Vacuum-blotting: a new simple and efficient transfer of proteins from sodium dodecyl sulfate–polyacrylamide gels to nitrocellulose

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1. INTRODUCTION

The transfer of proteins from polyacrylamide gels to nitrocellulose paper has been of great interest since a wide variety of analytical procedures can be applied to the immobilized proteins.

Renart et al. [7] adapted the technique of transferring DNA fragments from agarose gels onto nitrocellulose paper (Southern blotting [8]) or onto diazobenzyloxymethyl cellulose (Northern blotting [1]) to transfer proteins from polyacrylamide gels to diazobenzyloxymethyl paper. In these techniques buffer passes through the gel, drawn by capillary force and carries the molecules which become trapped in an adsorbing paper. The time required for transfer could be reduced by applying a strong potential [2,9]. Burnette [3] adapted the method of Towbin et al. [9] to achieve quantitative transfer of most proteins from SDS gels to nitrocellulose: Western blotting. After 22 h of blotting, the polypeptides were almost completely removed from the gel.

We developed an alternative blotting method for polyacrylamide gels and SDS–polyacrylamide gels, which is very cheap, easy to do, reproducible and extremely fast. The suction power of a pump, connected to a slab gel dryer system, drives the separated polypeptides from the gel onto the surface of a nitrocellulose paper. The efficiency of this technique is demonstrated by blotting separated polypeptides from an egg homogenate of *Sarcophaga bullata* and from the hemolymph of *Leptinotarsa decemlineata*. The polypeptides were afterwards detected by immunostaining using a second antibody. The established tradition of geographic naming of transfer techniques (Southern, Northern and Western) is not continued. Being Europeans, we find it hard to describe this method as Eastern blotting. Therefore we suggest to refer it as vacuum-blotting.

2. MATERIALS AND METHODS

2.1. *Electrophoresis*

Polypeptides were separated by discontinuous electrophoresis [5] applied to SDS–polyacrylamide gradient (5–15%) gels.

2.2. *Transfer from the gel onto nitrocellulose paper (fig.1)*

Blotting buffer: 25 mM Tris, 150 mM glycine and 20% methanol.

After electrophoresis the stacking gel is removed and the separating gel is transferred to the blotting buffer for 20 min. The gel can be stocked in the freezer or immediately processed as follows. Dry

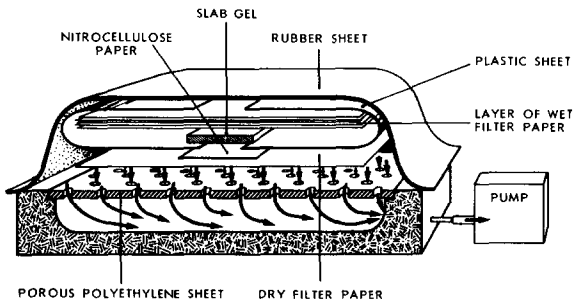

Fig.1. Cross-section of the vacuum-blotting apparatus.

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filter paper is layed on a porous polyethylene sheet. The nitrocellulose paper (Millipore, 0.45 μm) wet in blotting buffer, is put on the dry filter paper, making sure that no air bubbles are trapped in between these. A plastic sheet with a window somewhat smaller than the gel is put on the dry filter paper with the window on the nitrocellulose paper. The gel is placed on the nitrocellulose so that the edges of the gel overlap the plastic sheet. About 12 layers of filter paper are wet in blotting buffer and put on the gel. At least 3 cm of the outer border of the filter paper should rest on the plastic sheet. The outer margins of the plastic sheet are folded on top of the wet filter paper. Cover the whole system with a rubber or plastic sheet and connect it to a vacuum pump, so that the blotting buffer is sucked through the gel.

All vacuum-blottings were done with a pump of 250 W, 1390 l/min. We used the slab gel dryer of Biorad, but any slab gel dryer system can be used.

2.3. *Immunological detection of proteins on nitrocellulose*

The primary antisera were raised in rabbits.

Rinse buffer: 20 mM Tris, 150 mM NaCl adjust to pH 8.2 with HCl and add 2.5 ml pre-immune goat serum (PIG) to 1 liter buffer.

Pre-incubation buffer: 5 ml rinse buffer + 1 ml PIG. All incubations were done in plastic bags on a lab shaker at 35°C. The rinses between incubations were done in Petri dishes at room temperature. First antibodies, goat anti-rabbit serum and the peroxidase–anti-peroxidase complex (UCB, Brussels) were dissolved in rinse buffer (1/1500 to 1/3000, 1/20 and 1/300, respectively). Diaminobenzidine (30 mg) was dissolved in 100 ml of a 100 mM Tris buffer adjusted to pH 7.6 with HCl. The solution was filtered before adding 40 μl of a 30% H_2O_2 solution.

Procedure steps:

- Pre-incubation at 35°C for 1 h
- Rinse 15 min
- Incubation with the primary antiserum for 1 h
- Rinse 3 × 15 min
- Incubation with goat anti-rabbit serum for 30 min
- Rinse 3 × 15 min
- Incubation with the peroxidase–anti-peroxidase complex for 30 min
- Rinse 3 × 15 min
- Put nitrocellulose paper in diaminobenzidine–H_2O_2 solution until a good contrast is observed. The reaction is stopped by rinsing in water.

