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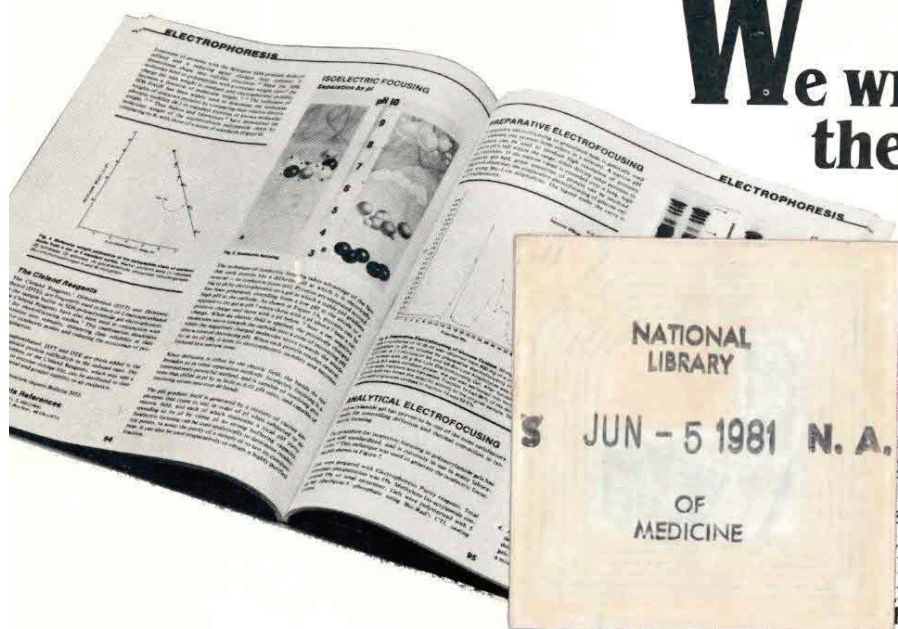
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a cumulative index for the year 1981.

"Western Blotting": Electrophoretic Transfer of Proteins from Sodium Dodecyl Sulfate-Polyacrylamide Gels to Unmodified Nitrocellulose and Radiographic Detection with Antibody and Radioiodinated Protein A

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Received May 20, 1980

A simple and efficient procedure was employed for the electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to sheets of pure, unmodified nitrocellulose. Immobilized proteins could then be radiographically visualized *in situ* by reaction with specific antibody and the subsequent binding of radioiodinated *Staphylococcus* protein A to the immune complexes. The detection of murine leukemia virus antigens in complex cellular lysates was used to demonstrate the efficacy of this technique.

A powerful tool in molecular genetics has been the blotting technique of Southern (1) in which electrophoretically fractionated DNA can be immobilized onto nitrocellulose filters and used to examine complementary sequences by hybridization *in situ*. An adaptation of the "Southern" blot is the covalent attachment of fractionated RNA (or DNA) to diazobenzyloxymethyl paper (DBM paper)² in order to probe for complementary DNA sequences ("Northern" blotting) (2). Although various proteins have been detected after fractionation on polyacrylamide gels using enzyme substrates (3) or specific antibody (4), only recently have attempts been made to immobilize gel-fractionated proteins on a solid phase (5-7). These techniques involve ei-

ther passive diffusion or the preparation and activation of DBM paper to achieve covalent immobilization.

Our laboratory has been interested in finding a simple yet reproducible transfer technique for use in the immunological detection and characterization of the products encoded by murine leukemia virus (MuLV) genomes. In this regard, Towbin *et al.* (8) recently described an elegant and straightforward method for the electrophoretic transfer of ribosomal proteins from polyacrylamide-urea gels to sheets of unmodified nitrocellulose and the radioautographic detection of specific antigens on such gel replicas with radiolabeled antibodies. Most types of biochemical and immunochemical analyses, however, make use of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis to identify and characterize proteins by their relative molecular weights. As described below, it was found that the rate of electrophoretic elution of proteins from SDS-containing gels was itself molecular weight dependent. By making certain adaptations to the transfer method of Towbin *et al.* (8), it was possible

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² Abbreviations used: DBM paper, diazobenzyloxymethyl paper; MuLV, murine leukemia virus; SDS, sodium dodecyl sulfate; IPA, radioiodinated *Staphylococcus* protein A; NP-40, Nonidet P-40; 2-DGE, two-dimensional gel electrophoresis; BSA, bovine serum albumin; IgG, immunoglobulin G.

to achieve essentially complete and quantitative elution of most proteins from SDS-gels to nitrocellulose with no loss in electrophoretic resolution. The use of radioiodinated *Staphylococcus* protein A (IPA), instead of radiolabeled primary or secondary antibodies, greatly simplified and enhanced the radioautographic detection of immune complexes on blots reacted with specific immune sera.

