Characterization of a zinc blotting technique: Evidence that a retroviral gag protein binds zinc

(protein blot/metalloprotein/zinc finger)

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ABSTRACT We have characterized a simple method that uses $^{65}$ZnCl$_2$ to detect zinc-binding proteins that have been immobilized on nitrocellulose. Conditions have been identified that permit the detection of as little as 1 μg of some zinc-binding proteins. The specificity of the binding is indicated by the ability of other divalent metal ions to compete with $^{65}$Zn(II) in this assay. We have used this technique to provide evidence that the nucleic acid-binding gag protein of retroviruses also binds zinc. This technique can be applied to biological mixtures of proteins and may be used in proteolytic mapping studies to identify protein fragments that have zinc-binding activity.

Modifications of the protein blotting technique (1) have provided information concerning the interaction of proteins with a variety of classes of ligands. These ligands include other proteins, such as antibodies (1) and toxins (2), nucleic acids (3), intact cells (4), and ions, such as calcium (5). Using a modification of the protein blotting technique, we identified a carboxyl-terminal double-stranded RNA-binding fragment of the mammalian reovirus outer capsid protein σ3 (6). Previous studies had shown that native σ3 possesses a double-stranded RNA-binding activity (7). The observation by Berg (8) of a potential correlation between metal binding sites and nucleic acid-binding domains, and the identification of a transcription factor IIIA (TFIIIA)-like zinc finger within the sequence of σ3, prompted us to examine σ3 for the presence of zinc. We were able to show by atomic absorption spectroscopy that both affinity-purified σ3 and the σ3 associated with purified virion particles contain stoichiometric amounts of firmly bound zinc (6).

To test the hypothesis that the double-stranded RNA- and zinc-binding activities of σ3 are linked, we developed a simple solid-phase zinc blotting assay and identified a zinc-binding proteolytic fragment of the σ3 protein. We probed a protein blot with $^{65}$ZnCl$_2$ and showed that $^{65}$Zn(II) bound to an amino-terminal fragment of σ3 that contains the TFIIIA-like zinc-binding sequence and is distinct from the carboxyl-terminal double-stranded RNA-binding fragment (6). These results indicated that the zinc–nucleic acid-binding paradigm might be relevant to the reovirus σ3 protein; however, the ability to detect $^{65}$Zn(II)-binding activity in a purified protein fragment that contains a proposed zinc finger sequence suggested that the zinc blotting assay might be used to identify other zinc-binding proteins and protein fragments.

In this report, we have characterized the zinc blot by using a panel of zinc metalloproteins. Assay conditions are described that permit the detection of some authentic zinc-binding proteins, and competition experiments with a variety of divalent cations suggest that the binding activity detected in this assay is specific for zinc. We have used the zinc blot to provide evidence that the nucleic acid-binding retroviral gag protein also binds zinc. This technique should prove useful for the identification and analysis of other zinc-binding proteins.

MATERIALS AND METHODS

Protein Samples. Stock solutions of purified proteins (Sigma, and Cooper Biomedical, Malvern, PA) were prepared in distilled deionized water. The molecular weight markers phosphorylase b ($M_r$, 94,000), bovine serum albumin ($M_r$, 67,000), ovalbumin ($M_r$, 43,000), carbonic anhydrase ($M_r$, 30,000), soybean trypsin inhibitor ($M_r$, 20,000), and α-lactalbumin ($M_r$, 14,000) were purchased from Pharmacia. Avian myeloblastosis virus (AMV) was obtained from Life Sciences (St. Petersburg, FL) and was partially purified by differential centrifugation (9). Purified reovirus virions were prepared as described (10). Prior to electrophoresis, protein samples were dissolved in sample buffer [100 mM Tris·HCl, pH 6.8/10% (vol/vol) glycerol/1% NaDodSO$_4$/5% 2-mercaptoethanol/0.005% bromophenol blue] and boiled for 2–5 min.

NaDodSO$_4$/PAGE and Electrophoretic Transfer. Discontinuous NaDodSO$_4$/PAGE was performed according to the protocol described by Laemmli (11). Gradient gels were prepared by the method described by Hames (12). Proteins were electrophoretically transferred to nitrocellulose (Bio-Rad Trans-Blot) by the protein blotting technique (1). The amount of protein per sample was adjusted to compensate for large differences in efficiencies of protein transfer. Proteins immobilized on the nitrocellulose were detected by staining with amido black (0.1% in 45% methanol/10% acetic acid). Protein staining with amido black was done after autoradiography and the membranes were rinsed in H$_2$O to preserve the dimensions of the nitrocellulose filter for densitometric analysis.

