Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7030–7034, September 1990
Biochemistry

Activity of synthetic tat peptides in human immunodeficiency virus type 1 long terminal repeat-promoted transcription in a cell-free system

(trans-activation/metal-binding proteins)

JANET JEYAPAUL, MOOLE R. REDDY, AND SHABBIR A. KHAN*

The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104

Communicated by Bruce Merrifield, June 25, 1990

ABSTRACT **The tat protein encoded by the human immunodeficiency virus type 1 is a potent trans-activator of gene expression from the viral long terminal repeat. The domains that are essential for trans-activation, a Pro-Xaa$_3$-Pro triad, a cysteine-rich metal-binding sequence motif, and a cluster of basic residues, are present within the N-terminal 57 residues of tat. To determine the structural requirements for tat function and the role of metal binding at the transcription level alone, tat-(1–86) (full-length tat peptide), tat-(1–57), and tat-(1–47) were chemically synthesized. These peptides as well as the Cd^{2+} and Zn^{2+} complexes of tat-(1–86) and tat-(1–57) were evaluated for stimulation of transcription from the human immunodeficiency virus type 1 long terminal repeat by using cell-free *in vitro* methods. All three peptides produced a 7- to 9-fold increase over the basal level of transcription at a peptide concentration of 0.4 μM. Interestingly, at 4 μM, both tat-(1–57) and tat-(1–86) inhibited even the basal level of transcription. In contrast, tat-(1–47), which lacks the basic domain (residues 49–57), exhibited full stimulatory activity at 4 μM. Our data suggest, therefore, that the basic region may be responsible for the observed inhibitory activity of tat-(1–86) and tat-(1–57). Furthermore, binding to Zn^{2+} and not to Cd^{2+} ions only slightly augments (≈2-fold) the activity of the tat peptides.**

The human immunodeficiency virus (HIV-1) contains at least six other genes in addition to the three characteristic retroviral genes, *gag*, *pol*, and *env*. Three of these genes, *tat*, *rev*, and *nef*, have been shown to play important regulatory roles in the viral gene expression (reviewed in refs. 1 and 2). The *tat* and *rev* gene products positively regulate the HIV-1 long terminal repeat (LTR)-directed gene expression in cultured cells (3) and are thus essential for viral replication and virus-mediated cytopathicity (4, 5). The mechanism by which tat mediates trans-activation of the HIV-1 LTR is not clear. The role of the tat protein appears to be bimodal. tat increases the steady-state level of viral mRNA by direct transcriptional activation from HIV-1 LTR (6, 7) or by acting as a transcriptional anti-terminator (8–10) and has also been shown to stimulate translation (11). tat functions through a trans-acting responsive region (TAR) located at +17 to +44 relative to the transcription start site (+1) in the viral LTR (12). The TAR sequence forms a stable stem–loop structure (13) and is present on the 5′ end of all HIV-1 transcripts. Mutants defective in TAR do not respond to tat. While it is not clear whether tat acts through the DNA or RNA form of TAR, there is evidence that TAR is recognized by tat as a nascent RNA (14). More recently, tat has been shown to specifically interact with TAR RNA (15). Several cellular factors have been shown to govern the trans-acting function of the tat protein (reviewed in ref. 2). However, the exact interplay of the regulatory elements of HIV-1 and the cellular factors that influence the trans-activation is not known.

The 86-residue tat protein (HTLV-III genomic clone HXb3, ref. 16) contains a triad of Pro-Xaa$_3$-Pro (positions 6–18), a Cys-rich metal-binding sequence motif (positions 22–37), and a cluster of basic residues (positions 49–57) (Fig. 1). Studies using recombinant mutants have shown that these regions are all required for the trans-acting function of tat (17–21). More recent experiments have shown that tat-(1–48) has an activity comparable to that of the wild-type tat when delivered to the cell nucleus (11). Further, the basic region is required for nuclear localization of tat (18, 20). The Cys-rich metal-binding sequence of tat could have a significant role in trans-activation (similar sequence motifs present in several proteins have been shown to play important roles in DNA binding and gene expression; reviewed in refs. 22–24). Mutations of the tat gene in the Cys-rich region resulted in a marked decrease in trans-activation (18, 20). However, conflicting results have been reported in studies with synthetic peptides in a cellular-uptake assay (25–27) using tat-(37–62), which lacks the Cys-rich region. The role of the Cys-rich region in trans-activation thus remains unclear. Bacterially expressed tat has been shown to bind Cd^{2+} and Zn^{2+} (28), but the role of metal binding in the function of tat and the oxidation states of the Cys residues in the native protein are not known. Analysis of chemically homogeneous tat and rev in cell-free *in vitro* transcription and translation systems offers an approach to a clear understanding of the mechanism of trans-activation of the HIV-1 LTR. In this report, using chemically synthesized tat-(1–86) (full-length tat peptide), tat-(1–57) (positions 1–57), and tat-(1–47) (positions 1–47) in a cell-free transcription system, we show that (*i*) transcriptional stimulation of HIV-1 LTR in HeLa cell extract requires optimal concentrations of tat, (*ii*) the basic region in tat may have a negative regulatory effect on transcription from HIV-1 LTR at high peptide concentrations, and (*iii*) although metal binding is essentially not required for tat function at the transcription level, complexation with Zn^{2+} but not with Cd^{2+} enhances further the activity of tat peptides to a small extent.

