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RNA pseudoknots that inhibit human immunodeficiency virus type 1 reverse transcriptase

(AIDS/reverse transcriptase inhibitors/directed ligand evolution/RNA secondary structure)

CRAIG TUERK*, SHEELA MACDOUGAL*, AND LARRY GOLD

Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309

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ABSTRACT **High-affinity ligands of the reverse transcriptase of human immunodeficiency virus type 1 (HIV-1) were isolated by the SELEX procedure (systematic evolution of ligands by exponential enrichment) from RNA populations randomized at 32 positions. Analysis of these ligands revealed a pseudoknot consensus with primary sequence bias at some positions. We demonstrated that at least one of the ligands inhibits cDNA synthesis by HIV reverse transcriptase but fails to inhibit other reverse transcriptases. These experiments highlight the power of SELEX to yield highly specific ligands that reduce the activity of target proteins. Such ligands may provide therapeutic reagents for viral and other diseases.**

One strategy for discovering effective treatments for acquired immunodeficiency syndrome (AIDS) has been to search for specific inhibitors of the reverse transcriptase (RT) activity of the human immunodeficiency virus type 1 (HIV-1), the probable causative agent (1–6). Two such inhibitors, 3′-azido-3′-deoxythymidine (AZT) and dideoxyinosine, are nucleoside analogues that have been shown to have clinical effectiveness against AIDS (7, 8). Unfortunately, dosages are limited by undesirable side effects (9) and mutations in the gene encoding the RT can arise that result in HIV-1 infections that are AZT resistant (10, 11).

SELEX (systematic evolution of ligands by exponential enrichment) is a protocol for isolating from a pool of variant RNA sequences high-affinity ligands to a target protein (12). Basically, this procedure involves cycles of affinity selection by a protein from a heterogeneous population of RNAs, replication of the bound species, and *in vitro* transcription to generate an enriched pool of RNA. We proposed that SELEX could supply ligands that selectively inhibit the activity of viral replicative proteins, thereby providing useful antiviral activity. We used HIV-1-RT as a target protein for SELEX to test this notion.

The RT activity of HIV-1 is composed of a heterodimer of two subunits (p51 and p66) that have common amino termini (13); the longer subunit contains the RNase H domain of RT (14). Initiation of retroviral cDNA synthesis involves annealing and extension of a tRNA primer; it has been shown previously that HIV-1-RT directly and specifically interacts with the cognate $tRNA^{Lys3}$, possibly at the anticodon stem and loop (15, 16). In addition to this specifically initiated replication, the HIV-1-RT is essential for continued polymerization of the cDNA of the viral genome (after template switching), hydrolysis of the copied RNA strand (RNase H activity), and synthesis of the second DNA strand (17). It has been hypothesized that a single primer–template recognition domain is used for these catalytic activities (6, 18).

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MATERIALS AND METHODS

SELEX. Initial templates for *in vitro* transcription were assembled by ligation as described (12) using the oligonucleotides listed in the legend to Fig. 1. [Templates for use in SELEX have a variable region (created by mixed nucleotide incorporation during oligonucleotide synthesis) flanked by 5′ and 3′ fixed regions. The 3′ fixed region is required for 3′ PCR primer annealing and cDNA synthesis; the 5′ fixed region is important for annealing the 5′ PCR primer to the cDNA to begin PCR. In addition to the sequences that complement the cDNA, the 5′ PCR primer contains sequences that signal transcriptional initiation by T7 RNA polymerase.] Five hundred picomoles of purified template (containing $\approx 10^{14}$ of the possible 10^{19} sequences) was transcribed with T7 RNA polymerase as described (12). The typical RNA concentration for rounds of SELEX was $\approx 30\ \mu M$. The concentration of target HIV-1-RT in SELEX was 20 nM in the first round and 10 nM in rounds 2–9. At every third round of selection, we purified the cDNA product to avoid anomalously sized species that typically appear during multiple rounds of SELEX. All RNA–protein binding reactions were done in a buffer of 200 mM KOAc/50 mM Tris·HCl, pH 7.7/10 mM dithiothreitol. RNA and protein dilutions were mixed and stored on ice for 30 min and then transferred to 37°C for 5 min. (In binding assays, the reaction volume was 60 μl, of which 50 μl was assayed; in SELEX rounds, the reaction volume was 100 μl.) Each reaction mixture was suctioned through a prewet (with binding buffer) nitrocellulose filter and rinsed with 3 ml of binding buffer, after which it was dried and counted for assays or subjected to elution as part of the SELEX protocol (12, 19).

