Interaction of HIV-1 Reverse Transcriptase with New Minor Groove Binders and Their Conjugates with Oligonucleotides

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Interaction of HIV-1 Reverse Transcriptase with New Minor Groove Binders and Their Conjugates with Oligonucleotides

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Abstract—The effect on polymerization catalyzed by reverse transcriptase (RT) of the human immunodeficiency virus type 1 (HIV-1) was studied for new nonnatural regular minor groove binders (MGBs) containing two to four imidazole, pyrrole, or thiazole residues and for MGB conjugates with oligonucleotides. Poly(A)–oligo(U), poly(A)–oligo(dT), poly(dA)–oligo(U), poly(dA)–oligo(dT), and activated DNA were used as model template–primer complexes. The half-inhibitory concentrations (I_{50}) of the oligopeptides were shown to strongly depend on the structure of the template–primer complex and on the number and type of heterocyclic rings in the MGB. With most compounds tested, I_{50} varied from 7.7×10^{-3} to 1.0×10^{-5} M. Minimal affinity of MGBs was observed with the poly(A)–oligo(U) complex. However, some imidazole- and pyrrole-containing MGBs showed unusually high affinity for the complex of RT with the template–primer duplex, I_{50} ranging from 3×10^{-9} to 4×10^{-8} M. In most cases, conjugates of thiazole-containing MGBs with oligonucleotides completely or partly complementary to the template had an affinity one to four orders of magnitude higher than free thiazole carboxamides. Possible causes of the dependence of I_{50} on the structure of template–primer complexes, MGBs, and their conjugates with oligonucleotides are considered.

Key words: human immunodeficiency virus, reverse transcriptase, thiazole-containing oligopeptides, inhibition

INTRODUCTION

Reverse transcriptase (RT) is the most important target of therapeutic agents affecting the human immunodeficiency virus (HIV). However, both HIV-1 and HIV-2 develop resistance to most of the well-known and new-generation anti-HIV drugs [1, 2]. That is why a search for new drugs and modification of the existing preparations are the focus of HIV pharmacology.

The natural compounds netropsin and distamycin are known as minor groove binders (MGBs): they bind to the minor groove of double-stranded B-DNA and thereby inhibit DNA-dependent enzymes. Distamycin, which contains pyrrole (Py) groups, has been initially isolated from *Streptomyces distallicus* [3] and then synthesized [4–6]. This agent suppresses the growth of Gram-positive and Gram-negative bacteria and inhibits replication of viral DNA, as demonstrated with the vaccinia virus, herpes virus [6], and HIV [7]. Netropsin derivatives are also active against the herpes virus [8]. Distamycin and netropsin analogs synthesized in recent years have benzene, pyridine, thiophene, thiazole (Tz), imidazole (Im), or pyrazole residues in place of Py groups [9–20]. MGBs as potential anticancer and antivirus agents are the subject of intensive studies [7, 19–29], and several compounds have already been put to clinical trials [30, 31]. Some distamycin derivatives suppress HIV reproduction and, therefore, have a therapeutic potential [32]. Studies of Tz-containing oligopeptides are few [13, 14, 18, 33–36], because Tz-containing MGBs are poorly soluble in water, which hinders their synthesis and application. Most studies consider distamycin and netropsin heteroanalogs with one of the methylpyrrole fragments replaced with a Tz ring.

A dipeptide consisting of thiazole carboxamide units has been shown to bind to the minor groove, interacting with GC-rich sites of 4–5 bp [13, 14]. Introduction of the methyl group into the Tz ring changes the properties of the compound: modified agents intercalate in DNA

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rather than acting as MGBs [37]. The biological activity of Py-containing MGBs depends on their sizes.

RT plays the major role in HIV-1 replication [38]. Provirus double-stranded DNA is synthesized on the template of genomic single-stranded RNA of a retrovirus as a result of reactions catalyzed by RT. RT has three enzymic activities: RNA-dependent DNA polymerase, DNA-dependent DNA polymerase, and RNase. To start DNA synthesis on genomic RNA of the virus, RT utilizes $tRNA^{Lys}$ as a natural primer. The resulting DNA strand is used as a template to synthesize the second strand and to yield provirus DNA. Thus, RT interacts with the RNA–RNA primer–template complex at the beginning of replication, with the RNA–DNA complex after adding deoxyribonucleotides to the primer tRNA, and with the DNA–DNA complex at the second step of replication.

