AIDS in Africa is characterized by the equal distribution of mortality between the two genders because of highly virulent human immunodeficiency virus type 1 (HIV-1) strains. The viral protein Tat trans-activates viral gene expression and is essential for HIV-1 replication. We chemically synthesized six different Tat proteins, with sizes ranging from 86 to 101 residues, from HIV-1 isolates located in different parts of the world including highly virulent African strains. Protein purification, mass spectroscopy, and amino acid analysis showed that the synthesis was successful in each case but with different yields. We show that all have the ability to bind the HIV long terminal repeat (LTR) RNA trans-activation response element (TAR) region, involved in Tat-mediated trans-activation, but structural heterogeneities are revealed by circular dichroism. These Tat synthetic proteins cross membranes but differ in their ability to trans-activate an HIV LTR-reporter gene in stably transfected HeLa cells. Two Tat proteins from virulent African HIV-1 strains were much more active than those from Europe and the United States. The interferon-induced kinase (PKR), involved in cell antiviral defense, phosphorylates only Tat variants corresponding to less or nonvirulent HIV-1 isolates. Our results indicate that the high virulence of some African HIV-1 strains could be related to Tat activity. The HIV-1 encodes regulatory proteins such as Tat, which profoundly affect the course of viral gene expression in infected cells. Tat stimulates the production of full-length viral transcripts by RNA polymerase II (1, 2). This function is generally associated with the ability of Tat to bind the nascent leader RNA hairpin TAR located at the 5'-end of all HIV-1 mRNAs (3). Tat presents a highly conserved basic region that can adopt an extended structure to fit into the TAR major groove (4). Furthermore, this basic region provides for Tat the capacity to cross membranes (5, 6).

However, the role of Tat in the HIV cycle is more than to facilitate the elongation of HIV mRNA. Tat is required for efficient HIV-1 reverse transcription (7). Activities of different cellular kinase is modulated by Tat, which results in the activation of viral transcription in infected cells (8, 9). Tat protein is released from HIV-1-infected cells and can be detected in sera from HIV-1-infected patients (10). Extracellular Tat may account for the decrease of the host immune response and cellular disorders connected to AIDS pathology. Tat in synergy with a basic fibroblast growth factor (bFGF) is involved in the induction of Kaposi's sarcoma lesions (11). Tat is able to repress the major histocompatibility complex (MHC) class I genes and provides the virus with a mechanism to evade the host immune response (12). Finally, Tat participates in the induction of apoptosis in lymphocytes and contributes to the depletion of the CD4+ T cells in AIDS (10, 13).

One of the primary cellular responses to viral infection is the production of interferon. The double-stranded RNA-dependent protein kinase (PKR) is induced by interferon and can be activated by double-stranded RNA such as the HIV RNA (14). Following its activation, PKR in turn inactivates the initiation factor eIF2α, leading to the inhibition of protein synthesis, which can be detrimental for the viral life cycle. Overexpression of PKR in HIV-1-infected cells was recently shown to inhibit the replication of the virus (15). However, interferon does not have a strong antiviral action during the course of natural infection. Tat was shown to inhibit the PKR activity in vitro and therefore Tat may contribute to the HIV resistance to interferon (16). Interestingly, once activated, PKR is able to phosphorylate Tat but the physiological significance of this phosphorylation is not known (17).

Tat is encoded by two exons and its second exon can be variable in size depending on the nature of the HIV-1 isolates (18). Most structural studies on Tat are made with a Tat protein 86 residues long, corresponding to the Bru HIV-1 isolate (19) or a closely related sequence from the HXB2 HIV-1 isolate (20). However, the analysis of Tat protein sequences allowed us to identify six structural groups (21). In this study, we have chemically synthesized six Tat proteins representative of each structural group. We show that all have the ability to bind the HIV LTR TAR region but structural differences observed between HIV-1 isolates from Africa and other continents*
heterogeneities appeared when analyzed by circular dichroism. Interestingly, these synthetic proteins cross membranes but differ in their ability to trans-activate an HIV LTR-reporter gene in stably transfected HeLa cells. We show a correlation between the rate of trans-activation, the Tat-PKR interaction, and HIV-1 virulence.