RESULTS

SELEX Using HIV-1-RT as a Target. Two RNA populations that were randomized at 32 nucleotide positions (shown in Fig. 1) were used in SELEX with the heterodimer HIV-1-RT (p51/p66) as the target protein. One population contained the same 5′ and 3′ flanking regions as described in ref. 12; the other population contained a 5′ fixed region that included the anticodon loop and stem sequence (unmodified) of $tRNA^{Lys3}$ at the 5′ end of all RNAs in the population. We included this sequence because of the reported evidence of sequence-specific binding of the RT to this part of the tRNA that is used to initiate HIV-1 replication (16). However, there was no difference in the affinity of the two RNA populations for HIV-1-RT (and, as we will show, the selection process was indifferent to either 5′ region for specific binding).

Abbreviations: RT, reverse transcriptase; HIV-1, human immunodeficiency virus type 1; AZT, 3′-azido-3′-deoxythymidine; SELEX, systematic evolution of ligands by exponential enrichment.

*Present address: NeXagen, Inc., 2860 Wilderness Place, Boulder, CO 80301.

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Starting RNA for:

SELEX Experiment 1

 5'-gggagcaucagacuuuuaaucugacaaucaag[ --------32 n's------ ]aucuaugaaagaauuuuauaucucuauugaaac-3'

SELEX Experiment 2

     5'-gggagccaacaccacaauuccaaucaag[ --------32 n's------ ]aucuaugaaagaauuuuauaucucuauugaaac-3'

SELEX Experiment 3

 5'-gggagcaucagacuuuuaaucugacaaucaagNNuuccgNNNNNNNNcgggaaaaNNNNcuaugaaagaauuuuauaucucuauugaaac-3'
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FIG. 1. Shown are the three starting RNA populations used in this work. The nucleotides that form the anticodon stem of $tRNA^{Lys3}$ in the starting RNA for SELEX experiment 1 are overlined. The following oligonucleotides were used in template construction, PCR, and cloning as identified: 5′-taatacgactcactatagggagcatcagacttttaatctgacaatcaag-3′ (bridging oligonucleotide for 5′ construction and 5′ PCR oligonucleotide for experiments 1 and 3), 5′-taatacgactcactatagggagccaacaccacaattccaatcaag-3′ (bridging oligonucleotide for 5′ construction and 5′ PCR oligonucleotide for experiment 2), 5′-atctatgaaagaattttatatctc-3′ (bridging oligonucleotide for 3′ ligation for experiments 1 and 2), 5′-tcagattaaaagtctgatgctccctatagtgagtcgtatta-3′ (template construction oligonucleotide for experiment 1), 5′-gaattgtggtgttggctccctatagtgagtcgtatta-3′ (template construction oligonucleotide for experiment 2), 5′-tttcatagatnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnnncttgattg-3′ (template construction oligonucleotide for experiments 1 and 2), 5′-ccggatccgtttcaatagagatataaaattc-3′ (3′ cloning oligonucleotide and template construction oligonucleotide), 5′-gtttcaatagagatataaaattctttcatag-3′ (3′ primer for PCR), 5′-agagatataaaattctttcatagnnnnttttcccgnnnnnnnncggaanncttgattgtcagattaaaagtc-3′ (random template for experiment 3), 5′-ccgaagcttctaatacgactcactatagggag-3′ (5′ PCR primer for cloning). All oligonucleotides were prepared as described (20).

Nine rounds of SELEX with each population were performed as described. We first completed the experiment with RNA containing the anticodon stem and loop sequence of $tRNA^{Lys3}$. Nitrocellulose filter binding assays performed at the ninth round revealed that the RNA population had increased ≈200-fold in affinity to HIV-1-RT when compared to the starting population but that the background binding to nitrocellulose filters in the absence of protein had increased from <2% of input RNA to 15% (data not shown). As a consequence, during SELEX experiment 2 the initial pool of RNAs was prefiltered through nitrocellulose before the first, third, sixth, and ninth rounds of selection. Individual isolates (49 of experiment 1 and 104 of experiment 2) were cloned from these populations and sequenced as described (12).

