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One-Step Electroblotting of 2- to 100-kDa Proteins onto a PVDF Membrane

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ABSTRACT

This article describes a method for electroblotting peptides and small proteins (< 100 kDa) from tricine gels onto a PVDF membrane. The major potential problem with these types of procedures is that proteins tend to stay in the gel under conditions where peptides are effectively eluted. The suggested protocol allows the complete transfer and binding of proteins and peptides in the range of 2–97 kDa.

Index Entries: Electroblotting; proteins; peptides; PVDF.

INTRODUCTION

The electrophoretic transfer of proteins from polyacrylamide gels to an immobilizing matrix is of great interest since a wide variety of analytical procedures can be applied to the immobilized proteins (*1–7*). Many different conditions of transfer have been tested during studies of various proteins, with the conclusion that there are no universally applicable conditions (*6,8*). Because elution is generally dependent on the molecular weight of the polypeptides, standard procedures that give excellent results for high-mol-wt proteins give a low and unreproducible signal with some low-mol-wt peptides (*9*). In addition, transfer and retention of low-mol-wt proteins (< 20,000 Dalton) are particularly troublesome (*1,9*).

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This limitation is especially important when trying direct and simultaneous identification and quantification on a membrane having separated components that differ greatly in their molecular weights.

Because the optimal transfer of polypeptides of a wide range of molecular weights in a single step would be of interest for research purposes, we have studied the conditions involved in achieving a simultaneous and high-efficiency transfer of these proteins and peptides from gels. The results show that addition of 0.05% SDS to the transfer buffer, together with mild transfer conditions (lower voltage and longer transfer time), are necessary for complete transfer and binding of proteins and peptides in the range of 2–97 kDa from 16% acrylamide gel electrophoresis to PVDF membrane in a single step. This approach would be especially useful when studying either the regulatory mechanisms governing proteolytic processing of peptide and hormone precursors, or peptide mapping after proteolytic cleavage of proteins.

MATERIALS AND METHODS

The Mini-PROTEAN II electrophoresis unit, Trans-Blot electrophoretic transfer cell, PVDF membrane (0.2 μm), SDS, acrylamide, bis-acrylamide, ammonium persulfate, and TEMED were obtained from Bio-Rad (Richmond, CA). Tricine, Tris and sucrose were purchased from Sigma Chemical Co. (St. Louis, MO). Peptide standards (range 2500–16,900 Dalton) were purchased from Pharmacia-LKB (Sweden), and low-mol-wt standard kit (range 14,400–97,400 Dalton) from Bio-Rad.

Peptides and proteins were resolved basically according to the procedure described by Schägger and Von Jagow (*10*). Sixteen percent acrylamide gels were used for the data presented (1 mm thickness). Samples were boiled for 5 min in 50 m*M* Tris-HCl, pH 6.8, containing 1% (w/v) SDS, 2% (v/v) β-mercaptoethanol, 5% (w/v) sucrose, and 0.05% (w/v) bromophenol blue before loading onto gel. The electrode buffer was 0.1% SDS in 0.1*M* tricine, pH 8.4.

On completion of electrophoresis, the gel was soaked in transfer buffer. The gel and the membrane were mounted in the transfer cell containing transfer buffer. Transfer was carried out overnight in a cold room. Towbin buffer was the base buffer for all transfers.

Total protein staining of PVDF was performed using Coomassie Brilliant Blue R-250. After transfer, the membranes were rinsed with water for 2–3 min, stained for 5 min with 0.025% Coomassie Brilliant Blue R-250 in 40% methanol, destained with 50% (v/v) methanol until the background was light blue, and air-dried. The destained time was kept uniform from blot to blot.

Samples electrotransferred into PVDF membrane were quantified by immersing the membranes in 50% (v/v) methanol. The membrane was

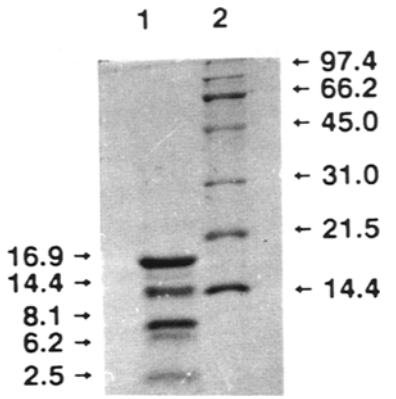

Fig. 1. Tricine-SDS-gel electrophoresis of standards. Gel was prepared as described in Materials and Methods section. Lane 1: peptide standards; lane 2: low-mol-wt standards.

directly scanned while still wet in a laser-computing densitometer from Molecular Dynamics (*11*).

