Design of a Cyclic Peptide that Targets a Viral RNA

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Figure 1. Imino proton region of 1-D proton spectra of BIV TAR RNA with increasing amounts of cyclic Tat peptide (stoichiometry shown at right). Spectra were recorded on a Varian Inova 500 MHz spectrometer at 288 K in 10 mM sodium phosphate pH 6.2 (10% deuterium oxide). RNA concentration was 1.3 mM.

An arginine-rich BIV Tat domain mediates recognition of a bulged stem region of BIV TAR. The similarity between the HIV and BIV Tat/TAR interaction suggests that lessons of peptide recognition in the BIV system may be directly applicable to the interaction in HIV. High-resolution NMR structure determination of a peptide–RNA complex has revealed details of RNA recognition. The peptide adopts an irregular β-hairpin conformation that recognizes the major groove of the RNA through specific hydrogen bonding, electrostatic, and hydrophobic interactions.

The structure of the RNA–peptide complex suggests that a cyclic peptide analogue of BIV Tat peptide should bind with high affinity to TAR RNA. Cyclic peptides represent a step toward small-molecule ligands. Cyclic peptides are conformationally constrained and therefore are less likely to adopt multiple alternative structures. Constraining a peptide into a conformation that is predisposed to adopt the bioactive conformation should also lead to increased binding affinity, since there is an entropic penalty to peptide folding on RNA binding. Finally, cyclic peptides are much more resistant to enzymatic degradation than linear peptides.

Here, we describe the design of a cyclic BIV Tat peptide and use NMR spectroscopy to demonstrate that this cyclic peptide binds tightly to BIV TAR RNA, forming a 1:1 complex that is strikingly similar in structure to the linear peptide–RNA complex.

Formation of a reverse turn from residues 74 to 76 is critical for TAR RNA recognition; glycine residues (Gly74 and Gly76) are required for the φ torsion angle necessary to accommodate the sharp reversal of the turn. Moreover, the backbone of the peptide near the N- and C-termini are close together in space, suggesting that the two termini could be covalently attached to constrain the conformation of the peptide such that it is predisposed to adopt a hairpin-like structure. Critical residues for the peptide–RNA interaction are primarily located proximal to the reverse turn, while the residues at each terminus (Arg68, Pro69, Arg80, and Arg81) do not play a direct role in the RNA recognition. This suggests that omitting the terminal residues may not have a significant effect upon RNA recognition.

Analogs of the naturally occurring cyclic peptide gramicidin S provide an interesting model for the design of cyclic peptide mimics of the BIV Tat peptide. Using the experiences in cyclic peptide design on gramicidin S, we designed a 14-amino acid peptide cyclized in a head-to-tail fashion (cyclo-dYGRGTRGKGR-RIVN). The sequence of the cyclic peptide is derived from the arginine-rich motif of BIV Tat protein and contains all of the critical residues for RNA recognition. In addition, a d-amino acid (d-Tyr) and a glycine are placed to facilitate formation of a reverse turn at a position equally spaced from the residues involved in turn formation observed in the linear peptide–RNA complex. While a d-proline may have been a better choice for type-I or type-II′ reverse turn, the d-Tyr provides the advantage of a spectroscopic handle for the peptide. Also, the amino acids asparagine and valine are incorporated to promote sheet stability.

The cyclized Tat peptide does not adopt a preformed structure. CD spectra of the unbound linear and cyclic peptides exhibit are characteristic of a random-coil conformation (data not shown). The amide region of a natural abundance 15N-resolved HSQC experiment reveals similar chemical shift dispersion for the linear and cyclic peptide; the cyclic peptide gives a distinct resonance for each of the 14 amino acids, while the linear peptide gives only 13, as is expected for a linear peptide with a protonated amino terminus. Furthermore, no medium- or long-range HN–HN NOEs were detected in NOESY or ROESY experiments for either peptide (data not shown). The cyclic and linear peptides do not form stable conformations.

Addition of either cyclic or linear peptide to BIV TAR induces similar dramatic changes in the RNA imino proton spectrum. The free and bound RNA are in slow exchange on the NMR time scale, and the NMR titration indicates formation of a 1:1 complex (Figure 1). A qualitative comparison of the complex formed between the RNA and the linear and cyclic peptides suggests that the cyclic peptide binds at least as tightly as does the linear peptide. Quantitative comparison of the binding affinities is not possible by NMR spectroscopy, since the concentration of the RNA (1.3 mM) is several orders of magnitude above the dissociation constant (~1 nM). An upper limit to the Kd is 1 µM.

Cyclic and linear peptides induce similar chemical shift changes to pyrimidine H5–H6 resonances (Figure 2). In particular, the H5–H6 cross-peak of C15, a residue that is proximal to the binding...
The striking similarity of the H5–H6 cross-peaks suggests that the RNA is forming a similar structure in the two complexes.

The HN–Hα region of the NOESY spectrum is also dramatically different for the cyclic peptide in complex with the RNA compared to that of the free peptide (data not shown). Although this region shows a high degree of overlap due to the presence of resonances of the RNA, the dispersion pattern of the peptide HN–Hα resonances is clearly much greater than that of the free cyclic peptide. An amide resonance at 8.95 ppm gives NOEs to two α-protons (3.99 and 3.56 ppm respectively), suggesting that this is a glycine residue. The chemical shifts of these resonances are nearly identical to those of Gly76 (8.91, 4.05, and 3.57 ppm for HN, Hα1, and Hα2, respectively) in the linear peptide complex. Gly 76 is one of the critical residues involved in the formation of the reverse turn. The cyclic peptide likely adopts a structure similar to that of the linear peptide when bound to BIV TAR.

Observed RNA–peptide NOEs demonstrate the similarities of the linear and cyclic peptide–RNA complexes. The side chain of Ile79 gives rise to a characteristic NOE pattern between the δ-methyl group of the isoleucine and the H5 of U10 (Figure 3A) in both complexes. Another characteristic NOE for the linear complex is between the methyl group of the threonine residue (Thr72) and the H8 proton of nucleotide G22 of the RNA (Figure 3B). Once again, in the cyclic peptide complex, an identical NOE is observed.

The high-resolution structure of a linear peptide in complex with BIV TAR RNA reveals that the peptide forms a β-hairpin-like structure that binds in the widened major groove of the RNA making specific electrostatic, hydrophobic, and hydrogen bonding interaction with the RNA. The backbone conformation of this linear peptide suggests that cyclization of the peptide may lead to a compound that retains its ability to specifically recognize BIV TAR RNA while improving the stability to enzymatic degradation and increasing the affinity. Cyclization did not lead to a preformed structure of the peptide, which is not unsurprising. The structure of the peptide in the RNA complex is irregular, and its folding is dominated by RNA–peptide interactions. However, cyclization does not dramatically affect the conformational flexibility of this peptide or its ability to form the proper conformation when bound to RNA.

The cyclic peptide binds tightly and specifically to BIV TAR RNA, forming a 1:1 complex with the RNA. The RNA binding affinities of the cyclic and linear peptides are similar, and the dissociation constant for the interaction is on the order of micromolar or tighter, in agreement with prior studies. Given the similarity of HIV and BIV TAR, investigation of such cyclopeptide analogues may lead to smaller, more stable molecules that may be effective anti-HIV agents.

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References


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