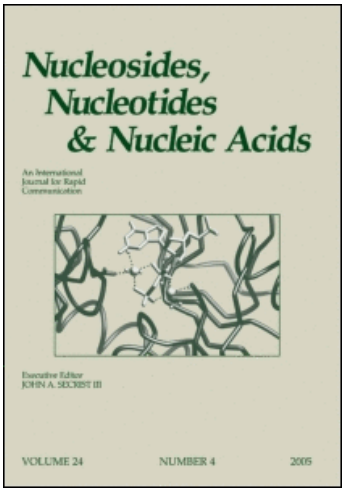

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

6-OXOCYTIDINE CONTAINING OLIGONUCLEOTIDES INHIBIT THE HIV-1 INTEGRASE *IN VITRO*

P. Brodin[a]; M. Pinskaya[b]; U. Parsch[c]; J. Bischerour[a]; H. Leh[a]; E. Romanova[b]; J. W. Engels[c]; M. Gottikh[b]; J. F. Mouscadet[a]
[a] Institut Gustave Roussy, Villejuif, cedex, France [b] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia [c] Institut für Organische Chemie, Johann-Wolfgang-Goethe Universität, Frankfurt, Germany

Online publication date: 31 March 2001

To cite this Article Brodin, P. , Pinskaya, M. , Parsch, U. , Bischerour, J. , Leh, H. , Romanova, E. , Engels, J. W. , Gottikh, M. and Mouscadet, J. F.(2001) '6-OXOCYTIDINE CONTAINING OLIGONUCLEOTIDES INHIBIT THE HIV-1 INTEGRASE *IN VITRO*', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 481 — 486
To link to this Article: DOI: 10.1081/NCN-100002322
URL: http://dx.doi.org/10.1081/NCN-100002322

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 481–486 (2001)

6-OXOCYTIDINE CONTAINING OLIGONUCLEOTIDES INHIBIT THE HIV-1 INTEGRASE *IN VITRO*

P. Brodin,[1] M. Pinskaya,[2] U. Parsch,[3] J. Bischerour,[1] H. Leh,[1] E. Romanova,[2] J. W. Engels,[3] M. Gottikh,[2] and J. F. Mouscadet[1,*]

[1]UMR 8532, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif cedex, France
[2]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119899 Moscow, Russia
[3]Institut für Organische Chemie, Johann-Wolfgang-Goethe Universität, Marie-Curie Strasse 11, 60439 Frankfurt, Germany

ABSTRACT

Integration of the proviral DNA into the genome of infected cells is a key step of HIV-1 replication. Integration is catalyzed by the viral enzyme integrase (IN). 6-oxocytidine-containing oligonucleotides were found to be efficient inhibitors of integrase *in vitro*. The inhibitory effect is sequence-specific and strictly requires the presence of the 6-oxocytidine base. It is due to the impairment of the integrase binding to its substrate and does not involve an auto-structure of the oligonucleotide.

Durable suppression of HIV-1 replication is achieved by the use of drug combinations (1). However, drug toxicity and rapid emergence of drug-resistant isolates necessitate the search for new drugs that can be added to the multi-drug regimen referred to as the highly active anti-retroviral therapy (HAART). Drugs included in this regimen are mainly directed against two viral enzymes, the reverse transcriptase (RT) and the protease (PR). Alongside RT and PR, the HIV-1 genome encodes a third enzyme integrase (IN) that carries out the integration of the viral

*Corresponding author.

DNA into the host cell genome. This replicative step is critical for the HIV-1 replication. Therefore, IN constitutes an attractive target for new pharmacological approaches. Integrase directs two distinct reactions: the endonucleolytic cleavage of the proviral DNA or 3′-processing, removing a dinucleotide and the strandtransfer which results in the joining of each processed 3′-end to 5′-phosphates in the target DNA (2). Both reactions can be modeled *in vitro* with purified recombinant integrase and appropriate substrates.

