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An α-Helical Peptidomimetic Inhibitor of the HIV-1 Rev−RRE Interaction

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While RNA molecules provide potentially useful targets for drug design, there are few examples of small, relatively rigid molecules able to discriminate between RNA sites with high specificity. One such target is the HIV-1 Rev-Responsive Element (RRE) RNA, which is recognized by the Arginine-Rich Motif (ARM) of the Rev protein,[2,3] primarily via insertion of the α-helical ARM into the widened major groove of an asymmetric internal bulge in stem-loop IIB.[4] Binding specificity for RRE correlates with peptide helicity,[5] suggesting that correctly positioning a set of interacting side chains within a rigid structural framework can lead to the desired discrimination. Given that the Rev−RRE interaction facilitates the nuclear export of incompletely processed viral transcripts and is essential for viral replication,[6] it provides an attractive target for anti-HIV therapy.

Previous work has shown that prestabilizing the Rev ARM α-helix within a zinc finger scaffold generates tight RRE binders.[7] We wished to create a new class of conformationally constrained α-helical peptidomimetics that are short, relatively rigid, and that might be effective competitors of the Rev−RRE interaction. Previously, peptidomimetics of a BIV Tat peptide, which binds to its cognate TAR RNA in a *β*-turn conformation,[8] were designed using head-to-tail cyclization to enforce the conformational constraint.[9,10] Here we report the first α-helical peptidomimetics targeted to a viral RNA using macrolactam constraints between amino acid side chains (*i*, *i*+4), which have previously been shown to induce helicity in small peptides.[11]

We generated peptidomimetics based upon a highly specific RRE binding peptide, R_6QR_7, identified in a genetic selection experiment.[12] When helically stabilized, this peptide binds the RRE with an affinity similar to the Rev ARM and contains a glutamine in place of the asparagine at the central position in the Rev ARM (Figure 1). The R_6QR_7 α-helix is presumably oriented further from the RNA major groove than the Rev ARM, potentially allowing more space for a macrolactam constraint.

A series of macrolactam-constrained R_6QR_7 peptidomimetics (Figure 1 and Table 1) was synthesized to investigate both the helical propensity and the specific binding of molecules with different length, location, and orientation of the lactam ring. All peptides were synthesized using Fmoc solid phase synthesis with orthogonal Pd(0) labile protecting groups on the relevant lactam precursors, followed by on-resin lactam formation.[13] Only one peptidomimetic (**8**) recognized the RRE with high affinity and specificity. Interestingly, peptide **8** exhibits a 2-fold higher affinity for RRE than a helical Rev peptide stabilized by terminal modifications (**17**).[4] Importantly, the unconstrained analogue of **8**, peptide **14**, does not specifically recognize the RRE, and thus adding the conformational constraint results in a >25-fold increase in binding specificity. Peptide **6**, which, like peptide **8**, contains a lysine−glutamate macrolactam straddling the essential glutamine, also specifically recognizes the RRE, albeit not as tightly, due to lack of helicity as described below. A helical (Figure S3) asparagine variant (**15**) constrained with the same linkage as **8** does not specifically bind the RRE. This is consistent with previous results showing the importance of the extra methylene group in the unconstrained R_6QR_7 peptide context.[11]

(a) **Rev17** **Suc- TRQARRNRRRRWRERQR AAAAR-am**
R_6QR_7 **Suc-AAAA RRRRRRQRRRRRRR AAAAR-am**

(b)

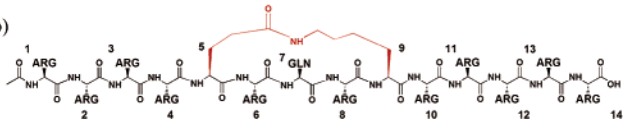

Figure 1. (a) Sequence comparison of Rev17 and R_6QR_7 peptides, helically stabilized by terminal modifications as previously reported.[4,11] (b) Schematic of R_6QR_7 peptidomimetic **8** with (*i*, *i*+4) macrolactam constraint in red.

