Identification of a Novel Cell Attachment Domain in the HIV-1 Tat Protein and Its 90-kDa Cell Surface Binding Protein*

(Received for publication, December 17, 1991)

Benjamin S. Weeks‡‡, Ketan Desai‡, Paul M. Loewenstein‡, Mary E. Klotman‡, Paul E. Klotman‡, Maurice Green‡, and Hynda K. Kleinman‡

From the ‡Laboratory of Developmental Biology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892; †Institute for Molecular Virology, St. Louis University Medical Center, St. Louis, Missouri 63110, and ‡Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892.

The HIV-1 transactivator protein Tat is essential for viral gene expression and replication. Tat is taken up by cells and transactivates the HIV-LTR promoter in the cell nucleus. The present studies show that cells adhere to both synthetic and recombinant Tat, and, using synthetic peptides, we localize the binding site to a region spanning amino acid residues 49–57 (peptide Tat49-57). Tat49-57 also inhibited cell attachment to solid phase full-length Tat peptide and to recombinant Tat protein. Using Tat peptide affinity chromatography, we identified a 90-kDa cell surface protein that binds to Tat. The 90-kDa protein could be eluted from the Tat column using the Tat49-57 peptide. A 90-kDa cell surface Tat binding protein was also identified by co-precipitation with Tat after incubation with radiolabeled cell membrane preparations. Co-precipitation of the 90-kDa protein was inhibited by competition with a Tat49-65 peptide, but not with Tat56-65. Our findings suggest that cellular attachment to Tat is mediated through a 90-kDa cell surface protein that binds to a Tat domain between amino acids 49 and 57.

The HIV-1 Tat protein is a strong transactivator of the HIV-LTR promoter (Green and Loewenstein, 1988; Frankel and Pabo, 1988) and may also regulate cellular genes and cell behavior (Brake et al., 1990; Roy et al., 1990; Ensoli et al., 1990). Tat transactivation of the HIV-LTR promoter is essential for both viral gene expression and virus replication (for reviews see Green (1991) and Sharp and Marciniak (1990)). Tat can be rapidly taken up by HeLa cells and localizes in the nucleus (Green and Loewenstein, 1988; Frankel and Pabo, 1988). Tat has been found to stimulate the growth of cultured cells and transactivates the HIV-LTR promoter in artificial constructs. Tat transactivation of the HIV-LTR promoter is essential for both viral gene expression and virus replication (for reviews, see Ruoslahti and Pierschbacher (1986), Hynes (1987), and Yamada (1990)). Indeed, mutation of the RGD sequence reduced cell attachment to Tat (Brake et al., 1990).

Here, we localized a cell binding domain within a 9-amino acid sequence of Tat (amino acid residues 49–57) lacking the RGD sequence. By Tat peptide-affinity chromatography and co-immunoprecipitation with Tat, we identified a 90-kDa cell surface binding protein which binds to Tat within the 49–57-amino acid sequence.

**EXPERIMENTAL PROCEDURES**

Tat, Peptides, and Antibodies—HIV-1 (BRU isolate) Tat26, Tat1-46, Tat49-57, Tat58-65, Tat66-72, and Tat65-72 peptides were chemically synthesized by the solid-phase method and purified by HPLC as previously described (Green and Loewenstein, 1988). A scrambled Tat49-57 peptide containing the sequence RRKQRKRKR was custom synthesized by Research Genetics using the same method and also purified by HPLC. Antibody to Tat amino-terminal peptide was prepared in rabbits by methods previously described (Green et al., 1983). Anti-Tat amino-terminal antibody was affinity purified on Tat49-Sepharose columns. Recombinant Tat was purchased from Advanced Biotechnologies. Antibodies to β1, β2, β3, αV, and αVβ3 integrins were purchased from Telios. Antibodies to β3 integrin were purchased from AMAC Inc., and control IgG was purchased from Kirkegaard-Perry.