Development of these assays have made possible the identification of several classes of integrase inhibitors (for a recent review see reference Pommier et al. (3)) and among them, nucleotides and oligonucleotides (for summary see Table I. Modified and phosphorylated nucleotides as well as dinucleotides have been reported to exhibit antiviral properties against integrase (4,5), thus leading to the characterization of a mononucleotide binding site to which the three lysines of the catalytic core domain, Lys-156, Lys-159 and Lys-160 contribute (6). Strikingly, structure-forming oligonucleotides such as tetrad-forming oligonucleotides (7), structured branched compounds (8) and triplex-forming oligonucleotides (9) are the most potent oligonucleotidic inhibitors identified to date *in vitro*. In each case, the fact that the oligonucleotides were capable of forming either a self-structure or an intermolecular structure with the DNA substrate played a key role in their inhibitory effect.

In order to improve their inhibition efficiency, optimization of such oligonucleotides with modified heterocyclic bases was investigated. Here, we report the inhibitory effect on integration of short oligonucleotides containing modified bases. Oligonucleotides harboring 6-oxo cytosine modification turned out to be potent integrase inhibitors with IC_{50} in the micromolar range. We observed that these

Table I. Most Representative Modified Oligonucleotides Described as HIV-1 IN Inhibitors

Compound	Reference
Self-structured oligonucleotides	
Guanosine quartet	Jing et al. 2000
3′-3′ branched oligonucleotides	Brodin et al. 1999
Triplex forming oligonucleotides	
Oligonucleotide-oxazolopyridocarbazole	Mouscadet et al. 1994
Modified nucleotides and dinucleotides	
$5N_3$-AZTMP	Mazumder et al. 1994
pdApdC & isodApdC	Taktakishvili et al. 2000
Single stranded oligonucleotide	
21-*mer*: 5′-GTGTGGAAAATCTCTAGCAGT-3′	Caumont et al. 1999
Phosphorothioate polydeoxycytidine	Tramontano et al. 1998
6-oxocytidine containing oligonucleotide	This report

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

compounds act by altering the binding properties of integrase to its substrate although we failed to demonstrate any self-or intermolecular structure of the oligonucleotides.

MATERIALS & METHODS

Oligonucleotides

Double-stranded and dumbell oligonucleotides used for integrase assays were purchased from Eurogentec. 2′-O-Methyl-3′-O-phosphoramidite building blocks of 6-oxocytidine were synthesized as described by Berressem & Engels (10) and incorporated via phosphoramidite chemistry in oligodeoxynucleotides having various lengths and nucleotide sequences. Concentrations were determined spectrophotometrically.

Assays for Integrase Activities

Recombinant integrase protein was purified as previously described (11). The reaction conditions which were used for 3′-processing, strand transfer and disintegration are precisely described in Brodin et al. (8). Different amounts of single stranded oligonucleotides were incubated in 20 μl with 0.3 pmol of the corresponding ^{32}P-labelled DNA substrate in the presence of 2 pmol of integrase in a buffer containing 20 mM Tris, pH 7.5, 50 mM NaCl, 2% (w/v) glycerol, 10 mM DTT supplemented with 8 mM $MnCl_2$ and 2 mM $MgCl_2$ at 37°C. Reaction was stopped by adding 80 μl of a stop solution (0.3 M sodium acetate, pH 7.5). Products were ethanol precipitated, resuspended in 7 M urea and separated on a 18% polyacrylamide/7M urea gel. Gels were analyzed on a STORM 840™ Phosphorimager (Molecular Dynamics) and quantified with Image QuaNT™ 4.1 software.

RESULTS AND DISCUSSION

Oligonucleotides containing modified heterocyclic bases were synthesized and tested as inhibitors of integrase-mediated reactions *in vitro*. Integrase inhibition assays were performed with recombinant integrase and different substrates mimicking viral DNA ends as described in Materials & Methods.