In addition to the general technique of electrophoretic transfer and immunological detection, various applications of the method are discussed. Certain problems are also identified, particularly those regarding the reuse of the gel blots, replica distortion, and antigen denaturation. With due respect to Southern (1), the established tradition of "geographic" naming of transfer techniques ("Southern," "Northern") is continued; the method described in this manuscript is referred to as "Western" blotting.

MATERIALS

AKR MuLV-infected C57BL/6 E δ G2 cells and Moloney MuLV-infected C3H cells were donated by R. Nowinski of this Center. ¹⁴C-Labeled molecular-weight-marker proteins and sodium [¹²⁵I]iodide were purchased from New England Nuclear (Boston, Mass.). *Staphylococcus* protein A from Pharmacia (Piscataway, N. J.) was radioiodinated as described by Hunter (9). Generally, IPA can be stored in aliquots for up to 2 weeks at -70°C in solutions containing 5% bovine serum albumin without significant loss in reactivity toward immunoglobulin. Acrylamide and bisacrylamide were from Polysciences (Warrington, Pa.) and Sequanal-grade SDS was from Pierce Chemical Company (Rockford, Ill.). Grade 470 filter paper and sheets of nitrocellulose of various pore sizes were purchased from Schleicher and Schuell (S&S, Keene, N. H.). The Canalco gel destaining apparatus is sold by Miles Laboratories

(Elkhart, Ind.); only the electrophoresis chamber and plate electrodes (sold separately) are necessary for the transfer technique. Gel electrophoretic apparatuses were manufactured to specifications by Biocraft (Peekskill, N. Y.). Rabbit anti-p30 sera were generously provided by H. Fan of the Salk Institute and by J. Ihle of the Frederick Cancer Research Center.

METHODS

Sample preparation. AKR MuLV-infected C57BL/6 E δ G2 cells and Moloney MuLV-infected C3H cells were washed three times in phosphate-buffered saline. Cell pellets, collected by centrifugation, were suspended at a density of no greater than 20-30 $\times 10^6$ cells/ml in a lysis buffer (10) containing 0.5 M urea, 2% Nonidet P-40 (NP-40; Shell Chemical Co., West Orange, N. J.), 2% Ampholine pH 3.5-10 (LKB Instruments, Stockholm) and 5% 2-mercaptoethanol. The cells were frozen and thawed three times and insoluble debris removed by centrifugation for 10 min in an Eppendorf centrifuge. Lysates were stored at -70°C until used. These samples could be applied directly to isoelectric focusing gels for two-dimensional gel electrophoresis (2-DGE). For SDS gel electrophoresis, small sample volumes could be diluted directly into SDS-gel sample buffer (11) or, for larger sample volumes, could be precipitated with cold trichloroacetic acid, the precipitates collected by centrifugation, and washed in cold acetone before resuspension in sample buffer.

Gel electrophoresis. SDS gel electrophoresis was performed in 10, 12.5, or 5-20% linear gradient acrylamide slab gels as previously described (11,12). 2-DGE is a cathode-directed isotachophoretic step coupled with SDS gel electrophoresis which has been used in this laboratory for the analysis of monoclonal immunoglobulins (13).

Electrophoretic transfer. The transfer procedure has been adapted from Towbin

et al. (8) for the quantitative recovery of proteins from SDS-containing polyacrylamide gels. Briefly, a "sandwich" is prepared with the following successive layers: (i) a porous polyethylene sheet (Bel-Art), 13.5 × 14 cm and 1.6 mm thick; (ii) three thicknesses of filter paper (S&S grade 470), 13.5 × 10 cm; (iii) the SDS-polyacrylamide slab gel (13.5 × 9.5 cm with the stacking gel removed); (iv) a nitrocellulose sheet (S&S BA83, 0.2 μ m) cut to the size of the gel; (v) three more thicknesses of filter paper; and finally (vi) another sheet of porous polyethylene. All components in contact with the slab gel are prewetted in the electrode solution composed of 20 mM Tris base, 150 mM glycine, and 20% methanol (8). This solution should be degassed briefly under vacuum before use. The sandwich is secured with thick rubber bands and inserted between the electrodes of the Can-alco gel destainer with the nitrocellulose toward the anode. The chamber is filled with electrode solution and electrophoretic transfer accomplished at 6–8 V/cm (with respect to electrode separation) for 16–22 h using a Buchler 3-1155 power supply. Recirculation of the electrode solution is not necessary.