3. RESULTS AND DISCUSSION

In fig.2 a vacuum-blotting of an egg homogenate of *Sarcophaga bullata* is shown. In this species the vitellogenin consists of 3 polypeptides: 50 000, 53 700 and 56 100 M_r [4]. The gel was blotted for 45 min and stained using an antiserum against the vitellogenin of *Sarcophaga*. As one can see, even after a very short time, the transfer is extremely efficient.

Mixtures of proteins with great variety of M_r-

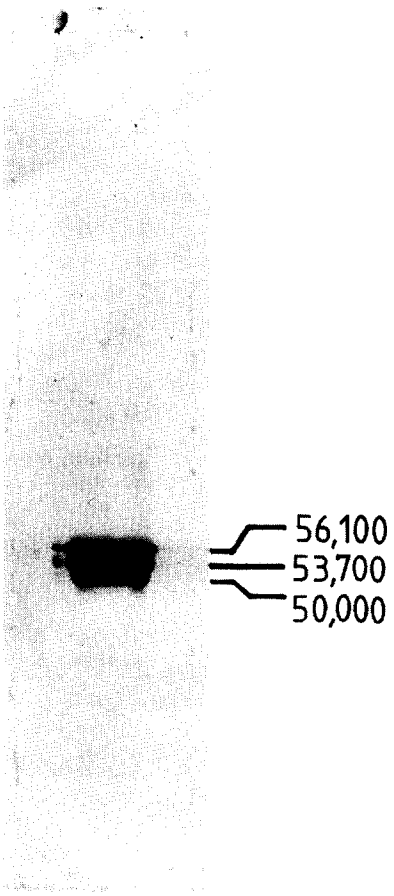

Fig.2. Vacuum-blotting of an egg homogenate of *Sarcophaga bullata* from SDS–polyacrylamide gel to nitrocellulose paper and stained with an antiserum against the vitellogenin.

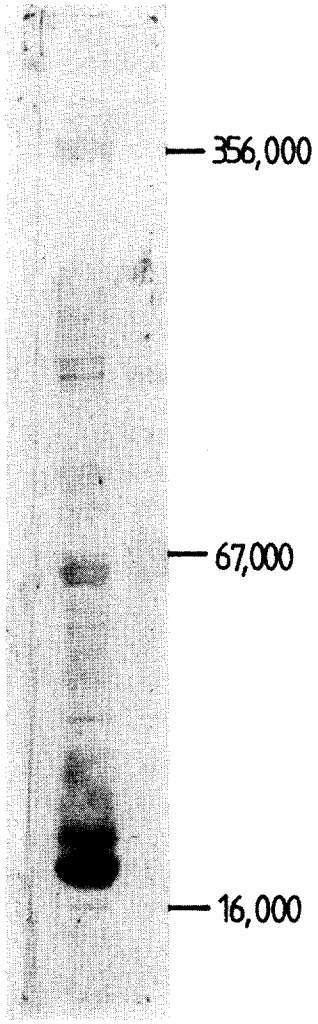

Fig.3. Vacuum-blotting of male hemolymph of *Leptinotarsa decemlineata* from SDS–polyacrylamide gel to nitrocellulose paper and stained with a total antiserum against male hemolymph.

values are also efficiently blotted. In *Leptinotarsa decemlineata*, the polypeptides of the hemolymph range from 12 000 to 356 000 M_r [6]. Figure 3 shows a vacuum-blotting of male hemolymph which has been stained using a total antiserum against male hemolymph. After 45 min, the 356 000 M_r polypeptide is detectable on the nitrocellulose. So, it is clear that polypeptides with a high M_r are easily transferred. On the other hand, we must keep in mind that polypeptides of low M_r ($\pm$ 14 000 M_r) are less adsorbed by the nitrocellulose paper with a pore size of 0.45 μm [3]. Vertebrate neurophysins ($\pm$ 10 000 M_r) separated by electro-focusing in polyacrylamide gels (5%) were completely removed from the gel and transferred to the back of the nitrocellulose after 45 min of vacuum-blotting. After 20 min of blotting time, we obtained a very good transfer but the nitrocellulose paper (0.45 μm) did not immediately immobilize the proteins, as a result there was some diffusion. When small polypeptides are transferred, we therefore advise the use of nitrocellulose with a small pore size (0.20 or 0.10 μm).

In our immuno-staining method we pre-incubated the nitrocellulose with pre-immune goat serum since this gave much better signal-to-noise ratios than pre-incubation with bovine serum albumin. The sensitivity of immunodetection is of coarse primarily a function of the specific antibody titer of the serum being used.

We do not see many restrictions to the application of the vacuum-blotting to polyacrylamide gels other than we used. In some cases very low concentrated polyacrylamide gels may stick the nitrocellulose after the transfer. Re-hydrating the gel makes it swollen and the nitrocellulose can be easily removed. For some proteins it might be necessary to use an other blotting buffer. When polyacrylamide gels without SDS are used, the methanol may precipitate the proteins. It is obvious that a buffer in which the proteins are very soluble should be used. If necessary, the blotting time can be extended, but in that case, make sure that there is enough blotting buffer, otherwise, the gel may dry and stick to the nitrocellulose.

So, without new investments, laboratories doing slab gel electrophoresis routinely can, by using their slab gel dryer system, very efficiently transfer their separated proteins to adsorbing paper.

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