Zinc Blotting and Autoradiography. After transfer of proteins to nitrocellulose, the filters were washed in metal-binding buffer (100 mM Tris·HCl, pH 6.8/50 mM NaCl) for at least 1 hr. The nitrocellulose was probed for 30 min to 1 hr with 5–10 μCi of $^{65}$ZnCl$_2$ per lane (2 Ci/g; 1 Ci = 37 GBq; New England Nuclear) in 5–20 ml of metal-binding buffer. The filter was washed with metal-binding buffer for 15–30 min with three changes of buffer. In competition experiments, divalent metal ions were included in the metal-binding buffer for all steps. Nitrocellulose filters were wrapped in SaranWrap and exposed to XAR film (Eastman Kodak) with an intensifying screen (Cronex Lightning Plus, E.I. du Pont de Nemours).

Densitometry. To determine the relative amount of different proteins that had been transferred to nitrocellulose by densitometry, stained nitrocellulose filters were dried between filter paper at 37°C, cleared with immersion oil, and

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Abbreviations: TFIIIA, transcription factor IIIA; AMV, avian myeloblastosis virus.
dried again between filter paper. Stained filters and autoradiograms were analyzed densitometrically with an Ultrascan laser densitometer and on-line integrator (LKB).

RESULTS

Identification of Zinc Metalloproteins by Zinc Blotting. To determine whether the zinc blot could be used to detect zinc-binding proteins other than reovirus σ3, we analyzed a panel of proteins that included several zinc metalloproteins (for a review, see ref. 13). Proteins were subjected to NaDodSO_4/PAGE and transferred to nitrocellulose by the protein blotting technique (1). After transfer, the nitrocellulose filter was equilibrated in metal-binding buffer containing 100 mM Tris-HCl (pH 6.8) and 50 mM NaCl. The filter was probed with ^65ZnCl_2, washed with metal-binding buffer, and wrapped in Saran Wrap for autoradiography. After autoradiography, the proteins on the filter were stained with amido black.

An example of such an analysis is shown in Fig. 1. A stained filter (Fig. 1A) revealed approximately equal amounts of the proteins phosphorylase a, enolase, alkaline phosphatase, alcohol dehydrogenase, carboxypeptidase A, carbonic anhydrase, superoxide dismutase, and lysozyme (lanes 1–8, respectively), as well as a sample of purified reovirus virions (lane 9). Autoradiography of the membrane (Fig. 1B) indicated that ^65Zn(II) was bound to the zinc metalloproteins alkaline phosphatase (lane 3), carbonic anhydrase (lane 6), and superoxide dismutase (lane 7) and to the reovirus capsid protein σ3 (lane 9) but not to the control proteins phosphorylase a, enolase, or lysozyme (lanes 1, 2, and 8, respectively). Alcohol dehydrogenase (lane 4) and carboxypeptidase A (lane 5), both of which are zinc metalloproteins, failed to bind significant amounts of ^65Zn(II) in this assay. As previously shown (6), the zinc blot suggests that one or more of the reovirus λ proteins also binds ^65Zn(II) (lane 9), consistent with the results of atomic absorption spectroscopy, which revealed the presence of zinc in the reovirus core (6).

Relative Affinity of Proteins for ^65Zn(II) in the Zinc Blot. The results of the previous experiment suggested that equivalent amounts of alkaline phosphatase, carbonic anhydrase, and superoxide dismutase present on the nitrocellulose filter bound different amounts of ^65Zn(II). To determine whether zinc metalloproteins exhibit different relative affinities for ^65Zn(II) in the zinc blot, we used densitometry to quantitate the ability of increasing amounts of several zinc-binding proteins to bind ^65Zn(II). Assuming that the proteins stain equivalently with amido black, the amount of protein bound to the filter was quantitated after staining the filter with amido black and clearing it with immersion oil. Because the apparent differences in affinity for ^65Zn(II) could be explained by differences in molar amounts of zinc-binding sites in equivalent amounts of stained protein, values for ^65Zn(II) binding obtained from this analysis were corrected for protein size. The relationship between ^65Zn(II) binding and protein concentration (Fig. 2) revealed that the amount of ^65Zn(II) bound to zinc metalloproteins on nitrocellulose increased linearly with