Staining, radioautography, and fluorography. For direct visualization of unlabeled proteins, the nitrocellulose sheet may be stained for 5 min in a solution containing 0.2% Coomassie brilliant blue R-250, 40% methanol, and 10% acetic acid. Rapid destaining is accomplished in 90% methanol and 2% acetic acid as described (8,14). Care must be exercised during destaining because the nitrocellulose will disintegrate if left in the acidic methanol for much longer than 5 min. The destained sheet is rinsed in water and blotted dry for 1–2 h between sheets of S&S grade 470 paper. Hot air drying should be avoided. Protein preparations radiolabeled with [^{35} S]methionine, ^{125} I, or to high specific activities with ^{14} C-labeled precursors may be directly visualized by radioautography after drying the nitrocellu-

lose replica. ^{14} C-Labeled and ^{3} H-labeled proteins may be visualized by fluorography utilizing the fluorographic cocktail "Enhance" (New England Nuclear), followed by intensifying screen-enhanced radioautography (15).

Binding of antibody and IPA to nitrocellulose-immobilized proteins. Immediately following transfer, the nitrocellulose sheet is immersed in a solution containing 0.9% NaCl, 10 mM Tris-HCl, pH 7.4 (Tris-saline), and 5% fraction V bovine serum albumin (BSA), and incubated at 40°C for 30 min on a rocking platform. The sheet is transferred to a fresh solution of Tris-saline with BSA containing the appropriate antiserum and incubated for 90 min at room temperature on a rocking platform. The nitrocellulose is then washed with rocking for 10 min in 200 ml of Tris-saline without BSA, for 20 min in two changes of 200 ml each of Tris-saline containing 0.05% NP-40, and again for 10 min in 200 ml of Tris-saline alone. The sheet is immersed in fresh Tris-saline with 5% BSA containing $2-5 \times 10^5$ cpm of IPA/ml. Binding of IPA is allowed to occur for 30 min with rocking at room temperature. The radioactive solution is aspirated and the nitrocellulose sheet again rinsed as described above. The sheet is briefly blotted with paper towels, wrapped in Glad-Wrap, and exposed at -70°C to Kodak XR film utilizing a GAFMED Rarex Mid Speed intensifying screen.

RESULTS AND DISCUSSION

Efficiency of Electrophoretic Transfer and Immobilization of Proteins

Figure 1 shows the indirect radioautograph of ^{14} C-labeled proteins on unmodified nitrocellulose following their transfer at 8 V/cm for 22 h from a 12.5% polyacrylamide-SDS gel. Compared to the electrophoretic transfer of proteins to DBM paper, Western blotting with unmodified nitrocellulose gives markedly better resolution, is signifi-

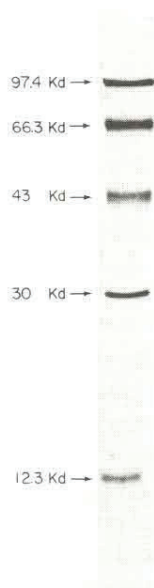


FIG. 1. Western blot of ^{14}C -labeled proteins. Approximately 6 nCi each of ^{14}C -labeled phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, and cytochrome *c* were fractionated in a 12.5% polyacrylamide SDS-gel. Following electrophoresis, the proteins were transferred to 0.20 μm nitrocellulose paper as described in the text and the blot exposed to Kodak XR film.

