Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7593–7597, October 1990
Biochemistry

Purification and characterization of recombinant Rev protein of human immunodeficiency virus type 1

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Communicated by John Burns, June 21, 1990 (received for review April 18, 1990)

ABSTRACT **Recombinant Rev protein of human immunodeficiency virus type 1 has been expressed in *Escherichia coli* and purified by ion-exchange and gel-filtration chromatography. Specific binding of the purified protein to the Rev-responsive element of the viral RNA is demonstrated. Physical characterization of the purified protein by circular dichroism and intrinsic fluorescence spectroscopy indicate that the protein preparation is suitable for structural analysis. Circular dichroism measurements show that the protein is approximately 40–45% α-helix. Tryptophan fluorescence measurements suggest that the single tryptophan residue is located near the surface of the protein. Gel-filtration chromatography of the protein indicates that it has an apparent molecular mass of 53,000 daltons. This suggests that the protein in solution forms a stable tetramer consisting of monomers having molecular mass of 13,000 daltons.**

Replication of human immunodeficiency virus type 1 (HIV-1) is regulated by a complex set of virally encoded proteins (Tat, Rev, Nef, Vif, and Vpu) at the levels of transcription, post-transcription, and maturation (reviewed in ref. 1). The mechanisms of action of these regulatory proteins and their interplay with cellular factors have been subjects of intense study. HIV-1 Rev protein induces the transition from expression of early regulatory genes (*tat*, *rev*, and *nef*) to late structural genes (*gag*, *pol*, and *env*) (2, 3). HIV-1 mutants that lack Rev fail to synthesize the viral structural proteins, thereby preventing formation of mature virion (4–6). Recently, it was demonstrated that HIV-1 Rev, a nuclear phosphoprotein (7–9), controls the export ratio of constitutively expressed spliced and unspliced viral mRNAs from the nucleus (10–17). This is the first protein to be identified with such a function. Although the mechanism by which Rev controls the cytoplasmic appearance of these viral RNA species is not clear at the present time, Chang and Sharp (18) reported that Rev may regulate the dissociation of pre-mRNA from splicing complexes.

The site of Rev binding on the RNA, the Rev-responsive element (RRE) has been mapped within the *env* coding sequence of the viral genome (5, 12). Purified recombinant HIV-1 Rev protein was shown to bind specifically to RRE RNA (19–21), and the binding site was mapped by site-directed mutagenesis (22, 23) and by RRE fragment binding (24). While this demonstrates that recombinant Rev retains binding activity, no physical or biochemical characterization of the protein has been reported, so far as we know.

The importance of this protein in the life cycle of the virus and the uniqueness of its apparent functional mechanism make it attractive for structural studies. Progress in solving the structure of HIV-1 Rev and directly assessing its interaction with viral RNA will depend on having a suitable supply of functional and structurally uniform protein. Cochrane *et al.* (25) reported the purification under denaturing conditions of a recombinant HIV-1 Rev having an N-terminal extension containing six histidine residues. Although the renatured HIV-1 Rev protein prepared by this method was shown to be active in a qualitative cell-based assay, this material was not suitable for structural analysis due to aggregation of the protein that occurs during refolding. It is not known whether the histidine cluster contributes to protein aggregation. More recently, Daly *et al.* (20) have reported purification of recombinant HIV-1 Rev by using ion-exchange and gel-filtration chromatography, also under denaturing conditions, followed by protein refolding. While refolded Rev was shown to bind RRE RNA *in vitro*, no analysis of the protein structure was performed.

We have developed an alternative method of purifying recombinant HIV-1 Rev from *Escherichia coli* that maintains the protein in a stable homogeneous form suitable for structural analysis. The purified protein binds specifically to RNA containing the RRE. Circular dichroism and intrinsic fluorescence spectroscopy indicate that the purified protein is structured. Protein characterization by gel-filtration chromatography and SDS/PAGE suggest that HIV-1 Rev forms a stable tetramer.

EXPERIMENTAL PROCEDURES

Cloning and Expression of HIV-1 *rev*. A synthetic *rev* gene segment for the first 56 amino acids of HIV-1 Rev (strain HXB2) was prepared from oligonucleotides; codons frequently used in *E. coli* were used (25). This gene segment was fused into the *Bam*HI/*Pvu* II fragment from a cDNA clone of the HXB3 strain to produce a hybrid Rev protein (21). Expression of the *rev* gene was under the control of the λPL promoter and was induced in midlogarithmic phase culture by temperature shift to 42°C. After induction, the cells were allowed to accumulate HIV-1 Rev for 4 hr prior to harvesting by centrifugation. Cells were stored at −80°C until needed for purification.