![Fig. 1](image1.png)

**Fig. 1.** ^65Zn(II) binds to some zinc metalloproteins in a zinc blot. Phosphorylase a (3 μg) (lane 1), enolase (6 μg) (lane 2), alkaline phosphatase (8 μg) (lane 3), alcohol dehydrogenase (22 μg) (lane 4), carboxypeptidase A (10 μg) (lane 5), carbonic anhydrase (9 μg) (lane 6), superoxide dismutase (6 μg) (lane 7), lysozyme (8 μg) (lane 8), and purified reovirus T3 Dearing virions (4 × 10^11) (lane 9) were separated on a 5–20% NaDodSO_4/polyacrylamide gel and transferred to nitrocellulose. The filter was equilibrated in metal-binding buffer for 3 hr, probed with 80 μCi of ^65ZnCl_2 in 20 ml of metal-binding buffer for 45 min, and washed for 15 min. (A) Nitrocellulose stained with amido black. (B) Autoradiograph of nitrocellulose exposed to XAR film (Kodak) for 6 hr at -70°C with an intensifying screen.

![Fig. 2](image2.png)

**Fig. 2.** Relative affinity of proteins for ^65Zn(II). Proteins were separated on two 5–20% NaDodSO_4/polyacrylamide gels and transferred to nitrocellulose. Filters were equilibrated for 2 hr in metal-binding buffer, probed with 100 μCi each of ^65ZnCl_2, in 20 ml of metal-binding buffer for 30 min, washed for 15 min, and exposed to XAR film for 18 hr at -70°C with an intensifying screen. After autoradiography, the filters were stained with amido black and cleared with immersion oil. The autoradiograph and the stained nitrocellulose were analyzed by using an Ultrascan laser densitometer (LKB). The amount of protein per lane on the nitrocellulose filter was determined from the amido black stain and expressed as densitometric units. The amount of ^65Zn(II) bound, corrected for the molecular weight of the protein analyzed, is expressed as densitometric units (determined from the autoradiograph) × M_r × 10^-7. Protein samples were as follows: (A) 2, 6, and 9 μg of carbonic anhydrase (CA); 4, 7, and 10 μg of alkaline phosphatase (AP); 2, 4, and 6 μg of enolase (EN); and 2 × 10^11, 4 × 10^11, and 6 × 10^11 purified reovirus T3 Dearing virions (σ3, λ); (B) 2, 6, and 9 μg of carbonic anhydrase (CA); 4, 8, 12 μg of alkaline phosphatase (AP); 2 × 10^11, 4 × 10^11, and 6 × 10^11 purified reovirus T3 Dearing virions (σ3, λ); 2, 5, and 8 μg of bovine serum albumin (BSA); 5, 8, and 11 μg of ovalbumin (OVA).
the amount of protein on the filter. These results also suggested that zinc metalloproteins can exhibit different relative affinities for $^{65}\text{Zn(II)}$ in the blotting assay. Superoxide dismutase (data not shown) and carbonic anhydrase bound $^{65}\text{Zn(II)}$ significantly better than other zinc-binding proteins such as alkaline phosphatase and reovirus $\sigma 3$ (Fig. 2). Some nonmetalloproteins such as enolase (Fig. 2A) and lysozyme (data not shown) exhibited negligible $^{65}\text{Zn(II)}$ binding, while others such as bovine serum albumin (Fig. 2B) bound $^{65}\text{Zn(II)}$ when large amounts of protein were present on the filter.

The Effect of pH on the Binding of $^{65}\text{Zn(II)}$. The ability of $^{65}\text{Zn(II)}$ to bind to proteins immobilized on nitrocellulose was dependent on the pH of the binding buffer (Fig. 3). Molecular weight marker proteins (Fig. 3A) and reovirus virion proteins (Fig. 3B) were subjected to NaDodSO$_4$/PAGE, and the separated proteins were transferred to nitrocellulose. All of the proteins that were present on the filters were evident on the panels stained with amido black (lanes S). Identical filters were equilibrated for 1 hr in metal-binding buffer that had been adjusted to pH 8, 7.5, 7, 6.5, or 6. These buffers were used for all incubation and washing steps. The filters were probed with $^{65}\text{ZnCl}_2$ and washed for 15 min. Autoradiography of the probed filters revealed $^{65}\text{Zn(II)}$ bound to all of the proteins on the filters that had been incubated at pH 8. At pH 7 and below, $^{65}\text{Zn(II)}$ was bound to only the zinc metalloproteins carbonic anhydrase and reovirus $\sigma 3$. Because $^{65}\text{Zn(II)}$ binding to zinc metalloproteins was diminished at pH 6.5, metal-binding buffer at pH 6.8 was used to maximize the specificity of the assay.