We have recently described the synthesis of new MGBs containing from one to four Tz residues and shown that these compounds efficiently inhibit HIV-1 RT [39, 40] and integrase [41]. Like distamycin, the Tz-containing MGBs only slightly inhibited the polymerization reaction catalyzed by HIV-1 RT in the case of RNA–RNA template–primer complexes [39, 40]. The inhibitory effect was comparable with that of distamycin in the case of DNA–DNA substrates and about two orders of magnitude higher in the case of RNA–DNA and DNA–RNA template–primer complexes.

In this work, we studied the inhibitory effect of new Py-, Im-, and Tz-containing nonnatural oligopeptides (MGBs) on the polymerization reaction catalyzed by HIV-1 RT with different primer–template complexes. In addition, we explored the possibility of using oligonucleotide conjugates of these MGBs as highly efficient RT inhibitors.

EXPERIMENTAL

Reagents. We used dNTPs, poly(dA), poly(A) of 300–400 nt, Tris, DTT, magnesium acetate, distamycin (Sigma, United States), and [^{3}H]dNTPs with specific activity 700–900 TBq/mol (Izotop). Other reagents were of high purity grade.

Preparations. Homogeneous oligonucleotides were synthesized as in [42, 43]. Homogeneous HIV-1 RT (the wild-type p66/p51 dimer) was obtained according to a published protocol [44].

Derivatives of Tz- [45], Py-, and Im-containing MGBs, distamycin, and netropsin were synthesized and characterized as in [45, 46]. Structural formulas are given below. Oligonucleotide–MGB conjugates, which were homogeneous by ion exchange and reversed-phase chromatography, were synthesized and characterized as in [46].

RT-dependent polymerization was carried out at 37°C [47]. The reaction mixture (50–100 μl) contained 50 mM Tris-HCl (pH 8.0), 1 mM DTT, 80 mM KCl (except in the cases of DNA–DNA and DNA–RNA template–primer complexes), 5 mM magnesium acetate (pH 7.5), 0.5 mM EDTA, 50 μM [^{3}H]dTTP (or dATP, dCTP, dGTP, and [^{3}H]dTTP). The specific activity of [^{3}H]dTTP was 60–90 TBq/mol in all cases. In addition, the reaction mixture contained a primer–template complex in a concentration optimal for polymerization. The concentration was 0.5 A_{260}/ml for poly(A)–d(pT)$_{16}$, 1.5 A_{260}/ml for poly(A)–(pU)$_{16}$ and poly(dA)–(pU)$_{16}$, or 2.0 A_{260}/ml for poly(dA)–d(pT)$_{16}$ and activated DNA. The primer/template concentration ratio (in terms of mononucleotides) was 1:5 in all but one case (activated DNA). Calf thymus DNA was hydrolyzed with DNase I by 7% [47].

The reaction was initiated by adding the enzyme (0.02–2 units of RT) and carried out for 2 h. Aliquots (5–10 μl) were taken every 5–20 min and applied on FN-16 paper filters, which were impregnated with 5% trichloroacetic acid (TCA) and dried before application. The filters were washed seven times with 5% TCA at 0°C for 10 min, washed with chilled acetone to remove TCA, and dried. Radioactivity was measured with a MINI-Beta counter by toluene scintillation counting.

The half-inhibitory concentration (I_{50}) was estimated with the standard polymerization mixture in the presence of oligonucleotides, which were used at various concentrations. The concentration dependence of the initial reaction rate was used to obtain I_{50}. All measurements were performed under conditions allowing a linear dependence between product accumulation and time or RT concentration.