Pseudoknot RNAs That Bind HIV-1-RT. Two motifs were found in this collection of sequences. One sequence motif that was found by both SELEX experiments is that of a pseudoknot (21) with conserved and nonconserved regions. A pseudoknot is a structure in which there is intramolecular base pairing of the "loop" sequences of an RNA hairpin to sequences either 5′ or 3′ to that hairpin. All sequences that have this motif (representing 27 of the 49 sequences of experiment 1 and 85 of the 104 sequences of experiment 2) are shown in Fig. 2; each of the 18 sequences is followed by the number of isolates that were found having that sequence [some with minor sequence variation (mostly single base changes) attributable to mutation during SELEX]. Seventeen of the 104 sequences found in experiment 2 were identical to ligand 1.1 (from experiment 1), except with different 5′ ends, and probably represent cross-contamination between the two experiments followed by misannealing of the 5′ primer during PCR.

The consensus secondary structure and primary sequence that can be derived from the 18 sequences are shown in Fig. 3. There are two regions of primary sequence conservation, a UCCG sequence followed at some spacing by CGGGANAA (where N is any nucleotide, although U is most frequently found at this position). The distance between these two conserved elements (d in Fig. 2) is variable, although there is a clear preference for a spacing of 8 nucleotides (8 of 18 isolates) or 7 nucleotides (4 of 18 isolates). The UCCG sequence can pair with the CGGG to form the 5′ helix (stem 1 in Fig. 3) of the pseudoknot. The two internal C·G pairs and the closing G·C pair of stem 1 are highly conserved. In three examples (ligands 1.7, 1.6, and 2.2b) other base pairs are substituted for the closing G·C pair. The U·G pair of stem 1 is also conserved; a C·G pair is substituted for it only in isolate 1.17, whose stem 1 also contains an unusual internal A·U pair.

HIV-RT ligands

Sequence Number		Frequency
	d=8	
1.1	ucaagAAUUCCGUUUUCAGUCGGGAAAAACUGAACAa	13
1.3a	gAAUAUCUUCCGAAGCCGAACGGGAAAACCGGCaucu	5
2.9	ucaagGUUUCCGAAAGAAAUCGGGAAAACUGucu	1
2.4a	agUAGAUAUCCGAAGCUCAACGGGAUAAUGAGCaucu	7
2.7a	agAUAUGAUCCGUAAGAGGACGGGAUAAACCUCAa-c	4
2.11	gUCAUAUUACCGUUACUCCUCGGGAUAAAGGAGaucu	1
1.7	agGAAUCGACCCAAGCCAAAGGGGAUAAUGCGGCauc	2
1.17	agUAAUGACCAGAGGCCCAACUGGUAAACGGGCGGuc	1
	d<8	
2.1b	caagAAUAUAUCCGAACUCGACGGGAUAACGAGAAGaGc	19
2.10	agUAAAUGAGUCCGUAGGAGGCGGGAUAUCUCCaAcu	1
1.8	ucaagGAUUAACCGACGCCAACGGGAGAAUGGCAGGGau	2
1.6	gAAGAAGAUUACCCAAGCGCAGGGGAGAAGCGCaucu	2
1.9b	gAGAGUAUCAUCCGUGCCGGCGGGAUAUCGGCGaucu	2
2.12	ucaagAAUAAUCCGACUCGCGGGAUAACGAGAAGaGc	1
	d>8	
2.5a	ucaagAUAGUAUCCGUUCUUGAUCAUCGGGACAAAUGaucu	9
2.6b	agUGAAACUUAACCGUUAUCAUAGAUCGGGACAAaucuauga	6
2.2b	gacaaucaagUACCUAGGUGAUAAAAGGGAGAACACGUGUGa	2
2.3a	gUUAAACAUAAUCCGUGAUCUUUCACACGGGAGaucuaugaaaga	17

Nitrocellulose Filter Retention Sequences

1.2	ucaagCGUAGGUUAUGAAUGGAGGAGGUAGGGUCGUAaucuaug	5
1.4	aucugacaaucaagGGCAUCUGGGAGGGUAAGGGUAAGGUUGUCGGau	4

FIG. 2. Shown are groupings of sequences from SELEX experiments 1 and 2 into two classes of motifs. The first motif is a pseudoknot that has specific affinity for HIV-1-RT. The second is an RNA hairpin with a relatively long helical region and a purine-rich loop, which we have found to be retained by nitrocellulose filters in the absence of protein. The number of isolates having each sequence is shown. The inverted repeat sequence indicative of base pairing for the conserved 5′ helix of the pseudoknot motif is overlined with boldface arrows. Inverted repeat sequences indicative of base pairing for the 3′ helix of the pseudoknot and for the stem of the nitrocellulose retention motif RNAs are overlined with regular arrows. The pseudoknots are grouped into three types: d = 8, the 5′ helix has a loop of 8 nucleotides; d < 8, a loop of <8 nucleotides; d > 8, a loop of >8 nucleotides. The minimal sequence required for high-affinity binding to HIV-1-RT is boxed for isolates 1.1 and 1.3a.