Table 1. Composition and Binding of R_6QR_7 Peptidomimetics[a]

#	compound	K_{sp}	K_{nonsp}	specificity
1	Ac-RRR**K**RRQ**D**RRRRRR-OH	800	1200	1.5
2	Ac-RRR**K**RRQ**E**RRRRRR-OH	1200	1600	1.3
3	Ac-RRR**D**RRQ**K**RRRRRR-OH	1200	1600	1.3
4	Ac-RRR**E**RRQ**K**RRRRRR-OH	700	1200	1.7
5	Ac-RRRR**K**RQR**D**RRRRR-OH	1200	1600	1.3
6	Ac-RRRR**K**RQR**E**RRRRR-OH	200	1200	6
7	Ac-RRRR**D**RQR**K**RRRRR-OH	700	1200	1.7
8	Ac-RRRR**E**RQR**K**RRRRR-OH	45	1200	26
9	Ac-RRRRR**K**QRR**D**RRRR-OH	1200	1600	1.3
10	Ac-RRRRR**K**QRR**E**RRRR-OH	1200	1200	1
11	Ac-RRRRR**D**QRR**K**RRRR-OH	1200	1200	1
12	Ac-RRRRR**E**QRR**K**RRRR-OH	700	1200	1.7
13	Ac-RRRRRRQRRRRRRR-OH	1200	1200	1
14	Ac-RRRRERQRKRRRRR-OH	1600	1600	1
15	Ac-RRRR**E**RNR**K**RRRRR-OH	800	1200	1
16	R_6QR_7	150	1200	8
17	Rev17	100	1600	16

[a] Compounds **1**−**12** are the designed peptidomimetics; red residues are involved in the macrolactam constraint. Peptide **13** is a general linear R_6QR_7 control peptide. RRE binding K_d's were determined by electrophoretic mobility shift assays, with specificity defined as the ratio of dissociation constants of wild-type RRE IIB and a C46−G74 mutant RRE, shown not to specifically recognize Rev or R_6QR_7 peptides.[4,11]

Circular dichroism (CD) spectra of only three peptidomimetics (peptides **4**, **8**, and **12**) exhibited a classical α-helical curve shape with dual minima at 222 and 208 nm, whereas the unconstrained R_6QR_7 peptide **13**, along with remaining peptidomimetics, exhibited a single minimum at 202 nm, typical of short, unstructured peptides (Figure 2a and Figure S3).[14] Only these three peptidomimetics, having a glutamate−lysine linkage, with glutamate on the N-terminal side, showed any evidence of helicity, with peptide **8** exhibiting 33% helicity. Although all three peptidomimetics are helical by CD, only peptide **8** is able to specifically recognize the RRE, illustrating that only the linkage positioned exactly on the opposite side of the essential glutamine is viable for binding. The difference in binding between peptides **8** and **6** is most likely due to the lack of helical induction in peptide **6**, based only on the orientation of the macrolactam.

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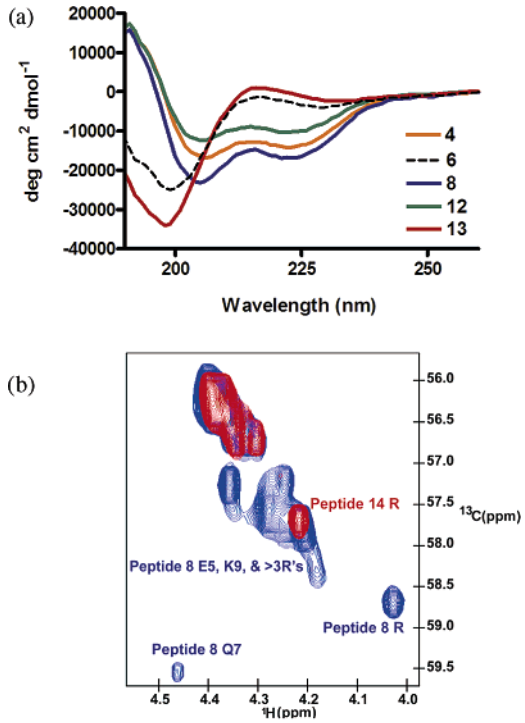