Purification of Recombinant HIV-1 Rev. *E. coli* cells (400 g, wet weight) expressing HIV-1 Rev were suspended in 50 mM Tris·HCl, pH 8.0/1 mM EDTA/0.5 mM phenylmethylsulfonyl fluoride (buffer A) at 0.25 g/ml and disrupted by sonication or by passing through a Manton–Gaulin homogenizer. Insoluble material was removed by centrifugation at 15,000 × *g* for 30 min. The soluble fraction was diluted 6-fold with buffer A containing 0.48 M NaCl and was applied onto a Q-Sepharose column (4.4 × 20 cm; Pharmacia) equilibrated with buffer A containing 0.4 M NaCl. The majority of cellular

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Abbreviations: HIV-1, human immunodeficiency virus type 1; RRE, Rev-responsive element.

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proteins and nucleic acids passed through the column and were discarded. The column was washed with 50 column volumes of buffer A containing 0.4 M NaCl over 48 hr. During this time, the absorption of the eluant decreased to less than 0.1 unit at 280 nm.

Protein was eluted from the column by using buffer A containing 0.8 M NaCl. Fractions equal to 1/5th of the column volume were collected, and the protein and nucleic acid contents were determined as described below. Fractions containing HIV-1 Rev protein were pooled, and solid NaCl was added to give 4 M. Nucleic acid was precipitated by addition of 2 vol of ice-cold ethanol, and the sample was stored at −20°C overnight. After centrifugation to remove the nucleic acids, the soluble fraction was dialyzed twice against 30 vol of 50 mM Tris·HCl, pH 7.5/0.5 M NaCl/1 mM EDTA/0.1 mM phenylmethylsulfonyl fluoride (buffer B) and concentrated by using an Amicon YM-30 membrane. When required, the protein was further purified by chromatography on a Sephacryl S-200 column (Pharmacia) in the same buffer. The purified protein was sterile filtered and stored at 4°C. Purity of the protein was determined by electrophoresis on SDS-PAGE. Analytical gel filtration was performed on a Sephacryl S-200HR column (Pharmacia).

UV Absorption. UV spectra were obtained by using a Perkin–Elmer Lambda 7 spectrophotometer with buffer B in the reference cell. DNA content of the samples was measured by the 280/260 absorbance ratio (26). Protein concentration was determined from absorption, using an extinction coefficient of 1.03 $M^{-1}\cdot cm^{-1}$ at 280 nm in buffer B or by colorimetric assay (Pierce) based on an HIV-1 Rev standard whose concentration was determined by amino acid composition.

Binding Assay. A plasmid containing RRE sequence (nucleotides 7296–7562) downstream from a T7 promoter (Bluescript vector, Stratagene) was a gift of C. A. Rosen (Roche Institute of Molecular Biology). By using a heterologous gene construct, the RRE-containing sequence was shown to respond to Rev induction *in vivo* (21, 22).

The plasmid was linearized and transcripts were generated according to the protocol recommended by the supplier of T7 RNA polymerase (BRL). Transcripts were purified by DNase I treatment (10 min, 1 unit/μg of DNA), gel filtration through Sephadex G-50 (Pharmacia), extraction with chloroform/phenol, and precipitation with ethanol. Quality of the purified RNA was judged by PAGE, which gave a single band of correct size (data not shown).

The mobility shift RNA protection assay was performed according to published procedures (19–23, 27). Approximately 1 ng of ^{32}P-labeled RNA (≈100,000 cpm) and 100 ng of Rev protein were combined in 20 μl of binding buffer [5 mM Hepes, pH 7.6/2 mM $MgCl_2$/25 mM KCl/3.8% (vol/vol) glycerol] containing 10 μg of tRNA, a nonspecific competitor. The mixture was incubated at 30°C for 15 min, followed by addition of 5 units of RNase T1 for 10 min at 37°C. Electrophoresis of the RNA-protein complex was performed at 4°C on a 4% acrylamide gel under nondenaturing conditions (12 V/cm, 3 hr). Autoradiography was used to identify the binding complex on the dried gel. Competitive binding assays were performed in binding buffer containing 0.4 M KCl as described (28).

