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Targeting RNA with Peptidomimetic Oligomers in Human Cells

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Abstract—Replication of human immunodeficiency virus type 1 (HIV-1) requires specific interactions of Tat protein with the *trans*-activation responsive region (TAR) RNA, a 59-base stem-loop structure located at the 5′-end of all HIV mRNAs. Here we report that two TAR RNA-binding peptidomimetics, oligourea and oligocarbamate, inhibit transcriptional activation by Tat protein in human cells with an IC_{50} of ~0.5 and 1 μM, respectively. Peptidomimetics that can target specific RNA structures provide novel molecules that can be used to control cellular processes involving protein–RNA interactions in vivo.

Readily accessible synthetic molecules that can bind with high affinity to specific sequences of single- or double-stranded nucleic acids have the potential to interfere with protein–nucleic acid interactions in a controllable way, thus making them attractive tools for molecular biology and medicine. Successful approaches used thus far include duplex-forming (antisense)[1] and triplex-forming (anti-gene) oligonucleotides,[2–4] peptide nucleic acids (PNAs),[5] and pyrrole-imidazole polyamide oligomers.[6,7] Each class of compounds employs a readout system based on simple rules for recognizing the primary or secondary structure of a linear nucleic acid sequence. Another approach employs carbohydrate-based ligands, calicheamicin oligosaccharides, which interfere with the sequence-specific binding of transcription factors to DNA and inhibit transcription in vivo.[8,9] While antisense oligonucleotides and PNAs employ the familiar Watson–Crick base-pairing rules, two others, the triplex-forming oligonucleotides and the pyrrole-imidazole polyamides, take advantage of straightforward rules to read the major and minor grooves, respectively, of the double helix itself.

In addition to its primary structure, RNA has the ability to fold into complex tertiary structures consisting of such local motifs as loops, bulges, pseudoknots and turns.[10,11] It is not surprising that, when they occur in RNAs that interact with proteins, these local structures are found to play important roles in protein–RNA interactions.[12] This diversity of local and tertiary structure, however, makes it impossible to design synthetic agents with general, simple-to-use recognition rules analogous to those for the formation of double- and triple-helical nucleic acids. Since RNA–RNA and protein–RNA interactions can be important in viral and microbial disease progression, it would be advantageous to have a general method for rapidly identifying synthetic compounds for targeting specific RNA structures. A particular protein-binding RNA structure can be considered as a molecular receptor not only for the protein with which it interacts but also for synthetic compounds, which may prove to be antagonists of the protein–RNA interaction.

The mechanism of *trans*-activation of human immunodeficiency virus type 1 (HIV-1) gene expression that requires the interaction of Tat protein with the *trans*-activation responsive region (TAR) RNA, a 59-base stem-loop structure located at the 5′-end of all nascent HIV-1 transcripts.[13] Inhibition of Tat–TAR interactions is a potential approach for anti-HIV therapeutics. Here we report that two peptidomimetic oligomers can interfere with Tat-mediated *trans*-activation in human cells.

We have recently begun to examine TAR RNA recognition by unnatural biopolymers containing urea and carbamate backbone structures.[14,15] Small Tat-derived oligocarbamates and oligoureas bind TAR RNA specifi-

Abbreviations: CAT, chloramphenicol acetyl transferase; Fmoc, 9-fluorenylmethyloxycarbonyl; FAB, fast atom bombardment; MALDI, matrix-assisted laser desorption ionization.

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cally with high affinities in vitro.[14,15] TAR RNA-binding oligo-carbamates and oligoureas are also resistant to protease degradation in vitro.[14,15]

Can these unnatural peptides target TAR RNA in vivo and inhibit Tat–TAR interactions? To address this question, we synthesized TAR RNA-binding oligocarbamate and oligourea (Fig. 1). The oligocarbamate was synthesized on an ABI 431 peptide synthesizer by using *N*-α-Fmoc-protected *p*-nitrophenyl carbonate monomers.[16] After cleavage from the resin, the oligocarbamate was purified by HPLC on a Zorbax 300 SB-C_8 column.[17] The mass of fully deprotected and purified oligocarbamate was confirmed by FABMS; 1831.3 (M + H). To synthesize Tat-derived oligourea on solid support, we used activated *p*-nitrophenyl carbamates of protected amines in the form of azides, which were reduced with $SnCl_2$–thiophenol–triethylamine on solid support.[18,19] After HPLC purification, the oligourea was characterized by MALDI mass spectrometry; 1850.0 (M + H).