Cells and Culture—PC12 cells (from G. Guroff, NICHD, NIH) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 7.5% fetal calf serum and 7.5% horse serum. The NG108-15 cells (from M. Nirenberg, NINCDS, NIH) were grown in DMEM supplemented with 0.1 mM hypoxanthine, 0.4 mM aminopterin, 16 mM thymidine, and 10% fetal calf serum. Kaposi's sarcoma cells (Ensoli et al., 1989). Recently, Tat was shown to promote lymphoctic and skeletal muscle cell attachment (Brake et al., 1990). The ability of Tat to be taken up and affect cell behavior suggests a cellular receptor for Tat. In this regard, Tat contains an amino acid sequence, Arg-Gly-Asp (RGD), at residues 72–74, which is a well known integrin receptor recognition sequence (reviewed in Akiyama et al., 1990), Ruoslahti and Pierschbacher (1987), and Hynes (1987) in a variety of adhesive proteins including fibronectin, laminin, thrombospondin, and fibrinogen (for reviews, see Ruoslahti and Pierschbacher (1986), Hynes (1987), and Yamada (1990)). Indeed, mutation of the RGD sequence reduced cell attachment to Tat (Brake et al., 1990).

Here, we localized a cell binding domain within a 9-amino acid sequence of Tat (amino acid residues 49–57) lacking the RGD sequence. By Tat peptide-affinity chromatography and co-immunoprecipitation with Tat, we identified a 90-kDa cell surface binding protein which binds to Tat within the 49–57-amino acid sequence.

**EXPERIMENTAL PROCEDURES**

**Cell Attachment Assays**—Cell attachment was determined using a modification of a previously described method (Klebe et al., 1974). Various amounts of synthetic Tat, Tat deletion peptides or recombinant Tat protein diluted in 200 μl of water were added to 16-nm diameter wells in 24-well cell culture plates (Costar) and air dried overnight at 4 °C. After drying, each well was treated with 3% bovine serum albumin for 1 h at 37 °C. The bovine serum albumin was then removed by aspiration. Next, PC12 and NG108-15 cells were detached by agitation, and H9 cells were collected in suspension. Cells were then washed three times in phosphate-buffered saline, pH 7.4 (PBS), and resuspended in DMEM (PC12 and NG108-15 cells) or RPMI (H9 cells) containing 100 μg/ml transferrin, 100 μM putrescine, 20 mM progestosterone, 30 mM NaH2PO4, and 5 μg/ml insulin. 1 × 106 cells were seeded in each well. Cells were incubated for 60 min at 37 °C in

*The abbreviations used are: HPLC, high performance liquid chromatography; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline.
lysine. the total number of cells which adhered as described under “Experimental Procedures.”

incubated at 37 °C for 1 h prior to determination of the percent of mm diameter wells coated with the indicated amounts of Tat substrate and polylysine each dissolved in DMEM at a concentration of 1 mg/ml. Competitive peptides were added at the time of cell seeding. For the antibody inhibition of attachment assays, Telios and AMAC monoclonal antibodies were tested at a final 1/10 dilution on wells coated with 20 μg of recombinant Tat. Using 1 mg/ml stock, 50 μl of control mouse IgG were added to the 450-μl assay well at the time of H9 cell seeding. Telios polyclonal rabbit anti serum to αvβ3 integrin and heat inactivated normal rabbit serum were tested at a final 1/25 dilution. Attachment was determined as described above.

Cell Surface Iodination—Cells were isolated by trypsinization, suspended, and washed twice with 0.5 ml of 0.02 M sodium phosphate containing 0.15 M NaCl (PBS). The attached cells were fixed with methanol and stained with Diff Quick (Baxter Scientific Products). 1.6% (1/64) of the cells which remained adherent so the average value arose 4B according to the manufacturer’s instructions. Briefly, cells (3–5 × 10^6) were washed in PBS three times and resuspended in 100 μl of PBS. Two ml of [125I]iodine (Amersham Corp.), 60 μl of 2 mg/ml lactoperoxidase (Sigma) in PBS, and 20 μl of 0.0125% H2O2 were added and the cell suspension was gently agitated for 5 min. Next, 30 μl of 0.05% H2O2 were added, and cells were incubated for an additional 10 min, followed by the addition of 40 μl of 0.05% H2O2 and a 15-min incubation. Cells were washed three times with PBS and either used for (i) Tat competition and immunoprecipitation analysis or (ii) frozen on dry ice and stored at −20 °C until used for affinity chromatography.