RESULTS AND DISCUSSION

Gradient gels are often more effective than single percentage gels for resolving proteins of a wide mol-wt range (*6,12*). However, the buffer system described by Schägger and Von Jagow (*10*) permits the use of single-percentage SDS-gel electrophoresis for molecular weights down to at least 2000 Dalton. Figure 1 shows single-dimensional separation of a low-mol-wt standard kit (14,400–97,400 Dalton) from Bio-Rad and peptide standards from Pharmacia (2500–16,900) using this system. As can be seen, the system gives good resolution and sensitivity for proteins and peptides in the range of 2–97 kDa. In order to increase the transfer yield of larger polypeptides, the optimal amount of acrylamide was found to be 16%.

To avoid the problem of distorting bands, it is common practice to preswell the gel for 30–60 min in transfer buffer to equilibrate the gel prior to transfer (*1,7*). This equilibration allows any changes in the size of the gel owing to swelling or shrinking to occur at this stage rather than during the transfer. We have studied the time-course polypeptide loss from the gel after the start of equilibration of the gel. Equilibration buffer and tranfer buffer were the same. As can be seen in Fig. 2, proteins diffuse out of the gel, and there was a loss of polypeptides after long periods in equilibration buffer. Therefore, this equilibration of the gel in the buffer should be avoided, above all when working with peptides, and simple rising of the gel in transfer buffer gives best results.

PVDF membrane was the matrix used in our experiments for the following reasons:

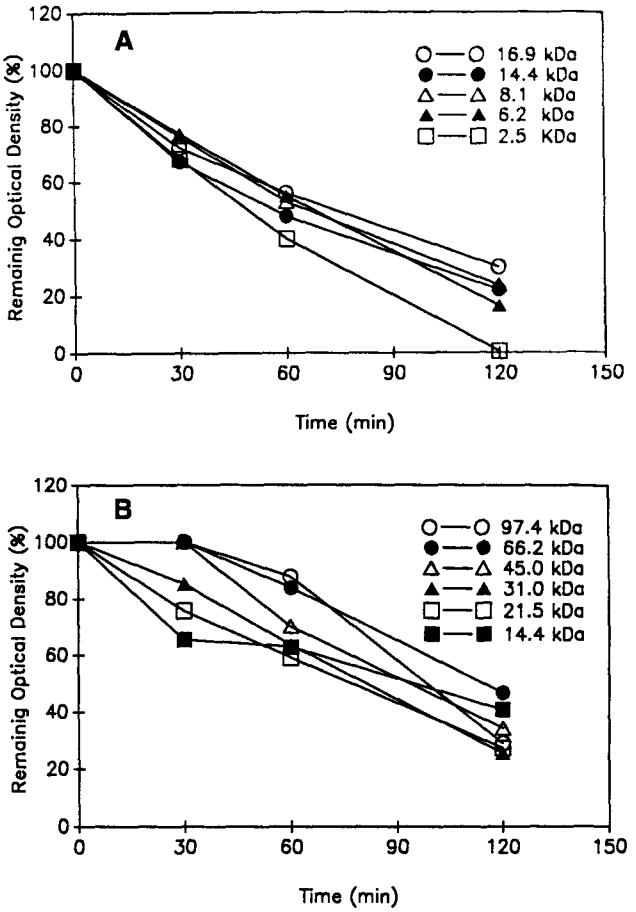

Fig. 2. Time-course of polypeptide loss from the gel after the start of equilibration of the gel. Polypeptides were separated on different lanes of a 16% SDS polyacrylamide gel, cut into strips corresponding to the lanes, and immersed in equilibration buffer. At different times, corresponding strips of gel were removed, stained, and destained. Bands were quantified using the computing densitometer. A. Peptides. B. Low-range mol-wt standards.

1. It is suitable for staining with anionic dye (*5*);
2. Peptides below 16 kDa are transferred from the gel to PVDF membrane with high efficiency (*11*); and
3. It is possible to quantify bands directly on membranes using a computing densitometer (*11*).