To determine whether the RNA-binding oligocarbamate and oligourea could be used to control HIV-1 gene expression in vivo, we used HL3T1 cells, a HeLa cell line derivative containing an integrated HIV-1 LTR promoter and CAT reporter gene.[20] We added different amounts of the oligocarbamate and oligourea during transfection of pSV2-Tat[21] and pAL[22] plasmids into HL3T1 cells. Plasmids pSV2Tat and pAL express the first exon of Tat protein and luciferase enzyme, respectively. Luciferase reporter gene provides an internal control. Transfection of HeLa cells with pSV2Tat enhanced transcription as determined by CAT activity. As shown in Figure 2, increasing amounts of the oligocarbamate and oligourea resulted in a decrease of CAT activity while luciferase activity was not affected. In the presence of 1 μM concentrations of oligourea, more than 80% of Tat *trans*-activation was inhibited. The oligocarbamate inhibited ~50% Tat *trans*-activation at 1 μM concentrations. These results show that peptidomimetics containing urea backbone structures are more potent in vivo as compared with carbamate oligomers. In vitro RNA-binding studies showed earlier that the oligourea binds to TAR RNA with 10-fold higher affinity than the oligocarbamate.[14,15] It is interesting that there is a direct correlation between in vitro RNA-binding and in vivo efficacy of peptidomimetics in inhibiting

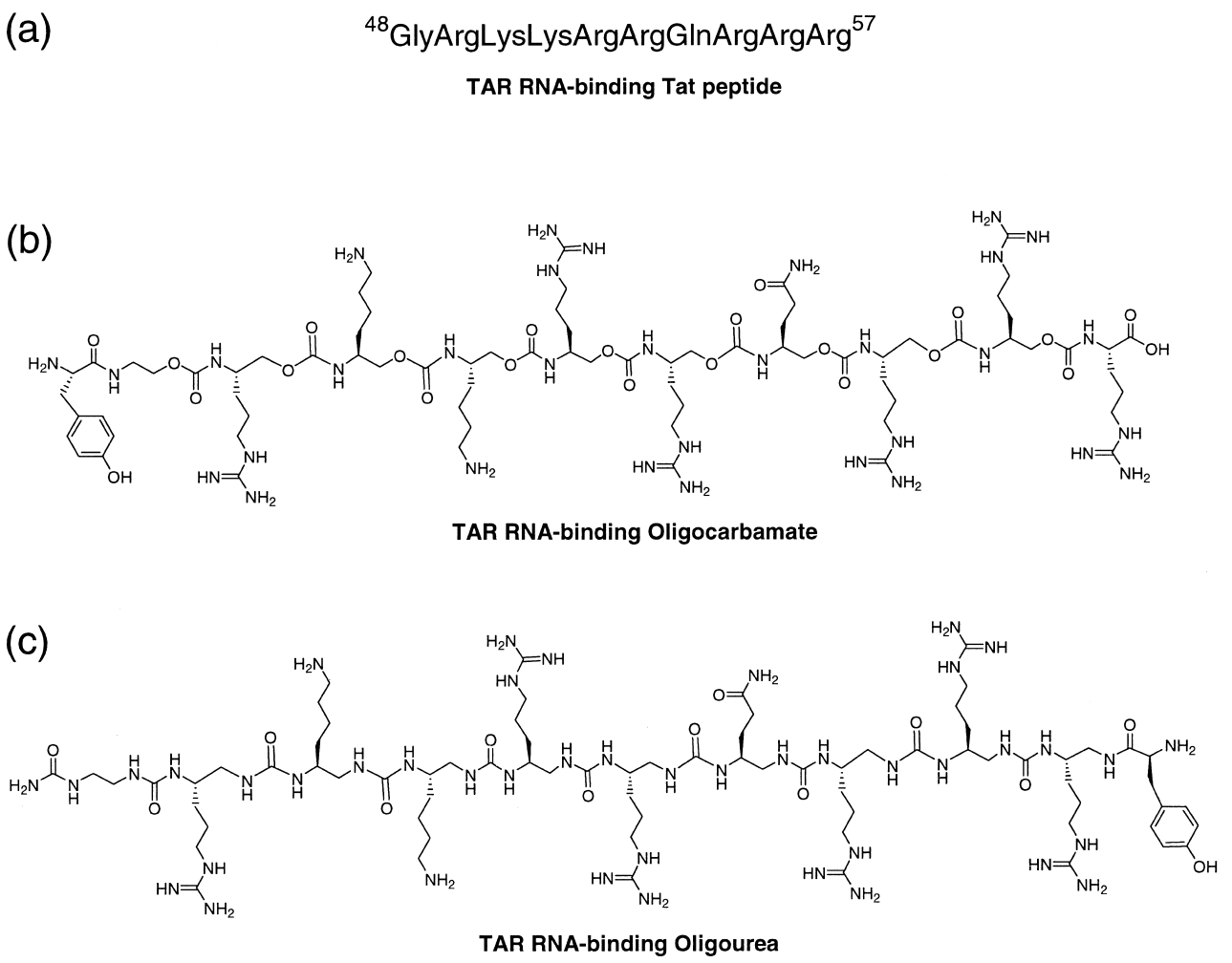

Figure 1. (a) The Tat-derived peptide, amino acids 48–57, contains the RNA-binding domain of Tat protein. (b) Structure of the TAR RNA-binding oligocarbamate. Sequence of the oligocarbamate corresponds to the Tat peptide shown in (a). (c) Structure of the TAR RNA-binding oligourea. Sequence of the oligourea corresponds to the Tat peptide shown in (a). L-Tyr was coupled as the last residue during synthesis of both oligomers.