Affinity Chromatography—Peptide affinity columns were prepared at 4 °C by incubating peptide (1 mg/ml) with CNBr-activated Sepharose 4B according to the manufacturer’s instructions. As control columns, both an unrelated 20-amino acid peptide from the laminin A chain or bovine serum albumin were coupled to Sepharose 4B. Cell surface-labeled cells (see above) were lysed and sonicated in binding buffer (100 mM Tris-HCl, pH 7.4, containing 25 mM N-octyl β-D-glucopyranoside, 150 mM NaCl, 2 mM phenylmethylsulfonyl fluoride, and 1 mM MnCl2). The lysate was clarified by centrifugation, applied to the column, and circulated overnight. In some experiments the bound protein fractions were eluted with 20 mM EDTA. In other experiments elution of the 90-kDa protein off the Tat column was carried out using the Tat49-57 peptide. For elution with the peptide, columns were prepared as described above and then 1 mg of Tat was added to 5 ml of binding buffer and circulated overnight. A parallel column was tested using the scrambled 49-87 peptide to try to elute the 90-kDa protein. In all experiments, eluates were dialyzed against water at 4 °C, lyophilized, and resuspended in SDS sample buffer for resolution on SDS-polyacrylamide gels and autoradiography, or resuspended in PBS containing 1% Triton X-100 for immunoprecipitation analysis.

Immunoprecipitation of Tat column fractions from [35S]leucine-labeled H9 cell lysates was performed as previously described (Roberts et al., 1988) using antibodies to αv, β4, and xαvβ3 integrin, applied to the column, and circulated overnight. In some experiments the bound protein fractions were eluted with 20 mM EDTA. In other experiments elution of the 90-kDa protein off the Tat column was carried out using the Tat49-57 peptide. For elution with the peptide, columns were prepared as described above and then 1 mg of Tat was added to 5 ml of binding buffer and circulated overnight. A parallel column was tested using the scrambled 49-87 peptide to try to elute the 90-kDa protein. In all experiments, eluates were dialyzed against water at 4 °C, lyophilized, and resuspended in SDS sample buffer for resolution on SDS-polyacrylamide gels and autoradiography, or resuspended in PBS containing 1% Triton X-100 for immunoprecipitation analysis.

Co-immunoprecipitation Analysis of Tat Protein Complexes—To analyze Tat interactions with cell surface proteins of intact cells, Molt3 cells were surface-labeled with [35S]leucine and incubated in 500 μl of PBS containing 400 ng of Tat/ml PBS or PBS alone for 60 min at 37 °C with shaking. Cells were washed twice with PBS and lysed in 1 ml of PBS containing 0.5% Nonidet P-40, 0.5% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride at 4 °C. After 15 min, nuclei were removed by centrifugation and 200-pl aliquots of the supernate were immunoprecipitated with anti-Tat antibody as follows. Samples were made up to 1.5 ml with immunoprecipitation buffer containing a final concentration of 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.1% BSA, 0.05% Nonidet P-40, and, in initial studies, 10 mM EDTA. In order to determine if the Tat-90-kDa protein interaction could occur in the absence of EDTA, EDTA was eliminated from the immunoprecipitation buffer in later studies and the Tat-90-kDa protein interaction was unaffected. Samples were incubated in the immunoprecipitation buffer overnight at 4 °C with 50 μl of affinity purified anti-Tat antibody. For the deletion peptide competition of the co-immunoprecipitation, either 1 mg of the Tat6*-%, or Tat4*-% peptides were added. Washed Protein A Sepharose beads (30 μl) were added and the mixture was rotated at 1 h at 4 °C. Beads were washed five times by brief centrifugation in buffer containing 500 mM NaCl, 50 mM Tris-HCl, pH 7.4, 0.5% Nonidet P-
40, and 5 mM EDTA in initial studies, with the EDTA eliminated in later experiments. Bound protein was dissociated by heating the beads at 100 °C for 5 min in 25 µl of SDS-sample buffer, and then the eluted proteins were electrophoresed on an 8% SDS-polyacrylamide gel. Gels were stained with Coomassie Blue, dried, and exposed to x-ray film with an intensifying screen at −70 °C.