cantly easier to perform, and does not require preequilibration of the gel nor diazotization of the nitrocellulose (R. Eisenman and W. Mason, personal communication). Six different grades of S&S nitrocellulose paper, with pore sizes ranging from 0.025 to 0.45 μm , were examined for efficiency of protein immobilization. No significant difference was found among them in resolution or in effective adsorption of marker proteins phosphorylase *b* (97.4 kdalton) bovine serum albumin (66.3 kdalton), ovalbumin (43 kdalton), and carbonic anhydrase (30 kdalton). However, the adsorption of cytochrome *c* (12.5 kdalton) to 0.45 μm nitrocellulose was consistently 20–30% less than to other nitrocelluloses. Consequently, nitrocellulose with a pore size of 0.2 μm (S&S BA83) was employed for all subsequent studies. This nitrocellulose paper exhibited a protein adsorption capacity of about 2 $\mu\text{m}/\text{mm}^2$, far exceeding the

amount necessary for detection by even less-sensitive staining methods (e.g., Coomassie brilliant blue, amido black).

In preliminary experiments it was apparent that an electrophoretic blotting time of 1 h at 6–8 V/cm, as suggested by Towbin *et al.* (8) and Bittner *et al.* (7), was not sufficient for complete transport of most proteins out of SDS-containing polyacrylamide gels. The rate of transfer is, in fact, dependent upon the apparent molecular weight of individual proteins, i.e., lower-molecular-weight polypeptides leave the gel and are deposited on the nitrocellulose faster than higher-molecular-weight proteins at a given accelerating voltage. Figure 2 qualitatively illustrates the necessity of longer blotting times. The upper panels of Fig. 2 show the residual radioactivity remaining in 10% polyacrylamide–SDS gels after transfer at 8 V/cm for 0, 1, 4, 12, and 22 h. The lower panels are the corresponding nitrocellulose blots. Quantitative transfer as a function of molecular weight can be demonstrated by cutting the appropriate radiolabeled bands

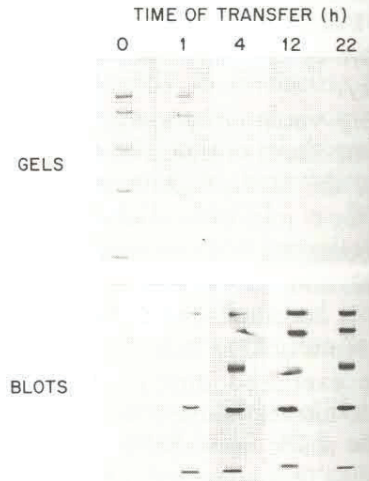


FIG. 2. Transfer of proteins as a function of electrophoresis time. ^{14}C -Labeled marker proteins were fractionated by 10% SDS gel electrophoresis and electrophoretically transferred to nitrocellulose at 8 V/cm for the times indicated. The upper panels are radioautograms showing the residual radioactivity remaining in the gels after transfer. The lower panels are the corresponding blots for each time point.

from each of the blots and determining radioactivity by liquid scintillation counting. Figure 3 illustrates the kinetics of transfer for two proteins, phosphorylase *b* (97.4 kdalton) and carbonic anhydrase (30 kdalton). It can be seen that quantitative transfer of both proteins is only approached after 12 h. Mass dependence of transfer is not affected by presoaking the gel before blotting to remove residual detergent. Indeed, equilibration in a solution other than the electrode solution can result in swelling or shrinking of the gel during blotting with consequent distortion of the replica pattern (see below). For 1.5-mm-thick gels, therefore, 20–22 h of electrophoresis at 6–8 V/cm is sufficient for greater than 90% transfer of all proteins up to about 100,000 daltons.

Accelerating voltages higher than 10 V/cm are not recommended for electrophoretic transfer because of the attendant joule heating of the gel. Constant-current electrophoresis is also satisfactory if the voltage limit is not exceeded. Although there is no separation of the cathodic and anodic chambers in the Canalcro destainer,

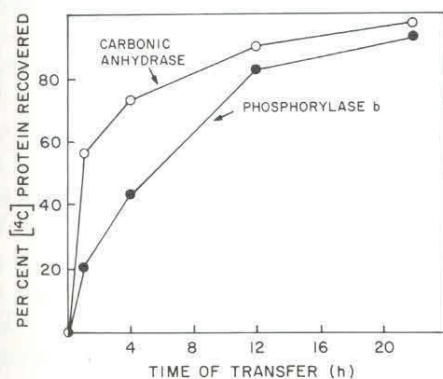


FIG. 3. Electrophoretic transfer time as a function of molecular weight. ^{14}C -Labeled phosphorylase *b* (97.4 kdalton) and carbonic anhydrase (30 kdalton) were fractionated by 10% SDS gel electrophoresis and subjected to electrophoretic blotting for 1, 4, 12, and 22 h at 8 V/cm. The individual protein bands were visualized by radioautography, cut from the nitrocellulose blots, and the radioactivity at each time point determined by liquid scintillation counting.