The Proposed Zinc-Binding Protein AMV p12gag Binds $^{65}\text{Zn(II)}$ in a Zinc Blot. It appeared from the study of the reovirus capsid protein $\sigma 3$ that the zinc blot could be used to detect proposed zinc-binding proteins or protein fragments within biological mixtures (6). We have used the zinc blot to test the prediction that nucleic acid-binding retroviral gag proteins also bind Zn(II) (8) (Fig. 6). The avian retroviral gag

![Fig. 3](image-url) Effect of pH on $^{65}\text{Zn(II)}$ binding. Samples containing 3 µl of a mixture of phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and $\alpha$-lactalbumin each at 1 µg/µl (A) and 3.5 $\times$ 10$^{11}$ purified reovirus T3 Dearing virions (B) were separated on a 5–20% NaDodSO$_4$/polyacrylamide gel. The proteins were transferred to nitrocellulose and the filter was cut into strips such that pairs of samples containing molecular weight marker proteins and reovirus virion proteins were equilibrated, probed, and washed in metal-binding buffer adjusted to pH 8, 7.5, 7, 6.5, and 6. The filters were probed with $^{65}\text{ZnCl}_2$ (20 µCi per 10 ml) for 1 hr and washed for 15 min. The filters were exposed to XAR film (Kodak) for 23 hr at −70°C with an intensifying screen. A duplicate (lane S) was stained with amido black. CA, carbonic anhydrase.

![Fig. 4](image-url) Competition of $^{65}\text{Zn(II)}$ binding. Samples containing 2 µg of superoxide dismutase and 4 µg of carbonic anhydrase (A) and 3.5 $\times$ 10$^{11}$ purified reovirus T3 Dearing virions (B) were separated on a 5–20% NaDodSO$_4$/polyacrylamide gel and the separated proteins were transferred to nitrocellulose. Strips of the nitrocellulose containing both samples were equilibrated for 1 hr in metal-binding buffer either in the absence (lane $-$) or in the presence of competing ions (10 mM Ca(II), 10 mM Mg(II), 0.1 mM Cd(II), or 0.01 mM Zn(II)). The strips were exposed for 30 min with 10 µCi of $^{65}\text{ZnCl}_2$ in 5 ml of metal-binding buffer containing the competitor and washed for 15 min in the same buffer. The filters were exposed to XAR film (Kodak) for 13 hr at −70°C with an intensifying screen. CA, carbonic anhydrase; SOD, superoxide dismutase.
FIG. 5. Competition of $^{65}$Zn(II) binding to superoxide dismutase. Samples containing 2 µg of superoxide dismutase were subjected to electrophoresis on 5–20% NaDodSO4/polyacrylamide gels and the proteins were transferred to nitrocellulose. The filters were cut into strips and then equilibrated, probed, and washed in metal-binding buffer containing a competing metal ion. The following competitors were used: ZnCl2, and CuCl2 (0.001, 0.01, and 0.1 mM), CoCl2, and FeCl3 (0.01, 0.1, 1, and 10 mM), CdCl2 (0.01, 0.1, and 1 mM), MnCl2 (1 and 10 mM), CaCl2, and MgCl2 (10 mM). Filters were probed for 30 min with 10 µCi of $^{65}$ZnCl2 in 5 ml of metal-binding buffer containing competitor and washed for 15 min in the same buffer. Filters were exposed to XAR film (Kodak) for 13 hr at −70°C with an intensifying screen. The amount of $^{65}$Zn(II) bound to superoxide dismutase in the presence of competitors was quantitated by using an Ultrascan laser densitometer with an on-line integrator (LKB) and is expressed as a percentage of that bound to the protein in the absence of a competitor.

protein p12 is a nucleic acid-binding core protein that forms ribonucleoprotein complexes (14). p12 contains two sequences with similarity to the TFIIIA-like zinc fingers (8, 15). AMV core proteins (Fig. 6, lanes 1) and molecular weight marker proteins including carboxy anhydrase (lanes 2) were separated by NaDodSO4/PAGE and transferred to nitrocellulose. The filter was equilibrated in metal-binding buffer, probed with $^{63}$ZnCl2, and washed. Staining the nitrocellulose with amido black (Fig. 6A) revealed the AMV gag proteins.