Addition of the 6-oxocytidine containing oligonucleotides led to a significant inhibition of both 3′-processing and strand transfer (see Table II). The position of 6-oxocytidine (C*) was found to be important for the inhibitory effect since oligonucleotides containing two neighboring C* nucleosides at their 3′- or 5′-end displayed the strongest activity. Moreover, 9-*mer* and 11-*mer* oligonucleotides inhibited both integration steps with about the same efficiency. The 7-*mer* mainly inhibited strand transfer reaction whereas 5-*mer* and 3-*mer* oligonucleotides had

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table II. 3′-Processing and Strand Transfer Inhibitory Effect of Different Oligonucleotides Containing Modified Bases

Compound	IC_{50} 3′-Processing	IC_{50} Strand Transfer
C*C*TTTTAGAGA	0.3	0.3
C*C*TTTTAGA	1	0.5
C*C*TTTTA	>5	1
C*C*TTT	>10	3
C*C*T	>10	5
C*	>1000	>20
C	25000	ND
CCTTTTAGAGA	100	>10
AGAGATTTTC*C*	0.5	0.5
GGAAAATC*TC*T	1.5	ND
TC*TC*TAAAAGG	3	ND
CCTTTTAG*AGA	10	ND
CG*TTTTAGACA	10	ND
CCTTTTAG^{d}AGA	10	ND

CI_{50} are in μM. C*: 6-oxocytosine, G*: 8-oxoguanosine, G^{d}: 7-deazaguanosine. ND: non-determined, (>) means that upper concentrations have not been checked.

no inhibitory activity. 6-oxocytidine alone demonstrated a slight inhibitory effect although at high concentration. In fact, the 11-*mer* C*C*TTTTAGAGA mimicking the viral DNA end LTR U5 was the most potent compound with a IC_{50} of 0.3 μM whereas the non-modified 11-*mer* oligonucleotide with identical sequence showed no activity against integrase at concentration up to 50 μM. Finally, disintegration, the reverse reaction catalyzed by the isolated catalytic sub-domain was also strongly inhibited in the presence of increasing concentrations of 6-oxocytidine-containing oligonucleotide, thus suggesting that this domain is part of the target of the inhibitor. It is also worthy to note that a similar inhibition effect was observed when the modified oligonucleotide was mixed with IN before or after addition of its DNA substrate. The presence of other modified bases such as 7-deazaguanosine or 8-oxoguanosine at different positions in the oligonucleotide with identical sequence did not lead to a comparable inhibitory effect against integrase activity. Thus, the inhibitory effect happens to be both sequence specific and dependent on the presence of the 6-oxocytidine residue.

Circular dichroism studies, thermal denaturation experiments and gel retardation assays were carried out to address the possibility of either a self- or an intermolecular structure formed by the 6-oxocytidine containing oligonucleotides. Surprisingly, we found that such a conjugate did not form any secondary structure either with the substrate DNA or at high salt concentrations (7). It has already been described that long single stranded oligonucleotides such as poly$(dC)_{50}$ or 21-*mer* mimicking the viral DNA ends are efficient inhibitors of 3′-processing reaction (12;13). However, this is the first report to our knowledge of an inhibition

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

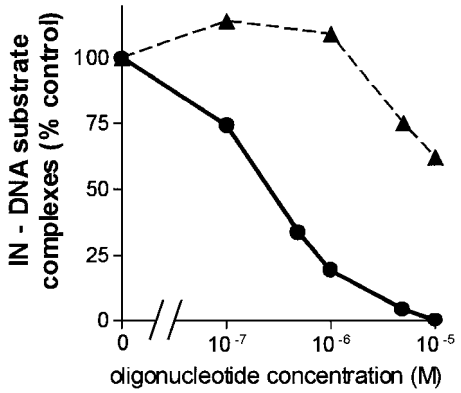

Figure 1. Filter binding assay. Quantification of retained integrase-DNA substrate complexes on filter in the presence of increasing concentrations of the 6-oxocytidine containing oligonucleotide C*C*TTTTAGAG (—●—) and its non-modified counterpart CCTTTTAGAGA (- -▲- -). The control is the quantity of retained IN-DNA substrate complexes without inhibitor.

of integrase in the micromolar range by short 11-*mer* single stranded oligonucleotide.