Circular Dichroism. Circular dichroism (CD) measurements of purified HIV-1 Rev were performed on a Jasco J-500A spectropolarimeter at room temperature. Protein samples at concentrations between 0.3 and 0.5 mg/ml were analyzed in 50 mM Tris·HCl, pH 7.5/500 mM NaCl/1 mM EDTA. The instrument was calibrated with *d*-10-camphorsulfonic acid in water. CD results are expressed in molar ellipticity, $[\theta]$ ($deg\cdot cm^2\cdot dmol^{-1}$). Calculations of the percent helical structure were based on the 221 nm and 208 nm Cotton effects as described (29).

Fluorescence Spectroscopy. Protein fluorescence measurements were made at room temperature in a Perkin–Elmer MPF-66 fluorescence spectrophotometer operated in corrected mode. Protein samples were diluted to 5–15 μg per ml in 50 mM Tris·HCl, pH 7.5/1 mM EDTA/500 mM NaCl (buffer C). Tryptophan fluorescence was excited by using 295-nm light from a xenon arc lamp, with slits set at 5 nm for both excitation and emission. Contribution by tyrosine to the fluorescence at 344 nm was negligible and was ignored in this analysis. All emission scans were obtained at scan speed of 60 nm/min. Fixed-wavelength measurements were integrated for 16 sec to minimize drift and increase reproducibility. Steady-state fluorescence polarization was measured by using horizontally and vertically polarized light as described (30).

Fluorescence quenching by chemical agents was determined by the method of Eftink and Ghiron (31). Aliquots of acrylamide (4 M) or KI (2.5 M) in buffer C were added to Rev (5 μg/ml in buffer C) in the cuvette and the fluorescence at 344 nm was determined immediately. Sodium thiosulfate (10 mM) was added to the KI stock to prevent the formation of I_2. Fluorescence measured after addition of quencher was corrected for dilution. Acrylamide quenching was also corrected for inner filter effects as described by Lakowicz (30). Stern–Volmer plots of fluorescence quenching were constructed as described (31).

RESULTS

Expression and Purification of HIV-1 Rev. A recombinant gene encoding HIV-1 Rev was expressed in *E. coli* at high levels (Fig. 1, lane 1). The protein is found predominantly in the cytosolic fraction of *E. coli*, where it binds to nucleic acid with high affinity. We have capitalized upon this strong interaction for protein purification since most cellular proteins and DNA do not bind to an anion-exchange column in high concentrations of salt. Ion-exchange chromatography under conditions for RNA purification (i.e., binding and elution at high ionic strength) yields a protein preparation that is >90% HIV-1 Rev (Fig. 1, lane 5).

While the majority of protein contaminants are removed by the anion-exchange step, on a per-weight basis, the preparation retains a large fraction of cellular nucleic acids as measured by the absorbance of the sample (Fig. 2, trace A). Note that to compensate for the strong UV absorbance by nucleic acids present in the sample, it was necessary to dilute the protein to 8 μg/ml for the absorbance measurements. At this concentration of protein and nucleic acid, the spectrum is dominated by nucleic acid at 260 nm. RNase and DNase

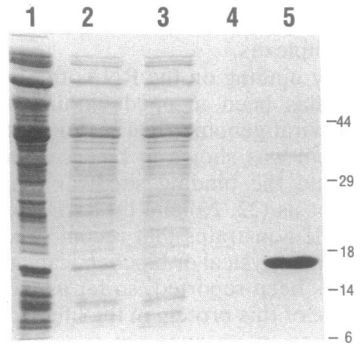

FIG. 1. SDS/PAGE of HIV-1 Rev. Aliquots from steps of the purification of HIV-1 Rev were subjected to electrophoresis on a 15% acrylamide gel. Lane 1, total *E. coli* protein extract; lane 2, cytosolic fraction used for Q-Sepharose column load; lane 3, column flow-through; lane 4, final column wash; lane 5, step elution with 0.8 M NaCl in buffer A. Molecular weight × 10^{-3} is indicated on the right.

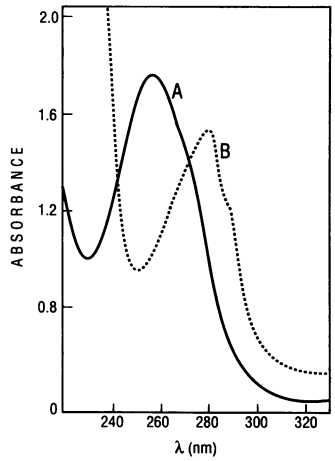

FIG. 2. Absorption spectra of HIV-1 Rev. Protein spectra were measured in 50 mM Tris·HCl, pH 7.5/0.5 M NaCl/1 mM EDTA/0.1 mM phenylmethylsulfonyl fluoride. Trace A, partially purified protein after ion-exchange chromatography. Protein was 8 μg/ml. Trace B, protein after precipitation of nucleic acid with ethanol. Protein was 1 mg/ml. Spectra were obtained in 1-cm pathlength cells measured against buffer in the reference cell.