MATERIALS AND METHODS

**Protein Synthesis, Purification, and Characterization**—Peptides were assembled according to the method of Barany and Merrifield (22) on an H-0002 carousel, with resin (0.5–0.65 mmol) (Perkin-Elmer, Applied Biosystem Inc., Forster City, CA) on an automated synthesizer (ABI 433A, Perkin Elmer, Applied Biosystem Inc.). To avoid derivatives with deletion, the N-terminal extremities without Fmoc were capped with a 4.75% acetic anhydride (Merck), 6.25% DIPA 2.0 M, 1.5% 1-hydroxybenzotriazol 1 M (HOBt) (Perkin Elmer, Applied Biosystem Inc., War- rington, UK), and 95.75% N-methylpirrolidinone (Merck Elmer). Each deprotection step was monitored with a conductivity device. The peptides were deprotected and removed from the resin with trifluoroacetic acid (TFA) complemented with 10% methylen sulfonyl fluoride (Merck) and 5% ethanedithiol (Merck) as scavenger. Purification was done with Beckman high pressure liquid chromatography (HPLC) apparatus and a Merck C8 reverse-phase column (10 × 250 mm). Buffer A was water with 0.1% TFA and buffer B was acetonitrile with 0.1% TFA. Gradient was buffer B from 20 to 40% in 40 min with a 2 ml/min flow rate. HPLC analysis was done with a Merck C8 reverse phase column (4 × 125 mm) with similar buffers but a gradient from 10 to 50% B in 40 min and a 0.8 ml/min flow rate. Electrospray mass spectrometry was carried out with a Perkin Elmer single quad PE-SCIEX API 150ex. Amino acid analyses were performed on a model 6300 Beckman analyzer.

**Circular Dichroism**—CD spectra were measured in a 50-μm path length cell from 240 to 185 nm on a Jobin-Yvon spectrophotometer (Mark VI). The instrument was calibrated length cell from 260 to 178 nm on a Jobin-Yvon (Long-Jumeau, France) UV CD spectrophotometer (Mark VI). The instrument was calibrated with similar buffers but a gradient from 10 to 50% B in 40 min and a 0.8 ml/min flow rate. Electrospray mass spectrometry was carried out with a Perkin Elmer, Applied Biosystem Inc.). To avoid derivatives with deletion, the N-terminal extremities without Fmoc were capped with a 4.75% acetic anhydride (Merck), 6.25% DIPA 2.0 M, 1.5% 1-hydroxybenzotriazol 1 M (HOBt) (Perkin Elmer, Applied Biosystem Inc., War- rington, UK), and 95.75% N-methylpirrolidinone (Merck Elmer). Each deprotection step was monitored with a conductivity device. The peptides were deprotected and removed from the resin with trifluoroacetic acid (TFA) complemented with 10% methylen sulfonyl fluoride (Merck) and 5% ethanedithiol (Merck) as scavenger. Purification was done with Beckman high pressure liquid chromatography (HPLC) apparatus and a Merck C8 reverse-phase column (10 × 250 mm). Buffer A was water with 0.1% TFA and buffer B was acetonitrile with 0.1% TFA. Gradient was buffer B from 20 to 40% in 40 min with a 2 ml/min flow rate. HPLC analysis was done with a Merck C8 reverse phase column (4 × 125 mm) with similar buffers but a gradient from 10 to 50% B in 40 min and a 0.8 ml/min flow rate. Electrospray mass spectrometry was carried out with a Perkin Elmer single quad PE-SCIEX API 150ex. Amino acid analyses were performed on a model 6300 Beckman analyzer.

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The protein concentration (ng/µl) is indicated at the top of each gel lane. Complexes and free RNA are identified as c and f, respectively. The same RNA preparation was used for the titration with the six proteins. A Tat Bru derivative with carboxymethylated cysteines (Bru Cm-Cys) was also tested. The equilibrium dissociation constants ($K_d$) were measured directly from these electrophoretic mobility shift assays. The $K_d$ values vary from approximately 50 nM for both Tat Eli and Tat Mal to about 140 nM for Tat Oyi and Tat JR. In addition we noted that, beyond the variation of the $K_d$ values, the binding profiles with the various proteins were somewhat different. For example, low concentrations of Tat Bru produced a well resolved single complex and only with rather high concentrations (>4.5 ng/µl) aggregates which were formed and which failed to enter the acrylamide gels. In contrast, multimeric complexes were easily identified with Tat Eli even when using low concentrations. Up to three retarded bands could be seen with Tat Eli and to a lower extent with Tat Jr. No such effects were observed with the shorter proteins such as Tat Bru and Tat Mal, for example, as well as with the longer protein Tat Oyi.