MATERIALS AND METHODS

Materials. Reagents for peptide synthesis were from Peninsula Laboratories and Fluka. DNA templates for the *in vitro* transcription assays were obtained by *Eco*RI digestion of recombinant plasmids pCD12 (a gift of Flossie Wong-Staal, National Institutes of Health), pLTRCAT/D (kindly provided by A. Srinivasan, The Wistar Institute), and pSV40-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV-1, human immunodeficiency virus type 1; LTR, long terminal repeat; TAR, trans-acting responsive region.

*To whom reprint requests should be addressed.

```
MetGluProValAspProArgLeuGluProTrpLysHisProGlySerGlnProLys
            """"""""""""""""""""""""""""""""""""""""""""""""

ThrAlaCysThrAsnCysTyrCysLysLysCysCysPheHisCysGlnValCysPhe
      ***************************************************

IleThrLysAlaLeuGlyIleSerTyrGlyArgLysLysArgArgGlnArgArgArg
                              ++++++++++++++++++++++++++

ProProGlnGlySerGlnThrHisGlnValSerLeuSerLysGlnProThrSerGln

SerArgGlyAspProThrGlyProLysGlu
```

FIG. 1. Predicted primary structure of the tat protein of HIV-1 from the cDNA sequence. Regions containing the Pro-Xaa$_3$-Pro triad ("""""), the Cys-rich region (****), and the basic nuclear localization signal (++++) have all been demonstrated to be required for full trans-acting function of the tat protein.

CAT (Promega). pCD12 contains the U3 and R regions of the HIV-1 LTR fused to the bacterial chloramphenicol acetyltransferase (CAT) gene, and pLTRCAT/D is similar to pCD12 except that it lacks nucleotides +14 to +18 of the TAR. After *Eco*RI digestion, the sizes of the runoff transcripts are 332, 327, and 294 bases from pCD12, pLTRCAT/D, and pSV40CAT, respectively. HeLa whole-cell lysate was from Bethesda Research Laboratories, and HeLa nuclear extracts were prepared in our laboratory (29).

Peptide Synthesis. Peptides were assembled on phenylacetamidomethyl (PAM) resin (30) on an automated synthesizer (Advanced ChemTech). Trifluoroacetic acid/methylene chloride (7:3, vol/vol) was used for removal of the N^{α}-butoxycarbonyl (Boc) group, and neutralization and couplings were carried out in *N*-methylpyrrolidinone. The benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate/1-hydroxybenzotriazole-mediated (31) coupling reactions of Boc-protected amino acids [except for His, which was incorporated by using symmetric anhydride of Boc-His(tosyl)] were repeated to ensure maximum yield (>99.5%) at each step, and the coupling efficiency was monitored by a quantitative ninhydrin analysis. The progress of the synthesis was also monitored by amino acid analysis of aliquots of peptide resin withdrawn after incorporation of every 10 residues. Acid digestion of peptide resin was done by using 12 M hydrochloric acid/propionic acid (1:1, vol/vol; 130°C, 10 hr), and samples were analyzed by the Pico·Tag method (Waters). Side-chain protecting groups used were benzyl ester (Asp and Glu), benzyl ether (Ser and Thr), 4-bromobenzyloxycarbonyl (Tyr), tosyl (His and Arg), formyl (Trp), 2-chlorobenzyloxycarbonyl (Lys), and 4-methylbenzyl (Cys). Removal of the side-chain protecting groups and cleavage of the assembled peptide from the resin was achieved by using the low/high hydrogen fluoride procedure (32). The crude peptides were treated with 0.5 M dithiothreitol at 50°C for 10 min, to ensure complete reduction of the Cys residues, and then purified by HPLC on a C_8 column with a water/acetonitrile gradient containing 0.1% trifluoroacetic acid. The purity of peptides was >90% by HPLC analysis. The amino acid composition of the peptides as determined by the Pico·Tag method was consistent with the expected ratios.