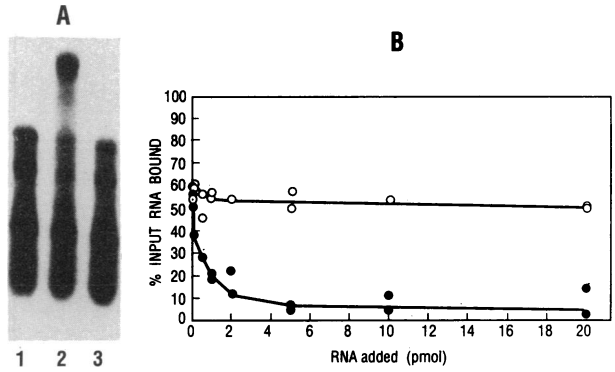

FIG. 3. RNA binding assay for HIV-1 Rev. (*A*) Purified HIV-1 Rev was incubated with radiolabeled RNA transcripts containing RRE and then digested with RNase T1. Aliquots were subjected to electrophoresis on a 4% acrylamide gel. RNA was visualized by autoradiography. Lane 1, no protein; lane 2, HIV-1 Rev; lane 3, Rev denatured by boiling 5 min. (*B*) Filter binding assay of Rev with RRE. HIV-1 Rev (0.2 pmol) was incubated in 40 μl with 0.5 pmol of RRE (containing 60,000 cpm) and unlabeled RRE (●) or tRNA (○) for 20 min. Duplicate aliquots (19 μl) were transferred to moist nitrocellulose filters and washed, and radioactivities were measured.

sensitivity of the sample indicates that the contaminant is mainly RNA, although some cellular DNA remains with the preparation as well (data not shown).

Separation of the protein from the nucleic acid is possible only at very high ionic strength. At NaCl concentrations below 2.0 M, gel filtration of protein from the anion-exchange step revealed a broad elution of Rev along with nucleic acid (not shown). At 4.0 M NaCl, the protein separates from high molecular weight RNA and migrates as a homogeneous peak. [The strong ionic interaction between Rev and RNA is likely mediated through an arginine-rich region of the protein (residues 35–50), a characteristic also found in HIV-1 Tat and other nucleic acid-binding proteins (32, 33).] While the sample is at high ionic strength, ethanol is added to precipitate nucleic acid, and the soluble protein is subjected to centrifugation and dialysis.

After removal of the ethanol, Rev is sufficiently pure for RNA-binding assays and protein characterization by spectrophotometric methods. When required, chromatography on a Sephacryl S-200 column was used to remove minor contaminants that remain with Rev after RNA precipitation. HIV-1 Rev protein prepared by this method is greater than 95% pure, based on amino acid sequencing and composition analysis (not shown). The protein yield when this purification protocol is used is approximately 5 mg of HIV-1 Rev per gram of total cellular protein.

SDS/polyacrylamide gels show that HIV-1 Rev protein has an apparent molecular weight of 17,000 (Fig. 1). While this is larger than the coding molecular weight (13,000), this anomalous behavior on SDS/PAGE is consistent with that observed for other proteins rich in basic residues (16% arginine and lysine residues) (34). Migration of the protein in SDS/PAGE was unaffected by incubation with dithiothreitol, indicating that the protein does not form interchain disulfide bonds (data not shown).

RNA Binding. Binding of HIV-1 Rev to RNA transcripts containing the RRE was studied by a gel shift assay (19–23, 27). Purified HIV-1 Rev was incubated with radiolabeled RNA to form an RNA·protein complex. After digestion with RNase T1, the protein·RNA fragments were fractionated on a polyacrylamide gel and visualized by autoradiography. The mobility of an RNA fragment was retarded in the presence of Rev protein (Fig. 3*A*, lane 2). Retardation was not seen in the absence of added protein (lane 1) or when the protein was denatured by boiling prior to incubation with RNA (lane 3). In a filter-binding assay, unlabeled RNA containing the RRE is able to compete for Rev binding, whereas unlabeled tRNA at the same concentrations has no effect on binding of labeled RRE, demonstrating the specificity of the RRE–protein interaction (Fig. 3*B*). The dissociation constant (K_d), which is equivalent to the protein concentration at 50% binding of the input RNA, was determined to be 3.5×10^{-9} M (not shown).