RESULTS AND DISCUSSION

The six Tat proteins representative of each structural group were synthesized with a Fast Fmoc chemistry in solid phase synthesis (22). Tat Bru (86 residues) and Tat Jr (101 residues) have similar sequences and are representative of HIV-1 isolates found in Europe and North-America (20, 23). Tat Oyi (101 residues) also has similarities with European and North-American Tat sequences but was identified from a healthy Gabones man bearing a defective HIV-1 strain (24). Tat Z2 (86 residues) came from a Zairian HIV-1 isolate and is closely related to an ancestral HIV-1 isolate (ZR59) identified recently (25). Tat Z2 is the closest of the ancient forms of Tat proteins. In contrast, Tat Mal (87 residues) and Tat Eli (99 residues) appeared more recently and came from highly virulent HIV-1 isolates recovered, respectively, from a 24-year old woman and a 7-year old boy in Zaire (now Democratic Republic of Congo) following a dramatic increase of AIDS in central Africa during the 1980s (26).

Each chemical synthesis was done in a single run using a Fast Fmoc chemistry. We use an HBTU activator in each case, and deprotection was monitored by the measurement of the conductivity. The first sequence to be synthesized was Tat Bru (Fig. 1) with carboxymethylated cysteines (Cm-Cys Tat Bru). Previous peptide syntheses were carried out with Tat HXB2 (86 residues), which has a sequence very close to Tat Bru (27, 28). After purification of Cm-Cys Tat Bru (data not shown), our synthetic protein showed that it could bind TAR RNA (Fig. 3) but could not trans-activate in our HeLa cell assay. The position of the seven cysteine residues is strictly conserved among Tat variants and the importance of cysteine residues for trans-activation is well described in the literature (18). This explains why Cm-Cys Tat Bru does not trans-activate in our HeLa cells assay. Another synthesis of Tat Bru was done with free cysteines this time. This synthetic protein turned out to be able to bind TAR (Fig. 3) and trans-activates in our HeLa cell assay (Fig. 5). To avoid precipitation that occurred at neutral pH, the

![Fig. 3. Electrophoretic mobility shift analysis for the synthetic Tat proteins.](image-url)
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Table I
Secondary structure analyses

| Tat variants | Solvent | H  | B  | T  | O  | Total |
|--------------|---------|----|----|----|----|-------|
| Tat Bru      | Aqueous | 2  | 22 | 32 | 44 | 1.00  |
|              | TFE     | 23 | 6  | 32 | 39 | 1.00  |
| cmc Tat Bru  | Aqueous | 0  | 22 | 32 | 46 | 1.00  |
|              | TFE     | 50 | 5  | 20 | 25 | 1.00  |
| Tat JR       | Aqueous | 1  | 20 | 36 | 44 | 1.00  |
|              | TFE     | 30 | 14 | 22 | 33 | 0.99  |
| Tat Oyi      | Aqueous | 1  | 21 | 33 | 46 | 1.01  |
|              | TFE     | 38 | 9  | 22 | 30 | 0.99  |
| Tat Z2       | Aqueous | 10 | 30 | 14 | 46 | 1.00  |
|              | TFE     | 24 | 20 | 22 | 34 | 1.00  |
| Tat Mal      | Aqueous | 10 | 14 | 33 | 43 | 1.00  |
|              | TFE     | 19 | 26 | 22 | 33 | 1.00  |
| Tat Eli      | Aqueous | 0  | 28 | 28 | 44 | 1.00  |
|              | TFE     | 22 | 29 | 25 | 25 | 1.01  |

* Aqueous solvent with 20 mM phosphate buffer, pH 4.5.

** Solvent composed of 50% trifluoroethanol and 50% water.

Tat proteins were dissolved in phosphate buffer at pH 4.5 and then directly mixed to the cell medium that has a neutral pH. Probably the different ions present in cell medium neutralized the cysteines. In these conditions, which were rather close to those encountered by Tat in the blood of HIV infected patients, we observed a much higher trans-activation activity (Fig. 5) compared with what was previously reported with synthetic Tat proteins. Therefore, all the other synthetic Tat variants were done with free cysteines.

