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Interaction of primer $tRNA^{Lys3}$ with the p51 subunit of human immunodeficiency virus type 1 reverse transcriptase: a possible role in enzyme activation

Olga D. Zakharova[a], Laura Tarrago-Litvak[b], Michel Fournier[b], Marie Line Andreola[b], Marina N. Repkova[a], Alija G. Venyaminova[a], Simon Litvak[b], Georgyi A. Nevinsky[a,*]

[a]*Institute of Bioorganic Chemistry, Siberian Division of the Academy of Sciences of Russia, Novosibirsk 630090, Russian Federation*
[b]*Institut de Biochimie et Génétique Cellulaires du CNRS, 1 rue Camille Saint Saëns, 33077 Bordeaux cedex, France*

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Abstract In the interaction between HIV-1 RT and $tRNA^{Lys3}$ each subunit of the heterodimer interacts with tRNA showing a different affinity: K_d (p66) = 23 nM, K_d (p51) = 140 nM. Preincubation of heterodimeric RT with tRNA, at concentrations similar to that of the K_d value for p51, leads to an increase of the catalytic activity on poly(A)-oligo(dT). These results were compared to those using different tRNA analogs: oxidized tRNA, tRNAs lacking one, two or three nucleotides from the 3'-end, or ribo- and deoxyribonucleotides mimicking the anticodon loop sequence. In all cases, tRNA analogs were weaker activators of HIV-1 RT than natural tRNA. A possible mechanism of RT p66/p51 activation by tRNA and its analogs, mediated through the p51 subunit, is discussed.

Key words: HIV-1 reverse transcriptase; $tRNA^{Lys3}$ derivative; Enzyme activation

1. Introduction

RT plays an essential role in the life cycle of HIV-1. In virions HIV-1 RT has been isolated as a heterodimer consisting of 66 and 51 kDa polypeptides [1,2]. HIV-1 RT utilizes $tRNA^{Lys3}$ as a primer for the initiation of cDNA synthesis, as deduced from the retroviral genome nucleotide sequence [3]. Using different approaches, a complex between mammalian $tRNA^{Lys3}$ and HIV-1 RT has been demonstrated (for a recent review see [4]). The recognition of tRNA by HIV-1 RT occurs through the interaction of the anticodon and dihydrouridine loop [5,6]. The binding of tRNA induces significant structural changes in RT, as followed by fluorescence emission or by the accessibility of p66/p51·tRNA complex to chymotrypsin [7].

In yeast, recombinant HIV-1 RT has been isolated either as a heterodimer p66/p51 [8] or as a homodimer p66/p66 [9]. We have shown before the stimulation of RT p66/p66 by primer $tRNA^{Lys3}$ [10]. Preincubation of RT p66/p66 with tRNA led to stimulation of the reaction of polymerization on poly(A)-oligo(dT) template-primer. Maximal activation was reached at equimolar amounts of tRNA and RT. tRNA did not change enzyme affinity to the template, primer or dNTP, but increased the V_{max} of the reaction. In this work we show that the heterodimeric form of HIV-1 RT can also be activated by $tRNA^{Lys3}$. We have determined the kinetic parameters that characterize the activation. A model is proposed where activation of the p66 catalytic subunit would result from the interaction of $tRNA^{Lys3}$ with the p51 subunit of RT p66/p51.

*Corresponding author.

Abbreviations: RT, Reverse transcriptase; HIV-1, Human immunodeficiency virus type 1; tRNA(−1), tRNA(−2), tRNA(−3), tRNA derivatives shortened by one, two and three nucleotides from the 3'-end.

2. Materials and methods

Nucleotides and polynucleotides were obtained from Sigma or Pharmacia. Radioisotopes were purchased from Amersham or Radioisotop (Russia). Oligonucleotides were synthesized as in [11,12]. HIV-1 RT p66/p51 was obtained as before [8]. tRNA was prepared according to [10]. Oxidation of tRNA was carried out as previously described [13]. tRNA(−1), tRNA(−2) and tRNA(−3) were prepared by subjecting tRNA to three reaction cycles of the Whitfield degradation according to Paulsen and Wintermeier [14] and purified using reverse phase chromatography [15]. Fluorescence determination was performed as described in [10].

RT assay and activation experiments were performed as in [10]. The increase of RT activity was estimated from the tangent of the curves, time vs. polymerization. Relative values of enzyme activation were calculated comparing the activity of RT after and before preincubation with tRNA. The activation of RT during preincubation with tRNA (or its derivatives) is described by the linear dependence of the log of enzyme activity on time. The apparent rate constant of activation, k_{app}, was determined as the slope of the semilogarithmic plot of the activation kinetics. The activation rate constant (k) and the dissociation constant (K_d) for the reversible complex enzyme·tRNA (or analogs of tRNA) were determined according to Kitz and Wilson [16] and to Eisenthal and Cornish-Bowden [17].