RESULTS AND DISCUSSION

Inhibition of Reverse Transcriptase by Nonnatural Oligopeptides

It is known that various MGBs interact efficiently with double-stranded DNA and poorly with RNA–RNA in aqueous solutions [21, 45]. We could not find any data on their interaction with RNA–DNA duplexes. The difference in interaction with DNA–DNA or RNA–RNA duplexes is probably explained by the considerable structural difference of these duplexes, which are in the B and A forms, respectively [21, 45]. At the same time, X-ray analysis of DNA and RNA complexes with various enzymes and other proteins have revealed a striking difference in structural characteristics of nucleic acids in these complexes and in solution [48–51]. Thus, MGBs may interact differently with DNA and RNA in solution and with their complexes with proteins.

As already mentioned, HIV RT can interact with three template–primer complexes: RNA–RNA

(genomic RNA/tRNA^{Lys}), RNA–DNA (genomic RNA/DNA (–) strand synthesized on the RNA template), and DNA–DNA (synthesis of the (+) strand on the template of the (–) strand) [38]. Thus, complexes of RT with various duplexes provide an adequate model for studying the interaction of MGBs with specific structures of protein-bound DNA–DNA, RNA–RNA, and RNA–DNA. To study the efficiency of interaction with various duplexes for distamycin (Fig. 1) and several new nonnatural oligopeptides (see below), we used the following model primer–template complexes: poly(A)–oligo(U), poly(A)–oligo(dT), poly(dA)–oligo(U), poly(dA)–oligo(dT), and activated DNA.

We examined new nonnatural MGBs, which contained Tz, Py, or Im residues. Most of these derivatives harbored 2–4 heterocyclic residues and were regular in structure. All MGBs had an aminocapronic acid residue at the N end, while their C-terminal resides differed (H-Aca; Table 1, Fig. 2). Structural formulas of some oligothiazole derivatives are shown in Fig. 2. Regular oligopyrrole- and oligothiazole-containing derivatives had the same additional groups at the C end (Table 1). In addition, we used a mixture of 27 tripeptides, which contained all three residues (Tz, Py, and Im) in a random order (Table 1).

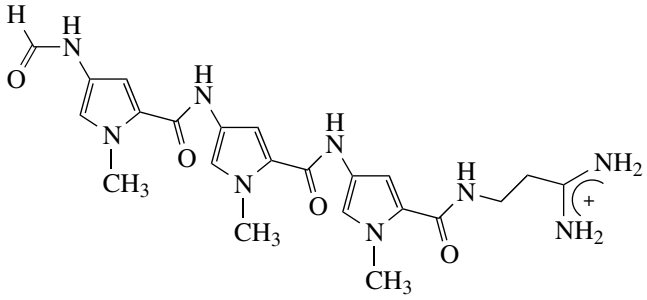


Fig. 1. Structure of the natural MGB distamycin.

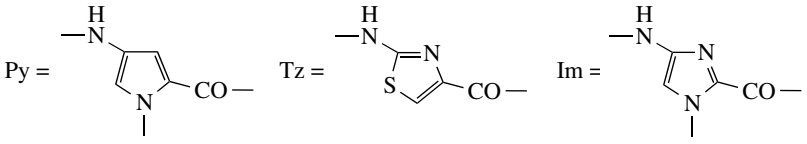
Using the dependences of initial reaction rate on inhibitor concentration, we estimated I_{50} for various compounds (Table 1). Most Tz-containing oligopeptides (Table 1, compounds 2–11) and distamycin (control compound 1) were characterized previously [39, 40] and are given for comparison with the new Py- and Im-containing MGBs and Tz_n conjugates with oligonucleotides.

Distamycin inhibited polymerization with all template–primer complexes when used at relatively high concentrations, 4–7 mM. Its I_{50} was two orders of magnitude lower (5×10^{-5} M) only in the case of

Table 1. Nonnatural oligopeptide concentrations halving the rate of the reaction catalyzed by HIV RT

