# Interactions with $tRNA^{Lys}$ induce important structural changes in human immunodeficiency virus reverse transcriptase

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Retroviral RNA-dependent DNA polymerase (reverse transcriptase or RT) uses the 3'OH end of a cellular tRNA as primer to initiate DNA synthesis. Previous work with avian retrovirus has shown that reverse transcriptase is implicated in the selection of cellular virion-encapsidated tRNAs and has shown that the primer tRNA is positioned on the primer binding site near the 5' end of the viral RNA. These mechanisms support the idea that the retroviral polymerase should form complexes with primer tRNA and the specific encapsidated ones. The genomic sequence of human immunodeficiency virus (HIV) allows the prediction that $tRNA^{Lys}_3$ is the natural primer. In this article we show, using the mobility shift assay, that recombinant HIV reverse transcriptase is able to form a complex with bovine $tRNA^{Lys}$. By fluorescence studies and α-chymotrypsin analysis we have observed a modification of the enzyme conformation when reverse transcriptase is bound to the putative primer tRNA. This structural change is specific for $tRNA^{Lys}$ although the retroviral polymerase is able to interact with other tRNAs.

HIV; Reverse transcriptase; Primer tRNA recognition

## 1. INTRODUCTION

Human immunodeficiency virus is the causative agent of AIDS [1,2]. As is the case for all retroviruses, HIV genomic single stranded RNA is reverse transcribed into double stranded DNA by a virus encoded and virion-encapsidated RNA-dependent DNA polymerase [3]. Retroviral RT initiates DNA synthesis in vivo from the 3'OH end of a host tRNA which is partially annealed to a complementary region near the 5' end of the viral genome (primer binding site or PBS) [4]. Previous work of our laboratory has dealt with the specific recognition of bovine primer $tRNA^{Trp}$ by avian myeloblastosis virus (AMV) RT. The avian heterodimeric enzyme seems to be involved in the binding, in the partial unwinding and positioning of host $tRNA^{Trp}$ on the retroviral PBS [5,6]. More recently it was shown that a retrovirus-encoded small basic nucleocapsid protein is also involved in primer-template annealing [7].

Little is known about the interactions of RT and primer tRNA other than those in avian retroviruses. Recently, we have been involved in the study of the mechanism of action of recombinant HIV RT produced in transformed yeast [8,9]. The human retroviral DNA polymerase, as in the case of the avian RT, is an heterodimer. HIV RT is formed by a p66 subunit and a p51 subunit derived by proteolytic cleavage near the carboxyl-end of the p66 peptide [10]. This partial maturation is probably performed by the HIV encoded acid protease. HIV genomic nucleotide sequence determination [11] allowed the prediction that the host tRNA that serves as the primer for HIV RT is human $tRNA^{Lys}_3$ [12]. Animal cells contain several isoacceptor forms of $tRNA^{Lys}$. This tRNA is highly conserved as evidenced by sequence studies of $tRNA^{Lys}$ and $tDNA^{Lys}$ genes from rat, rabbit and human cells [13]. Our recent results demonstrate that HIV RT is able to form a complex with a highly purified bovine $tRNA^{Lys}$ fraction containing the three $tRNA^{Lys}$ isoacceptors [9]. We used both a direct approach by centrifugation in glycerol gradients and an indirect one, by the specific protection of enzyme activity by bovine $tRNA^{Lys}$ against thermal denaturation and trypsin digestion. Moreover, $tRNA^{Lys}$ is able to affect HIV RT DNA polymerase activity, the effect being different when the heterodimer (p66/p51) or the homodimer (p66/p66) enzyme forms are incubated with primer tRNA [14]

In this article we show, by gel retardation, that recombinant HIV RT expressed in yeast is able to form a complex with bovine $tRNA^{Lys}$ with a higher affinity than with $tRNA^{Trp}$, while other tRNAs are not recognized by the enzyme. By following the fluorescence emission of the enzyme in the presence of different tRNAs, as well as by the study of the ac-

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cessibility of the HIV RT-tRNA$^{Lys}$ complex to α-chymotrypsin, we show that the formation of the enzyme–primer tRNA complex can lead to dramatic protein structural changes.