3. Results and discussion

The interaction between the p66/p51 form of RT and tRNA was quantitatively analyzed by fluorescence quenching. The stoichiometry and K_d values were obtained from the Scatchard plot using titration data. HIV-1 RT p66/p51 bound two molecules of tRNA with different affinities: K_{d1} = 23 nM; K_{d2} = 140 nM (Fig. 1).

Since both subunits of the heterodimer can bind tRNA it was interesting to determine which of the subunits has higher affinity towards its natural primer. To answer this question different facts were considered: (a) Cross-linking studies have shown that in some conditions (high *trans*-diaminedichloroplatinum concentration) tRNA is associated with both the p66- and 51-kDa polypeptides of heterodimer [5]. However, under conditions of limiting cross-linking agent, there would be a preferential binding of $tRNA^{Lys3}$ to the p66 subunit of the heterodimer (as discussed in [18]); (b) Selective tRNA binding is in part supported by data from Cheng et al. [19]. They showed that

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cross-linking of template-primer and thymidine triphosphate substrates occurred exclusively to the p66 subunit of the heterodimeric RT; (c) The affinity of p66 to a template-primer oligo(A)·d(pT)$_{10}$ is higher than that of the p51 subunit [20]; (d) The different forms of HIV-1 RT were affinity labeled using a 5′-modified primer, covalently linked, that was further elongated in the presence of a radioactive dNTP precursor. The three forms were labeled, but with different efficiencies. In the case of the heterodimer the analog preferentially modified the p66 subunit [21]. These results indicate that the active site for the reaction of polymerization resides on the p66 subunit of the heterodimer. They support the fact that primer tRNA must have a higher affinity for p66 (K_d = 23 nM) than for p51 (K_d = 140 nM).

When analyzing the influence of tRNA on the activity of RT p66/p51, two factors should be taken into account: the competition of tRNA and poly(A)-oligo(dT) for the catalytic subunit of the heterodimer and the higher affinity of tRNA for p66. These data could explain why tRNA cannot activate RT p66/p51 when the ratio tRNA/RT is equal to one, while p66/p66 was stimulated at very low concentrations of tRNA. We observed activation of RT p66/p51 at concentrations of tRNA where, according to the fluorescence data, the complex p51·tRNA takes place. As for RT p66/p66 [10], preincubation of tRNA with the heterodimer p66/p51 led to the activation of the polymerizing activity of RT (Fig. 2A). But the activation level was lower than in the case of the homodimer p66/p66 [10].

All activation experiments were carried out using tRNA at concentrations higher than RT concentration. The dilution of the activated enzyme (10 to 30 times) and the following incubation for 1 h did not diminish the activation effect. These two conditions allowed the analysis of the activation process (at least during the length of time of enzyme activity determination, 10 min) as an irreversible reaction of pseudo-first order. The dissociation constant (K_d) of the complex RT·tRNA was determined on the basis of the following reaction scheme:

$$RT + tRNA\ RT \rightleftharpoons RT \cdot tRNA \qquad (K_d)$$

$$RT \cdot tRNA\ RT \rightarrow tRNA^* \qquad (k)$$

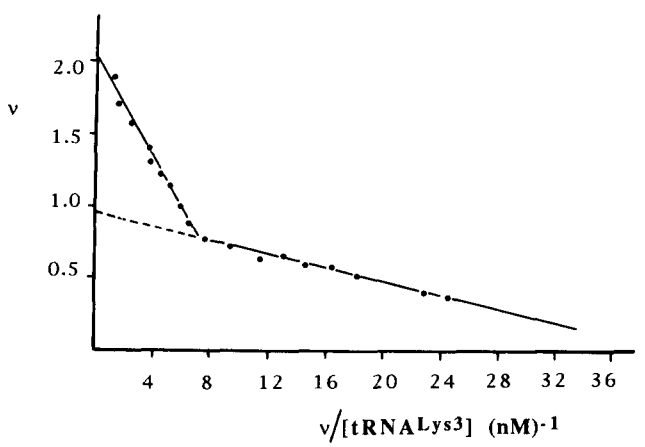


Fig. 1. Determination of K_d values of the complex between HIV-1 RT p66/p51 and tRNA^{Lys3} using Scatchard plot for the fluorescence data representation.