The choice of buffer composition is influenced by the type of gel, the membrane, and the physical nature of the protein of interest. Elution is generally dependent on the molecular weight of the polypeptide. Thus, lower-mol-wt polypeptides leave the gel and are deposited on the membrane support faster than higher-mol-wt proteins (*7*). Various suggestions have been made to overcome or reduce this problem, among them addition of detergent to Towbin's buffer (25 m*M* Tris, 192 m*M* glycine,

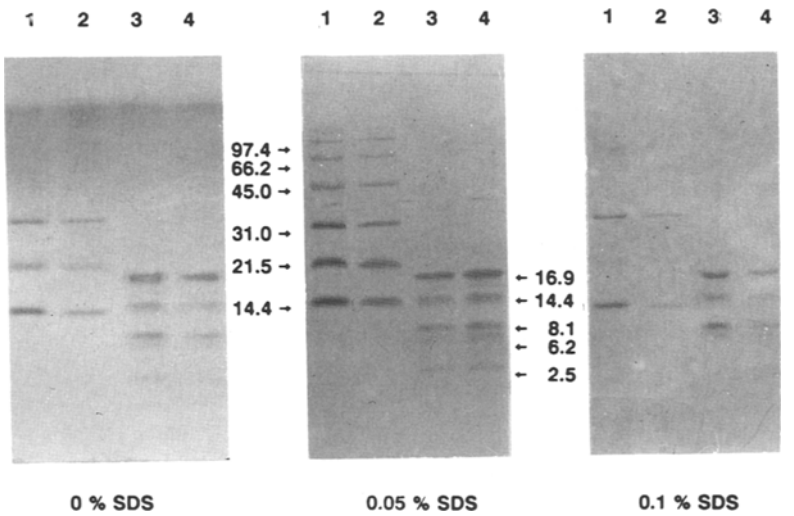

Fig. 3. Proteins bound to PVDF after transblotting with the various buffers. Left panel: 0% SDS; center panel: 0.05% SDS; right panel: 0.1% SDS. Membranes were stained as described in Materials and Methods.

20% methanol, pH 8.3) to facilitate elution of high-mol-wt proteins (*1*). As can be seen in Fig. 3, the addition of 0.05% SDS to the transfer buffer yielded the maximum transfer efficiency and sharpness of the band patterns. Using transfer buffer without SDS (Fig. 3), proteins of higher molecular weight (97.4, 66.2, and 45.0 kDa) were not transferred, and peptide transfer efficiency was lower than when using 0.05% SDS. Electrophoretic transfer using Towbin's buffer plus 0.1% SDS (Fig. 3) did not improve transfer, and binding to PVDF was inferior to that observed with buffer plus 0.05%. It is possible that the protein actually eluted from the gel and passed through the membrane to give the reduced transfer efficiency observed (this possibility was not tested).

In our study, mild power conditions (lower voltage and longer time) were used. As under high current density, movement of the low-mol-wt polypeptides is faster than the binding reaction between polypeptides and membranes. Therefore, the transfer was carried out for 16 h at 30 V constant voltage.

Figure 4 is a photograph of stained PVDF membrane after electrophoretic transfer of a dilution series of standard. This transfer was performed using the optimal conditions described above. Electrotransferred samples were quantified by scanning the gels and comparing the results with equivalent samples run on SDS gels not electrotransferred. For all peptides and proteins analyzed, it was possible to achieve essentially complete elution from SDS gels to PVDF membrane (Table 1).

To indicate that transfer occurred in a quantitative manner, samples electrotransferred onto PVDF were quantified as described previously (*11*). The data presented in Fig. 4 were used to generate the data presented

