Enhanced Capture of Small Histidine-Containing Polypeptides on Membranes in the Presence of ZnCl₂

BioTechniques 30:1224-1230 (June 2001)

Small hydrophilic polypeptides and proteins can be difficult to capture on membranes designed for western blot analysis using standard blotting protocols (2,6,10). The use of charged membranes, such as polyvinylidene difluoride (PVDF), and membranes with small pore sizes can improve capture, but, in some cases, even these membranes when used with standard transfer protocols do not give satisfactory results. We report a transfer protocol that results in the improved capture of three small histidine-containing polypeptides and is likely to be of use with other histidine-containing proteins, including proteins with histidine tags.

In the course of studying the 8-kDa nodulin from actinorhizal Alnus (3,11), we observed that immunostaining on western blots, if present at all, was more intense on the backside of the membrane than on the side facing the gel from which proteins were transferred. In subsequent experiments, Coomassie Brilliant Blue R-250 (Bio-Rad Laboratories, Hercules, CA, USA) staining of the Whatman® 3MM blotting papers (Whatman, Clifton, NJ, USA) used in semidry transfer revealed that most of the protein had transferred through the membrane to the blotting papers. Attempts at quantitative capture of the polypeptide on membranes by adjusting transfer buffer composition (pH, SDS, methanol, continuous vs. discontinuous buffers), blotting times and amperage, as well as using the lowest pore size membranes commercially available (0.1-μm nitrocellulose, 0.2-μm PVDF, and 0.2-μm nylon), were not successful.

A search of the literature led us to papers by McKeon and Lyman (8) and Mizzen et al. (9) that reported enhanced retention of calmodulin and metallothionein, respectively, in the presence of 2 mM CaCl₂. In light of these results, we tested the ability of CaCl₂ to improve capture on membranes of nodulin 84-6. Addition of 2 mM CaCl₂ to transfer buffers did not result in quantitative capture, nor did the presence of MgCl₂ or MnCl₂ in transfer buffers improve capture on any membranes tested.

Nodulin 84-6 is a histidine-containing polypeptide that binds the following divalent cations: Ni²⁺, Zn²⁺, Co²⁺, Cu²⁺, Cd²⁺, and Hg²⁺, but not Ca²⁺, Mg²⁺, and Mn²⁺ (data not shown). Therefore, we tested the ability of these divalent cations to enhance the capture of nodulin 84-6 on 0.2- and 0.45-μm PVDF, 0.1- and 0.45-μm nitrocellulose, and 0.2- and 0.45-μm nylon membranes (Schleicher & Schuell, Keene, NH, USA). The addition of 2 mM ZnCl₂, CdCl₂, and HgCl₂ separately to transfer buffer consistently enhanced the capture of nodulin 84-6 on 0.2-μm PVDF, 0.1-μm nitrocellulose, and 0.2-μm nylon membranes. Only a faint protein band of 84-6 was observed on PVDF and nitrocellulose membranes in transfers without these metals. When using nylon membranes under the same conditions without added metal, protein capture was undetectable with immunoblotting procedures. These same results were observed when a second small histidine-containing nodulin from Alnus, nodulin 164-20, was transferred with or without metals in the transfer buffer. Figure 1 shows the effect of the presence of 2 mM ZnCl₂ in the transfer buffer on the capture of nodulins 84-6 and 164-20 on PVDF, nylon, and nitrocellulose membranes. Density scans of the blots indicate that from 4 to 10 times more protein is captured on the membranes when electroblotting is done in the presence of 2 mM ZnCl₂. Addition of cadmium and mercury to transfer buffers results in equal enhancement of capture, but because of toxicity and disposal problems associated with these metals, we do not recommend their use in electroblotting.

In an effort to determine if the addition of ZnCl₂ to transfer buffers had a wider applicability, we applied the technique to another small difficult-to-capture histidine-containing polypeptide, the β amyloid peptide (fragment 1-40) associated with Alzheimer’s disease, which we had previously found passed through nylon, nitrocellulose,
and PVDF membranes using standard transfer protocols (data not shown). Figure 2 demonstrates the ability of transfer buffer containing ZnCl₂ to increase the capture efficiency of Aβ peptide on 0.2-μm PVDF membranes. With ZnCl₂ in the transfer buffer, Aβ is immunodetected at a loading amount of 5 ng, whereas, in the absence of ZnCl₂ in the transfer buffer, the lowest amount detected occurs at a loading amount of 50 ng. An identical set of gels was blotted on 0.45-μm PVDF membranes. In this case, the capture of Aβ peptide was not enhanced by the presence of ZnCl₂ in transfer buffer (data not shown). Spotting of the Aβ peptide on PVDF membrane in the presence of 2 mM ZnCl₂ did not interfere with subsequent microsequencing.

With the two nodulin polypeptides tested, capture on 0.2-μm PVDF, 0.1-μm nitrocellulose, and 0.2-μm nylon membranes in the presence of Zn⁺² appears to be quantitative. The same is true of the capture of the Aβ peptide on 0.2-μm PVDF membranes. Under the conditions used in the experiments described in this paper, no residual protein is detected in post-transfer gels electrobotted with or without ZnCl₂, indicating that essentially complete transfer from the gels has occurred (Figure 1D).

