previously reported[13]) and control β-peptide **2**, in which all arginine side chains have been replaced by lysine side chains, were carried out by automated solid-phase methods[15] from Fmoc-protected β-substituted β-amino acids ("β^3-amino acids") obtained enantiospecifically using Müller's modification[16] of Seebach's methodology.[17] Fmoc-β^3HArg(Pmc) obtained by homologation, as previously reported,[13] requires repeated column chromatography, with fraction-by-fraction HPLC analysis, to eliminate small ($\leq$1%) amounts of starting material Fmoc-Arg(Pmc) impurity. We have also detected α-Lys impurity in Fmoc-β^3HLys(Boc) and its oligomers, albeit at lower levels. If chromatography is performed only once on the building blocks, these impurities give rise in highly redundant sequences to a population of α-Arg- and α-Lys-containing contaminants, detectable by mass spectrometry but inseparable from the desired β-peptide by HPLC. Peptides used for the studies described here were synthesized using Fmoc-β^3HArg(Pmc) and Fmoc-β^3HLys(Boc) containing undetectable (<0.05%) amounts of Fmoc-α-amino acid. Analogous control α-peptides **3** and **4** were synthesized similarly from α-amino acids.

Electrophoretic mobility shift data (see Supporting Information) showed binding of **1** to TAR RNA. However, because partial dissociation of complexes during gel electrophoresis has been reported to interfere with assessments of peptoid−TAR binding,[8a] an alternative method for determining K_d was required. We therefore developed a fluorescence anisotropy (FA) assay for Tat−TAR binding.

When a fluorophore is excited by polarized light, the loss of polarization in the emitted light (FA) can be correlated with the mobility of the fluorophore. In our assay, wild-type and bulge-deleted TAR RNA were labeled with fluorescein. Binding of peptides **1**−**4** (MW $\approx$ 1400−1700) to labeled wild-type or bulgeless TAR (MW $\approx$ 9700 or 8900) increases the effective molecular weight of the fluorophore-bearing complex, decreasing the effective fluorophore mobility and, hence, increasing FPA. Fluorescence anisotropy was measured as a function of β-peptide concentration (Figure 1), and K_d was determined from these curves (Table 1).[18]

The affinity of Tat protein and Tat-derived peptides for TAR is known to depend sensitively on assay conditions. For example, electrophoresis-derived K_d values for extremely similar peptides can differ by 2 orders of magnitude with varying salt concentrations (70 mM NaCl vs 20 mM KCl) and experimental conditions.[6,19] Therefore, the difference in K_d values for **3** between our assay and one reported previously[6] is not completely unexpected. The K_d and binding mode of **3** to fluorescein-tagged TAR have been verified by FRET to rhodamine-tagged **3** (Cao, H.; Rana, T. M. Unpublished results).

Because of this variation in measured K_d values, a standard procedure in this field is to report dissociation constant ratios (K_{rel}), in which a value above 1 denotes stronger binding of the analogue relative to the native peptide. The K_{rel} value for **1** relative to **3** is 0.072, indicating a significant diminution of affinity when the backbone is altered to a β-peptide backbone. Interestingly, alteration of the backbone to an oligocarbamate gives $K_{rel} = 0.69$,[6] and alteration to an oligourea gives $K_{rel} = 7.1$,[7] indicating that affinity of Tat analogues for TAR cannot be explained as a simple function of side chain spacing.

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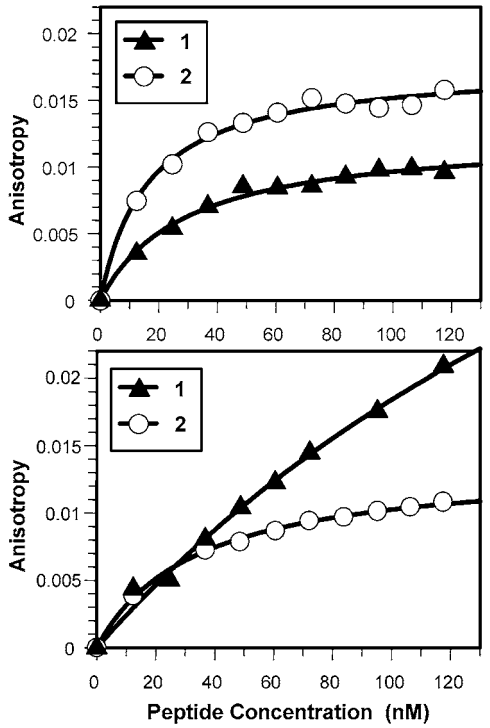

Figure 1. Fluorescence anisotropy data for β-peptides **1** and **2** binding to fluorescein-tagged wild-type TAR (top) and bulge-deleted TAR (bottom). Each data point represents five observations; curve fittings were performed using an equation that may be found, along with full procedural details, in Supporting Information.

Table 1. Dissociation Constants for Peptides **1**−**4** from Wild-Type or Bulge-Deleted TAR RNA as Determined by FA[a]

	K_d (nM)		
	wt TAR	bulgeless TAR	specificity for bulge
1	29 ± 4	281 ± 68	10
2	16 ± 2	33 ± 2	2
3	2.1 ± 0.8	1.1 ± 0.2	0.5
4	32 ± 4	72 ± 8	2

[a] Assays were performed in 20 mM KCl.

Surprisingly, the intended "negative control" β-peptide **2** binds more tightly to TAR in this assay than either designed β-peptide **1** or "negative control" α-peptide **4**, the affinity of which for TAR has not previously been reported. It is difficult, however, to attribute these differences to specific binding of the TAR bulge by an "arginine fork" motif, because similar trends are seen in the affinities of peptides **2**−**4** for the bulgeless control RNA. The lack of specificity of the α-peptide Tat 49−57 for wild-type TAR over bulgeless TAR has been previously reported.[20]

It is even more surprising to find that **1** displays an enhanced specificity for wild-type TAR RNA relative to **2**−**4**. Because the bulgeless hairpin is a common secondary structural motif in RNA, specificity for the bulged stem-loop is a requirement for any effective therapeutic directed at TAR. Although the affinity of β-peptide **1** for wild-type TAR is roughly 15-fold lower than that of α-peptide **3**, the affinity of β-peptide **1** for bulgeless TAR is reduced by more than 2 orders of magnitude compared to α-peptide **3**. Appending a minimum of eight random amino acids to Tat 49−57 has been reported to increase specificity,[20] but the increased length of β-peptide **1** (11 atoms, less than the length of 4 α-amino acids) cannot be solely responsible for the increased specificity. Similarly, including the 10-residue Tat "helical core region" N-terminal to the basic region gives a modest increase in peptide specificity for wild-type TAR over mutants,[20,21] but this effect may require four residues at the N-terminus of the core.[22] A full explanation of the complex effects of residue spacing and peptide length on specificity must await more detailed studies of the binding modes of peptides **1** and **2** to wild-type and bulgeless TAR.

β-Peptides have shown promise in a variety of biological applications, including inhibition of cholesterol uptake,[11a] somatostatin receptor binding,[11b] and antimicrobial activity.[11c,d] The data presented here suggest that β-peptide analogues of HIV-1 Tat, like other Tat-derived peptidomimetics,[5−8,19] may also be leads for antitranscriptive anti-HIV therapy. Although these compounds require further optimization, they represent a step forward in specificity and an exciting new direction in antiviral development.

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Supporting Information Available: Electrophoresis gel image of **1** binding to TAR RNA, procedures for fluorescence anisotropy assay, synthetic procedures for protected β^3HArg and β^3HGln monomers, and discussion of the synthesis and purification of peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

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