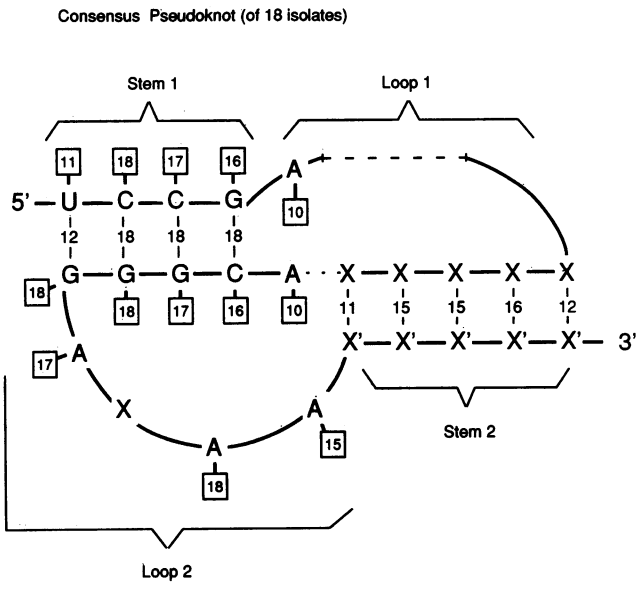

FIG. 3. Illustrated is the consensus primary and secondary structure of the combined sequences from SELEX experiments 1 and 2 with HIV-1-RT. The frequencies of the nucleotides and base-pairing patterns occurring at >50% at each position for the group that compose the aligned consensus are shown. Dashed lines indicate variably sized gaps in the alignment of conserved sequences.

The sequences that are immediately 3′ to the CGGGANAA sequence have a high frequency of base pairing to the d nucleotides of Fig. 2 to form stem 2 of the pseudoknot, leaving the ANAA sequence single stranded (loop 2 of Fig. 3). In addition to the A bias in this loop 2, there is a bias for A residues at other single-stranded positions within the pseudoknot as shown. The primary sequences involved in stem 2 are relatively nonconserved; the most variable part of the consensus is the base-pairing pattern that comprises stem 2.

Sequences that contain the pseudoknot motif were found to bind with high affinity to HIV-1-RT (K_d, ≈5 nM) as exemplified by clones 1.1 and 1.3a (Fig. 4). Clone 1.3b differs from clone 1.3a at the positions underlined in Fig. 2; the underlined A of 1.3a is a G in 1.3b and the underlined G is an A, a

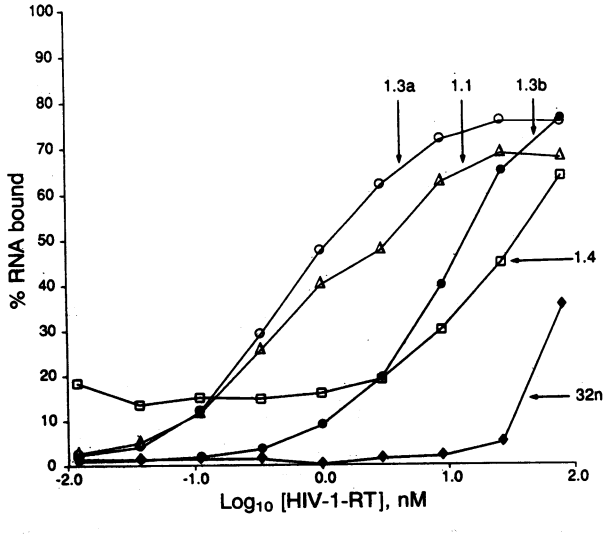

FIG. 4. Nitrocellulose filter binding assays of ligand affinity for HIV-1-RT. Shown is the percentage of input RNA that is bound to the nitrocellulose filter with various concentrations of HIV-1-RT performed as described (19). △, 1.1; ○, 1.3a; ●, 1.3b; □, 1.4; ◆, 32*n* starting population.

mutation that disrupts the base pairing of stem 2. As shown, 1.3b binds significantly less well than 1.3a to HIV-1-RT. Ligands 1.1 and 1.3a were further analyzed to determine the boundaries of the information required for high-affinity binding to HIV-1-RT (as in ref. 19; data not shown). These experiments establish that the pseudoknot motif is the high-affinity recognition site for HIV-1-RT within those ligands and that most of the fixed sequences used for cDNA synthesis or PCR amplification are dispensable. The minimal recognition domains thus determined of ligands 1.1 and 1.3a are boxed in Fig. 2.