Metal-Binding Studies. Spectra of the tat peptides and their metal complexes were recorded on a Shimadzu UV265 spectrophotometer. A solution of peptide (≈1 mg) in 0.5 M dithiothreitol (0.5 ml) was heated at 50°C for 10 min and passed through a Sephadex G-50 column equilibrated with molecular oxygen-free water, and the eluate fraction (2 ml) containing the peptide was diluted to 10 ml with 20 mM Tris·HCl (pH 7.5). To 1.0 ml of this solution in a cuvette, calculated amounts of $CdCl_2$ or $ZnCl_2$ dissolved in water were added, and the spectra were recorded. To prevent air oxidation of Cys thiol groups, all manipulations with purified peptides were performed in a nitrogen-purged anaerobic chamber.

***In Vitro* Transcription Assay.** Transcription of *Eco*RI-cut plasmids in HeLa nuclear or whole-cell extract was carried out following essentially the procedure described by Okamoto and Wong-Staal (33). A typical 50-μl reaction mixture consisted of 20 mM Hepes (pH 7.9), 60 mM KCl, 8 mM $MgCl_2$, 0.5 mM EDTA, 1.5 mM dithiothreitol, 7% glycerol, 30 μl of HeLa whole-cell extract (or 9 μl of nuclear extract), 1.5–2 μg of template DNA, 250 μM ATP, 250 μM GTP, 250 μM CTP, 25 μM UTP, 4 mM creatine phosphate, and 5 μCi (1 Ci = 37 GBq) of [α-^{32}P]UTP. After incubation at 30°C for 60 min, samples were successively treated with RNase-free DNase (50 μg/ml) for 5 min at 30°C and proteinase K (0.7 mg/ml) for 3 min at room temperature. The reaction mixture was extracted three times with phenol/chloroform/2-pentanol (20:20:1, vol/vol) and then diluted with cold ethanol. The precipitated RNA was denatured by treatment with glyoxal and analyzed by electrophoresis in an agarose gel (1.8%) containing SDS (1%). The recovery of the total RNA was monitored by visualization of the 28S and 18S rRNAs. The gel was fixed by treatment with streptomycin sulfate (1%) for 20 min, dried, and autoradiographed for 3–12 hr. The runoff transcript was quantitated by scintillation counting of gel slices containing the transcript bands and slices of equal size of gel immediately below the transcript as well as from scans of autoradiographs by using a laser densitometer (Ultroscan XL, LKB).

For evaluation of the peptide-induced transcription of HIV-1 LTR, peptide dissolved in the HeLa-cell lysate dilution buffer was directly added to the transcription mixture. Peptide (≈200 mg) was dissolved in the buffer (50 μl), and the pH was adjusted to 8.0 with 0.1 M Tris. Aliquots of this solution were diluted to the required concentrations with the buffer. The peptide content of the stock solution was determined by amino acid analysis of an aliquot after hydrolysis.

The Cd^{2+} and Zn^{2+} complexes of peptides were formed (monitored by UV spectroscopy) by treatment of pure dithiothreitol-reduced peptide in 10 mM Tris·HCl (pH 7.4) with calculated amounts of the corresponding metal chloride. After lyophilization, the complexes were dissolved in lysate dilution buffer to the required concentrations.

RESULTS AND DISCUSSION

Our interest is to study the structure–function relationships of the tat protein of HIV-1 independently at the transcription and translation levels by using cell-free *in vitro* methods. The objective of the present study was to ascertain the differences in the activity of full-length tat, tat-(1–57), and tat-(1–47) and to determine the role of the basic domain (positions 49–57) as well as metal binding in transcription from HIV-1 LTR. We evaluated synthetic tat peptides for stimulation of transcrip-

tion from HIV-1 LTR in a cell-free transcription system. An *in vitro* assay that uses a recombinant construct, pCD12, containing the U3 and R regions of HIV-1 LTR fused to the chloramphenicol acetyltransferase gene with *Eco*RI and *Nco* I restriction sites to permit a runoff transcription assay has been used to show the presence of trans-acting functions in the nuclear extracts of HIV-1-infected cells (33). The trans-acting activity of these extracts could have been due to tat and/or cellular factors. Results presented here show that this *in vitro* assay could be successfully used to evaluate the trans-acting function of tat at the transcription level. This assay may also serve in the elucidation of the mechanisms involving tat in the initiation of transcription, RNA processing, and RNA stability.

