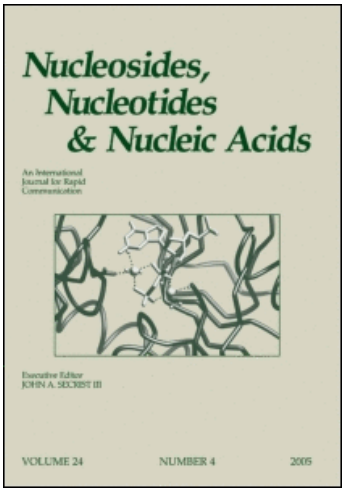

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EVIDENCE FOR THE INVOLVEMENT OF THE SMALL SUBUNIT OF HIV-1 REVERSE TRANSCRIPTASE (RT) IN THE TSAO-RESISTANCE.

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Brief Abstract

By introducing the TSAO-resistance mutation 138Glu→Lys in only the p51 subunit of HIV-1 RT, we obtained compelling evidence for a structural and functional role of the p51 subunit in the sensitivity and/or resistance of the enzyme to the TSAO-derivatives.

Introduction

The reverse transcriptase (RT) of the human immunodeficiency virus (HIV-1) occurs as a heterodimer, consisting a 66 kDa (p66) and a 51 kDa (p51) subunit. The functions of p66 are already well characterized, but the role of p51 is still unclear. According to cristallographic studies of the HIV-1 RT, p51 has a totally different folding than p66 (1). Through site-directed mutagenesis the polymerase active site is identified only on the p66 subunit (2).

Furthermore, the amino acid mutations, which are selected by the HIV-1-specific RT-inhibitors and which afford resistance to these compounds, are clustered in the palm domain of the HIV-1 RT p66 subunit (3). However, for the TSAO derivatives, the resistance mutation is at position Glu138 (4,5). On the p66 subunit this amino acid is distant from the binding site of the other HIV-1-specific RT-inhibitors.

We introduced the TSAO-specific resistance mutation either solely in the p66 subunit or in the p51 subunit of RT p66/p51 heterodimer. This allowed us to demonstrate clearly the importance of the p51 subunit of

HIV-1 RT in the interaction with, and resistance development against, TSAO-derivatives.

Results and Discussion

The coexpression system used for production of the wild-type HIV-1 RT in *E.coli* JM109 consisted of two compatible plasmids, pACYC66 and pKRT51, carrying the genetic information for the 66 kDa and the 51 kDa subunit, respectively. After introducing the 138Glu→Lys-mutation in the RT sequence by using site directed mutagenesis, the four possible combinations were accomplished by different transformations. The recombinant RT enzymes were purified up to 70-80 % from the bacterial lysates (7).

Comparable amounts of the different recombinant RTs (2 nM) were evaluated for their sensitivity to the TSAO derivative TSAO-m^3T, the TIBO derivative R82150 and ddGTP in the RT inhibition assay. The IC_{50} values are shown in Table I. The double mutant (p66MT138+p51MT138) HIV-1 RT was totally resistant, whilst the wild-type RT was sensitive to TSAO-m^3T. From these results it could be concluded that the substitution of the negatively charged glutamic acid by the positively charged lysine138 is sufficient to render HIV-1 RT resistant to TSAO. Since the 4"-NH_2-group of the 3'-spiro moiety of the TSAO derivatives has been suggested to interact with the COOH-group of Glu138, replacement of Glu138 by Lys138 should prevent the ionic interaction between the TSAO molecule and the HIV-1 RT and this may explain the complete lack of sensitivity of (p66MT138+p51MT138) RT to the TSAO derivative as is evident from our data.

Introduction of the 138Glu→Lys-mutation in only p66 did not alter the TSAO sensitivity as compared to the wild-type. However, introduction of this mutation in only the p51 subunit of the RT affords full resistance to TSAO-m^3T. These results indicate that inhibition of the HIV-1 RT by TSAO-m^3T results from an interaction with the Glu138 residue of the p51 subunit and not of the p66 subunit. As it has been hypothesized from the HIV-1 RT three-dimensional structure (3), Glu138 on the p51 subunit can be considered as a part of the hydrophobic pocket at which the HIV-1-specific RT inhibitors, including TSAO derivatives, bind.

The wild-type and the mutant recombinant RTs did not show significant differences in their sensitivity to the inhibitory effects of the TIBO derivative R82150 and ddGTP (Table I).

For the recombinant enzymes that were sensitive to TSAO-m^3T, i.e. p66WT+p51WT and p66MT+p51WT, we also determined the K_i values in the presence of varying concentrations of dGTP and poly(rC)oligo(dG). With each enzyme, inhibition by TSAO-m^3T was noncompetitive with respect to both the substrate and the template-primer (data not shown). The

TABLE 1 *Inhibition of the recombinant RT enzymes by TSAO-m^{3}T, TIBO R82150 and ddGTP.*

Recombinant RT	IC_{50} (μM)		
	TSAO-m^{3}T	TIBO R82150	ddGTP
p66WT+p51WT	2.6 ± 1.6	0.35 ± 0.14	0.075 ± 0.023
p66WT+p51MT138	>100	0.76 ± 0.42	0.015 ± 0.005
p66MT138+p51WT	2.6 ± 1.8	0.28 ± 0.10	0.028 ± 0.005
p66MT138+p51MT138	>100	0.83 ± 0.42	0.022 ± 0.008

K_i values calculated for the wild-type RT were 2.2 μg/ml and 2.9 μg/ml, respectively. Similar values were obtained for the recombinant RT with the 138Glu→Lys-mutation in the p66 subunit only, i.e. 3.1 μg/ml and 1.9 μg/ml respectively. Thus, the introduction of the TSAO-specific resistance mutation in the p66 subunit did not alter the kinetics of inhibition by TSAO-m^{3}T. Therefore, amino acid residue Glu138 of the p66 subunit may be considered as irrelevant to the interaction of the TSAO derivatives with HIV-1 RT as well as to HIV-1 RT resistance development towards TSAO.

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