## 2. MATERIALS AND METHODS

### 2.1. *Materials*

Unlabeled nucleotides, oligonucleotides and polynucleotides were obtained from Sigma Chem. Co or Pharmacia. Radio-isotopes were from the C.E.A.-Saclay (France) and Amersham. Beef tRNAs were purified as previously described [15]. Recombinant HIV RT expressed in yeast cells was purified as described before [9].

### 2.2. *Gel retardation experiments*

Binding reactions for bandshift assays contained, in 15 μl, 50 mM Tris-HCl, pH 8.0, 100 mM KCl, 15 mM $MgCl_2$, 6% glycerol (v/v), 0.5 mM 2-mercaptoethanol and tRNAs labeled at the 5′ end with [γ-$^{32}$P]ATP. Binding reactions were initiated by adding the RT and complexes were allowed to form for 10 min at 4°C. Then, the mixtures were loaded onto a 6% non-denaturing polyacrylamide gel containing TBE 1X (89 mM Tris-HCl, pH 8.3, 89 mM boric acid, 1 mM EDTA), prerun for 30 min at 12 V/cm and electrophoresed until a suitable separation was achieved at the same voltage. Migration was followed by adding Bromophenol blue to a sample containing only radioactive tRNA used as a control. After electrophoresis the gel was fixed with 5% methanol, 5% acetic acid for 30 min, dried and autoradiographed.

### 2.3. *Fluorescence emission spectra of HIV RT*

HIV RT and tRNAs were incubated in 50 mM phosphate buffer, pH 6.0, 100 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 1% glycerol for 10 min before the spectra were recorded at 25°C on a thermostated SPF-500 Aminco spectrofluorimeter. Excitations were performed at 295 nm. The fluorescence from the buffer was substracted from the fluorescence data of the enzyme.

### 2.4. *Digestion of HIV reverse transcriptase by α-chymotrypsin*

Recombinant HIV RT (0.03 μM) was preincubated in a buffer containing 50 mM Tris-HCl, pH 7.5, 0.5 mM 2-mercaptoethanol, 0.01% Triton-X100, 5% glycerol in the presence or absence of 0.4 μM tRNA for 10 min, then α-chymotrypsin was added for 30 min at 25°C. The proteolytic reaction was stopped by addition of 6 mM phenylmethylsulfonyl fluoride and the remaining activity of the RT tested as described before using poly rA-$dT_{12}$ as template-primer [9].

### 2.5. *Immunoblotting of HIV RT*

Digestion of HIV RT (1.2 μM) by increasing amounts of α-chymotrypsin for 10 min at 0°C was performed as described in the previous paragraph. Production of rabbit anti-HIV RT antibodies and immunoblotting were performed as already described [14].

## 3. RESULTS AND DISCUSSION

The specific complex formed between nucleic acids and proteins can be studied by different techniques. The approach of gel retardation has been extremely useful to study this kind of interactions [16]. Using this technique, we have shown the formation of a complex between a highly purified fraction of tRNA$^{Lys}$ (containing the 3 isoacceptors) and HIV RT. In Fig. 1 it can be seen that HIV RT can retard labeled tRNA$^{Lys}$ and that the addition of various amounts (up to 10 μM) of unlabeled tRNA$^{Lys}$ was able to displace the retarded radioactive tRNA$^{Lys}$ (lanes 1–4). We have also observed

