Synthesis and properties of G-quartet oligonucleotide-HIV-1 tat peptide conjugate

Tetsuya Kumashiro¹, Toru Otake², Takuya Kawahata², Masao Akagi¹ and Hidehito Urata¹

¹Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan and ²Osaka Prefectural Institute of Public Health, 1-3-69 Nakamichi, Higashinari-ku, Osaka 537-0025, Japan

ABSTRACT

Zintevir is a single strand DNA that forms an intramolecular quadruplex structure and shows potent anti-human immunodeficiency virus type 1 (HIV-1) activity. Zintevir was discovered as a potent inhibitor for HIV-1 integrase. Recently, the primary molecular target of Zintevir, however, was shown to be the HIV-1 gp120. In fact, in our previous study, Zintevir was shown to inhibit the only processes of the viral adsorption and the entry into the cell. This result suggests that Zintevir is not able to penetrate through the cell membranes. Therefore, we designed and synthesized the complex of D-17mer with HIV-1 tat peptide that has the cell membrane permeability.

INTRODUCTION

The molecular targets of most of approved anti-HIV drugs are HIV reverse transcriptase (RT) and protease. Although these drugs achieved the inhibition of HIV replication with the high active anti-retroviral therapy, the emergence of drug-resistant viruses has resulted in many therapeutic failures. Therefore, the development of a novel inhibitor which has a different molecular target from HIV RT and protease is required. Zintevir (Figure 1) is an oligodeoxynucleotide analogue which has the phosphorothioate modification at both termini to raise its in vivo stability, and forms a highly stable four-stranded DNA structure in the presence of potassium cations¹. At first, Zintevir has been discovered as an effective inhibitor of HIV-1 integrase in vitro², and then was found to inhibit the interaction between HIV-1 gp120 glycoprotein and host CD4 receptor to show anti-HIV-1 activity³. Integration of viral DNA into host cell chromosomal DNA to form a provirus is an essential step in HIV replication, which is mediated by viral integrase. In our previous study, Zintevir and its all-phosphodiester analogue (D-17mer) were shown to inhibit the viral adsorption onto the cells and the entry into the cells, however, they did not inhibit integration in the HIV replication⁴. These results suggest that Zintevir and D-17mer can not penetrate through the cell membranes. HIV-1 tat peptide is a highly basic and hydrophilic peptide which easily transports macromolecules such as oligopeptides and proteins into the cell⁵. Recently, it was reported that HIV-1 tat peptide delivers antisense oligonucleotides into the cytoplasm and nucleus⁶.

Here, we report the design and synthesis of the complex of G-quartet oligonucleotide with HIV-1 tat peptide to afford the inhibitory activities for integrase as well as gp120.

RESULTS AND DISCUSSION

We used a hetero-bifunctional cross-linker, N-(6-maleimidocaproyloxy)succinimide (MCS) to connect D-17mer with HIV-1 tat peptide (Figure 2A). This reagent possesses two selectively reactive groups. One is the N-hydroxysuccinimidyl ester which reacts with the amino group, and the other is the maleimide group which reacts with the thiol group. Figure 2B shows the synthetic scheme of D-17mer-HIV-1 tat peptide conjugate. D-17mer-NH₂ which has the long chain alkylamino modification at the 3’-end of D-17mer was synthesized according to solid-phase phosphoramidite chemistry by using the solid-phase support, 3’-Amino-Modifier C7 CPG. After deprotection and

![Figure 1. Structures and sequences of Zintevir and D-17mer.](https://academic.oup.com/nass/article-abstract/50/1/177/1341294 by guest on 21 March 2019)
desalting, the amino group of D-17mer-NH$_2$ was reacted with MCS to produce D-17mer-MC. After the removal of the unreacted MCS by gel-filtration on a Sephadex G-25 column, the thiol group in the additional cysteine residue which was incorporated into the C-terminus of HIV-1 tat peptide (RKKRRQRRRPPQ) was allowed to couple with the maleimide moiety of D-17mer-MC, and then the unreacted peptide was removed by gel-filtration. The obtained D-17mer-tat was purified by an anion exchange column and its structure was confirmed by MALDI-TOF MS spectrum: calc’d m/z: 7577.97 [M$^+$]; found 7577.01 (positive). The properties of the D-17mer-tat conjugate will be reported.

REFERENCES


