22. IC_{50} is the concentration of R subunit required to inhibit the catalytic activity of C subunit by 50%.
23. J. Kuret, K. E. Johnson, C. Nicolette, M. J. Zoller, *J. Biol. Chem.*, in press.
24. R(Ala^{145}) is the mutant regulatory subunit of yeast cAdPK that contains alanine at position 145.
25. R. Roskoski, *Methods Enzymol.* 99, 3 (1983).
26. A. Fersht, *Enzyme Structure and Mechanism* (Freeman, New York, ed. 2, 1985), p. 107.
27. We thank M. Ockler and J. Duffy for artwork and D. Greene for photography. Supported by NIH grants GM33986 (M.J.Z.) and R35 CA39829-02 (M.W.), the American Cancer Society (M.W.), the American Business Foundation for Cancer Research, and Pfizer Biomedical Research Award.

16 October 1987; accepted 29 January 1988

Tat Protein from Human Immunodeficiency Virus Forms a Metal-Linked Dimer

ALAN D. FRANKEL, DAVID S. BREDT, CARL O. PABO

Tat, the transactivating protein from HIV, forms a metal-linked dimer with metal ions bridging cysteine-rich regions from each monomer. This novel arrangement is distinct from the "zinc finger" domain observed in other eukaryotic regulatory proteins. Ultraviolet absorption spectra show that Tat binds two Zn^{2+} or two Cd^{2+} ions per monomer, and electrophoresis of the Tat-metal complexes demonstrates that the protein forms metal-linked dimers. Partial proteolysis and circular dichroism spectra suggest that metal binding has its primary effects in the cysteine-rich region and relatively little effect on the folding of other regions. These results suggest new directions for biological studies and new approaches to drug design.

THE HUMAN IMMUNODEFICIENCY virus (HIV) encodes several regulatory proteins that are not found in simpler retroviruses. The Tat protein, which is one of these regulatory proteins, transactivates genes that are expressed from the HIV long terminal repeat (LTR) (*1*) and Tat is essential for viral replication in vitro (*2, 3*). The mechanism of transactivation is unclear. Tat seems to cause accumulation of messenger RNA (mRNA) by acting as a transcriptional anti-terminator (*4*), by increasing the rate of transcription from the LTR promoter (*5*), or by stabilizing viral mRNAs (*6–9*). Tat may also increase translational efficiency (*6–11*). Deletion analysis has shown that a region of 50 to 100 base pairs near the 3′ end of the LTR is required for transactivation by Tat (*12*). RNA from this site (which is called the TAR site) forms two stable stem-loop structures, and it has been suggested that these double-stranded regions may be binding sites for Tat (*9, 13, 14*).

The Tat protein contains 86 amino acids (*15, 16*). Its sequence includes a highly basic region (two lysines and six arginines within nine residues) that might participate in nucleic acid binding. Tat also contains a cysteine-rich region (seven cysteines within 16 residues), and sequence comparisons from several HIV isolates show that the cysteines are perfectly conserved. Genetic studies have shown that fragments of Tat are biologically active (*8, 9, 16, 17*), but each of the active fragments (1 to 72 as a protein and 1 to 56 or 1 to 58 as fusion proteins) retains both the cysteine-rich region and the basic region. The cysteine-rich region is strikingly similar to the metallothioneins (*18, 19*) and also matches a test filter that was devised to locate potential metal-binding sites (*20*). Although this filter has often been used to identify possible "zinc finger" domains, the high density of cysteines in Tat suggested that this protein might have a novel structure. We show that Tat forms a metal-linked dimer and that its structure is distinct from the zinc finger motif found in other eukaryotic regulatory proteins.