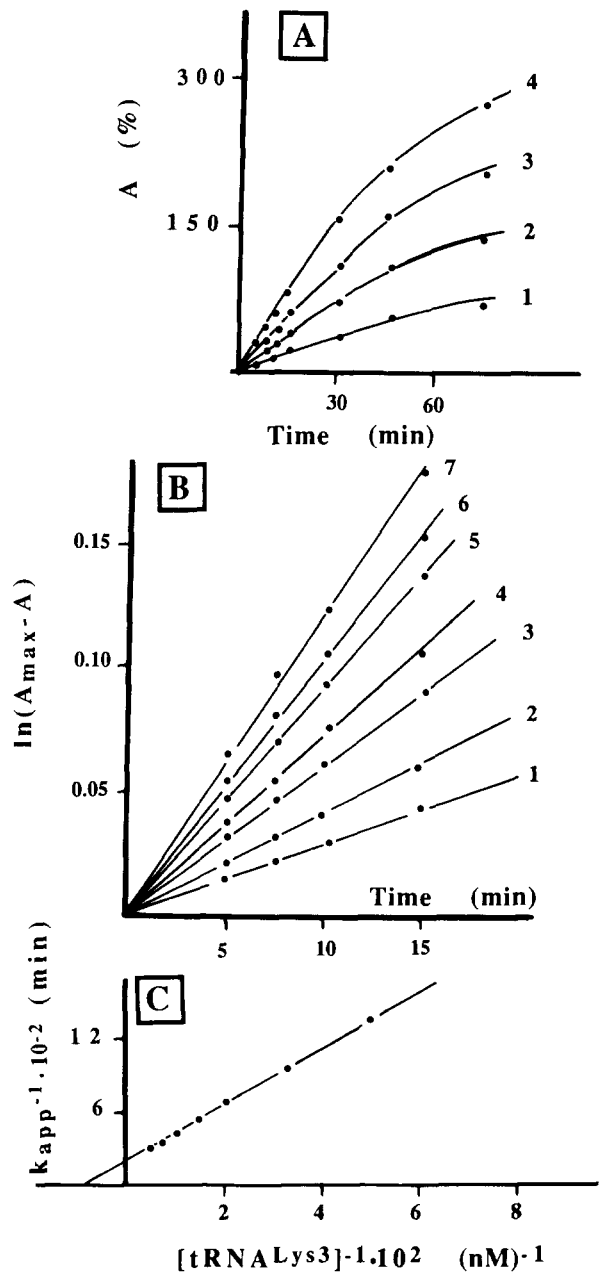


Fig. 2. (A) Time dependence of the activation, A, of RT p66/p51 by tRNA^{Lys3}. RT (4 nM) was preincubated with 20 nM (1), 50 nM (2), 100 nM (3) or 200 nM (4) tRNA. After preincubating for different times, RT activity was determined in the presence of poly(A)-oligo(dT). (B) Data from Fig. 2A is presented as a function of ln (A_{max}−A) upon the time. The level of maximal activation (A_{max}) = 300%, was taken as 1.0. tRNA^{Lys3} concentration, expressed in nM, was: 20 (1), 30 (2), 50 (3), 67 (4), 100 (5), 133 (6) and 200 (7). (C) The dependence of k_{app} of RT activation rate on the concentration of tRNA^{Lys3} in inverse coordinates: determination of the K_d value for the complex of the enzyme and tRNA.

where k is the rate constant of the transformation of RT·tRNA to an activated form RT·tRNA*.

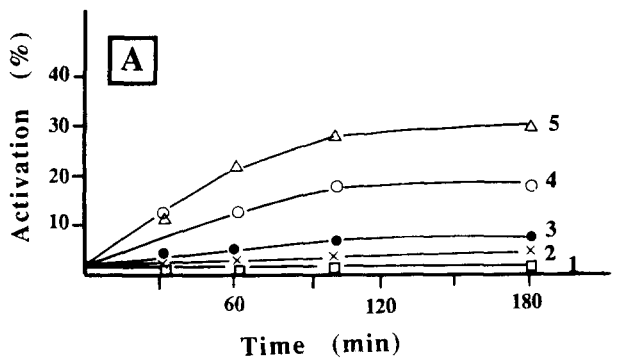
For the treatment of the experimental data, the graphical plot $1/k_{app} = 1/k + K_d/k_0$ was used, where k_{app} is the constant of enzyme activation at a fixed concentration of tRNA (x_0).

From the kinetic curves of enzyme activation, the (A_{max}−A) values were calculated, where A_{max} is the maximum level of

enzyme activation and A is the relative increase of RT activity during the time of enzyme preincubation with tRNA. The values of k_{app} were estimated using the linear part of the curve, dependence of ln $(A_{max}-A)$ upon the time (Fig. 2B). The K_d value of the complex RT·tRNA (130 ± 30 nM) and the value for the constant of maximal rate of activation (1.0×10^{-2} min^{-1}) were found from the linear dependence of k_{app} of enzyme activation upon the concentration of tRNA (Fig. 2C).