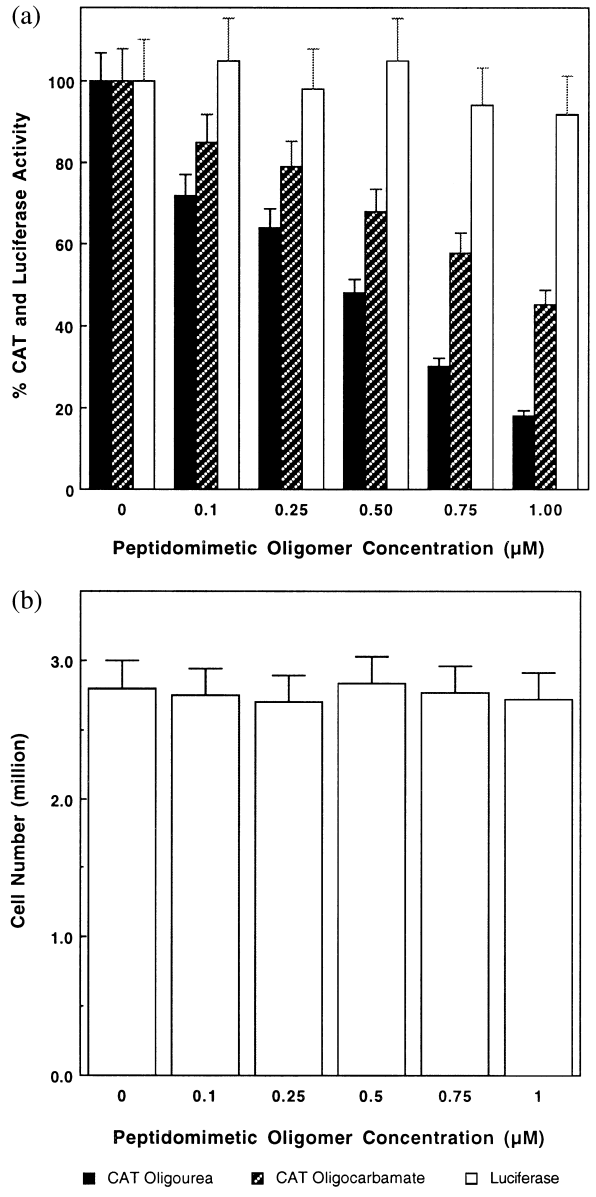

Figure 2. (a) Inhibition of Tat transactivation by the TAR RNA-binding oligocarbamate and oligourea in vivo. CAT activity expressed from the integrated HIV-1 LTR of HL3T1 cells with increasing amounts of the oligocarbamate and oligourea is shown. Monitoring luciferase activity was a control experiment to quantify the transfection efficiency and nonspecific inhibition of gene expression by the addition of the oligocarbamate and oligourea. Transfection and enzymatic activity (CAT and luciferase) assays were performed as described previously.[21,22] CAT and luciferase activities were measured from three experiments and normalized to 100%. (b) Cell viability assay after 48 h under experimental conditions described in (a), showing that oligourea and oligocarbamate were non-toxic to cells at 1 μM concentration.

protein–RNA interactions. However, cellular uptake and half-lives of the compounds in the cell are other important factors which determine in vivo efficacy. Although both the oligourea and oligocarbamate are protease resistant and could have longer in vivo half lives, it is possible that the cellular uptake of the oligourea is more efficient than the oligocarbamate.

To rule out the possibility that the observed inhibition of transactivation could be due to some nonspecific toxicity of peptidomimetic oligomers or reduction of the pSV2Tat plasmid uptake, transcription of luciferase gene was monitored (Fig. 2). Transcription of luciferase gene was not affected by peptidomimetic oligomers. Further control experiments showed that scrambled oligocarbamate and oligourea had no inhibitory effect on Tat transactivation. Cell viability assays showed that oligocarbamate and oligourea treatment was not toxic to the cells (Fig. 2b). These results demonstrate that the TAR RNA-binding oligocarbamate and oligourea inhibit Tat–TAR interactions in vivo.

We have described that TAR RNA-binding oligocarbamate and oligourea can be used for controlling HIV-1 gene expression in vivo. These results also establish an example of the application of peptidomimetic oligomers as artificial regulators of cellular processes involving protein–RNA interactions in vivo.

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