Tat has been overexpressed in *Escherichia coli* (*21*), and protoplast fusion experiments show that the protein produced in *E. coli* allows a virus deficient in the Tat protein to replicate (*2*). On SDS gels, this protein migrates as a 15-kD band and thus is indistinguishable from Tat expressed in mouse cells or HIV-infected cells (*7, 21*). The *E. coli* cells induced for Tat expression (*22*) were sonicated in lysis buffer (50 m*M* tris-HCl, *p*H 8.0, 200 m*M* KCl, 2 m*M* EDTA) and the initial lysate was cleared by centrifugation. Polyethyleneimine was added to 0.5%; the mixture was stirred at 4°C for 30 minutes and then centrifuged. This pellet was resuspended in lysis buffer containing 700 m*M* KCl (which was required to resolubilize Tat). Protein was precipitated again with 40% ammonium sulfate, and the pellet was resuspended in a small volume of S-Sepharose washing buffer (20 m*M* tris-HCl, *p*H 7.5, 50 m*M* KCl, 2 m*M* EDTA). This material was placed on a S-Sepharose column and eluted with a 0.05*M* to 1.0*M* KCl gradient. Tat was tentatively identified by SDS gel electrophoresis as a protein with an apparent molecular size of 15,000 daltons, and it eluted as a very broad peak at 0.3 to 0.6*M* KCl. The fractions containing Tat were pooled, concentrated by pressure filtration, and treated with 0.5*M* dithiothreitol in 6*M* guanidine hydrochloride. The reduced protein was loaded onto a C_4 reversed-phase high-performance liquid chromatography (HPLC) column in 0.1% trifluoroacetic acid and eluted with an acetonitrile gradient. The Tat protein eluted with approximately 32% acetonitrile and was immediately lyophilized. Western blotting of the SDS gels, with a previously described antibody (*21*), confirmed that the purified protein was Tat. Amino acid analysis of the purified sample also confirmed the identity of Tat and allowed us to quantitate the amount of protein. Tat has an absorption maximum at 278 nm with an extinction coefficient of 1.32 optical density units per milligram of pure protein in 20 m*M* tris-HCl, *p*H 7.2.

To study the metal binding of Tat, we needed to ensure that the protein was reduced. After HPLC purification and lyophilization, Tat was resuspended in water and reaction with 5,5′-dithiobis(2-nitrobenzoic acid) (*23*) indicated the presence of 7 mol of free thiol per mole of Tat. Careful handling was required to prevent reoxidation. Under nonanaerobic or nonreducing conditions, Tat oxidized to form disulfide-linked multimers that could be seen by gel electrophoresis (see below). To prevent oxidation, we carried out all manipulations in an anaerobic chamber, and all solutions were degassed with helium and equilibrated in the chamber before use.

Optical absorption spectra gave the first evidence that Tat binds metals. The spectra obtained with reduced Tat and 0 to 2.0 molar equivalents of $CdCl_2$ (Fig. 1A) show that the absorbance increases as Cd^{2+} is added. There is a maximum at 248 nm in the difference spectra, indicating charge transfer transitions between the metals and sulfur ligands. Addition of more than two equivalents of $CdCl_2$ did not further change the spectrum, suggesting that two Cd^{2+} ions bind to the protein. Titrating the Tat-Cd^{2+} complex with acid showed that the metals must be tightly bound because the complex remained stable until the *p*H fell below 4.0 (Fig. 1B). No metal binding to oxidized Tat was observed. Ultraviolet absorption spectra were also used to monitor the binding of

A. D. Frankel and D. S. Bredt, Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205.
C. O. Pabo, Howard Hughes Medical Institute and Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Zn^{2+} (Fig. 1C). Difference spectra, measured with 0.5 to 2.0 equivalents of $ZnCl_2$, show an absorption maximum at 218 nm. No further changes in the spectrum were seen when more than 2 mol of $ZnCl_2$ were added, demonstrating that Tat binds two zinc ions per mole of protein. Our Cd^{2+} and Zn^{2+} spectra are quite similar to those found for metallothionein-metal complexes, although metallothionein (which has 20 cysteines) binds 7 equivalents of metals (*19, 24–29*). Competition experiments show that Tat binds Cd^{2+} much more tightly than Zn^{2+}. Adding 2 equivalents of $CdCl_2$ to the Tat-Zn^{2+} complex immediately displaces the Zn^{2+}, and the Cd^{2+} spectrum is observed. Conversely, a 5000-fold excess of $ZnCl_2$ only partially displaces Cd^{2+} from the complex. Absorption spectra of Tat with $CoCl_2$ were also measured, since cobalt complexes have distinctive spectra which can give information about the conformation of the metal sites. Charge transfer bands near 300 nm were observed, but only very slight changes in absorption were seen between 600 and 700 nm [changes here are characteristic of tetrahedral complexes and are clearly seen in metallothionein-Co^{2+} complexes (*19, 26, 27, 30, 31*) and with a single zinc finger domain from TFIIIA (*32*)]. The spectroscopic changes seen with Tat did not saturate at two equivalents of $CoCl_2$ and this may reflect a low affinity for Co^{2+}. These optical absorption measurements suggest an order of metal affinities of Cd > Zn > Co, which is consistent with studies of metallothionein and other metal-binding proteins (*33, 34*).