To analyze Tat interactions with cell membrane-associated proteins, Mol3 cells were washed twice with RPMI lacking cysteine and methionine and were resuspended at 2 x 10^6 cells per ml in the same medium. After incubation at 37 °C for 1 h, 10 ml of cells were metabolically labeled by addition of 380 µCi of Tran^35S-label (ICN, 1.179 Ci/mmol, 15% Cys, 85% Met) and further incubated for 4.5 h. Cells were washed twice with cold PBS, resuspended in PBS containing 80 mM sucrose, and disrupted with four strokes of a Dounce homogenizer fitted with a B pestle. Nuclei were removed by centrifugation at 5,000 x g for 5 min. EDTA and KCl were added to 10 mM and 50 mM final concentration, respectively, to the supernatant fluid followed by centrifugation at 100,000 x g for 1 h. The membrane pellet was rinsed sequentially with 100 mM NaCl and distilled water, resuspended in 80 mM sucrose, 50 mM KCl, 10 mM EDTA, 10 mM Tris-HCl, pH 7.2, and recentrifuged. The pellet was solubilized in 1 ml of cold PBS, containing 0.5% Nonidet P-40, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and clarified by centrifugation after 15 min. To 500 µl of supernate were added either 500 µl of PBS containing 200 µg of Tat^45 or PBS alone as control. After 1 h at 37 °C, 200-µl aliquots were immunoprecipitated with either Tat^45 antibody or normal rabbit serum and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography as described above.

RESULTS

Binding of Cells to Immobilized HIV-1 Tat Peptide—We tested the ability of PC12 pheochromocytoma, NG108-15 neuroblastoma x glioma, and the H9 lymphocytic cell lines to attach to increasing concentrations of recombinant Tat and the full length, 86 amino acid synthetic Tat peptide (Tat^86) (Fig. 1). All cell lines attached to Tat^86 and to polylysine. Spreading and process formation was observed with the neuronal cells on Tat, but not on polylysine (Fig. 2). For both neuronal cell lines, approximately 40-50% attachment was reproducibly observed at 5 µg with 80-90% of cells attached at 30 µg. The H9 cell line was less adherent compared to the neuronal cells, with approximately 25% of cells adhering at 5 µg and less than 50% attachment at 30 µg.

The active binding region within Tat^86 was localized using Tat deletion peptides with overlapping amino acid sequences. PC12 cells bound Tat^45, Tat^37-72, and Tat^55-86, but did not bind Tat^1-45 or Tat^55-86 (Table 1). Cell attachment activity maps within a basic 9-amino acid sequence encoded in residues Tat^55-86. The Tat^45-57 peptide also promoted cell spreading in the neuronal cells (Fig. 2).

In the presence of 10 µg of soluble Tat^45-57 peptide, attachment to both recombinant and synthetic Tat was competitively inhibited by approximately 50% at various substrate levels (Fig. 3A). With a constant substrate level of 5 µg, inhibition of binding to both recombinant Tat and Tat^86 was not observed with the addition of 1 µg or less of soluble Tat^45-57. Inhibition was observed with addition of soluble Tat^45-57 peptide and reached 50% with the addition of 5 µg of soluble peptide (Fig. 3B). With both varying and constant substrate levels, polylysine and a scrambled Tat^45-57 did not

![Fig. 2. Morphology of PC12 and NG108-15 cells on Tat peptides and polylysine. PC12 (upper panel) and NG108-15 (lower panel).](image-url)
inhibit cell binding to Tat. These data demonstrate that the HIV-1 Tat protein contains a cell attachment site in the 49-57-amino acid sequence.