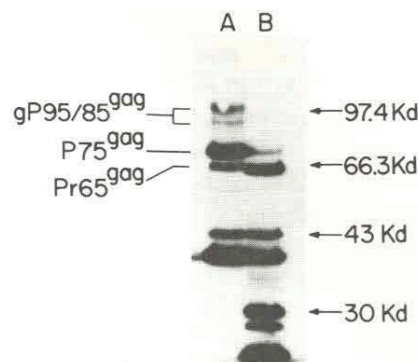


FIG. 4. Western blot and antibody-IPA detection of intracellular MuLV-specific antigens. Lysates of AKR MuLV-infected E δ G2 mouse cells and Moloney MuLV-infected C3H mouse cells were subjected to SDS gel electrophoresis in a 10% polyacrylamide slab gel and the fractionated proteins subsequently transferred to nitrocellulose paper. The blot was then reacted with rabbit antiserum directed against the major internal structural protein (p30) of MuLV and the immune complexes illuminated with IPA followed by radioautography. Lane A contained lysate equivalent to 3×10^5 E δ G2 cells and lane B to 1×10^5 C3H cells. Exposure time of this radioautogram was 15 min.

a pH gradient of 6.8–8.8 is set up across the plate electrodes during electrophoresis. However, this does not adversely affect transfer and recirculation of the electrode solution is not necessary. Transfer of proteins from isoelectric focusing slab gels (1.5 mm thick) containing urea can be accomplished in 4–6 h at the same voltage using an electrode solution of 0.7% acetic acid and reversing the electrodes.

Immunodetection of Blotted Proteins

Figure 4 is the Western blot and antibody-IPA illumination of MuLV-specific products found in AKR virus-infected E δ G2 mouse cells and Moloney MuLV-infected C3H mouse cells. The 65,000-dalton polypeptide precursor (Pr65^{gag}) of the major MuLV structural proteins (see Ref. (16)) is easily detected in both cell lines with a rabbit antiserum directed against a constituent of the polypeptide, the viral capsid polypeptide p30 (17,18). Here the intracellular viral proteins can be seen in a steady-state con-

dition not fully achievable by metabolic radiolabeling. The immunoreactive band identified as P75^{gag} in Fig. 4 is probably the glycopolyprotein precursor (gPr80^{gag}) to the glycosylated cell surface antigens gP95/85^{gag} (19,20). Although P75^{gag} is structurally and antigenically related to Pr65^{gag}, it is believed to be a separately initiated translation product of the viral *gag* gene (21). The lower-molecular-weight polypeptides in Fig. 4 are specific proteolytic cleavage products in the processing of Pr65^{gag} to the individual structural proteins of the virion (16).

Although radioiodinated antibodies (both primary and secondary) can be used to detect the immobilized antigens, IPA was chosen for the convenience and simplicity of preparing a single, standardizable radio-labeled reagent capable of detecting most types of immune complexes. The varying levels of radiographic "background" seen with different antisera preparations after reaction with IPA is due to nonspecific binding of immunoglobulin to nitrocellulose. The signal-to-noise ratio is therefore a function of the relative titers of specific immune antibodies and nonimmune IgG in each serum. Various protein solutions and protein admixtures have been used in efforts to "block" the nonspecific adsorption of immunoglobulin to the nitrocellulose and thereby increase the signal-to-noise ratio of the IPA-immune complexes. Although none of these mixtures (e.g., ovalbumin, avian globulins, gamma globulin-free bovine serum albumin) have duplicated the nitrocellulose binding of mammalian immunoglobulins, solutions of 5% bovine albumin (fraction V) have given satisfactory results and are the most convenient and inexpensive. Lengthening or increasing the number of washings after either antibody or IPA binding generally had no effect toward decreasing background. Since the Fc portion of rabbit IgG has a greater affinity for protein A than the immunoglobulins of most other animal spe-

cies (22), one can use much higher dilutions of rabbit antisera compared to the immune sera of other animals (e.g., goat); there is, however, no increase in the signal-to-noise ratio. Most antisera, diluted in Tris-saline-BSA and stored at -20°C , can be reused many times for reaction with blotted proteins. One such antiserum dilution was used more than 40 times over a 10-month period with no apparent diminution in specific immunoreactivity. Freshly radioiodinated protein A at $2-5 \times 10^5$ cpm/ml was routinely employed to illuminate the immune complexes since most results could be seen after 30-60 min of intensifying screen-enhanced radioautography. Smaller inputs of IPA require a consequent increase in radiographic exposure time. Neither the amount nor the specific activity of IPA had any significant effect on the signal-to-noise ratio.