	Oligopeptide structure*	I_{50} with various template–primer complexes, M			
		poly(A)–(pU)$_{16}$	poly(A)–d(pT)$_{16}$	poly(dA)–(pU)$_{16}$	activated DNA
		RNA–RNA	RNA–DNA	DNA–RNA	DNA–DNA
1	Distamycin A	4.8×10^{-3}	5.0×10^{-3}	7.5×10^{-3}	5.0×10^{-5}
2	H-Aca-Tz$_2$-NH(CH$_2$)$_6$NH$_2$	2.8×10^{-3}	2.5×10^{-4}	3.6×10^{-4}	5.0×10^{-5}
3	H-Aca-Tz$_2$-NHCH$_3$	6.0×10^{-3}	6.0×10^{-4}	5.0×10^{-4}	8.0×10^{-4}
4	H-Aca-Tz$_3$-NHCH$_3$	2.7×10^{-3}	3.0×10^{-4}	8.7×10^{-4}	3.0×10^{-4}
5	H-Aca-Tz$_4$-NHCH$_3$	3.5×10^{-4}	5.0×10^{-4}	–	2.0×10^{-4}
6	H-Aca-Tz$_2$-NH(CH$_2$)$_3$N(CH$_3$)$_2$	7.7×10^{-3}	2.5×10^{-4}	4.6×10^{-4}	1.0×10^{-5}
7	H-Aca-Tz$_3$-NH(CH$_2$)$_3$N(CH$_3$)$_2$	5.5×10^{-3}	4.5×10^{-5}	2.3×10^{-5}	1.0×10^{-5}
8	H-Aca-Tz$_4$-NH(CH$_2$)$_3$N(CH$_3$)$_2$	6.8×10^{-3}	2.5×10^{-5}	3.5×10^{-5}	1.0×10^{-5}
9	H-Aca-X$_3$-NH(CH$_2$)$_3$N(CH$_3$)$_2$ (X = Py, Tz, or Im)	–	1.0×10^{-4}	–	7.3×10^{-5}
10	H-Aca-Py$_4$-NH(CH$_2$)$_3$N(CH$_2$CH$_3$)$_2$	3.5×10^{-8}	7.5×10^{-5}	–	7.0×10^{-5}
11	H-Aca-Py$_4$-Aca-Py$_4$NH(CH$_2$)$_3$N(CH$_2$CH$_3$)$_2$	2.5×10^{-9}	6.0×10^{-5}	–	1.3×10^{-5}
12	H-Aca-Py$_4$-Aca-Py$_4$NH(CH$_2$)$_3$N(CH$_2$CH$_3$)$_2$	5.0×10^{-8}	5.0×10^{-5}	–	7.0×10^{-5}
13	H-Aca-Im$_3$-NH(CH$_2$)$_3$N(CH$_2$CH$_3$)$_2$	3.0×10^{-8}	6.0×10^{-5}	–	2.0×10^{-5}

Note: Monomeric units of Py-, Tz-, and Im-containing MGBs had the following structural formulas:



Each MGB had an aminocapronic acid residue (Aca = –NH(CH$_2$)$_5$CO–) at the N end. The C-terminal residues are indicated.

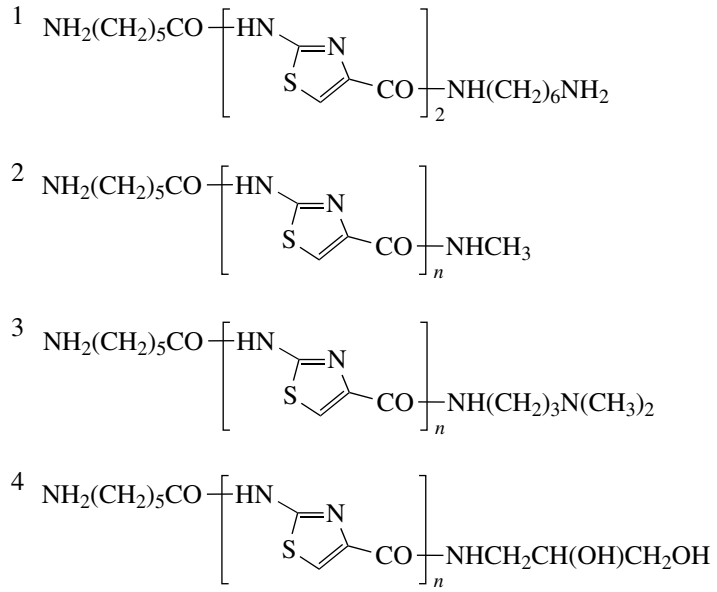


Fig. 2. Structures of some typical nonnatural MGBs containing one–four thiazole residues (n = 1–4). The MGBs each had an aminocapronic acid residue at the N end and varied in structure of the C-terminal residue.