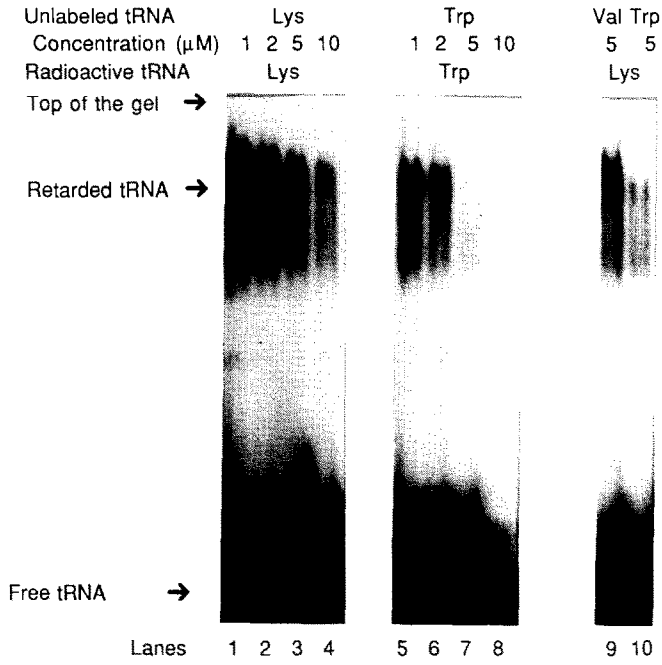


Fig. 1. Detection of tRNAs binding by HIV RT visualized by gel retardation electrophoresis. Each lane contains 2.5 μM recombinant HIV RT, the indicated concentrations of unlabeled tRNAs and 10 000 cpm of [$^{32}$P]tRNA$^{Lys}$ (lanes 1–4, 9–10) or 10 000 cpm of [$^{32}$P]tRNA$^{Trp}$ (lanes 5–8). Lanes 9–10 also contains 2 μM tRNA$^{Lys}$.

a complex between HIV RT and tRNA$^{Trp}$ by using the same technique (lanes 5–8). Unlabeled tRNA$^{Val}$ did not compete with tRNA$^{Lys}$ (lane 9) while 5 μM of unlabeled tRNA$^{Trp}$ can partly displace tRNA$^{Lys}$ (lane 10). In competition experiments, we have observed that the HIV RT/tRNA$^{Trp}$ complex is displaced by lower concentrations of tRNA$^{Lys}$ than the HIV RT/tRNA$^{Lys}$ complex by tRNA$^{Trp}$ (results not shown). This may indicate that HIV RT has a better affinity for its putative primer tRNA$^{Lys}$. The migration position of the retarded tRNA$^{Lys}$ or tRNA$^{Trp}$ is the same suggesting an equivalent stoichiometry of the complex between HIV RT and these two tRNAs.

HIV RT is extremely rich in aromatic residues. Thus, analysis of the *pol* domain sequence shows the presence of 19 tryptophan residues [11]. This property allowed us to study by spectrofluorometry the interactions between the enzyme and its primer tRNA. When RT was excited at 295 nm (mainly affecting the tryptophan residues), it showed an emission spectrum with a maximum at 338 nm (Fig. 2). The addition of tRNA$^{Lys}$ to RT induced an important shift of this maximum to about 355 nm. The new emission spectrum induced specifically by tRNA$^{Lys}$ is characteristic of the exposure to the solvent of tryptophan residues previously buried in the enzyme [17], suggesting that the complex between the retroviral DNA polymerase and its specific primer leads to important protein structural changes. Other tRNAs (Trp or Val) were not able to induce such a shift;