Peptide synthesis starts from the C-terminal extremity and the yield of synthesis for the first 50 residues was from 90 to 98% as a function of the Tat sequence. This yield decreased significantly beyond this level for Tat Bru, Tat Jr, and Tat Oyi and are revealed by the HPLC analysis of the synthesis before purification (Fig. 2). The conductimetry analysis of the deprotection steps revealed that the sequence just after the basic region was critical (data not shown). Tat Eli is the sequence that gave the best synthesis yield and a significant decrease in conductimetry was observed only from step 88. For Tat Mal, the final deprotection was not carried out, and half of the crude peptide was cleaved from the resin with an Fmoc on at the N terminus. This gave a 10-min shift for the major fraction in the analytical HPLC and could have been helpful for the purification (Fig. 2E). Unfortunately it was not possible to have soluble Tat after the removal of the Fmoc even in the presence of a solution containing 6 M guanidine and dithiothreitol (data not shown). The Fmoc was removed before cleavage for the other Tat and in the crude peptide remaining from Tat Mal (data not shown). The final yield after synthesis and purification varied from 5% for Tat Jr to 16% for Tat Eli. The purity and characterization of the Tat proteins were determined by analytical HPLC (Fig. 2), mass spectroscopy, and amino acid analysis (data not shown). In each case, the results indicated that we obtained a pure protein with the correct molecular weight and the expected amino acid composition.

The six synthetic Tat proteins can bind to TAR RNA (Fig. 3) and can inhibit PKR (data not shown). Nevertheless, structural changes exist among these proteins and are revealed by circular dichroism in two different solvents (Fig. 4), and Table I shows the results of the CD data analysis (31). In aqueous buffer (Fig. 4A), it can be deduced from the intensity of the negative 200 nm band that nonorganized structures are predominant (29). Table I reveals that there is no α helix in aqueous buffer excepted for Tat Mal and Tat Z2, which could have a short helix of eight to nine residues. In the presence of trifluoroethanol that mimic the membrane environment (30), three CD bands characteristic of α helix (29) appeared in all spectra (Fig. 4B). Table I shows that 19 to 30 residues are able to adopt an α helix structure as a function of the Tat variants. It is possible that this conformational heterogeneity helps Tat to cross the membrane barrier because most membrane proteins contain amphipathic α helix. It was shown that peptides derived from Tat can adopt such a conformation (32). Interest-
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The major result of trans-activation tests was the high activity of Tat Mal and Tat Eli, which correlates with the high virulence of these two HIV-1 isolates. Interestingly, the long C-terminal extremity of Tat Eli seems to improve the trans-activation compared with Tat Mal (Fig. 5). However, the long C-terminal effect was not always observed with Tat Jr compared with Tat Bru as a function of the concentration (Fig. 5). In a different assay, the ability of Tat to be phosphorylated by PKR was verified (Fig. 6). Previous studies have shown only the phosphorylation to Tat Bru by PKR. Here we have further analyzed the six synthetic Tat variants with regard to their relation to PKR. When analyzed for their ability to serve as substrates for PKR, they proved to be different (Fig. 6). The Tat residues which are important for phosphorylation by PKR are Ser-62, Thr-64, and Ser-68 (17). The position of these three residues is well conserved in Tat corresponding to poorly virulent or nonvirulent HIV-1 isolates, such as Bru, Oyi, and Jr. In contrast to this, the virulent Tat variant Mal and Eli do not have these residues in similar positions. Fig. 6A shows that when PKR is activated with a synthetic RNA (poly(I)-poly(C)), Tat Bru and Tat Oyi can be clearly phosphorylated but not Tat Mal and Tat Eli. Phosphorylation level is low for Tat Z2, which also differs from the STS consensus (Gly-62, Pro-68) but has kept the central Thr-64. Tat Jr also has a low phosphorylation level although it has the STS consensus. In a previous study, we mentioned that the C-terminal extremity of Tat Jr was unable to fit in a groove made by the N-terminal and the basic region as the C-terminal extremity of other long Tat variants (21). This structural particularity may disturb the interaction of Tat Jr with PKR. Fig. 6B shows that if PKR and Tat concentrations are increased, there is still no phosphorylation for Tat Mal. This experiment shows the high specificity of the PKR phosphorylation for Tat Bru compared with Tat Mal.