Protein Spectroscopy. The UV absorption spectrum of purified HIV-1 Rev is typical of a protein containing 1 tryptophan and 2 tyrosine residues: there is a peak at 279 nm and a shoulder at 290 nm (Fig. 2, trace B). The 280/260 absorption ratio indicates less than 0.25% nucleic acid (the lower limit for detection) present in the purified protein (26).

A circular dichroism spectrum of HIV-1 Rev shows a characteristic α-helical conformation for the purified protein (Fig. 4). On the basis of the intensities of the 221 nm and 208 nm Cotton effects, the α-helix content is estimated to be 47% and 39%, respectively (29). The negative CD bands in the aromatic region of the spectrum (260–300 nm) are relatively strong. This may reflect an interaction between the arginine

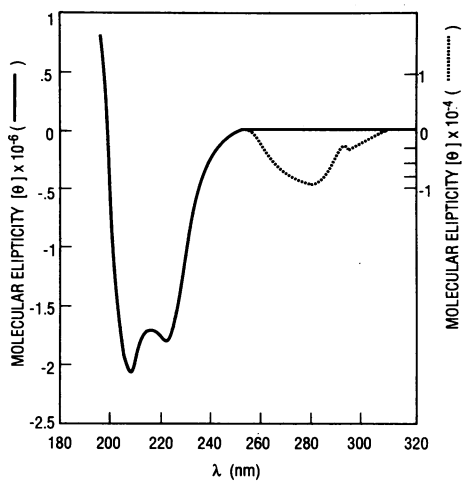

FIG. 4. Circular dichroism spectrum of HIV-1 Rev. The spectrum was obtained at 0.35 mg of protein per ml in 50 mM Tris·HCl, pH 7.5/500 mM NaCl/1 mM EDTA. Measurements were recorded at room temperature.

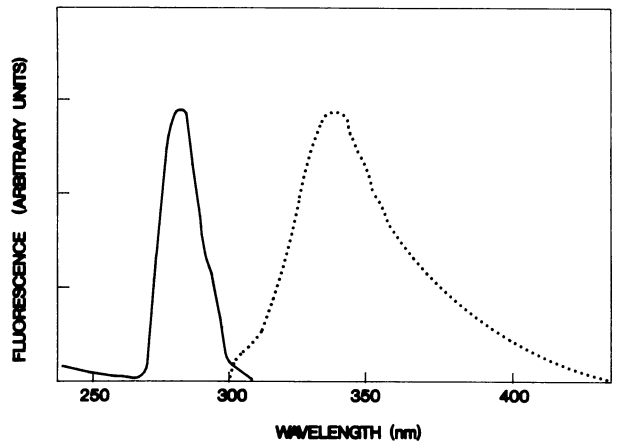

FIG. 5. Fluorescence excitation and emission spectra of HIV-1 Rev. Corrected spectra are shown. Protein concentration was 10 μg/ml in buffer C. ——, Excitation; ....., emission.

residues and the phenolic hydroxyl group of a vicinal tyrosine residue (35).

HIV-1 Rev is one of only a few proteins that contain a single tryptophan residue (Trp-45). This feature allows us to characterize the environment around the tryptophan residue by using fluorescence spectroscopy. While the two tyrosine residues in the protein (Tyr-23 and Tyr-63) contribute to the fluorescence spectrum, the quantum yield of tyrosine is small compared with that of tryptophan and can be ignored for this analysis. A scan of the emission spectrum of HIV-1 Rev excited at 280 nm shows a corrected emission peak at 344 nm (Fig. 5). In comparison to other proteins, Trp-45 of HIV-1 Rev has a long-wavelength fluorescence that is characteristic of tryptophan residues exposed to an aqueous environment. Considering the amino acid residues that immediately surround Trp-45, it is likely that this residue is at or near the surface of the protein.

The relative location of Trp-45 within the Rev structure can also be inferred from analysis of fluorescence polarization and chemical quenching. The measured polarization and anisotropy (0.15 and 0.10, respectively) are characteristic of a fluorophor that is relatively unconstrained within the protein structure, as would be expected for a fluorophor near the surface of a protein. Also consistent with this analysis, the effects of chemical quenchers on fluorescence further support assignment of Trp-45 near the protein surface. More than 50% of the fluorescence is quenched by addition of acrylamide, a permeant quencher, or iodide, a nonpenetrating reagent (Fig. 6).