Three peptides, tat-(1–86), tat-(1–57), and tat-(1–47), were synthesized by the solid-phase method and purified to homogeneity by liquid chromatographic procedures. The peptides were evaluated for stimulation of transcription from HIV-1 LTR by using *Eco*RI-linearized pCD12 in HeLa nuclear or whole-cell extract. All three peptides showed transcriptional enhancement: a 7- to 9-fold increase in the level of the 332-base transcript was observed in the presence of 0.4 μM peptide. The amount of the transcript formed depended on the type of HeLa extract, nuclear or whole cell, and varied with different preparations. This difference was apparent even in the basal level of transcription (in the absence of peptide). However, in all experiments, the amount of the transcript clearly increased in the presence of peptide compared with the control.

A further analysis of the stimulatory effect revealed that the peptides enhanced transcription in a dose-dependent manner.

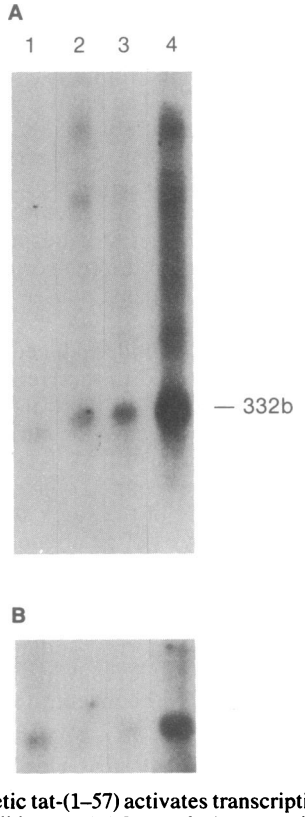

FIG. 2. Synthetic tat-(1–57) activates transcription of HIV-1 LTR in HeLa whole-cell lysate. (*A*) Lanes 2–4, respectively, show levels of transcription from *Eco*RI-cut pCD12 (40 μg/ml) in the presence of 0.004, 0.04, and 0.4 μM tat-(1–57) compared with the basal level of transcription in the absence of the peptide (lane 1). (*B*) Transcript levels in the absence (lane 1) or presence of 40 μM (lane 2), 4 μM (lane 3), or 0.4 μM (lane 4) tat-(1–57). Quantitation of the 332-base (332b) transcript was done by using a laser densitometer as well as by scintillation counting of gel slices containing the bands.

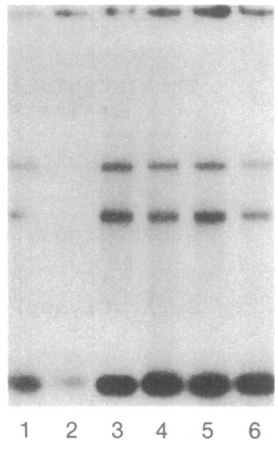

FIG. 3. Transcriptional stimulatory activity of tat-(1–47) compared with tat-(1–86) in HeLa nuclear extracts. The levels of transcripts in the presence of 4, 0.4, and 0.04 μM tat-(1–86) (lanes 2–4) or 4 and 0.4 μM tat-(1–47) (lanes 5 and 6) are compared with basal level transcription in the absence of peptide (lane 1).

Compared with the control, tat-(1–57) showed <2-fold enhancement at 0.004 and 0.04 μM and about a 25-fold increase at 0.4 μM when analyzed in HeLa whole-cell lysate (Fig. 2*A*). In HeLa nuclear extract, tat-(1–86), tat-(1–57), and tat-(1–47) produced a 7- to 9-fold increase at 0.4 and 0.04 μM (Fig. 3). A very interesting finding was that at a concentration of 4 μM tat-(1–86) and tat-(1–57) inhibited even the basal level of transcription (Figs. 2*B* and 3). This inhibitory effect was specific to tat-(1–86) and tat-(1–57) as an unrelated peptide (Lys-Glu-Gly-Cys-Val-Glu-Lys-Ile-Gly-Gly-Trp-Leu-Arg-Lys-Asn-Tyr) at concentrations as high as 40 μM did not show a similar effect (data not shown). In addition, tat-57S, a synthetic analogue of tat-(1–57) in which all the seven Cys residues were substituted with Ser, did not stimulate transcription at 0.4 and 0.04 μM concentrations and at 4 μM inhibited the basal level of transcription (data not shown). Furthermore, the inhibitory effect was not observed in the presence of 4 μM tat-(1–47) (Figs. 3 and 4). Our data indicate that optimal concentrations of tat-(1–86) and tat-(1–57) are required for a maximum stimulation of transcription from HIV-1 LTR in HeLa cell extracts.