Identification of a Cell Surface Binding Protein for Tat of 90 kDa in Various Cell Lines by HIV-1 Tat Peptide Affinity Chromatography—The data suggest that Tat can interact with a cell surface receptor protein. Since integrins comprise a large family of adhesion receptor molecules, we first tested whether antibodies to various integrin subunits could block cell adhesion to recombinant Tat. Monoclonal antibodies to β1, β2, β3, and αV did not inhibit attachment to Tat (Table II). Rabbit antisera to αVβ3 inhibited attachment by approximately 50%, but control normal rabbit sera also inhibited attachment. A monoclonal antibody to β4 integrin inhibited attachment to Tat by over 90% without inhibition by the control mouse IgG (Table II). These data suggest involvement of the β4 integrin subunit in cell attachment to Tat.

Affinity chromatography of cell surface labeled proteins

![Graph showing inhibition of PC12 cell attachment to Tat86 and recombinant Tat by addition of soluble Tat49-57. A, 10⁵ PC12 cells were plated in 16-mm diameter wells coated with various amounts of either Tat86 or recombinant Tat and incubated at 37 °C for 1 h prior to determination of cell attachment. For competition, 10 μg of Tat49-57 were added in a 10-μl solution of DMEM. B, 10⁵ PC12 cells were plated in 16-mm wells coated with 5 μg of either Tat86 or recombinant Tat and competed for attachment with 0.1-5 μg of soluble peptides.]

![Graph showing inhibition of PC12 cell attachment to Tat86 and recombinant Tat by addition of soluble Tat49-57. A, 10⁵ PC12 cells were plated in 16-mm diameter wells coated with various amounts of either Tat86 or recombinant Tat and incubated at 37 °C for 1 h prior to determination of cell attachment. For competition, 10 μg of Tat49-57 were added in a 10-μl solution of DMEM. B, 10⁵ PC12 cells were plated in 16-mm wells coated with 5 μg of either Tat86 or recombinant Tat and competed for attachment with 0.1-5 μg of soluble peptides.]
was carried out using Tat66 immobilized on Sepharose 4B to identify a cellular binding protein. A major protein band of 90 kDa from PC12 cells was eluted from the Tat peptide column with 20 mM EDTA and could be visualized by Coomassie Blue staining (Fig. 4A). Additional 90-kDa protein could be detected eluting from the column with higher concentrations of EDTA (not shown). Tat binding proteins which stained with Coomassie Blue, but were not cell surface associated, were also observed. A similar cell surface iodinatable 90-kDa Tat binding protein was found in Kaposi’s sarcoma cells (Fig. 4B). Furthermore, the presence of a protein with the same electrophoretic mobility that bound Tat was detected in H9 cells by Coomassie Blue staining (Fig. 4C). In addition to the 90-kDa protein, a protein band of 180–200 kDa was observed in some of the preparations in variable and lesser amounts.

The 90-kDa protein could also be eluted from the Tat column using the Tat49 peptide (Fig. 5). The scrambled Tat49 peptide did not elute the 90-kDa protein.