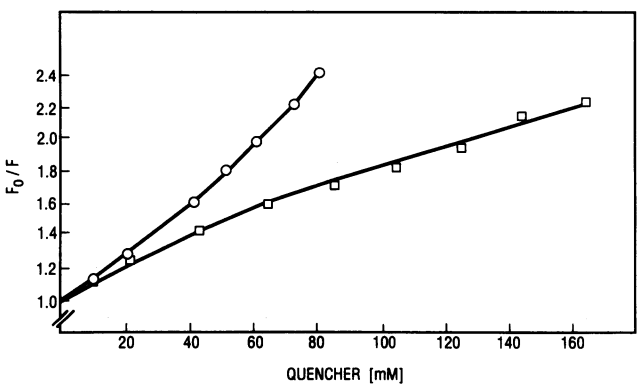

FIG. 6. Stern–Volmer plot of quenching of purified HIV-1 Rev fluorescence by acrylamide (○) or KI (□). F_0 and F, Fluorescence in the absence and presence of quencher.

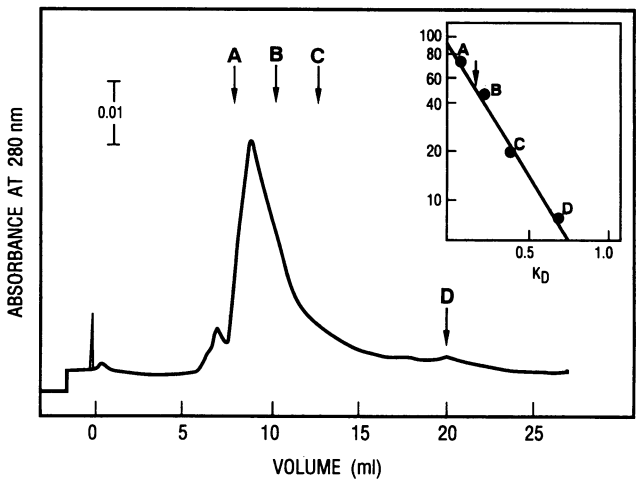

FIG. 7. Gel filtration of HIV-1 Rev on Sephacryl S-200. HIV-1 Rev (150 μg) was subjected to gel filtration on 1 × 25 cm S-200HR column equilibrated in 50 mM Tris·HCl, pH 7.5/500 mM NaCl/1 mM EDTA/0.1 mM phenylmethylsulfonyl fluoride. Arrows mark the position of gel filtration molecular weight standards run separately: A, bovine serum albumin (68,000); B, ovalbumin (43,000); C, myoglobin (17,000); and D, vitamin B_{12} (1350). (*Inset*) Semilogarithmic plot of elution profile for molecular weight markers. K_D, partition coefficient; ↓, HIV-1 Rev.

Gel-Filtration Chromatography. Molecular sizing of HIV-1 Rev by analytical gel filtration was performed to determine the overall structure of the protein in solution. The elution profile relative to standard globular proteins indicates that Rev has an apparent molecular weight of 53,000 (Fig. 7). Since the coding molecular weight of Rev is 13,000, this suggests that the protein forms a tetramer. No peak corresponding to monomer was observed in the chromatography, nor was Rev observed in the filtrate of a 30,000 molecular weight cutoff ultrafiltration (not shown), implying that the equilibrium strongly favors an oligomeric structure.

Since HIV-1 Rev contains a high percentage of charged amino acid residues, care was required to eliminate possible effects of aggregation in determining the molecular weight of the protein. In addition to chromatography in buffers at moderate ionic strengths, gel filtration in 2 M NaCl was performed. The size of the protein was unaffected by the high salt concentration, an indication of the stability of the tetramer. Moreover, incubation with 20 mM dithiothreitol prior to gel filtration had no effect on Rev, consistent with SDS/PAGE, where the protein appears as a monomer under nonreducing, denaturing conditions.

DISCUSSION

Progress in determining the structure of HIV-1 Rev will require a protein preparation that retains biological activity and has a homogeneous structure suitable for analytical study. In this paper we have described the expression, purification, and characterization of a recombinant form of HIV-1 Rev that retains these properties.