To investigate further the mechanism of inhibition, we carried out filter binding experiments to determine the influence of the modified oligonucleotides on the binding of IN to its substrate. For this experiment, the ^{32}P-labelled DNA substrate and the modified oligonucleotide were added simultaneously to the integrase solution in standard reaction conditions. After incubation at 37°C, samples were spotted on nitrocellulose filters which are capable of retaining specifically the protein/DNA complexes. In the presence of increasing concentration of 6-oxocytidine containing oligonucleotide, the amount of the ^{32}P-labelled IN/DNA substrate complex which was retained on the nitrocellulose filter decreased (Fig. 1). This result strongly suggests that the 6-oxocytidine containing oligonucleotide prevents the fixation of IN to its substrate. We observed a 50% binding diminution for 0.3 μM of modified oligonucleotide which correlates strongly with the inhibitory effect. Conversely, the non-modified oligonucleotide devoid of inhibitory activity, did not influence the integrase binding. Altogether, these results indicate that the anti-integrase activity that was observed specifically with 6-oxocytidine containing oligonucleotide can be ascribed to its capability of preventing the formation of stable DNA-integrase complexes. From this viewpoint, it was previously reported that the presence of single stranded oligonucleotide may induce a conformational change of the enzyme (13). Furthermore, the activity of the integrase is enhanced and the integration profile is altered in the presence of short non-modified oligonucleotide (13). It can be hypothesized that 6-oxocytidine containing oligonucleotides exhibit a higher affinity either for IN or for IN/DNA complexes than their non modified counterpart. Studies are currently carried out to determine the precise mechanism of inhibition of 6-oxocytidine containing oligonucleotides.

ACKNOWLEDGMENTS

This work was supported by funds from INTAS grant No. 96-1216, from the Agence Nationale de Recherche sur le Sida (ANRS) and SIDACTION. P.B. gratefully acknowledges fellowship support from SIDACTION.

REFERENCES

1. Palella, F. J. Jr; Delaney, K. M.; Moorman, A. C.; Loveless, M. O.; Fuhrer, J.; Satten, G. A.; Aschman, D. J.; Holmberg, S. D. *N. Engl. J. Med.* **1998**, *338*, 853–60.
2. Brown, P. O. *Retroviruses,* Coffin, J. M.; Hughes, S. H. Varmus, H. E., Eds.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 1997; Chapter 5.
3. Pommier, Y.; Marchand, C.; Neamati, N. *Antiviral Res.* **2000**, *47*, 139–48.
4. Mazumder, A.; Cooney, D.; Agbaria, R.; Gupta, M.; Pommier, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5771–75.
5. Taktakishvili, M.; Neamati, N.; Pommier, Y.; Nair, V. *Bioorg-Med. Chem. Lett.* **2000,** *10*, 249–51.
6. Drake, R. R.; Neamati, N.; Hong, H.; Pilon, A. A.; Sunthankar, P.; Hume, S. D.; Milne, G. W.; Pommier, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4170–75.
7. Jing, N.; Marchand, C.; Liu, J.; Mitra, R.; Hogan, M. E.; Pommier, Y. *J. Biol. Chem.* **2000**, *275*, 21460–67.
8. Brodin, P.; Pinskaya, M.; Volkov, E.; Romanova, E.; Leh, H.; Auclair, C.; Mouscadet, J. F.; Gottikh, M. *FEBS Lett.* **1999**, *460*, 270–74.
9. Mouscadet, J. F.; Carteau, S.; Goulaouic, H.; Subra, F.; Auclair, C. *J. Biol. Chem.* **1994**, *269*, 21635–38.
10. Berressem, R.; Engels, J. W. *Nucleic Acids Res.* **1995**, *23*, 3465–72.
11. Leh, H.; Brodin, P.; Bischerour, J.; Deprez, E.; Tauc, P.; Brochon, J. C.; LeCam, E.; Coulaud, D.; Auclair, C.; Mouscadet, J. F. *Biochemistry* **2000**, *39*, 9285–94.
12. Pemberton, I.K.; Buc, H.; Buckle, M. *Biochemistry* **1998**, *37*, 2682–90.
13. Caumont, A.; Jamieson, G.; de, Soultrait VR; Parissi, V.; Fournier, M.; Zakharova, O. D.; Bayandin, R.; Litvak, S.; Tarrago-Litvak, L.; Nevinsky, G. A. *FEBS Lett.* **1999**, *455*, 154–58.
14. Tramontano, E.; Colla, P. L.; Cheng, Y. C. *Biochemistry* **1998**, *37*, 7237–43.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Request Permission or Order Reprints Instantly!