Tat affinity column unbound flow-through and 20 mM EDTA-eluted fractions from 35S-labeled H9 cells were immunoprecipitated using anti-β3, β4, and αV antibodies to identify the 90-kDa band. While the Tat unbound fractions contained immunoprecipitable proteins, the Tat binding proteins did not immunoprecipitate with these antibodies (data not shown). These data suggest that the Tat binding proteins isolated by our column chromatography are not β1, β3, or αV integrins and suggests the possibility of multiple cell recognition sites on Tat and multiple Tat binding proteins.
Detection of a Complex between Tat and a 90-kDa Protein by Co-immunoprecipitation with Anti-Tat Antibody—To provide additional direct evidence that Tat can associate with a 90-kDa cell surface protein in vitro, cells were metabolically labeled with [35S]Met/[35S]Cys, and the membrane fraction was isolated, lysed with detergent, and incubated with Tat. Immunoprecipitation with anti-Tat antibody (Fig. 6B, lane 3) but not with normal serum (Fig. 6B, lane 1) precipitated a major band of 90 kDa. The 90-kDa protein was not immunoprecipitated in the absence of Tat (Fig. 6B, lane 2).

We also determined in co-immunoprecipitation studies that Tat can associate with the 90-kDa cell surface protein using cells surface labeled with [35S]Met/[35S]Cys and incubated with Tat. The cells were then lysed with detergent, and the extract was immunoprecipitated with anti-Tat antibody. A major labeled band of 90 kDa was found in the immunoprecipitate (Fig. 6A, lane 3), indicating that Tat added to intact cells can form a complex with the 90-kDa cell surface protein. The 90-kDa protein was not detected when normal rabbit serum was used instead of anti-Tat antibody (Fig. 6A, lane 1) or in the absence of added Tat (Fig. 6A, lane 2). In addition to the 90-kDa protein, a protein band of 180–200 kDa was observed by co-immunoprecipitation analysis in Molt3 cells (Fig. 6).

That the 90-kDa protein binds Tat within the 49–65 sequence was demonstrated by competing co-immunoprecipitation of the iodinated 90-kDa cell surface protein with Tat deletion peptides. In the absence of Tat, no immunoprecipitable complex was formed (Fig. 7, lane 5). Tat formed an immunoprecipitable complex with a 90-kDa protein (Fig. 7, lanes 1 and 6). Tat did not inhibit Tat interaction with the 90-kDa protein (Fig. 7, lane 3), while Tat almost completely blocked Tat interaction with the 90-kDa protein (Fig. 7, lanes 4 and 8). These data demonstrate that Tat binds in solution to the 90-kDa cell surface protein via residues 49–65 on Tat.

**DISCUSSION**

We have demonstrated that the HIV-1 transactivator protein, Tat, is active for cell attachment with a variety of cell lines, and using synthetic Tat peptides we mapped the cell attachment site to amino acid residues 49–57. A synthetic Tat peptide comprised of residues 49–57 (Tat) promoted cell attachment and spreading and competitively inhibited PC12 cell attachment to recombinant Tat protein and to a full-length synthetic Tat peptide. Using Tat peptide affinity chromatography and co-immunoprecipitation studies, we identified a novel 90-kDa cell surface protein which binds Tat. The specificity of the Tat-90 kDa binding protein binding was confirmed in co-immunoprecipitation studies where Tat, but not Tat, blocked co-immunoprecipitation. These data demonstrate that Tat recognizes a 90-kDa cell surface binding protein on various cell types and that the cell attachment site is within amino acid residues 49–65.

Although integrins are members of a related family of cell adhesion receptors and one of the tested antibodies against a member of the integrins reduced attachment to Tat, it is unlikely, for several reasons, that a known integrin is involved in Tat-mediated cell adhesion. First, antibodies to α1, β2, β3, αV, and αVβ3 did not inhibit cell attachment to Tat. Second, while anti-β4 inhibited attachment to Tat, antibodies to β4 (and α1 and αV) integrin did not immunoprecipitate any protein from the 20 mM EDTA Tat affinity column fraction which contains the 90-kDa Tat binding protein. Third, it is known that integrins change their mobility after reduction in SDS gels (Tamura et al., 1990) and the mobility of the 90-kDa protein was unaffected by reduction.