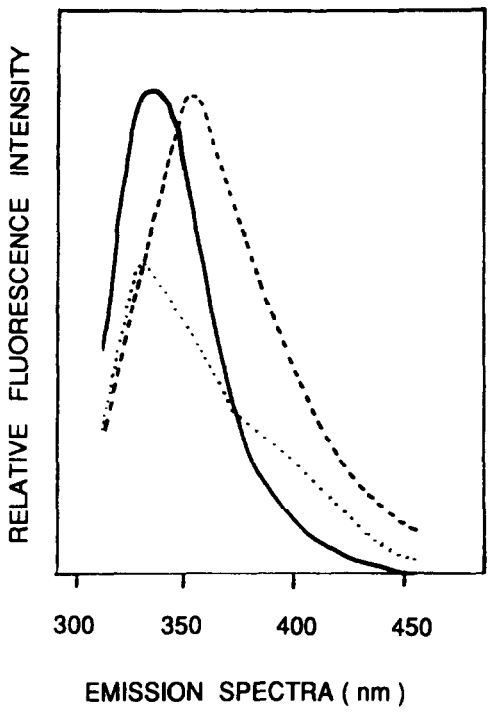


Fig. 2. Fluorescence emission spectra of HIV RT. HIV RT and tRNAs were incubated as described in section 2. Excitations were performed at 295 nm on 0.5 μM RT alone (——); 0.5 μM RT plus 0.5 μM $tRNA^{Lys}$ (-----); 0.5 μM RT plus 0.5 μM $tRNA^{Trp}$ (····).

in the presence of $tRNA^{Trp}$ we only observed a noticeable fluorescence quenching at 338 nm.

In order to verify that the binding of $tRNA^{Lys}$ could affect the enzyme structure, we studied the protease accessibility of HIV RT in the presence or absence of tRNA. We chose chymotrypsin, a protease hydrolyzing peptide bonds on the carboxyl side of aromatic amino acids. Moreover, it has been described that, under mild incubation conditions, α-chymotrypsin digests the p66 subunit to give a p66-p51 dimer very similar to the virion encapsidated polymerase [18]. Using those conditions, we found (Fig. 3a) that RT was relatively resistant to α-chymotrypsin (50% of enzyme activity was lost at 4 μM protease); but when RT was preincubated with $tRNA^{Lys}$, it became strongly sensitive to α-chymotrypsin digestion since a 50% inactivation was observed at 0.2 μM protease. No effect of $tRNa^{Val}$ on the activity of HIV RT in the presence of protease was observed. These results indicate that the accessibility of RT to the protease is greatly increased in the presence of the specific primer tRNA, thus confirming the fluorescent data that indicated important structural changes in the enzyme structure induced by $tRNA^{Lys}$.

The digestion of RT by α-chymotrypsin was also followed by immunoblotting after SDS-PAGE using an anti-HIV RT polyclonal antibody. Fig. 3b shows the protein bands obtained after incubation with different concentrations of α-chymotrypsin. Discrete degradation bands were observed. The results obtained when the enzyme was incubated in the presence or in the absence of $tRNA^{Lys}$ (Fig. 3c) showed that with the putative tRNA primer, fainter bands were observed. As equal amounts of protein were added to each lane, there are probably smaller peptides running out of the gel that can not be visualised. It is interesting to note that the enrichment in the p51 band, at low protease concentration does not lead to an important decrease in en-