Sensitivity of Immunodetection

It must be emphasized that sensitivity in Western blotting is primarily a function of the specific antibody titer of the immune serum being utilized. From studies of avian leukosis viruses (see Ref. (23)) it can be calculated that as little as 1-2 ng of a specific viral protein (p27) is detectable on blots with the appropriate hyperimmune rabbit serum (1:50 final dilution) exhibiting low nonspecific binding to nitrocellulose. This particular antiserum was capable of precipitating approximately 1 ng of ^{125}I -labeled avian myeloblastosis virus p27 at a final dilution of 1:20,000 in a standard 100- μl radioimmunoprecipitation assay (24). The rabbit antiserum used for the experiments shown in Fig. 4 had a radioimmunoprecipitation titer of about 1:12,000 for 0.8 ng of AKR MuLV p30. At a final dilution of 1:250 in Western blotting and a radiographic exposure time of 15 min after reaction with 2×10^5 cpm of IPA/ml, this hyperimmune serum detected the viral polyproteins in lysates equivalent to 3×10^5 E δ G2 cells

(Fig. 4, lane A) and 1×10^5 C3H cells (lane B). Other experiments with this same anti-serum showed it easily capable of detecting Moloney MuLV antigens from as little as 10^3 C3H cells.

Other Applications of the Western Blot

Figure 5 is the Western blot of the 2-DGE of E δ G2 and C3H cell lysates. There is no loss of resolution in the focused spots after transfer and reaction with antibody-IPA. Western blotting used in conjunction with 2-DGE offers high sensitivity, two independent parameters of fractionation, maintenance of point-to-point resolution, simplicity of technique, and the absence of a requirement for costly *in vivo* metabolic radiolabelings. For these reasons it is an ideal way to characterize complex mixtures of antigens from biopsies of animal tissues.

Electrophoretic transfer coupled with the sensitivity of antibody-IPA illumination makes the Western blot a suitable method for analysis of products synthesized *in vitro* by messenger-dependent cell lysates (25). Rather than relying upon the incorporation of expensive translation-grade radiolabeled amino acids and the individual immunoprecipitation reactions for detection of synthetic polypeptides, it is possible to visualize these products *in situ* on a gel blot with specific antibody and IPA. *In vitro* translations of approximately 1 μ g of MuLV genomic RNA resulted in sufficient Pr65^{gag} to be detected after 5–6 h of direct radiographic exposure following antibody-IPA treatment of a gel blot; parallel translation with incorporation of [³⁵S]methionine allowed comparable visualization of Pr65^{gag} after overnight exposure (12–16 h) of a fluorographed gel. Western blotting has also been applied to studies concerned with the segregation of mRNA molecules and subsequent compartmentalized protein synthesis on free and membrane-bound polyribosomes. In this regard, certain nascent virus-specific polypeptides associated