Studies have shown that trans-activation of HIV-1 LTR by the tat protein requires the TAR sequences (12, 34). To determine whether the observed enhanced transcription in the presence of the tat peptides is TAR dependent, we studied the

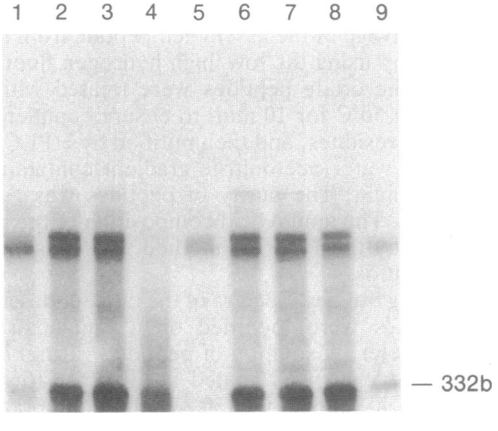

FIG. 4. Relative activities of tat-(1–86), tat-(1–57), and tat-(1–47) in transcription from HIV-1 LTR in HeLa cell nuclear extract. The levels of the 332-base (332b) transcript in the presence of 4, 0.4, 0.04, and 0.004 μM tat-(1–86) (lanes 1–4), 4, 0.4, and 0.04 μM tat-(1–57) (lanes 5–7), or 4 μM tat-(1–47) (lane 8) are compared with basal level transcription in the absence of peptide (lane 9).

Table 1. Enhancement of HIV-1 LTR-directed transcription by tat-(1–86) in HeLa cell extracts requires TAR

Plasmid	tat-(1–86), μM	cpm from transcript*	Fold increase of transcription
pCD12	0	1,289	—
	0.4	12,082	8
	0.04	9,344	6
pSV40CAT	0	894	—
	0.4	923	0
	0.04	1,026	0
pLTRCAT/D	0	1,125	—
	0.4	942	0
	0.04	906	0

*Radioactivity of gel slices containing each transcript was measured by scintillation counting. The background cpm were subtracted from the cpm of the specific transcript. Variability among different experiments was ±12% (SD).

effect of tat-(1–86) on transcription from pLTRCAT/D, which is deficient in nucleotides +14 to +18 of TAR. Studies have shown that pLTRCAT/D does not respond to constitutively expressed tat protein (34). Consistent with this, in our assays tat-(1–86) did not stimulate transcription from pLTRCAT/D, and also it did not trans-activate the simian virus 40 promoter. The results of these studies are summarized in Table 1.