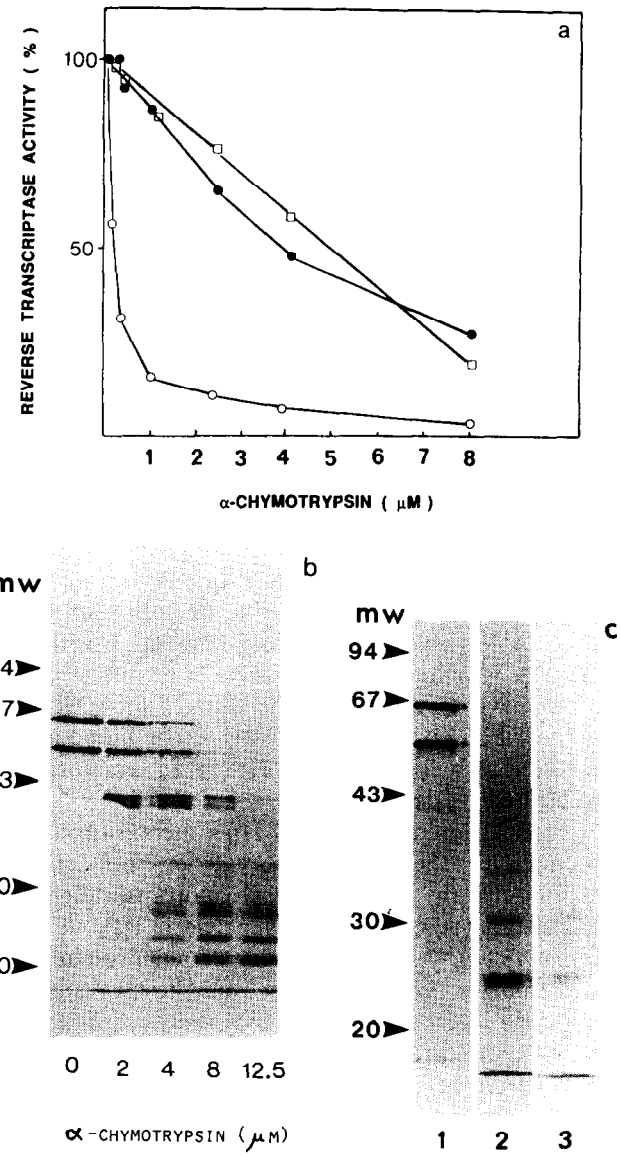


Fig. 3. Partial α-chymotypsin digestion of HIV RT. Panel a: α-chymotrypsin digestion of HIV RT (0.03 μM) in the absence of tRNA (□—□), in the presence of 0.4 μM of $tRNA^{Lys}$ (○—○), or 0.4 μM of $tRNA^{Val}$ (●—●). Results are expressed as the percentage of the remaining activity of the RT using polyrA-$dT_{12}$ as template-primer. Panel b: immunoblotting of HIV RT (1.2 μM) after digestion in the presence of increasing amounts of α-chymotrypsin. Panel c: comparison of the product of digestion of HIV RT (1.2 μM) by α-chymotrypsin (17 μM) visualized by immunoblotting. Lane 1, non-digested RT; lane 2, a 15 min digested RT without rTNA; lane 3, a 15 min digested RT after being preincubated in the presence of 20 μM $tRNA^{Lys}$. mw: molecular weight (94, 67, 43, 30, 20 kDa).

zyme activity. This is in agreement with the results described using the activity gel technique [19], as well as those observed when active recombinant HIV RT was expressed after extensive deletions at the 3′ end of the coding region of the RT gene [20], indicating that both the p51 and the p66 subunits carry the DNA polymerase activity.

It seems that three families of tRNA can be distinguished regarding their ability to form a complex with HIV RT: a first class with a high affinity, like $tRNa^{Lys}$, the natural primer, which is the best recognized and which induces an important change in conformation of the enzyme; a second class, including $tRNA^{Trp}$ and probably other tRNAs, which form a lower affinity complex with the enzyme inducing minor changes in HIV RT structure, and a third family of tRNA which is too poorly recognized by the enzyme to allow complex detection by the usual methods. The lower affinity of the HIV RT/$tRNA^{Trp}$ complex may explain that using glycerol gradient or nitrocellulose binding we did not detect this complex [9]. Association is also observed by gel retardation between HIV RT and each $tRNA^{Lys}$ isoacceptor purified by two-dimensional electrophoresis (D.R., unpublished results). Given the structural similarities between the 3 bovine isoacceptors these results are not surprising. It remains to be established if the affinity between the retroviral enzyme and each $tRNA^{Lys}$ isoacceptor is quantitatively different. It is interesting to point out that crosslinking studies showed very recently that the anticodon region of $tRNA^{Lys}$ seems to be in close interaction with HIV RT [21]. Whether this observation is related to the effect we have shown of the anticodon region of $tRNA^{Lys}$ on the DNA polymerase activity of the homodimeric and heterodimeric forms of HIV RT, remains to be established [14].

In conclusion, two different experimental approaches described in this work (fluorescence shift and accessibility of the enzyme to α-chymotrypsin digestion) indicate that the interactions between HIV RT and animal $tRNA^{Lys}$ can lead to important protein structural changes. Work performed on the mechanism of retrovirus replication may be extremely relevant to arrest retroviral proliferation. A significant part of these studies have been devoted to the interactions between RT and primer tRNA. Work is in progress to map the enzyme region(s) involved in $tRNA^{Lys}$ binding, as well as on the tRNA regions recognized by the retroviral polymerase.

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