Direct interaction between HIV-1 Rev and a target site within the viral mRNA has been previously demonstrated by using crude extracts of recombinant protein (19) or a purified, refolded protein (20–23). It is not known if the methods used in these studies for purification and refolding produce a homogeneous preparation of folded protein. Protein purified by metal chelate chromatography under denaturing conditions did not refold into a homogeneous structure, although it apparently recovered activity when "scrape-loaded" into cells (25).

The purification method described in this report efficiently produces a purified HIV-1 Rev protein without the need for denaturants. Purified Rev retains RNA-binding activity *in vitro*. In addition, physical characterization by circular dichroism, intrinsic fluorescence, and gel-filtration chromatography indicate that the recombinant protein assumes a folded homogeneous structure in solution. Moreover, we can conclude that the ethanol extraction method used to separate HIV-1 Rev from nucleic acid does not irreversibly denature the protein.

Our initial studies of the protein structure have concentrated on the single tryptophan residue in HIV-1 Rev, which is located within a cluster of basic amino acids (10 arginine residues between amino acids 35–50). It has been proposed that this basic domain is essential for both nucleolar localization and RNA binding (14, 16, 36). Site-directed mutagenesis of HIV-1 Rev has been used to suggest that Trp-45 is also essential for RNA binding, since arginine substitution at residue 45 inactivates the protein (36). Recently, Lazinski *et al.* (37) observed that many proteins that recognize RNA hairpin structures contain a conserved arginine-rich motif within the primary sequence. Although an aromatic residue is found within the arginine-rich motif of some of the proteins cited, the deduced consensus sequence does not include an aromatic residue. It is possible that nonconsensus residues within the cluster may provide the determinants that confer specificity to RNA binding. While the fluorescence data reported here indicate that the tryptophan residue is located near the surface of the protein, further experiments will be needed to determine whether Trp-45 is directly involved in RRE RNA recognition. An alternative interpretation of the mutagenesis results that demonstrate an essential role of Trp-45 in RNA binding would be that alterations at that residue may affect the conformation of adjacent amino acid residues which directly bind RNA.

Gel-filtration chromatography of purified HIV-1 Rev indicates that the folded protein has a molecular weight of 53,000. When compared with the coding molecular weight of Rev (i.e., 13,000), the chromatography molecular weight suggests that HIV-1 Rev forms a tetramer in solution. It should be noted that such a structure presumes that HIV-1 Rev is globular, as is characteristic of the proteins used as molecular weight standards. If Rev assumes a structure in solution that is not globular, an apparent molecular weight determined by gel filtration could be inaccurate. The circular dichroism spectrum does not indicate that the protein structure is nonglobular. Moreover, preliminary experiments using chemical crosslinking also suggest that HIV-1 Rev can form multimers (C.M.N., unpublished results).

While it remains to be seen whether the tetramer structure for HIV-1 Rev has biological significance, it is worth noting that Daly *et al.* (20) have reported a binding stoichiometry of 8 Rev per RRE RNA. One interpretation of this stoichiometry would be that two copies of HIV-1 Rev tetramer can bind to RRE RNA simultaneously. In our own preliminary studies, protein purified by the current method binds RRE RNA at a stoichiometry of 1:1 based on the monomer molecular weight; however, this measurement is sensitive to salt and buffer composition in the binding assay (38). Additional studies, including isolation of a stable protein·RNA complex, will be required to fully address this interaction.

Availability of HIV-1 Rev in a stable form devoid of nucleic acid is important in determining the structure of this viral protein. Information gained on Rev and its interaction with the other proteins and the target RNA may prove useful in modeling therapeutics for AIDS intervention.

The authors thank Dr. C. A. Rosen for providing us with plasmids used to initiate this study, and J. Hulmes, M. C. Miedel, and Dr. Y.-C. Pan for performing amino acid sequencing and composition analysis of the purified protein.