Tat contains an RGD sequence at residues 72–74. The RGD sequence is found in many adhesive proteins including fibronectin, laminin, thrombospondin, and fibrinogen (for reviews, see Ruoslahti and Pierschbacher (1986), Hynes (1987), and Yamada (1990). Here, we localized by two methods a Tat binding domain within a 9-amino acid sequence encoded in residues 49–57. Tat and Tat promoted cell attachment, whereas Tat and Tat peptides did not promote cell attachment. Attachment to Tat was inhibited using the Tat peptide and Tat blocked Tat binding to the 90-kDa protein in co-immunoprecipitation experiments. Tat did not disrupt Tat-90-kDa protein co-immunoprecipitation. Our experiments may appear at odds with a recent report supporting the role of the Tat RGD (residues 72–74) sequence in cell attachment (Brake et al., 1990). In the latter study, however, multiple cell recognition sequences on Tat are implicated as the sole partial reduction in attachment was observed (42–58%) in T lymphocyte cell lines HUT-78 and MOLT-4 and monocyte line THP-1 when the RGD site in Tat was modified to RGE. Similar evidence of multiple cell binding sites in Tat is present in our studies since only partial inhibition (50%) of cell binding to Tat was observed by addition of soluble Tat peptide. Taken together, these data imply the existence of multiple active cell binding sites on Tat.

AIDS patients suffer from a variety of syndromes which may not be related directly to immunodeficiency. These syndromes include dementia and Kaposi's sarcoma. While the exact pathogenesis of these syndromes remains unclear, a more direct role for the Tat viral protein has been suggested (Brake et al., 1990; Roy et al., 1990; Ensoli et al., 1990). Cell adhesion to Tat, and possibly Tat activity within host cells, may play a role in AIDS pathogenesis. Here we show that Tat interacts with a cell surface binding protein of 90 kDa which may facilitate Tat binding and possibly entry into cells.

**Acknowledgments**—We thank Jerry Stute for peptide synthesis, Janey Symington for preparation of anti-Tat antibody, Frances Cannon for the column chromatography, and George R. Martin for the helpful suggestions.

**REFERENCES**

Aikawa, S. S., Nagata, K., and Yamada, K. M. (1990) *Biochim. Biophys. Acta* 1031, 91–110

Brake, D. A., Debock, C., and Biesecker, G. (1990) *J. Cell Biol.* 111, 1275–1281

Ensoli, B., Barillari, G., Salahuddin, S. Z., Gallo, R. C., and Wong-Staal, F. (1990) *Nature* 345, 84–86

Frankel, A. D., and Pabo, C. O. (1988) *Cell* 55, 1189–1193

Green, M., and Loewenstein, P. M. (1988) *Cell* 55, 1179–1188

Green, M., Brackmann, K. H., Locher, L. A., and Symington, J. S. (1983) *J. Virol.* 46, 694–695

Green, W. P. (1991) *N. Engl. J. Med.* 324, 306–318

Hynes, R. O. (1987) *Cell* 48, 549–554

Klebe, P. (1974) *Nature* 250, 248–251

Low, A. M., Margalies, D. H., Mab, W. L., Lileloep, P. M., McClosky, J., and Coligan, J. E. (1986) *Proc. Natl. Acad. Sci. U.S.A.* 83, 6934–6938

Nakamura, S., Salahuddin, S. Z., Hoberfeld, P., Ensoli, B., Markham, P. D., Wong-Staal, F., and Gallo, R. C. (1988) *Science* 242, 426–430

Roberts, C. J., Burkemeyer, T. M., McQuillan, J. J., Aikawa, S. K., Yamada, S. S., Chen, W., Yamada, K. M., and McDonald, J. A. (1986) *J. Biol. Chem.* 261, 4584–4592

Roy, S., Katze, M. G., Parkin, N. T., Edery, A., Hovanesian, A. G., and Soengen, N. (1990) *Science* 247, 245–248

Ruoslahti, E., and Pierschbacher, M. D. (1985) *Cell* 44, 517–518

Sharp, P. A., and Marcinuk, R. A. (1990) *Cell* 60, 229–230

Yamada, K. M. (1990) *Curr. Opin. Cell Biol.* 1, 906–925