DNA–DNA duplexes. All thiazolecarboxamides (Table 1, compounds 2–11) only slightly inhibited polymerization in the RNA–RNA duplex poly(A)–$(pU)_{11}$: I_{50} = 1.5–7.8 mM. Their inhibitory effect was virtually independent of the number of Tz monomers or the structure of additional groups and was comparable with the effect of distamycin. In the case of DNA–DNA duplexes, the effect of Tz-containing oligopeptides was similar to that of distamycin, differing by no more than one order of magnitude. In the case of the RNA–DNA poly(A)–$d(pT)_{16}$ and DNA–RNA poly(dA)–$(pU)_{11}$ duplexes, the extent of inhibition depended on the structure of additional groups. The Tz-containing dipeptides that had N- and C-terminal groups charged positively at neutral pH (Table 1; compounds 2, 3, and 6) inhibited RT with an efficiency one order of magnitude higher than with the RNA–RNA duplex; the difference was about two orders of magnitude in the case of similar tri- and tetrapeptides (Table 1; compounds 4, 5, 7, and 8). The tri- and tetrapeptides inhibited the RT reaction with RNA–DNA and DNA–RNA duplexes 1–2.5 orders of magnitude more efficiently than distamycin.

It is of interest that, with the RNA–DNA and DNA–DNA duplexes, the mixture of 27 randomized tripeptides (X_3), containing Py and Im along with Tz, inhibited RT as efficiently as the homologous Tz-containing tripeptide with the C-terminal NH–CH_3 group (Table 1, compound 4) and 2–7 times less efficiently than the Tz-containing tripeptide with the C-terminal $NH(CH_3)_2$ group (compound 7).

With activated DNA, oligopeptides containing four or eight Py groups (Table 1, compounds 10–12) inhibited RT at much the same concentrations (13–70 μM) as distamycin (50 μM) and $Tz_{2–4}$-containing compounds 6–8 (10 μM). Similar results were obtained for the Im-containing tripeptide (Table 1).

With the RNA–DNA poly(A)-$d(pT)_{16}$ duplex, all Py- and Im-containing oligopeptides (compounds 10–13) inhibited RT two orders of magnitude more efficiently (50–75 μM) than distamycin (5 mM). The inhibitory effect was comparable with that of some Tz-containing tri- and tetrapeptides (25–45 μM, Table 1).

It is of special interest that, with the RNA–RNA duplex, Py- and Im-containing oligopeptides inhibited RT at extremely low concentrations: I_{50} = 2.5–50 nM. By contrast, Py-containing distamycin and all Tz-containing derivatives exerted a weak inhibitory effect (0.35–7.7 mM) in the case of the poly(A)-$(pU)_{16}$ template–primer duplex (Table 1). This result is surprising, because analysis of the melting temperature of RNA–RNA duplexes in the presence of Py-containing oligopeptides has suggested their weak interaction [45].

Since a strong inhibitory effect was observed for Py- and Im-containing oligopeptides (compounds 10–13), it was necessary to check whether the poly(A)-$(pU)_{16}$ duplex is precipitated or RNA hydrolyzed in their presence. When the poly(A)-[^{32}P]$(pU)_{16}$ duplex was incubated with Py-containing oligopeptides (50 nM) for 1 h and the mixture was centrifuged at 15000 rpm for 20 min, radioactivity was almost totally contained in the supernatant. Thus, the RNA–RNA duplex remained well soluble in the presence of MGBs.

It is known that some compounds containing two Im groups mimic the RNase active center and hydrolyze RNA [52]. To check this possibility, the reaction mixture with a ^{32}P-labeled template, a primer, and the Im- or Py-containing peptides under study was examined by denaturing PAGE. We did not detect any appreciable hydrolysis of the template or the primer. Thus, inhibition of RT by Py- and Im-containing oligopeptides is due to their high affinity for the RNA–RNA duplex that has assumed a specific conformation in complex with the enzyme. These compounds provide the first example of MGBs having extremely high affinity for nucleic acids, especially for the RNA–RNA duplex bound with the enzyme.