1. Cullen, B. R. & Green, W. C. (1989) *Cell* **58,** 423–426.
2. Sodroski, J. G., Goh, W. C., Rosen, C. A., Dayton, A., Terwilliger, E. & Haseltine, W. A. (1986) *Nature (London)* **321,** 412–417.
3. Feinberg, M. B., Jarrett, R. F., Aldovini, A., Gallo, R. C. & Wong-Staal, F. (1986) *Cell* **46,** 807–817.
4. Knight, D. M., Flomerflet, F. A. & Ghrayeb, J. (1987) *Science* **236,** 837–840.
5. Rosen, C. A., Terwilliger, E., Dayton, A., Sodroski, J. G. & Haseltine, W. A. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2071–2075.
6. Terwilliger, E., Burghoff, R., Sia, R., Sodroski, J. G., Haseltine, W. A. & Rosen, C. A. (1988) *Cell* **62,** 655–658.
7. Hauber, J., Bouvier, M., Malim, M. H. & Cullen, B. R. (1988) *J. Virol.* **62,** 4801–4804.
8. Cochrane, A., Kramer, R., Ruben, S., Levine, J. & Rosen, C. A. (1989) *Virology* **171,** 264–266.
9. Cullen, B. R., Hauber, J., Campbell, K., Sodroski, J. G., Haseltine, W. A. & Rosen, C. A. (1987) *J. Virol.* **62,** 2498–2501.
10. Hammarskjold, M.-L., Heimer, J., Hammarskjold, B., Sangwan, I., Albert, L. & Rekosh, D. (1989) *J. Virol.* **63,** 1959–1966.
11. Sadaie, M. R., Benter, T. & Wong-Staal, F. (1988) *Science* **236,** 837–840.
12. Malim, M. H., Hauber, J., Fenrick, R. & Cullen, B. R. (1989) *Nature (London)* **335,** 181–183.
13. Malim, M. H., Hauber, J., Le, S.-Y., Maizel, J. V. & Cullen, B. R. (1989) *Nature (London)* **338,** 254–257.
14. Malim, M. H., Bohnlein, S., Hauber, J. & Cullen, B. R. (1989) *Cell* **58,** 205–214.
15. Ahmad, N., Maitra, R. K. & Venkatesan, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6111–6115.
16. Felber, B. K., Hadzopoulou-Cladaras, M., Cladaras, C., Copeland, T. & Pavlakis, G. N. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1495–1499.
17. Emerman, M., Vazeux, R. & Peden, K. (1989) *Cell* **57,** 1155–1165.
18. Chang, D. D. & Sharp, P. A. (1989) *Cell* **59,** 789–795.
19. Zapp, M. L. & Green, M. R. (1989) *Nature (London)* **342,** 714–716.
20. Daly, T. J., Cook, K. S., Gray, G. S., Maione, T. E. & Rusche, J. R. (1989) *Nature (London)* **342,** 816–819.
21. Cochrane, A. W., Chen, C.-H. & Rosen, C. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1198–1202.
22. Olsen, H. S., Nelbock, P., Cochrane, A. W. & Rosen, C. A. (1990) *Science* **247,** 845–848.
23. Malim, M. H., Tiley, L. S., McCarn, D. F., Rusche, J. R., Hauber, J. & Cullen, B. R. (1990) *Cell* **60,** 675–683.
24. Heaphy, S., Dingwall, C., Ernberg, I., Gait, M. J., Green, S. M., Karn, J., Lowe, A. D., Singh, M. & Skinner, M. A. (1990) *Cell* **60,** 685–693.
25. Cochrane, A. W., Chen, C.-H., Kramer, R., Tomchak, L. & Rosen, C. A. (1989) *Virology* **173,** 335–337.
26. Layne, E. (1957) *Methods Enzymol.* **3,** 447–454.
27. Leibold, L. A. & Munro, H. N. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2171–2175.
28. Butler, J. S. & Clark, J. M. (1984) *Biochemistry* **23,** 809–815.
29. Jibson, M. D. & Li, C. H. (1981) *Int. J. Peptide Protein Res.* **18,** 297–301.
30. Lakowicz, J. R. (1983) *Principles of Fluorescence Spectroscopy* (Plenum, New York), pp. 258–305.
31. Eftink, M. R. & Ghiron, C. A. (1976) *Biochemistry* **15,** 672–680.
32. Sperling, R. & Wachtel, E. J. (1981) *Adv. Prot. Chem.* **34,** 1–60.
33. Arya, S., Guo, C., Josephs, S. F. & Wong-Staal, F. (1985) *Science* **229,** 69–74.
34. Gabriel, O. & Wong, S. F. (1969) *Anal. Biochem.* **27,** 545–553.
35. Strickland, E. H. (1974) *CRC Crit. Rev. Biochem.* **2,** 113–175.
36. Perkins, A., Cochrane, A. W., Ruben, S. M. & Rosen, C. A. (1989) *J. Acquired Immune Defic. Syndr.* **2,** 256–263.
37. Lazinski, D., Grzadzielska, E. & Das, A. (1989) *Cell* **59,** 207–218.
38. Antelman, D., Nalin, C. M. & Hsu, M.-C. (1990) *FASEB J.* **4,** 2253.