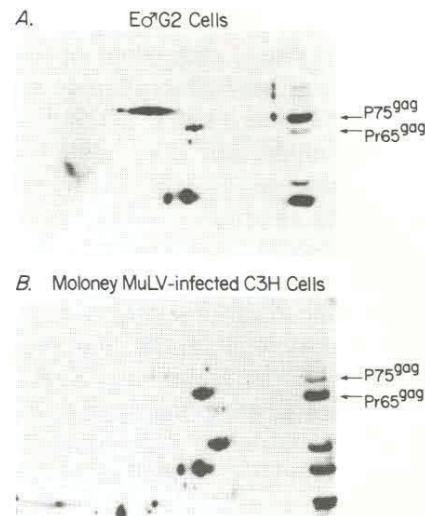


FIG. 5. Western blot and antibody-IPA detection of intracellular MuLV-specific antigens fractionated by 2-DGE. Lysates containing either 3×10^5 E δ G2 cells or 1×10^5 Moloney-C3H cells were first fractionated by cathode-directed isotachopheresis in cylindrical polyacrylamide-urea gels containing a pH 3.5–10 ampholyte. Following isotachopheresis for 3000 V · h, the gels were electrophoresed at right angles in 10% polyacrylamide-SDS slab gels. The slab gels were subjected to electrophoretic blotting and the blots subsequently reacted with rabbit anti-p30 serum and IPA as described in the text. Radiographic exposure time was 30 min. In both panels the direction of isotachopheresis was from right to left. Marker lanes to the right on the second dimension gels show the authentic samples run in a single dimension. A, E δ G2 cell lysate; B, Moloney-C3H cell lysate.

with such purified polysome preparations from infected cells have been detected by antibody-IPA illumination on nitrocellulose blots.

Problems Encountered in Western Blotting

Distortion due to unequal volume expansion of the gel and the nitrocellulose may arise during long-term electrophoresis, particularly at high accelerating voltages. This may lead to diffuse bands (in SDS gel electrophoresis) or spots (in 2-DGE). Joule heating can be minimized by keeping voltages below 10 V/cm. Further swelling and shrinking can be prevented by empirically

matching the methanolic composition of the electrode solution with the amount of crosslinking in the polyacrylamide matrix. Gels prepared from a stock solution of 30% (w/w) acrylamide and 0.8% (w/w) bisacrylamide undergo no significant volume changes in the electrode solution described. Gels constructed with different matrix ratios may require slight modification in the methanol concentration or short (less than 1 h) preswelling in electrode solution to inhibit distortion. Failure to have good initial contact between the gel and nitrocellulose can lead to "skewing" of certain bands; this artifact can be seen with the bovine albumin and ovalbumin markers in Fig. 2.

A disadvantage of the Western blot from SDS-gels is the tendency of antigenically reactive sites (epitopes) on the fractionated molecules to be irreversibly denatured by the detergent (26,27). Although not generally a problem when utilizing polyvalent monospecific antisera for detection, this limitation is quite severe when screening panels of monoclonal antibodies. Since such antibodies are directed against single epitopes within an antigenic molecule (28), there is a likelihood that the reaction of interest may be abolished by the denaturing effect of the detergent. Therefore, when selecting monoclonal antibodies for use in Western blotting analysis it is necessary to test the SDS sensitivity of the specific epitopes. Although it is possible to achieve a greater degree of protein renaturation by substitution of various commercial grades of SDS in the SDS electrophoretic electrode buffer ((29,30), unpublished observation), it is best to avoid this detergent altogether if suitable fractionation of sensitive antigens can be accomplished by other gel methods (e.g., slab gel isoelectric focusing).

Unlike blotting to diazotized paper (1,2,6), the radiolabeled probes of Western blotting are not readily removed from non-derivatized nitrocellulose for reuse of the replicas with different probes. Treatment of blots with detergent (5% SDS), chaotropic

agent (3 M NaSCN), or acid (pH 3) fails to preferentially remove probe and background radioactivity relative to the desorption of immobilized antigens.

CONCLUSIONS

The technique of Western blotting should prove valuable for the analysis of proteins fractionated on the basis of molecular weight in SDS gel electrophoresis. Use of unmodified nitrocellulose sheets and an electrophoretic mode of transfer provide speed and a simplicity of technique that, combined with essentially complete and quantitative protein transfer, is not achievable by other blotting methods. Coupled with antigen detection by antibody and IPA, the Western blot is a very sensitive method for visualizing specific proteins in complex antigenic mixtures. The use of radioiodinated protein A allows such analyses to be accomplished from cells in culture as well as from necropsied animal organs and biopsied tissues without having to resort to metabolic radiolabeling of the cellular antigens (N. Burnette, R. Tao, and R. Nowinski, unpublished). The blotting method can also be adapted to a large variety of other problems employing SDS gel electrophoresis for analysis. These include, for example, two-dimensional gel fractionation of cellular components, analysis of products synthesized *in vitro* in response to exogenous messenger RNA, and studies of nucleic acid binding proteins using appropriate radiolabeled nucleic acid probes (5). Realizing the limitations imposed by SDS denaturation, investigations utilizing monoclonal antibodies for detection may be undertaken if these probes are first selected on the basis of their reactivity against detergent-insensitive epitopes.

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