Gel electrophoresis with radioactive metals revealed that Tat formed metal-linked dimers and that the Cd^{2+} dimers were stable even in the presence of SDS. Electrophoresis of the Tat-Cd^{2+} complex on an SDS gel shows that the predominant species is a dimer, and complexes formed with $^{115m}CdCl_2$ show that most of the radioactivity is present in the dimer band (Fig. 2A). Analysis on native gels (discussed below) suggests that the other bands result from oxidation (by ammonium persulfate) during electrophoresis. The Tat-Zn^{2+} complex was not stable on an SDS gel; significantly more oxidation was observed, and no ^{65}Zn was seen in the protein bands. To minimize oxidation, the complexes were subjected to electrophoresis on native gels from which the ammonium persulfate had been removed. [Under these conditions, apo-Tat gives a ladder of oxidized oligomers (Fig. 3B, lane 1) and these were used to calibrate the relative mobility of a monomer and dimer on the native gels.] A complex with $^{115m}CdCl_2$ gave a single dimer band whereas a complex with $^{65}ZnCl_2$ gave a mixture of monomers and dimers. To test whether Tat dimers formed with Zn^{2+} might be dissociating during electrophoresis, native gels were run in the presence of 0.1 m*M* $ZnCl_2$ (Fig. 2B). Under these conditions, only dimers of the Tat-Zn^{2+} complexes were observed, and the Tat-Cd^{2+} dimers were also stable in the presence of $ZnCl_2$.

Two experiments confirmed that the dimer seen on native gels was metal-linked and

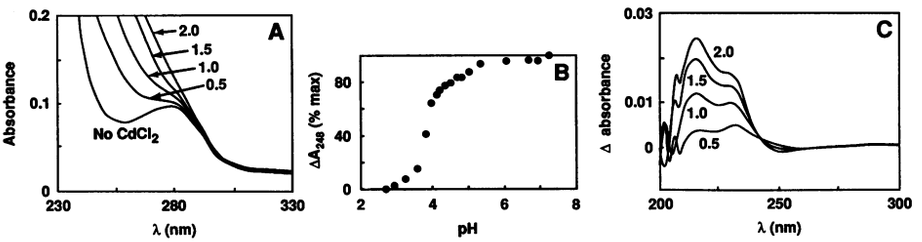

Fig. 1. (**A**) Ultraviolet absorption spectra of reduced Tat and of Tat with 0.5 to 2.0 molar equivalents of $CdCl_2$. (**B**) The *p*H dependence of $CdCl_2$ binding to Tat. (**C**) Difference ultraviolet absorption spectra of Tat with 0.5 to 2.0 molar equivalents of $ZnCl_2$. Protein samples (20 to 94 μg/ml in 20 m*M* tris-HCl, *p*H 7.2) were prepared in a nitrogen-purged anaerobic chamber, and metals were added directly to the cuvette. All spectra were recorded and analyzed on a Hewlett-Packard 8451A diode array spectrophotometer, and the sample compartment was purged with nitrogen for all measurements. For the *p*H titration, 2.0 equivalents of $CdCl_2$ were added to reduced Tat at *p*H 7.24, and the absorption difference calculated at 248 nm was used as the maximum value. Hydrochloric acid was added to acidify the complex, and spectra were measured down to *p*H 2.71.