Fig. 6. AMV p12 gag binds $^{65}$Zn(II). AMV virion cores were partially purified from infected chicken plasma. Partially purified AMV cores (20 µl) (~1 µg/µl) (lane 1) and molecular weight marker proteins (2 µl) including carboxy anhydrase (1 µg/µl) (lane 2) were separated on a 5–20% NaDodSO4/polyacrylamide gel, and the separated proteins were transferred to nitrocellulose. The filter was equilibrated in metal-binding buffer for 2 hr, probed with $^{65}$ZnCl2 (15 µCi per 10 ml) for 30 min, and washed. (A) Nitrocellulose filter stained with amido black. (B) Autoradiograph of filter exposed to XAR film (Kodak) for 18 hr at −70°C with an intensifying screen.

p27, p19, p12. An autoradiograph of this filter probed with $^{65}$ZnCl2 (Fig. 6B) indicated that $^{65}$Zn(II) was bound to AMV p12, but not to p19 or p27 (lane 1). These results provided evidence that the retroviral nucleic acid-binding gag proteins also bind zinc; however, a comparison of the binding of $^{65}$Zn(II) to p12 (lane 1) and carbonic anhydrase (lane 2) suggested that p12 bound $^{65}$Zn(II) relatively poorly in this assay, given its size and the presence of two proposed zinc-binding sequences.

**DISCUSSION**

In this report, we have characterized the zinc blot technique (6) by using a panel of zinc metalloproteins. We have demonstrated that some of these proteins are able to bind $^{65}$Zn(II) after being denatured, subjected to NaDodSO4/PAGE, and immobilized on nitrocellulose filters. The zinc metalloproteins carboxy anhydrase, superoxide dismutase, alkaline phosphatase, and retrovirus o3 bind $^{65}$Zn(II) in our assay conditions, as does thermolysin (data not shown); however, carboxypeptidase A and alcohol dehydrogenase do not.

The zinc-binding sites of a number of zinc metalloproteins have been characterized. Those of carboxypeptidase A (16) and alkaline phosphatase (17, 18), for example, consist of amino acid residues that are widely separated on the peptide chain but are brought close together in the tertiary structures of the native proteins (residues 69, 72, and 196 in carboxypeptidase A; residues 331, 372, and 412 and residues 51, 369, and 370 in alkaline phosphatase). We think of these binding sites as "conformational." Other zinc-binding sites use residues that lie within a short stretch of primary amino acid sequence. A motif of this sort has been described for TFIIIA and a set of apparently related proteins, in which structural elements referred to as "fingers" are formed by the coordination of zinc ions by invariant pairs of closely spaced cysteine and histidine residues (19, 20). Many other well-described zinc-binding proteins may be considered to contain zinc-binding fingers in the sense that the zinc ion is coordinated by residues that are closely spaced in their primary amino acid sequences. These proteins include carboxy anhydrase (residues 94, 96, and 119), superoxide dismutase (residues 61, 69, 78, and 81), and thermolysin (residues 142, 146, and 166).

The ability or inability of a zinc metalloprotein to bind $^{65}$Zn(II) in the zinc blot is likely to reflect the structure of its zinc-binding site. Most zinc metalloproteins whose zinc-binding sites may be characterized as fingers appear to bind $^{65}$Zn(II) well in our assay. This observation suggests that these proteins require minimal renaturation to reconstitute a functional zinc-binding site. On the other hand, one protein with a conformational zinc-binding site (carboxypeptidase A) was unable to bind significant amounts of $^{65}$Zn(II) in the zinc blot, while another (alkaline phosphatase) appeared to have some zinc-binding activity. The observation that alkaline phosphatase, which contains two conformational zinc-binding sites (17, 18), has a lower relative affinity for zinc in the blotting assay than carboxy anhydrase or superoxide dismutase, which each contain a single zinc finger, may reflect different requirements for renaturation of one or the other of its conformational zinc-binding sites. Alcohol dehydrogenase, which contains a zinc finger (residues 87, 100, 103, and 111) as well as a conformational zinc-binding site (residues 46, 67, and 174) (21), binds $^{65}$Zn(II) poorly in this assay. Attempts to prepare a stable apoprotein of alcohol dehydrogenase have been unsuccessful (22), suggesting that the removal of zinc causes the irreversible denaturation of the zinc-binding site of this protein. While denaturation and immobilization of proteins on a solid phase could alter or obscure zinc-binding sites that are present in native proteins,
these procedures might also reveal fortuitous zinc-binding activities not seen in native proteins. However, all of the well-characterized proteins we have tested that display a high affinity for $^{65}$Zn(II) in this assay are authentic zinc metalloproteins. Although our present experiments do not address the actual site at which $^{65}$Zn(II) is bound to any of the immobilized proteins, the study of mutant proteins and proteolytic fragments should provide insight into the binding sites for zinc in the immobilized proteins.