Some MGBs display certain specificity to the DNA primary structure or efficiently interact with DNA, but not with RNA, in solution [21, 37, 49]. However, the regularities of their interaction with nucleic acids in solution and in complex with enzymes may differ. According to X-ray data, binding with an enzyme leads to structural adaptation of nucleic acids, including partial or complete melting, structural changes in the sugar–phosphate backbone, stretching, compression, bending or kinking, base flipping, etc. Such changes have been observed for all enzymes tested, and their set is individual for each enzyme [49–51, 53–55]. Based on kinetic and thermodynamic data, we assumed that the nucleic acid structure is optimal for HIV RT when the template is in an A-like and the primer, in a B-like, conformation [50, 59–61]. As X-ray data demonstrate, the structure of nucleic acids in complex with RT is even more intricate: the template is in the A conformation in the vicinity of the polymerase center and in the B conformation in the RNase H active center, with the A- and B-DNA regions making a bend of 40°–45° [53–55].

DNA–DNA and DNA–RNA duplexes can assume the B as well as the A conformation in solution, while RNA–RNA duplexes are structurally conservative and occur in two highly similar forms, A and A', under various conditions [58]. It is clear that, like the other duplexes, the RNA–RNA duplex interacting with RT should undergo certain changes to achieve the conformation optimal for catalysis. These changes are probably far from optimal in the case of the RNA–RNA duplex as opposed to the DNA–DNA and DNA–RNA duplexes, because the RNA–RNA duplex is the least efficient substrate of RT [47, 57]. It is possible, however, that the structural characteristics of the RNA–RNA double helix in complex with RT are most optimal for its interaction with the Py- and Im-containing oligopeptides under study.

Thus, new Py- and Im-containing oligopeptides are more potent and universal inhibitors of HIV RT than distamycin and efficiently suppress the RT activity with all template–primer complexes (Table 1, compounds 10–13).

Inhibition of Reverse Transcriptase by Oligopeptide–Oligonucleotide Conjugates

In the infected cell, RT initiates DNA synthesis on the viral RNA template by partly melting the primer $tRNA^{Lys}$, which is bound in the tRNA recognition center, and hybridizing its 18-nt 3'-terminal region with the template [59]. In addition, RT can bind and efficiently extend template-complementary RNA and DNA primers of one or more nucleotides [47, 59]. It is of particular interest that RT efficiently binds with any oligonucleotide regardless of its sequence, provided that its 3'-terminal nucleotide is complementary to the template [59]. This is due to the fact that, after the binding of the 3'-terminal nucleotide, the 5'-terminal region of a noncomplementary oligonucleotide efficiently interacts with the tRNA recognition site [59]. Owing to the additional contacts between an oligonucleotide and the tRNA recognition site, RT differs from other DNA and RNA polymerases in efficiently binding oligonucleotides partly complementary to the template [59–61]. In view of this, we linked oligopeptides to the 5' and/or 3' end of oligonucleotides completely or partly complementary to the template.

Oligopeptides containing one to four Tz residues were linked to the 3'-terminal phosphate of various oligonucleotides through the N-terminal amino group of aminocapronic acid (Aca; Fig. 1, Table 2). Most conjugates had the $–NH(CH_2)_3N(CH_3)_2$ group (R, Table 2) at the C end of the Tz-containing oligopeptide (Fig. 1). One oligonucleotide was 3'-modified with a Tz-containing oligopeptide having $NHCH_2CH_2OH–CH_2OH$ in place of the above group at the C end (Fig. 2, *4*). The resulting derivative is designated as dT_{16}-p-Aca-Tz_4-$NHCH_2CH(OH)CH_2OH$ in Table 2. In addition, we used a dT_{16} bifunctional derivative having a Tz-containing peptide both at the 3' and at the 5' ends of the oligonucleotide: R-Tz_4-Aca-p-dT_{16}-p-Aca-Tz_4-R, where R is $NH(CH_2)_3N(CH_3)_2$ (Table 2).