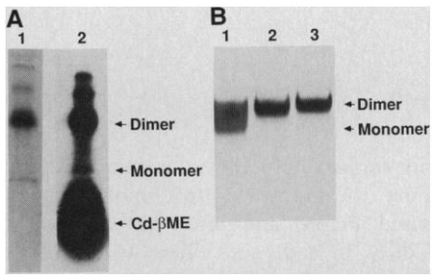

Fig. 2. (**A**) SDS gel electrophoresis of Tat-Cd^{2+} complexes. (Lane 1) Three micrograms of Tat with 3 molar equivalents of $CdCl_2$, stained with Coomassie blue. (Lane 2) Autoradiograph of 3 μg of Tat with 3 molar equivalents of $^{115m}CdCl_2$. Samples were incubated with $CdCl_2$ in buffer (20 m*M* tris-HCl, *p*H 7.2, 10 m*M* β-mercaptoethanol) for 30 minutes at 22°C before electrophoresis. The gel contained 20% polyacrylamide, and the electrophoresis buffers were those described by Laemmli (*39*). Positions of Tat monomers and dimers are indicated, and the large band below the monomer in lane 2 results from complexes of ^{115m}Cd with β-mercaptoethanol. (**B**) Native gel electrophoresis of Tat on a 20% polyacrylamide gel containing 0.1 m*M* $ZnCl_2$. (Lane 1) Apo-Tat; (lane 2) Tat with 3 molar equivalents of $ZnCl_2$; (lane 3) Tat with 3 molar equivalents of $CdCl_2$. Each lane contained 8 μg of protein, and the gel was stained with Coomassie blue. The gel was thoroughly prerun to remove ammonium persulfate, and the buffer was replaced with fresh running buffer (30 m*M* tris-acetate, *p*H 7.0, 0.1 m*M* $ZnCl_2$).

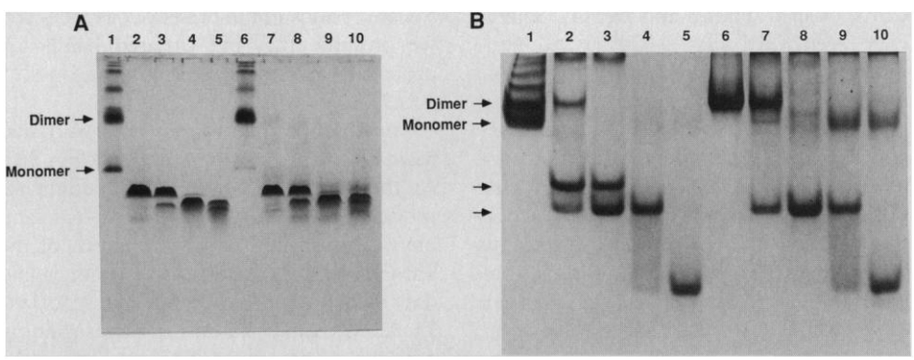

Fig. 3. Partial proteolysis of Tat by chymotrypsin. (**A**) Apo-Tat (lanes 2 to 5) and Tat-Cd^{2+} complexes (lanes 7 to 10) were digested for various times with chymotrypsin and analyzed on a 20% SDS-polyacrylamide gel. Digestions were carried out for 1 minute (lanes 2 and 7), 5 minutes (lanes 3 and 8), 30 minutes (lanes 4 and 9), and 120 minutes (lanes 5 and 10). Lanes 1 and 6 contain apo-Tat and Tat-Cd^{2+} complexes before proteolysis. Reactions were carried out at 22°C in 20 m*M* tris-HCl, *p*H 7.2, 10 m*M* β-mercaptoethanol, and had a chymotrypsin:Tat ratio of 1:60 (wt/wt). Proteolysis was stopped by addition of 10 μg of phenylmethylsulfonyl fluoride (PMSF) to each reaction. (**B**) Apo-Tat (lanes 2 to 5) and Tat-Cd^{2+} complexes (lanes 7 to 10) were digested for various times with chymotrypsin, the reaction was stopped with PMSF, and the samples were analyzed on a 20% polyacrylamide native gel. Lanes 1 and 6 contain apo-Tat and Tat-Cd^{2+} complexes before proteolysis. Reaction conditions and digestion times are the same as in (A), but the chymotrypsin:Tat ratio was 1:300 (wt/wt) in these experiments. The cleavage sites of two fragments (arrows) have been determined.