The trans-activation assays and the PKR-mediated phosphorylation assays demonstrate that our Tat variants behave as natural Tat proteins. Tat Bru can trans-activate HIV-1 LTR whereas Tat Oyi cannot, but they are both phosphorylated by PKR. Tat Mal and Tat Eli have a high trans-activation activity but are not phosphorylated by PKR. This demonstrates for the first time that the PKR-mediated phosphorylation of Tat is not related to the ability of Tat to trans-activate HIV-1 LTR. However, Tat Z2 and Tat Jr are poorly phosphorylated but donot have a high trans-activation activity. Only a low phosphorylation is perhaps enough to modify the Tat trans-activation activity, but it is possible that the correlation that we observed between the high trans-activation activity and virulent HIV strains could be also attributed to differences in uptake into the cell and/or phosphorylation by PKR. These studies show that few mutations can induce more efficient Tat proteins, which did correlate with the emergence of highly virulent HIV-1 strains in Africa. This result emphasizes the essential role played by Tat in the HIV infection and all the benefit that a Tat inhibitor could bring to AIDS therapies.

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REFERENCES

1. Fisher, A. G., Feinberg, M. B., Josephs, S. F., Harper, M. E., Marselle, L. M., Reyes, G., Genda, M. M., Adewunmi, A., Debouk, C., Gallo, R. C. & Wong-staal, F. (1986) Nature 320, 367–371

2. Peterlin, B. M., Liu, C., Paine, P. J. & Walker, M. D. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 9734–9738

3. Berkhout, B., Gauthier, A., Rabson, A. B. & Jeanie, K. T. (1990) Cell 62, 7257–7267

4. Lore, E. F., George, P., Johnson, W. C. & Ho, P. S. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9734–9738

5. Vives, E., Brodin, P. & Lehleu, B. (1997) J. Biol. Chem. 272, 16010–16017

6. Efthyrianades, A., Briggs, L. J. & Jans, D. A. (1998) J. Biol. Chem. 273, 1623–1628

7. Herrich, N., Ulrich, C., Garcia-Martinez, L. F. & Gaynor, R. B. (1997) EMBO J. 6, 1224–1235

8. Garcia-Martinez, L. F., Mavankal, G., Neve, J. M., Lane, W. S., Ivanov, D. & Gaynor, R. B. (1997) EMBO J. 16, 2836–2850
Correlation between HIV-1 Virulence and Tat Activity

9. Yang, X., Gold, M. O., Tang, D. N., Lewis, D. E., Aguilar-Cordova, E., Rice, A. P. & Herrmann, C. H. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 12331–12336

10. Westendorp, M. O., Frank, R., Ochsenbauer, C., Stricker, K., Dhein, J., Walczak, H., Debatin, K.-M. & Krammer, P. H. (1995) *Nature* **375**, 497–500

11. Ensoli, B., Gendelman, R., Markham, P., Fiorelli, V., Colombini, S., Raffeld, M., Cafaro, A., Chang, H. K., Brady, J. N. & Gallo, R. C. (1994) *Nature* **371**, 674–680

12. Howcroft, T. K., Strebel, K., Martin, M. A. & Singer, D. S. (1993) *Science* **260**, 1320–1323

13. Li, C. J., Friedman, D. J., Wang, C., Metelev, V., and Pardee, A. B. (1995) *Science* **268**, 429–431

14. Roy, S., Katze, M. G., Parkin, N. T., Edery, I., Hovanessian, A. G. & Sonenberg, N. (1997) *J. Biol. Chem.* **272**, 8388–8395

15. Benkirane, M., Neuveut, C., Chun, R. F., Smith, S. M., Samuel, C. E., Gatignel, A. & Jeang, K. T. (1997) *EMBO* **16**, 611–624

16. Brand, S., Kohayashi, R. & Mathews, M. B. (1997) *J. Biol. Chem.* **272**, 8388–8395

17. Benbrook, J. (1996) in *HIV-1 Tat: Structure & Function*, pp. 3–18, Los Alamos National Laboratory (Ed) Human Retroviruses & AIDS Compendium III

18. Clavel, F. & Charneau, P. (1994) *J. Virol.* **68**, 1179–1185

19. Laurent, A. G., Krust, B., Galabru, J., Svej, J. & Hovanessian, A. G. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 4341–4345