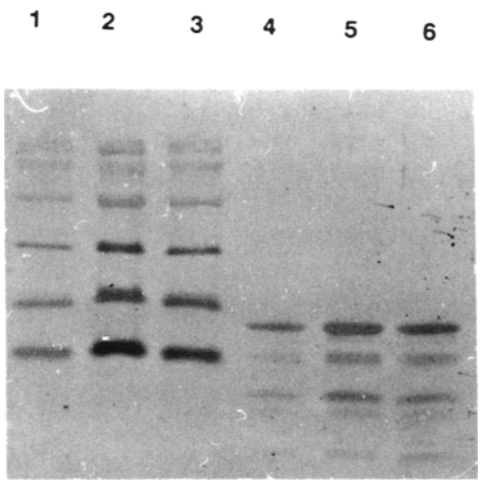

Fig. 4. Photograph of stained PVDF membrane after electrophoretic transfer of a dilution series of standard. Different amounts of standards were subjected to Tricine-SDS-gel electrophoresis, transferred to PVDF, and stained with Coomassie Brilliant Blue R-250 as described in Materials and Methods section. Lanes 1–3: 5, 20, and 10 μg of low-mol-wt standards; lanes 4–6: 5, 15, and 10 μg of peptide standards.

Table 1
Transfer Yield of Standards in PVDF Membrane[a]

Protein and peptide	Efficiency of transfer, %
Phosphorilase B	96
Serum albumin	94
Ovoalbumin	100
Carbonic anhydrase	100
Trypsin	100
Lysozyme	100
Myoglobin	100
Myoglobin I and II	100
Myoglobin I	98
Myoglobin II	100
Myoglobin III	100

[a]Quantification of sample electrotransferred was performed by scanning the gels and comparing these results with equivalent samples run on SDS gels, but not electrotransferred. Results are mean of two different experiments.

in Fig. 5. The relationship of the integrated optical density with the amount of peptides is shown in Fig. 5A. The response of the instrument to the band was linear and highly reproducible. Similar results were found in the case of low-mol-wt standard (Fig. 5B).

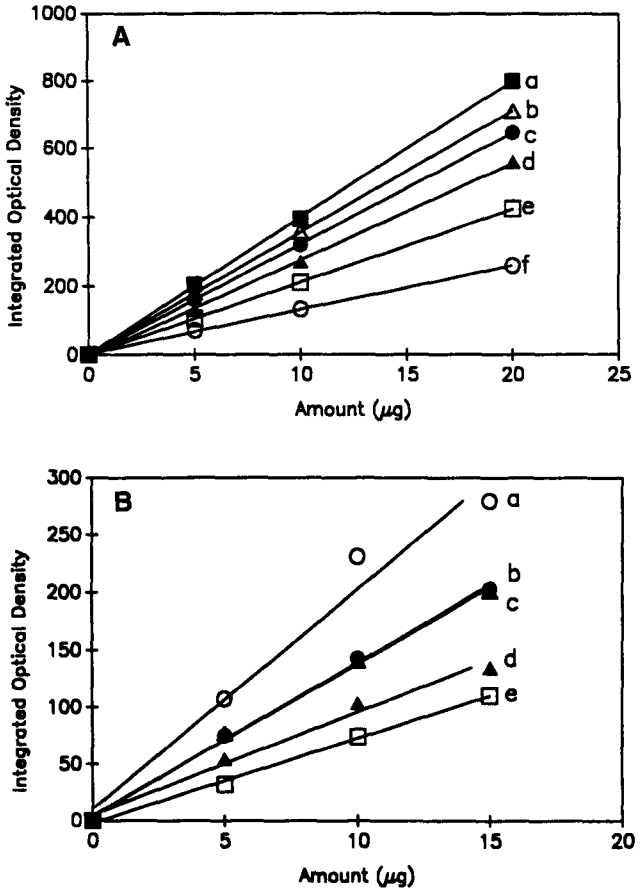

Fig. 5. Scanner optical response vs amount of low-mol-wt standards (A) and peptides (B) on PVDF membrane. Treatment of the PVDF membrane and the densitometric procedure are described in the text. The line is a least-squares fit to a straight line. Low-mol-wt markers: A, 14.4 kDa; B, 21.5 kDa; C, 31.0 kDa; D, 45.0 kDa; E, 66.2 kDa; F, 97.4 kDa. Peptide standards: A, 16.9 kDa; B, 14.4 kDa; C, 8.1 kDa; D, 6.2 kDa; E, 2.5 kDa. The labels on the x-axis reflect the amount of total protein in the mixture.

In conclusion, by using Towbin's buffer with 0.05% SDS and transferring for 16 h at 30 V, we obtained a maximum transfer of proteins and peptides in the range of 2–97 kDa onto PVDF membrane in one step from a single-percentage polyacrylamide gel.

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