Upon modification with oligopeptides at the 3' end or at both the 3' and 5' ends, $d(pT)_{16}$, complementary to the poly(A) template, lost its primer properties and inhibited polymerization at 4×10^{-8} (compound 14) or 8×10^{-8} M (compound 15). The conjugates demonstrated I_{50} four orders of magnitude lower than free thiazole carboxamides (Tables 1, 2). The oligonucleotide $d(pT)_{16}$ is characterized by K_M ($K_M \approx K_d$) of about 5×10^{-7} M [47, 59], one order of magnitude higher than I_{50} of $d(pT)_{16}$ conjugated with MGBs (Table 2). It is known that K_d and I_{50} of a ligand are associated with each other and that the I_{50} is usually higher than the K_d. Thus, addition of an oligopeptide to the 3' end of an oligonucleotide formally increases its affinity for RT by at least one order of magnitude. Addition of the second MGB to the 5' end reduces affinity, suggesting a steric hindrance to the interaction of the resulting derivative with the poly(A) template in complex with the enzyme.

Table 2. Effect of some oligonucleotides and their conjugates with thiazole carboxamides on polymerization catalyzed by HIV RT

Conjugate structure*	I_{50} with various template–primer complexes, M		
	poly(A)–$(pU)_{16}$	poly(A)–$d(pT)_{16}$	activated DNA
	RNA–RNA	RNA–DNA	DNA–DNA
dT_{16}	nd	5×10^{-7}**	
14. dT_{16}-**p**-$AcaTz_4$-$NHCH_2CH(OH)CH_2OH$	nd	4.0×10^{-8}	9.1×10^{-7}
15. R-Tz_4-Aca-**p**-dT_{16}-**p**-Aca-Tz_4-R	nd	8.0×10^{-8}	1.0×10^{-6}
TCCGCT	1.0×10^{-5}	5.0×10^{-5}	1.0×10^{-3}
16. TCCGCT-**p**-$AcaTz_1$-R	4.5×10^{-6}	2.0×10^{-6}	No inhibition
17. TCCGCT-**p**-$AcaTz_2$-R	2.0×10^{-6}	1.0×10^{-6}	No inhibition
18. TCCGCT-**p**-$AcaTz_3$-R	1.5×10^{-6}	3.0×10^{-6}	No inhibition
19. TCCGCT-**p**-$AcaTz_4$-R	1.5×10^{-6}	1.0×10^{-6}	No inhibition
AGCGGA	nd	9.0×10^{-6}	No inhibition
20. AGCGGA-**p**-$AcaTz_1$-R	nd	6.5×10^{-6}	No inhibition
21. AGCGGA-**p**-$AcaTz_2$-R	nd	6.0×10^{-6}	No inhibition
22. AGCGGA-**p**-$AcaTz_3$-R	nd	7.0×10^{-6}	No inhibition
23. AGCGGA-**p**-$AcaTz_4$-R	nd	5.0×10^{-6}	No inhibition

Note: The effect was not determined (nd) in some cases.

* The 3'-terminal phosphate of each oligonucleotide was linked to the amino group of the N-terminal aminocapronic acid residue (Aca) of a Tz-containing oligopeptide. The C-terminal groups of MGBs are indicated; R is $–NH(CH_2)_3N(CH_3)_2$.