Associations of metals and proteins have been classified as metalloproteins or metal–protein complexes on the basis of the apparent stability constants of their interactions (reviewed in ref. 23). Metalloproteins are distinguished by a small number of metal ions bound firmly to a limited number of specific binding sites ($K_d = 10^{-10}$ M for zinc binding by alkaline phosphatase, carbonic anhydrase, and carboxypeptidase A). Less stable metal–protein complexes have been described for numerous other proteins. Both enolase and bovine serum albumin, for example, form zinc–protein complexes ($K_d = 10^{-5}$ M for enolase). Except at higher pH, most nonmetalloproteins we have tested in the zinc blot do not bind significant amounts of $^{65}$Zn(II). Thus, it appears that the conditions we have described for the zinc blot are relatively specific for metalloproteins. Our ability to detect $^{65}$Zn(II) bound to bovine serum albumin suggests that, under conditions of high protein concentration, these assay conditions might detect some proteins that form zinc–protein complexes.

The ability of proteins to bind metals is sensitive to pH (reviewed in refs. 13 and 23). The fact that $^{65}$Zn(II) bound to all proteins at pH 8 in our assay presumably reflects an increased amount of nonspecific binding dictated by the proteins’ increased negative charge. Thus, the detection of specific binding in the zinc blot appears to depend on an ability to decrease nonspecific binding with decreased pH without eliminating the specific binding activities.

We have found that $^{65}$Zn(II) does not bind nonspecifically to all cation-binding sites in the zinc blot. For example, the calcium-binding proteins α-lactalbumin and parvalbumin are not detected in this assay. However, we have detected $^{65}$Zn(II) binding to the iron-binding protein catalase (data not shown). These data are consistent with the observation that Fe(II) but not Ca(II) has been shown to reconstitute some zinc metalloproteins. In addition, only those metallic cations that structurally substitute for Zn(II) in metalloproteins (13) effectively compete with $^{65}$Zn(II) in the zinc blot. These findings suggest that it is unlikely that nonspecific electrostatic interactions are entirely responsible for metal binding in this assay.

The zinc-binding activity of the reovirus σ3 protein was initially suggested by sequence homology between σ3 and the TFIIIA zinc fingers and was subsequently demonstrated by atomic absorption analysis (6). We then demonstrated that an amino-terminal fragment of σ3, which contained the TFIIIA-like zinc finger sequence, bound $^{65}$Zn(II) in a zinc blot (6). We have presented additional evidence in this report that the zinc blot may be used to detect other potential zinc-binding proteins. The nucleic acid-binding gag proteins of retroviruses contain one or two sequences that have been hypothesized to form metal-binding domains (8). Homology between these sequences and the nucleic acid-binding g32 protein of bacteriophage T4 had been previously noted (15), and the g32 protein has recently been shown to be zinc metalloprotein (24). We have shown that one nucleic acid-binding gag protein, AMV p12gag, binds $^{65}$Zn(II) in the zinc blot. Although the zinc-binding activities of AMV p12gag and reovirus σ3 are easily identified within samples containing mixtures of viral proteins, other experiments (data not shown) suggest that a high background of zinc-binding proteins in extremely complex biological mixtures, such as cellular or bacterial extracts, may make the identification of some zinc-binding proteins more difficult.

In summary, we have characterized the zinc blot technique by using a panel of zinc metalloproteins. Conditions have been identified that permit the identification of as little as 1 μg of some zinc-binding proteins. This analysis does not depend on purified protein samples, does not rely on specialized instrumentation, and does not require metal-free conditions. We have used this technique to test the hypothesis that the retroviral nucleic acid–binding gag proteins also bind zinc and have shown that AMV p12gag binds $^{65}$Zn(II) in the zinc blot. This technique should prove useful for the preliminary identification and characterization of other zinc-binding proteins and protein fragments.

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