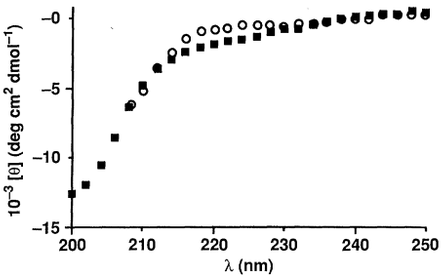

Fig. 4. Circular dichroism spectra of Tat. Solid squares show the spectrum of apo-Tat, and an indistinguishable spectrum was obtained when $ZnCl_2$ or $CdCl_2$ was added. The open circles show the spectrum measured for apo-Tat in 6*M* urea. Spectra were recorded at 27°C on an Aviv model 60DS spectropolarimeter (purged with nitrogen) and were averaged over five scans. Protein samples (0.45 mg/ml in 10 m*M* tris-HCl, *p*H 7.2) were prepared in an anaerobic chamber and placed in a 0.05-cm demountable quartz cuvette for these measurements.

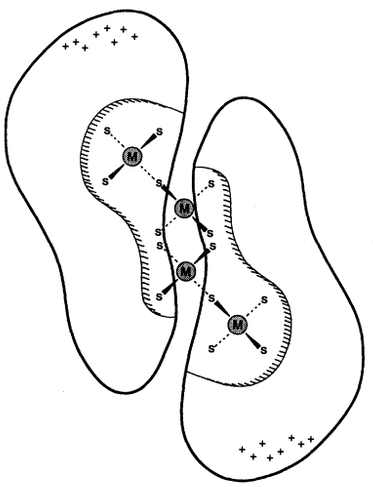

Fig. 5. Schematic representation of a Tat metal-linked dimer. Shown are four metal ions (M) coordinated in tetrahedral geometries by 14 cysteine thiolate ligands (S). Each Tat monomer contributes 7 cysteines. Two of the 14 cysteines are shown to bridge a pair of metal ions, but the bridging cysteines would be unnecessary if one histidine or one carboxylate from each molecule also serves as a ligand. The clusters of positive charges (+) represent the basic region of the protein and could constitute part of a nucleic acid binding site. This arrangement has twofold symmetry which might be used to recognize a symmetric nucleic acid binding site. Alternatively, Tat could interact with other proteins (cellular or viral) and the basic regions of the Tat dimer might contact acidic domains on DNA-binding proteins and alter their activity.

not some metal-induced oxidation product. First, the number of free thiols was measured before and after the addition of $CdCl_2$. In both cases, there were 7 mol of thiol per mole of Tat monomer, demonstrating that no oxidation occurred as a result of metal binding. Second, in experiments monitored by native gel electrophoresis, we found that adding EDTA dissociates the Tat-Cd^{2+} dimer. Native gels were also run with other metals, including $HgCl_2$, $CoCl_2$, $CuCl_2$, CuCl, $FeCl_3$, and $FeSO_4$. Dimers were seen with Hg^{2+}, Cu^{2+}, and Cu^{1+} whereas the Fe^{2+}, Fe^{3+}, and Co^{2+} samples were indistinguishable from samples containing no metal. (We note that Cu^{2+} is a strong oxidant and some of the dimer formation with $CuCl_2$ may result from oxidation.) These results probably reflect the relative affinities of the metals for Tat. Obviously, complexes with different metals could have important structural and functional differences.

Proteolytic digestion was used to monitor folding of Tat. Chymotryptic digests of apo-Tat and of Tat-Cd^{2+} complexes were analyzed by SDS–polyacrylamide gel electrophoresis (Fig. 3A). At first, proteolysis yields one predominant product band, regardless of whether or not Cd^{2+} is present (lanes 2 and 7). Digestions with subtilisin, elastase, and proteinase K (in the presence or absence of metals) also showed a similar band at early times. This part of the protein, which consists of residues 48 to 86 (see below), might be structured in the absence of metals. However, some differences in the digestion patterns of apo-Tat and Tat-Cd^{2+} complexes can be seen: in the presence of $CdCl_2$, a set of smeared bands migrate at or somewhat below the dimer position. In addition, a band of low molecular weight appears (lane 8), which is not present in the apo-Tat sample (lane 3). Subtilisin, elastase, and proteinase K also showed smeared bands in the presence of $CdCl_2$ and we presume that the smeared bands are proteolytic fragments which oxidize on SDS gels.