Figure 2. Structure of R_6QR_7 peptidomimetics. (a) CD spectra of selected peptidomimetics and unconstrained control peptide (**13**). CD spectra were recorded at 4 °C in 10 mM sodium phosphate, 100 mM KF, pH 7.5. (b) Natural abundance ^{13}C HSQC comparing chemical shifts in the Cα−Hα region between peptide **8** (blue) and uncyclized peptide **14** (red).

A comparison of the ^{13}C HSQC NMR spectra of the constrained peptide **8** and that of the unconstrained peptide **14** showed differences in dispersion in the Cα−Hα region (Figure 2b), indicating a difference in backbone conformation. Peptide **8** revealed numerous peaks with characteristic downfield ^{13}C shifts and upfield ^{1}H shifts consistent with an α-helical secondary structure.[15] Homonuclear 2D TOCSY experiments (see Supporting Information) provided unique assignments for the lysine, glutamate, and glutamine side chains, which all appeared helical based on the dispersion. At least three arginines also showed helical downshifts but could not be assigned definitively. The NMR data on peptidomimetic **8** are consistent with the ∼30% helicity observed by CD and further indicate that the helicity is most likely localized within the constrained region of the peptide.

A fluorescence polarization competition assay was used to test whether the conformationally constrained, tight RRE binding peptide **8** could efficiently compete for RNA binding with the wild-type Rev peptide.[16] Peptide **17**, which is ∼30% helical and has a high affinity for the RRE, was labeled with fluorescein at its C-terminus and titrated with unlabeled competitors (Figure S4). Of the three helical peptidomimetics (**4**, **8**, and **12**), peptide **8** was the most effective competitor, with an IC_{50} (∼150 nM) comparable to its K_d (∼40 nM) (Figure 3 and Table 1; note that the competition assay was conducted at an RNA concentration 4-fold above K_d). The other peptidomimetics showed minimal competition, also consistent with the direct binding assays (Figure 3 and Table 1).

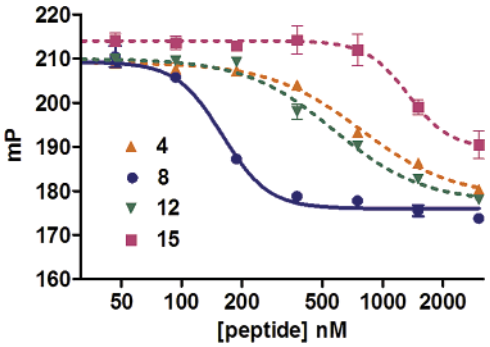

Figure 3. Fluorescence polarization competition assays with helical peptidomimetics. Assays were performed by titrating competitors into a mixture of RRE RNA and labeled Rev peptide in 30 mM HEPES pH 7.5, 100 mM KCl, 40 mM NaCl, 10 mM NH_4OAc, 10 mM guanidinium, 2 mM $MgCl_2$, 0.5 mM EDTA, and 0.01% NP-40.[16]

The results presented here indicate that a conformationally constrained α-helical peptidomimetic (peptide **8**) can bind the RRE RNA with high affinity and specificity when the constraint is properly located and oriented. The positioning of the constraint on the α-helical face opposite the RNA major groove proves essential, as about two-thirds of the α-helix is surrounded by RNA in the Rev ARM−RRE complex.[3] The observation that our designed, relatively short, constrained peptidomimetic is able to recognize the RRE IIB and compete for Rev binding suggests that the approach may be useful for generating other molecules that specifically target the Rev−RRE interaction for anti-viral therapy.

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Supporting Information Available: Details of the synthesis and purity of compounds **1**−**12**, experimental details for gel mobility shift assay, CD, fluorescence polarization and NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

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