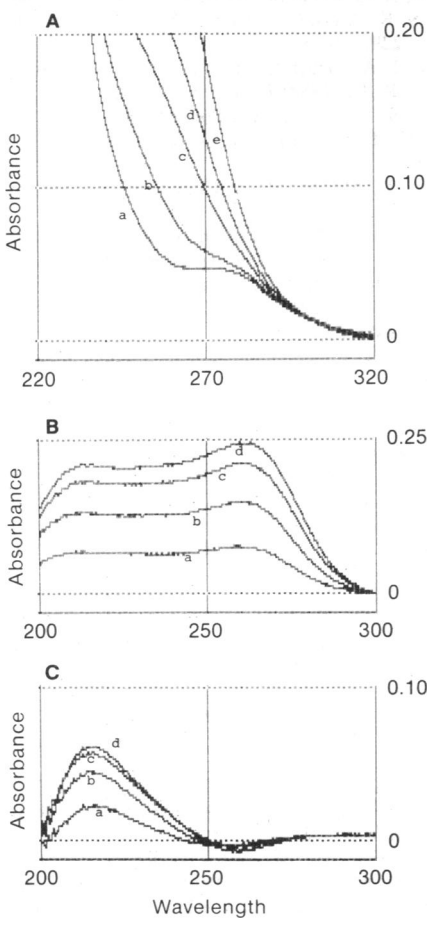

FIG. 5. Synthetic tat-(1–86) binds Zn^{2+} or Cd^{2+}. (*A*) UV absorption spectra of reduced tat-(1–86) (curve a) and tat-(1–86) with 0.5, 1.0, 1.5, and 2.0 molar equivalents (curves b–e, respectively) of $CdCl_2$. (*B*) Difference UV absorption spectra of tat-(1–86) with 0.5, 1.0, 1.5, and 2.0 molar equivalents (curves a–d, respectively) of $CdCl_2$. (*C*) Difference UV absorption spectra of tat-(1–86) with 0.5, 1.0, 1.5, and 2.0 molar equivalents (curves a–d, respectively) of $ZnCl_2$.

The inhibitory effect observed with tat-(1–86) and tat-(1–57) seems analogous to the "squelching" phenomenon observed in other systems (23). For example, in yeast overproduction of intact GAL4 has been observed to inhibit transcription of genes lacking the GAL4 binding site, such as the *CYC1* gene (35). The U3 region of HIV-1 LTR has been shown to contain functional binding domains for several transcription factors, including Sp1 and NF-kB (reviewed in ref. 2). In our assay system, inhibition of even the basal level of transcription by high concentrations of tat-(1–86) and tat-(1–57) but not by an unrelated peptide indicates that possibly tat interacts with one or more cellular factors off the DNA and that high concentrations of tat saturate a cellular factor(s) rendering incomplete the formation of the active transcription complex.

Mutation studies have shown the basic domain in tat to be a nuclear localization signal. Our data show that at 4 μM tat peptides containing the basic region of tat repressed transcription from the HIV-1 LTR. We found that deletion of the basic domain (positions 49–57) from tat-(1–57) eliminates the inhibitory activity of the peptide without diminishing its trans-acting function. It is possible that the basic region might interact with an acidic region(s) of a cellular factor(s) present in the HeLa cell extracts. The activation regions of several cellular factors have been shown to contain negatively charged residues, probably in amphipathic α-helical structures (reviewed in ref. 23). It is conceivable that at high concentrations the basic peptides could saturate the factor(s), present in limiting amounts in the *in vitro* assays, thereby inhibiting transcription. Alternatively, a nonspecific tat–DNA interaction at high concentrations of the peptides may also repress transcription (36).

We then investigated the role of Zn^{2+} and Cd^{2+} binding with tat-(1–86) and tat-(1–57) in transcription from HIV-1 LTR. The metal-binding activity of tat-(1–86) and tat-(1–57) was established by UV absorption spectroscopy. Both peptides complexed with Zn^{2+} and Cd^{2+}, and our spectra of the peptide–metal complexes (Fig. 5) are quite similar to those reported for bacterially expressed tat (28). Analysis of these

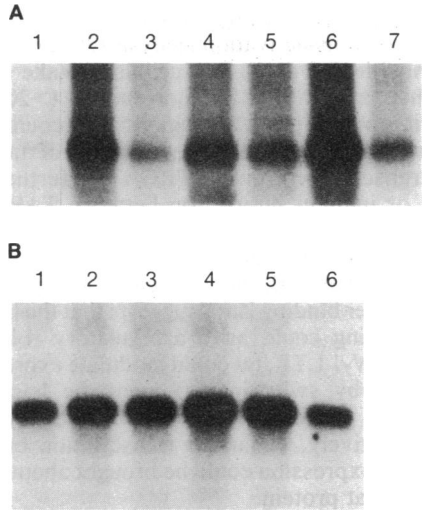

FIG. 6. The synthetic tat-induced transcriptional stimulation of HIV-1 LTR is further increased by binding to Zn^{2+}. (*A*) The level of the 332-base transcript in the presence of oxidized (lanes 2 and 3), Cd^{2+}-complexed (lanes 4 and 5), or Zn^{2+}-complexed (lanes 6 and 7) tat-(1–57). The final concentrations of peptide or peptide–metal complex were 0.4 μM (lanes 2, 4, and 6) or 0.04 μM (lanes 3, 5, and 7). Lane 1, transcription in the absence of peptide. (*B*) Transcription in the presence of 0.4 μM oxidized tat-(1–86) (lane 2), 0.4 μM tat-(1–86) complexed to Cd^{2+} (lane 3), or 0.4, 0.04, or 0.004 μM tat-(1–86) complexed to Zn^{2+} (lanes 4–6, respectively). Lane 1, transcription in the absence of the peptide.