The chymotryptic digests were also analyzed by native gel electrophoresis (Fig. 3B). At the first time point, two predominant product bands are seen in the apo-Tat sample (arrows, lane 2), whereas much of the Tat-Cd^{2+} complex remains as a dimer and only the lower product band is present (lane 7). At later times, additional bands running near the monomer position are seen in the samples containing $CdCl_2$ (lanes 9 and 10), but corresponding bands are not present at significant levels in the samples containing apo-Tat (lanes 4 and 5). This is additional evidence for proteolytic protection, and native gels with ^{115m}Cd show that these fragments contain Cd^{2+}.

To determine the actual cleavage sites, we purified and analyzed the two major bands seen early during chymotryptic digestion (arrows, Fig. 3B). Amino acid sequencing indicated that these fragments begin with residue 38 and residue 48, and amino acid analysis indicated that they both extend to the COOH-terminus of the protein. Thus, the fragment that appears in the presence or absence of metal (lower arrow, Fig. 3B) contains residues 48 to 86 and includes the highly basic region of Tat. We suspect that cleavage at this site, which is between the cysteine-rich and basic regions, is structurally significant. Four proteases (chymotrypsin, subtilisin, proteinase K, and elastase) all produce fragments with similar mobilities on SDS and native gels. In the absence of metal, chymotrypsin also cleaves at Phe^{38} (upper arrow, Fig. 3B), but this site, which is immediately adjacent to the cysteine-rich region, is not accessible in the Tat-Cd^{2+} complex. These proteolytic results suggest that Tat contains at least two domains; the cysteine-rich region shows metal-dependent folding, while the COOH-terminal region seems to be relatively resistant to proteolysis and may be folded in the absence of metal.

Circular dichroism (CD) spectra of apo-Tat and Tat-metal complexes are essentially indistinguishable, suggesting that metal binding does not have a dramatic effect on protein folding (Fig. 4). No change in the CD spectrum was observed when either $CdCl_2$ or $ZnCl_2$ was added, even though absorption spectra confirmed that the Tat-metal complexes were properly formed. The CD spectra at various urea concentrations suggest that apo-Tat may be partly folded (Fig. 4). A change in the spectrum of apo-Tat is seen as 4 to 5*M* urea is added, suggesting that some denaturation occurs at these concentrations, but this spectral change is unaffected by metals. These results are consistent with the proteolytic experiments and confirm that metal ions do not regulate a global folding-unfolding transition for the entire protein.

Although the TAR site is required for transactivation, it is not known whether Tat binds directly to this site or whether the effects are mediated by interactions with other proteins or sites. Our data suggest that apo-Tat, the Tat-Zn^{2+} complex, and the Tat-Cd^{2+} complex bind tightly but nonspecifically to both DNA and RNA. These studies focused on the TAR site, and several DNA fragments were synthesized for binding experiments. Gel shift experiments used DNA fragments containing base pairs +1 to +57 and base pairs +1 to +108 from HIV. At Tat concentrations of 10^{-7} to 10^{-8}*M*, these fragments remained in the wells of polyacrylamide or agarose gels, but Tat bound as tightly to unrelated fragments of similar lengths. Deoxyribonuclease I footprinting experiments confirmed that this

binding was nonspecific, and binding was not dependent on metals. However, these DNA fragments do not contain sequences upstream from the transcription initiation site which could be important for binding. We also checked for specific RNA binding. RNA fragments that spanned the TAR site were synthesized by in vitro transcription of constructs with an SP6 promoter. These fragments contained HIV sequences +1 to +57 and +1 to +108 (with nine additional bases at the 5′ end) and thus contain either one or two stem-loop regions from the TAR site (*9*). At Tat concentrations of 10^{-7} to $10^{-8}M$, all the RNA remained in the wells of polyacrylamide or agarose gels. However, control experiments showed similar results with unrelated RNA fragments, and the binding was not dependent on metals.