The second motif (found only in the population of SELEX experiment 1) is a long RNA hairpin with a loop of purine repeat elements (as shown in Fig. 2); this motif was found in a total of 16 of the 49 isolates that were sequenced. Nitrocellulose filter binding assays (Fig. 4) revealed that these sequences exhibit significant retention on nitrocellulose filters that is independent of added protein (exemplified by sequence 1.4 in Fig. 2).

With the exception of the other nitrocellulose retention motif sequences, all other unreported sequences (6 of experiment 1 and 19 of experiment 2) occurred only once. Some were tested and found to have nonspecific affinity to HIV-1-RT and no significant retention by nitrocellulose filters. The persistence of such sequences is expected according to the analysis of Irvine *et al.* (22). No other significant consensus patterns were detected in these populations by computer analysis (23, 24).

Secondary SELEX Experiments with HIV-1-RT Identify the Optimal Secondary Structure for HIV-1-RT Binding. There is significant variability in the pattern and extent of base pairing in the pseudoknots shown in Fig. 2. To find the optimal base-pairing patterns and the best primary sequence for the bases involved in stem 2 of the pseudoknot, we created RNAs with random nucleotides at 14 positions interspersed with fixed sequences common to ligands 1.1 and 1.3a. As shown in Fig. 1, the 8-base loop of the helix formed by the fixed sequences UUCCG and CGGGA is random, as are the two nucleotide positions that are 5′ to the sequence UUCCG and the four nucleotide positions that are 3′ to the fixed sequence CGGGAAAA. After eight rounds of SELEX, individuals were cloned and 45 sequences were compiled. The frequencies of the bases found at each variable position are listed in Table 1. No individual sequence predominated in this selected population (there was only one sequence that occurred twice in the population), suggesting that there are many sequence combinations that give similar affinities for HIV-1-RT and that no one primary sequence has a large competitive advantage over the others, as we have discussed elsewhere (22). Inspection of these sequences also revealed base pairing between the 8*n* variable region and the downstream 4*n* variable region and 3′ flanking sequences so that these ligands could virtually all be folded as an RNA pseudoknot.

Table 1. Nucleotide frequencies found at each varied position for SELEX experiment 3 from the collection of cloned sequences

	Position													
	5′ region		Variable loop region								3′ variable region			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A	17	31	36	24	12	2	10	4	29	42	13	11	9	19
C	2	0	1	3	0	6	7	18	7	0	9	8	16	10
G	10	9	0	7	29	23	16	7	6	0	9	21	13	11
U	16	5	8	11	4	14	12	16	3	3	14	5	7	5

Numbering refers to positions of the n sequences shown in the starting RNA in Fig. 1, so that the sequence of interest is . . . ucaag(position 1)(position 2)uuccg(positions 3–10)cgggaaaa(positions 11–14)cuau. . . .