complexes showed that binding of Cd^{2+} did not greatly influence the activity of tat-(1–86) and tat-(1–57) (Fig. 6). However, at 0.04 μM, tat-(1–57)–Cd^{2+} was found to be more active than tat-(1–57). Interestingly, binding to Zn^{2+} further enhanced (≈2-fold) the activity of tat-(1–86) and tat-(1–57). Addition of 10 mM Zn^{2+} or 1 mM Cd^{2+} increased the activity (2- to 3-fold) of bacterially expressed tat (26). Our results show that the peptide–Zn^{2+} complexes are operative at the transcription level and thus could contribute to the observed enhanced trans-activation.

There is strong evidence for several proteins (*Xenopus* transcription factor IIIA, Sp1, steroid hormone receptor, and yeast factors GAL4 and ADR1) that DNA binding is a property specifically conferred by complexation to Zn^{2+} (reviewed in refs. 22–24). In transcription factor IIIA, binding to Zn^{2+} has been shown to form an independent structural domain termed the "zinc finger" in which two Cys and two His ligands coordinate with the metal atom in a tetrahedral geometry to form a loop structure (37). Though Zn^{2+} is implicated in specific DNA binding, the role of the metal in transcription has been shown only in the case of transcription factor IIIA, where Zn^{2+} is shown to be necessary for both specific DNA binding and transcription of the 5S RNA gene (38). Metal-binding motifs in trans-activation could exert their effect either after binding to the DNA template or in a post-binding event by means of interaction with a target protein of the transcription system. Our results show that tat-(1–57) and tat-(1–86) were able to trans-activate in the absence of Zn^{2+} and complexation with Zn^{2+} only further enhanced the activity of the peptide. It is interesting that though bacterially produced tat has been shown to have a greater affinity for Cd^{2+} than Zn^{2+} (28), trans-activation by tat-(1–57) and tat-(1–86) was enhanced only by Zn^{2+} binding. Whether this is due to a particular structural feature conferred by Zn^{2+} needs further investigation.

Results presented in this report show that synthetic peptides can be used in cell-free transcription systems for structure–function studies of trans-acting factors. Our data that tat-(1–47) is as active as tat-(1–86) suggest that the N-terminal 47-residue sequence of tat has the required structural features for transcriptional activation of HIV-1 LTR. Since previous trans-activation analysis (25–27) and our own studies (unpublished data) in a cellular uptake assay have revealed that tat-(1–86) is far more active (>20-fold) than tat-(1–57), it is possible that the region 57–86 could play a role in posttranscription events. The inability of tat-(1–86) to stimulate transcription from pLTRCAT/D further indicates that direct or indirect interaction between TAR and tat is essential for tat function. The repression of transcription exhibited by tat-(1–86), tat-(1–57), and tat-57S at high concentrations together with the augmented activity of the first two peptides after binding Zn^{2+} suggests that the basic region and metal binding could have a regulatory role in trans-activation of HIV-1 LTR. tat could modulate expression from the viral LTR by switching off even the basal level of transcription, a property probably conferred by the basic region. Alternatively, enhanced transcription essential for extensive gene expression could be brought about by binding of Zn^{2+} to the tat protein.

We thank Professors E. Premkumar Reddy and A. Srinivasan (The Wistar Institute) for helpful suggestions and Ms. M. Desai for the preparation of the HeLa cell extracts. This work was supported by Grants AI25380 and AI27422 from the National Institutes of Health (to S.A.K.).