We have shown that Tat binds two metal ions per monomer and that it forms a metal-linked dimer. However, the proteolysis experiments and CD spectra indicate that metals do not regulate a global folding-unfolding transition. The primary effects of metals appear to be limited to structural changes in the cysteine-rich region and to subsequent dimerization. Although detailed structural studies of Tat will be required, our studies show that at least two metal-binding sites must be at the dimer interface. In attempting to further explore the structural significance of our results, we have used metallothioneins as a guide. X-ray crystallography and nuclear magnetic resonance have been used to determine the structure of two metallothioneins (*35, 36*). Each protein contains two domains. The first domain binds three metals using six cysteines that are liganded to a single metal and three cysteines that bridge pairs of metal ions. This allows each metal to have a tetrahedral arrangement of cysteine ligands. The second domain of metallothionein binds four metals. In this domain, tetrahedral binding sites are achieved when six cysteines are used as terminal ligands and five as bridging ligands. Counting the cysteines and metal ions in Tat allows us to propose a plausible arrangement for the Tat dimer (Fig. 5) although we must remember that the $CoCl_2$ spectrum does not give direct evidence for tetrahedral sites in Tat and we do not know whether histidines or carboxylates from Tat serve as metal ligands. Since Tat forms a metal-linked dimer, there are 14 cysteines available to bind the four metal ions. A tetrahedral arrangement can be constructed around each metal if 12 cysteines serve as terminal ligands (bind to just one metal) and 2 cysteines act as bridging ligands. Obviously, there are many plausible arrangements, but each Tat monomer must contact at least three metals, and some of the metal-binding sites must contain cysteines from each monomer.

Patarca and Haseltine proposed that a Tat monomer might bind a single metal ion in a manner analogous to the "zinc finger" proteins (*14*). We have shown that this model is unlikely and our results should be a deterrent to indiscriminate use of the term "zinc fingers" for any sequence that matches the test pattern devised by Berg for defining potential metal-binding sites (*20*). It is probably appropriate to restrict the term "zinc fingers" to those proteins that have multiple repeats of about 30 amino acids each, that conserve the cysteine and histidine residues at a proper spacing, and that conserve the two aromatic residues and the leucine found in the TFIIIA repeats. Other sequences containing sets of cysteine and histidine residues may be metal-binding sites, but there is no reason to believe that they will be structurally homologous to the "zinc finger" domain from TFIIIA.

Given the reducing environment and the relatively high metal concentrations in eukaryotic cells, we believe that Tat binds metals in vivo. However, we do not know which metals bind or how metals affect transactivation. Clearly, it will be necessary to isolate the protein from infected cells to determine which metals bind in vivo and whether the activity of Tat depends on which metal is bound. Zn^{2+} is a likely candidate since it is very abundant in the body and since many proteins bind zinc. Copper also is a reasonable possibility since it is relatively abundant and can form metal-linked Tat dimers. Although less abundant in the human body, Cd^{2+} could be important because it binds Tat so tightly.

Since Tat is essential for viral replication, it provides an attractive target for drug design. Our results suggest several possibilities for interfering with the action of Tat. It is conceivable that dietary intake of metals may affect Tat activity and chelators could also affect cellular metal concentrations. One chelator, D-penicillamine, blocks HIV replication in vitro (*37*) and appears to suppress viral replication in AIDS patients (*38*). It is conceivable that penicillamine affects Tat and inactivates the protein by chelating metals or by binding to the cysteine-rich region of Tat. Another approach would involve designing peptides or drugs that would prevent Tat dimerization by masking the normal dimerization site, perhaps by mimicking the cysteine-rich interface normally provided by the other monomer. Further studies of Tat are necessary to fully understand the significance of metal-linked dimerization, but our results clearly focus attention on the role of metal binding and the effects of metals on the structure and function of Tat.