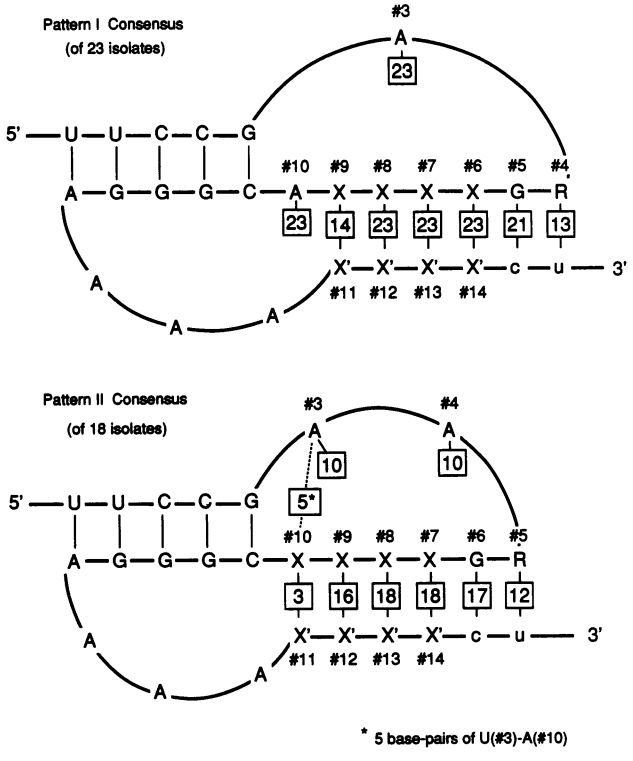

FIG. 5. Illustrated are the consensus pseudoknot structures and primary sequence biases found in SELEX experiment 3 identified as pattern I and pattern II (see text). The frequencies of canonical and G·U base pairing are indicated in the bases between X (any nucleotide) and X'. R, purine. The frequency of unpaired bases, which occur at >50%, is also indicated (all A residues).

As in the first two SELEX experiments, there is a significant bias for A residues at any single-stranded position. Further analysis showed a strong preference for one of two base-pairing alignments. If the variable nucleotides of the starting construct are labeled 1–14 as shown in Table 1, then nucleotide 13 of the 3' variable region base pairs to nucleotide 7 of the variable loop in pattern I for 23 of the 45 isolates, or to nucleotide 8 in pattern II for 18 isolates (see Fig. 5). Only 4 isolates did not fit one of these two patterns. Each consensus for these two patterns is illustrated in Fig. 5.

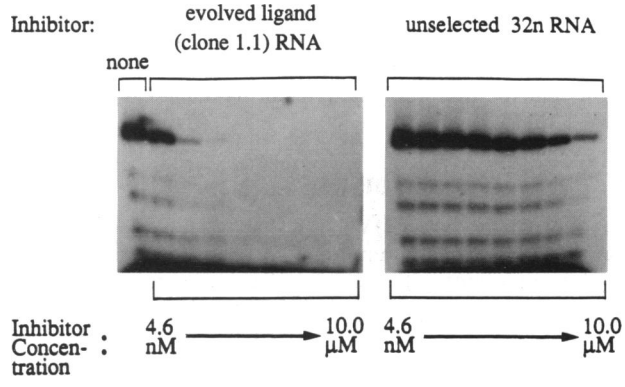

FIG. 6. Inhibition of HIV-1-RT by ligand 1.1. The RNA template for assaying RT activity was transcribed from a PCR product of pT7-1 obtained from United States Biochemical using oligonucleotides 5'-ccgaagcttctaatacgactcactatagggag-3' (5' primer) and 5'-gacgttgtaaaacgacggcc-3' (3' PCR and labeled extension primer). A series of 3-fold dilutions of 32*n* starting RNA and ligand 1.1 RNA ranging in final reaction concentration from 10 μM to 4.6 nM were premixed with HIV-1-RT and incubated for 5 min at 37°C in 6 μl of 200 mM KOAc/50 mM Tris·HCl, pH 7.7/10 mM dithiothreitol/6 mM $Mg(OAc)_2$/0.4 mM NTPs (all at final reaction concentration). In a separate tube, RNA template and ^{32}P-end-labeled primer were mixed and heated at 95°C for 1 min and cooled on ice for 15 min in 10 mM Tris·HCl, pH 7/0.1 mM EDTA. Four microliters of this template was added to each enzyme/inhibitor mixture to start the reaction; the mixture was incubated for a further 5 min at 37°C and the reaction was stopped as described (20). The final concentration of HIV-1-RT was 16 nM, that of RNA template was 13 nM, and that of labeled primer was 150 nM in all reaction mixtures. Shown are the extension products of each reaction (as prepared and visualized in ref. 20).

Selected RNA Ligands of HIV-1-RT Are Inhibitory. One of the purposes of these experiments was to find inhibitory ligands to HIV-1-RT. We compared the ability of ligand 1.1 to the ability of the starting RNA population to inhibit RT activity (Fig. 6). At equal concentrations of inhibitor RNA to HIV-1-RT (third lane from left), the RT is significantly inhibited by ligand 1.1. In contrast, only with 10 μM starting random RNA (or 200-fold excess) is there equivalent inhibition of HIV-1-RT. The same difference in affinity between the random populations and ligand 1.1 was observed in the binding assays. Thus, affinity is correlated with inhibition. To test the specificity of this inhibition, various concentrations