In the case of proteins transferred without ZnCl₂, protein has been detected on the backside of the primary membrane (Figure 1E), on backup membranes (Figure 1F), and on the 3MM blotting paper below (Figure 1, G and H). This is not the case with proteins transferred in the presence of Zn⁺². However, in the case of all three proteins tested, capture on the membrane in the presence of ZnCl₂ is not equivalent to retention on the membrane. In all three cases, the transferred polypeptides are not retained on the membranes in subsequent washing steps, as are required for immunodetection. If post-transfer processing of the membrane is to be done, air-drying and glutaraldehyde fixation steps, as described in the legend of Figure 1, are required.

McKeon and Lyman (8) conclude that the enhancement of calmodulin binding to PVDF membranes that is seen with the addition of CaCl₂ to semidy transfer buffers is due to the removal of residual SDS from the protein, allowing hydrophobic regions of the protein to better interact with the membrane. We are not certain of the mechanism by which ZnCl₂ enhances the capture of histidine-containing polypeptides, but it may be that Zn⁺² causes peptide aggregation such that the aggregates are less likely to pass through pores in the membrane and more likely to be captured. Nodulins 84-6 and 164-20 are small polypeptides with secondary structures that are predicted to be predominantly random coils in aqueous solutions (data not shown). Aβ amyloids are small polypeptides that have been shown by circular dichroism spectroscopy to be predominantly random coils in aqueous solutions (7). In the presence of Zn⁺², the Aβ peptide assumes a parallel β-sheet conformation (1) and is known to aggregate under these conditions.

![Figure 1. Comparison of nodulin 84-6 and 164-20 polypeptide capture on membranes with and without the addition of ZnCl₂ to the transfer buffer.](image)

![Figure 2. Comparison of Aβ peptide capture on PVDF membranes with and without the addition of ZnCl₂ to the transfer buffer.](image)
which are due, at least in part, to histidine-13 (7). In the presence of between 5 and 10 times molar excess of metals, nodulins 84-6 and 164-20 begin to precipitate (data not shown), which may facilitate their capture on membranes.

Utilization of the ZnCl$_2$ transfer protocol has allowed us to perform western blot analysis on complex mixtures of proteins containing small amounts of nodulin 84-6, something that we were not able to do before the development of this protocol. Likewise, this protocol has been useful in western blot analysis of $\beta\beta$ proteins, where the cross reactivity of antiserum for various $\beta\beta$ mutants needed to be tested. The presence of ZnCl$_2$ in transfer buffers does not appear to affect the transfer of other proteins, as shown by the nearly identical transfer of molecular weight marker proteins in the presence and absence of ZnCl$_2$ (Figures 1 and 2). We expect that this protocol will have even broader applicability for use with recombinant histidine-tagged polypeptides that do not bind well to membranes using standard transfer protocols.

REFERENCES

Sending Plasmid DNA by Mail

BioTechniques 30:1230 (June 2001)

It is quite common to send small samples of plasmid DNA or other relatively stable material to other researchers. The most convenient method of doing this is to include it in a plain envelope mailed to the recipient at the cost of a first-class stamp. We have observed that it is common practice to pipet a few microliters of DNA into a 1.5-mL microcentrifuge tube, tape the tube to a letter describing its contents, insert the tube into a standard envelope, and trust it to the postal service.

While conceptually simple, this plan can be thwarted by automated sorting machines that slide the envelope through machines that accept letters no thicker than 2 mm. Over the years, we have received empty envelopes containing a hole in the side, punctured envelopes containing a letter with a matching hole, and intact envelopes containing shattered plastic tubes that now fit through a 2-mm gap. Using the DNA that might be clinging to the tube is risky. We usually ask for another shipment, but the mishap delays experiments and further inconveniences both the sender and us. In the USA, padded envelopes are not put through sorting machines and are therefore more reliable, but they are also more expensive and cost more to mail.

As an alternative, we use a simple, reliable, and inconspicuous way of sending plasmid DNA via the postal service using a standard envelope. We cut a 2-inch length of Clay-Adams Intromedic thin-walled polyethylene tubing (1.14 mm I.D. × 1.57 mm O.D., 0.215 mm wall thickness) and push 10 μL of the solution to be shipped to the center of the tube using a micropipet, leaving an air space at both ends of the tube (Figure 1). The tube is sealed by holding one end close to a flame and quickly pinching them between the thumb and index finger. The recipient of the tube simply cuts off both sealed ends and draws the liquid out of the tubing into a plastic micropipet tip. We have never had the tubing fail to arrive intact at its destination.

Figure 1. Tube and sample preparation. In the first step, 10 μL of a plasmid solution are pushed into the center of a 2-inch section of polyethylene tubing using a micropipet. Next, the ends of the tubing are sealed by heating the ends over a flame and quickly pinching them between the thumb and index finger.