REFERENCES AND NOTES

1. J. G. Sodroski *et al.*, *Science* **227**, 171 (1985).
2. A. G. Fisher *et al.*, *Nature (London)* **320**, 367 (1986).
3. A. I. Dayton *et al.*, *Cell* **44**, 941 (1986).
4. S.-Y. Kao, A. F. Calman, P. A. Luciw, B. M. Peterlin, *Nature (London)* **330**, 489 (1987).
5. J. Hauber, A. Perkins, E. P. Heimer, B. R. Cullen, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 6364 (1987).
6. B. M. Peterlin, P. A. Luciw, P. J. Barr, M. D. Walker, *ibid.* **83**, 9734 (1986).
7. C. M. Wright, B. K. Felber, H. Paskalis, G. N. Pavlakis, *Science* **234**, 988 (1986).
8. B. R. Cullen, *Cell* **46**, 973 (1986).
9. M. A. Muesing, D. H. Smith, D. J. Capon, *ibid.* **48**, 691 (1987).
10. M. B. Feinberg, R. F. Jarrett, A. Aldovini, R. C. Gallo, F. Wong-Staal, *ibid.* **46**, 807 (1986).
11. C. A. Rosen *et al.*, *Nature (London)* **319**, 555 (1986).
12. C. A. Rosen, J. G. Sodroski, W. A. Haseltine, *Cell* **41**, 813 (1986).
13. T. Okamoto and F. Wong-Staal, *ibid.* **41**, 813 (1986).
14. R. Patarca and W. A. Haseltine, *AIDS Res. Hum. Retroviruses* **3**, 1 (1987).
15. S. K. Arya, C. Guo, S. F. Josephs, F. Wong-Staal, *Science* **229**, 69 (1985).
16. J. Sodroski, R. Patarca, C. Rosen, F. Wong-Staal, W. Haseltine, *ibid.*, p. 74.
17. L. J. Seigel *et al.*, *Virology* **148**, 226 (1986).
18. D. H. Hamer, *Annu. Rev. Biochem.* **55**, 913 (1986).
19. J. H. R. Kagi *et al.*, *Env. Health Persp.* **54**, 93 (1984).
20. J. M. Berg, *Science* **232**, 485 (1986).
21. A. Aldovini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6672 (1986).
22. These cells were provided by C. Debouck. Tat expression was induced with the use of nalidixic acid (*21*).
23. G. E. Means and R. E. Feeney, *Chemical Modification of Proteins* (Holden-Day, San Francisco, 1971).
24. K. B. Nielson and D. R. Winge, *J. Biol. Chem.* **258**, 13063 (1983).
25. M. Vasak, J. H. R. Kagi, H. A. O. Hill, *Biochemistry* **20**, 2852 (1981).
26. M. Vasak and J. H. R. Kagi, *Metal Ions Biol. Systems* **15**, 213 (1985).
27. M. Beltramini, K. Lerch, M. Vasak, *Biochemistry* **23**, 3422 (1984).
28. D. R. Winge, K. B. Nielson, W. R. Gray, D. H. Hamer, *J. Biol. Chem.* **260**, 14464 (1985).
29. M. J. Stillman, W. Cai, A. J. Zelazowski, *ibid.* **262**, 4538 (1987).
30. M. Vasak, J. H. R. Kagi, B. Holmquist, B. L. Vallee, *Biochemistry* **20**, 6659 (1981).
31. M. Good and M. Vasak, *ibid.* **25**, 3328 (1986).
32. A. D. Frankel, J. M. Berg, C. O. Pabo, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4841 (1987).
33. K. B. Nielson, C. L. Atkin, D. R. Winge, *J. Biol. Chem.* **260**, 5342 (1985).
34. M. P. Waalkes, M. J. Harvey, C. D. Klaassen, *Toxicol. Lett.* **20**, 33 (1983).
35. W. F. Furey *et al.*, *Science* **231**, 704 (1986).
36. W. Braun *et al.*, *J. Mol. Biol.* **187**, 125 (1986).
37. P. Chandra and P. S. Sarin, *Arzneim. Forsch.* **36**, 184 (1986).
38. P. Sarin *et al.*, Fifth International Meeting on AIDS Research (Washington, DC, 1987), abstract, p. 14.
39. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).

40. We thank C. Debouck for providing us with *E. coli* cells containing Tat, antibody to Tat, and suggestions about purification, and M. Rosenberg and Smith Kline & French Laboratories for their help in obtaining these materials; C. Riley, V. Vogel, and B. Byers for protein sequencing and oligonucleotide synthesis; P. Shenbagamurthi for amino acid analysis; J. Berg for advice on anaerobic handling of samples and for helpful comments on this manuscript; D. Shortle for the use of his Aviv spectropolarimeter; H. Dintzis for the use of his Hewlett-Packard spectrophotometer; and A. Mildvan for advice on preparing CuCl solutions. Supported by the Howard Hughes Medical Institute and by NIH grant GM31471.

16 November 1987; accepted 5 February 1988