** K_M is given.

In the case of activated DNA, the inhibitory effects of the mono- and bifunctional derivatives of $d(pT)_{16}$ did not significantly differ from each other and were 50–55 times higher than the effect of the free oligopeptide and 13–23 times lower than with the poly(A) template, complementary to $d(pT)_{16}$ (Table 2). To explain the decrease in affinity, it is possible to assume that $d(pT)_{16}$ cannot find completely or partly complementary regions in activated DNA and interacts with the RT–DNA complex mostly through its 3'-terminal nucleotide and additional contacts of the 5'-terminal region with the tRNA-binding site of the enzyme (see above). It is of interest that I_{50} of the mono- and bifunctional conjugates of $d(pT)_{16}$ with MGBs were lower than K_M (2–4 μM) of similarly sized primers having only one 3'-terminal nucleotide complementary to the template [59]. We have shown previously that oligonucleotides partly complementary to the template can act as primers and that their derivatives carrying additional groups at the 3' or 5' end compete with complementary primers for the binding with the RN–template complex [60, 61]. It is probable that $d(pT)_{16}$ conjugates also compete with the $d(pT)_{16}$ primer and that the embedding of the oligopeptide in the minor groove of the primer–template duplex makes the complex tighter. Addition of the second ligand to the 5' end does not increase inhibition because of the steric hindrance to simultaneous interactions of the two oligopeptide residues with the template and the enzyme.

In addition, we studied the interaction of RT with conjugates of short noncomplementary (dAGCGGA) or partly complementary (dTCCGCT) oligonucleotides containing one–three Tz rings at the 3' end (Table 2, compounds 15–19 and 20–23, respectively). Compared with extended complementary oligonucleotides (e.g., $d(pT)_{16}$), short oligonucleotides partly complementary to the poly(A) template (e.g., d(TCCGCT)) are less effective primers for polymerization, and their relative affinity for the enzyme can be estimated in experiments on inhibition, as in the case of noncomplementary oligonucleotides [51]. The nonmodified hexanucleotides d(TCCGCT) and d(AGCGGA) inhibited the RT reaction with poly(A)–$(pU)_{16}$ and/or poly(A)–$d(pT)_{16}$ and demonstrated $I_{50} \sim (1–5) \times 10^{-5}$ M. Addition of oligopeptides containing one–four monomers to partly complementary d(TCCGCT) reduced I_{50} by a factor of 17–50 or 2.2–6.7 in the case of the DNA–RNA or the RNA–RNA template–primer complex, respectively (Table 2). In these experiments, I_{50} of the conjugates were 1.5–2 orders of magnitude lower than I_{50} of free thiazole carboxamides (Tables 1, 2).

It is of interest that, in the case of activated DNA, d(TCCGCT) had two orders of magnitude lower affinity for the enzyme (I_{50} = 1 mM) and its conjugates

exerted virtually no inhibitory effect on polymerization when used at concentrations lower than ~0.5 mM (Table 2). Note that oligopeptides consisting of two–four monomers demonstrated I_{50} ranging from 10^{-4} to 10^{-5} M (Table 1). The decrease in conjugate affinity indicates that short oligonucleotides do not allow efficient interactions of the oligopeptide fragments with the minor groove in the case of heteropolymeric DNA. At the same time, it is clear that short oligonucleotides conjugated with oligopeptides cannot efficiently interact with enzyme molecules that have various heteropolymeric DNA sequences bound in the DNA-binding center. We think that, in the case of activated DNA, MGB conjugates with oligonucleotides bind mostly to the minor groove through the MGB residues, while the interactions characteristic of free MGBs are weak because of a steric hindrance determined by the oligonucleotide fragment.

It is of special interest in this connection that d(AGCGGA), which is completely noncomplementary to the poly(A) template, had the same or even higher affinity for the enzyme when conjugated with various Tz-containing oligopeptides (Table 2). We have observed previously that short G-containing oligonucleotides are rather inefficient as primers in the case of the poly(A) template [60, 61]. We assumed that such oligonucleotides are similar to oligo(dT) primers in the mode of interaction with the RT–poly(A) complex and form noncanonical A–G, rather than A–T, pairs, which can be relatively stable:

3'-A A A A A **A** A **A** **A** A A A A-5'
• • •
5' -p A **G** C **G** **G** A-OH-3'.

The higher inhibitory effect of the d(AGCGGA) derivatives compared with the free oligonucleotide was probably due to additional contacts of the MGB residues of the conjugate with the template and/or the enzyme.

To summarize, we showed that oligopeptide–oligonucleotide conjugates have several orders of magnitude higher affinity than the initial compounds. This finding opens the way for developing new selective inhibitors of RT on the basis of MGBs and their conjugates with oligonucleotides completely or partly complementary to the template.

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