1. Varmus, H. (1988) *Genes Dev.* **2,** 1055–1062.
2. Cann, A. J. & Karn, J. (1989) *AIDS* **3,** Suppl. 1, S19–S34.
3. Sodroski, J., Patarca, T., Rosen, C., Wong-Staal, F. & Haseltine, W. A. (1985) *Science* **229,** 74–77.
4. Fisher, A. G., Feinberg, M. B., Josephs, S. F., Harper, M. E., Marselle, M. L., Reyes, G., Gonda, M. A., Aldovini, A., Debouck, C., Gallo, R. C. & Wong-Staal, F. (1986) *Nature (London)* **320,** 367–371.
5. Sadie, R. M., Benter, T. & Wong-Staal, F. (1988) *Science* **239,** 910–913.
6. Wright, C. M., Felber, B. K., Paskalis, H. & Pavlakis, G. N. (1986) *Science* **234,** 988–992.
7. Muesing, M. A., Smith, D. H. & Capon, D. J. (1987) *Cell* **48,** 691–701.
8. Kao, S.-Y., Calman, A. F., Luciw, P. A. & Peterlin, B. M. (1987) *Nature (London)* **330,** 489–493.
9. Toohey, M. G. & Jones, K. A. (1989) *Genes Dev.* **3,** 265–282.
10. Laspia, M., Rice, A. & Mathews, M. (1989) *Cell* **59,** 283–292.
11. Braddock, M., Chambers, A., Wilson, W., Esnouf, M. P., Adams, S. E., Kingsman, A. J. & Kingsman, S. M. (1989) *Cell* **58,** 269–279.
12. Garcia, J. A., Harrich, D., Soultanakis, E., Wu, F., Mitsuyasu, R. & Gaynor, R. B. (1989) *EMBO J.* **8,** 765–778.
13. Feng, S. & Holland, E. C. (1988) *Nature (London)* **334,** 165–167.
14. Berkhout, B., Silverman, R. & Jeang, K.-T. (1989) *Cell* **59,** 273–282.
15. Dingwall, C., Ernberg, I., Gait, M. J., Green, S. M., Heaphy, S., Karn, J., Lowe, A. D., Singh, M., Skinner, M. A. & Valerio, R. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6925–6929.
16. Arya, S. K., Guo, C., Josephs, S. F. & Wong-Staal, F. (1985) *Science* **229,** 69–73.
17. Garcia, J. A., Harrich, D., Pearson, L., Mitsuyasu, R. & Gaynor, R. B. (1988) *EMBO J.* **7,** 3143–3147.
18. Ruben, S., Perkins, A., Purcell, R., Joung, K., Sia, R., Burghoff, R., Haseltine, W. A. & Rosen, C. A. (1989) *J. Virol.* **63,** 1–8.
19. Hauber, J., Malim, M. & Cullen, B. R. (1989) *J. Virol.* **63,** 1181–1187.
20. Kuppuswamy, M., Subramanian, T., Srinivasan, A. & Chinnadurai, G. (1989) *Nucleic Acids Res.* **17,** 3551–3561.
21. Seigel, L. J., Ratner, L., Josephs, S. J., Derse, D., Feinberg, M. B., Reyes, G. R., Obriez, S. J. & Wong-Staal, F. (1986) *Virology* **148,** 226–231.
22. Klug, A. & Rhodes, D. (1987) *Trends Biochem. Sci.* **12,** 464–469.
23. Ptashne, M. (1988) *Nature (London)* **335,** 683–689.
24. Mitchell, P. J. & Tjian, R. (1989) *Science* **245,** 371–378.
25. Green, M. & Loewenstein, P. M. (1988) *Cell* **55,** 1179–1188.
26. Frankel, A. D. & Pabo, C. O. (1988) *Cell* **55,** 1189–1193.
27. Frankel, A. D., Biancalana, S. & Hudson, D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7397–7401.
28. Frankel, A. D., Bredt, D. S. & Pabo, C. O. (1988) *Science* **240,** 70–73.
29. Shapiro, D. J., Sharp, P. A., Wahli, W. W. & Keller, M. J. (1988) *DNA* **7,** 47–55.
30. Barany, G. & Merrifield, R. B. (1980) in *The Peptides: Analysis, Synthesis, Biology,* eds. Gross, E. & Meienhofer, J. (Academic, New York), Vol. 2, pp. 1–284.
31. Hudson, D. (1988) *J. Org. Chem.* **53,** 617–624.
32. Tam, J. P., Heath, W. F. & Merrifield, R B. (1983) *J. Am. Chem. Soc.* **105,** 6442–6455.
33. Okamoto, T. & Wong-Staal, F. (1986) *Cell* **47,** 29–35.
34. Bohan, C. A., Robinson, R. A., Luciw, P. A. & Srinivasan, A. (1989) *Virology* **172,** 573–583.
35. Gill, G. & Ptashne, M. (1988) *Nature (London)* **334,** 721–724.
36. Levine, M. & Manley, J. (1989) *Cell* **59,** 405–408.
37. Diakun, G. P., Fairall, L. & Klug, A. (1986) *Nature (London)* **324,** 698–699.
38. Hanas, J. S., Hazuda, D. J., Bogenhagen, D. F., Wu, F. & Wu, C.-W. (1983) *J. Biol. Chem.* **258,** 14120–14125.