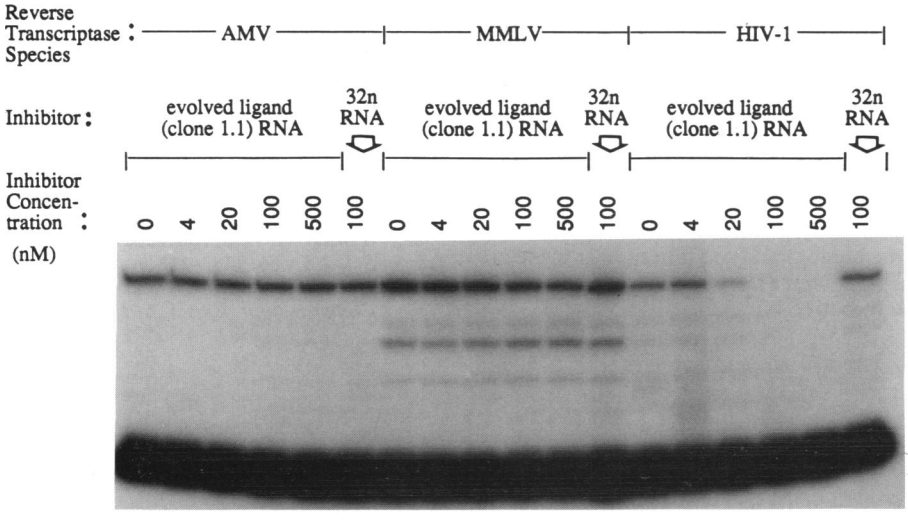

FIG. 7. Comparison of HIV-1-RT inhibition by ligand 1.1 to effects on Moloney murine leukemia virus RT and avian myeloblastosis virus RT. The experiments were performed as described in the legend to Fig. 6, except that 5-fold dilutions of inhibitor were prepared with the resultant concentrations as shown.

of ligand 1.1 were assayed for inhibition of the reverse transcriptases from Moloney murine leukemia virus, avian myeloblastosis virus, and HIV-1 virus. The results (Fig. 7) show that the inhibitory activity of ligand 1.1 is specific for HIV-1-RT.

DISCUSSION

The RNA ligands of HIV-1-RT that we have isolated have clearly evident characteristics. The consensus is that of an RNA pseudoknot with stem 1 rather stringently specified at the primary sequence level and stem 2 with little if any primary sequence preference. SELEX experiment 3 goes beyond the primary sequence preference of this ligand motif to yield two preferred secondary structure patterns as shown in Fig. 5. This pattern preference is probably a reflection of the physical constraints imposed by pseudoknot formation (as discussed in ref. 21). These constraints allow a single nucleotide bridge as loop 1 between stem 1 and stem 2 across the deep groove of the continuous stack formed by these two helices (nucleotide 3 in Fig. 5) and require at least two nucleotides to cross the shallow groove (accomplished by the fixed AAA of Fig. 5). The preference for G at variable positions 5 and 6 reflects the base pairing to the fixed C immediately 3′ to the variable region found in patterns I and II, respectively. There is, in addition to preferred base-pairing alignments, a strong A bias in the remaining single-stranded portions of the variable loop region, especially at positions 3 and 10. We have searched all available sequences in the GenBank data base and have found no sequence that fits the complete consensus.

We reasoned that the forces that attract and position primer–template junctions and nucleotide triphosphates at the active sites of replicative enzymes would likewise localize RNA ligands. We thus expected that the ligands we isolated against HIV-1-RT would also inhibit polymerase function. Not only do these ligands inhibit HIV-1-RT, but they do so specifically, failing to inhibit other RTs. Several other compounds, most notably nucleoside analogues, have been extensively tested for their ability to inhibit RT activity. The triphosphate derivative of AZT competitively inhibits the use of the natural substrate dTTP and not primer–template binding (5). Ribonucleoside–vanadyl complexes and certain quinone antibiotics inhibit presumably by interacting with the primer–template binding site of the enzyme (2, 4, 6). Cytotoxic or other unwanted side effects most likely result from inhibition of cellular polymerases, thus limiting the dose that can be administered. We expect that the relatively complex ligands we have isolated would present a rich mixture of intermolecular contacts with multiple sites on HIV-1-RT. Such contacts enhance the probability of specificity (which we have demonstrated), so that fewer cytotoxic effects resulting from inhibition of cellular polymerases would be expected. In addition, the likelihood that inhibitor-insensitive viruses could readily arise through mutation is reduced, especially if the primary contacts are involved with amino acids that are indispensable for catalytic function. If these or related ligands can be presented effectively to infected cells, they may mitigate the infective life cycle of HIV-1.