The K_d of the p51 subunit of the heterodimer evaluated from the fluorescence data (140 nM) was practically the same as the K_d found in the activation experiments (130 nM). These results suggest that the activation of the p66 catalytic subunit in the reaction of polymerization using poly(A)-oligo(dT) as template-primer can be due to the interaction of $tRNA^{Lys3}$ with the p51 subunit of HIV-1 RT p66/p51. Thus, activation could be regarded as the result of the induction of conformational changes in the interaction RT-tRNA.

We showed earlier the important role of the 3′-end OH group of the primer in its interaction with pro- and eucaryotic DNA polymerases [12,15,22–25]. Here our aim was to estimate the contribution of the 3′-terminal CCA of tRNA in the interaction with HIV-1 RT p66/p51. For this purpose a periodate-oxidized



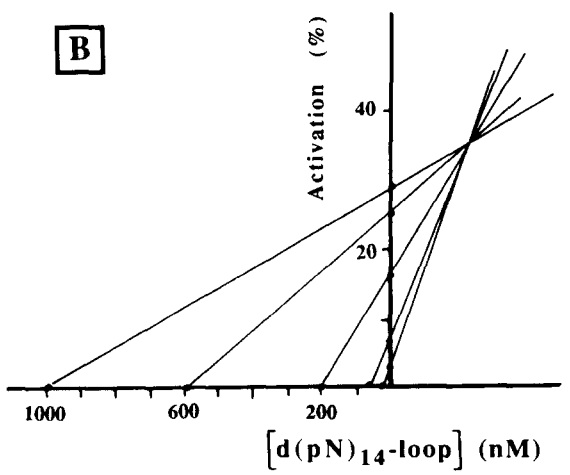


Fig. 3. (A) Activation of RT by the $d(pN)_{14}$-loop. 4 nM RT was preincubated with 0 (1), 20 (2), 50 (3), 100 (4) and 200 nM (5) $d(pN)_{14}$-loop. B: Determination of the K_d value for the complex RT·$d(pN)_{14}$-loop using the method of Eisenthal and Cornish-Bowden [17].

Table 1
Parameters characterizing the interaction of HIV-1 RT p66/p51 with $tRNA^{Lys3}$ and its derivatives determined from the activation experiments

Activator of RT	K_d (nM)	$\tau_{1/2}$ (min)	Maximum level of activation (%)*
tRNA	130 ± 40	21	300
tRNA analogs:			
$tRNA_{ox}$	150 ± 50	29	160
tRNA (–1)	200 ± 100	29	170
tRNA (–2)	300 ± 150	28	85
tRNA (–3)	300 ± 100	10	86
Anticodon analogs:			
$d(pN)_{15}$-loop	160 ± 70	10	38
$d(pN)_{14}$-loop	250 ± 50	22	37
$(pCpUpU)_5$	250 ± 50	30	50

*Errors of the value were within 5–30%.

tRNA without the native 3′-OH group ($tRNA_{ox}$) was used, as well as tRNA derivatives shortened by one, two or three nucleotides from the 3′-end. The activation level produced by the tRNA analogs was lower compared to natural tRNA (Table 1).

To evaluate the role of the anticodon loop (that has been shown to be important for the recognition of tRNA [5,6]) in the activation of RT, different oligonucleotides were used: (i) $d(pN)_{15}$-loop, a hairpin-like structure with an analogous sequence to that of the anticodon loop of $tRNA^{Lys3}$: d(CAG ACT **TTT** AAT CTG). The trinucleotide d(TTT) is an analog of the anticodon SUU, where S is thiouridine [26]. (ii) $d(pN)_{14}$-loop. This oligonucleotide has the sequence d(GGG ACT **CTT** AAC CC) and contains the trinucleotide d(CTT), an analog of CUU, the anticodon of $tRNA^{Lys1}$ and $tRNA^{Lys2}$. (iii) $ribo(CUU)_5$.

The preincubation of RT with each oligonucleotide led to a low but significant activation of the enzyme (Fig. 3A). The K_d values were calculated from activation experiments using the approach described above and the method of Eisenthal and Cornish-Bowden [17] (Fig. 3B). Both approaches gave the same values (Table 1). While the K_d and the half-time values of enzyme activation ($\tau_{1/2}$) for the oligonucleotides were similar to those for $tRNA^{Lys3}$ the activation level was significantly lower.

Summarizing the above facts the following model of interaction of tRNA with the enzyme can be proposed. At concentrations lower than K_{d2}, $tRNA^{Lys3}$ binds to p66. At higher concentrations, binding to p51 takes place inducing a conformational rearrangement of the heterodimer. As a consequence, the activity of the p66 catalytical subunit is enhanced. At the same time the 3′-CCA-terminal sequence, as well as most probably other structural elements of the tRNA may be important for the efficiency of HIV-1 RT structural changes.

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