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1. DeClerq, E. (1986) *J. Med. Chem.* **29,** 1561–1569.
2. Take, Y., Inouye, Y. & Nakamura, S. (1989) *J. Antibiot.* **42,** 107–115.
3. Ono, K., Nakane, H., Fukushima, M., Chermann, J.-C. & Barre-Sinoussi, F. (1990) *Eur. J. Biochem.* **190,** 469–476.
4. Goldman, M. E., Salituro, G. S., Bowen, J. A., Williamson, J. M., Zink, D. L., Schleif, W. A. & Emini, E. A. (1990) *Mol. Pharmacol.* **38,** 20–25.
5. Kedar, P. S., Abbotts, J., Kovacs, T., Lesiak, K., Torrence, P. & Wilson, S. H. (1990) *Biochemistry* **29,** 3603–3611.
6. Krug, M. S. & Berger, S. L. (1991) *Biochemistry* **30,** 10614–10623.
7. Yarchoan, R., Weinhold, K. J., Lyerly, H. K., Gelmann, E., Blum, R. M., Shearer, G. M., Mitsuya, H., Collins, J. M., Myers, C. E., Klecker, R. M., Markham, P. D., Durack, D. T., Lehrmann, S. N., Barry, D. W., Fischl, M. A., Gallo, R. C., Bolognesi, D. P. & Broder, S. (1986) *Lancet* **i,** 575–581.
8. Yarchoan, R., Mitsuya, H., Thomas, R. V., Pluda, J. M., Hartman, N. R., Perno, C.-F., Marczyk, K. A., Allain, J.-P., Johns, D. G. & Broder, S. (1989) *Science* **245,** 412–415.
9. Yarchoan, R., Mitsuya, H. & Broder, S. (1989) *Am. J. Med.* **87,** 191–200.
10. Larder, B. A., Darby, G. & Richmann, D. D. (1989) *Science* **243,** 1731–1734.
11. Larder, B. A. & Kemp, S. D. (1989) *Science* **246,** 1155–1158.
12. Tuerk, C. & Gold, L. (1990) *Science* **249,** 505–510.
13. diMarzo Veronese, F., Copeland, T. D., DeVico, A. L., Rahman, R., Oroszlan, S., Gallo, R. C. & Sarngadharan, M. G. (1986) *Science* **231,** 1289–1291.
14. Davies, J. F., II, Hostomska, Z., Hostomsky, Z., Jordan, S. R. & Matthews, D. A. (1991) *Science* **252,** 88–95.
15. Sallafranque-Andreola, M.-L., Robert, D., Barr, P. J., Fournier, M., Litvak, S., Sarih-Cottin, L. & Tarrago-Litvak, L. (1989) *Eur. J. Biochem.* **184,** 367–374.
16. Barat, C., Lullien, V., Schatz, O., Keith, G., Nugeyre, M. T., Gruninger-Leitch, F., Barre-Sinoussi, F., LeGrice, S. F. J. & Darlix, J. L. (1989) *EMBO J.* **8,** 3279–3285.
17. Gilboa, E., Mitra, S. W., Goff, S. & Baltimore, D. (1979) *Cell* **18,** 93–100.
18. Sobol, R. W., Suhadolnik, R. J., Kumar, A., Lee, B. J., Hatfield, D. L. & Wilson, S. H. (1991) *Biochemistry* **30,** 10623–10631.
19. Tuerk, C., Eddy, S., Parma, D. & Gold, L. (1990) *J. Mol. Biol.* **213,** 749–761.
20. Gauss, P., Gayle, M., Winter, R. B. & Gold, L. (1987) *Mol. Gen. Genet.* **206,** 24–34.
21. Pleij, C. W. A., Reitveld, K. & Bosch, L. (1985) *Nucleic Acids Res.* **13,** 1717–1731.
22. Irvine, D., Tuerk, C. & Gold, L. (1991) *J. Mol. Biol.* **222,** 739–761.
23. Stormo, G. D. & Hartzell, G. W., III (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1183–1187.
24. Hertz, G. Z., Hartzell, G. W., III, & Stormo, G. D. (1990) *Comput. Appl. Biosci.* **6,** 81–92.