COMPARISON OF THE BLOTTING EFFICIENCIES OF VARIOUS PVDF MEMBRANES

Paul Matsudaira

Whitehead Institute for Biomedical Research, and Dept. of Biology, Massachusetts Institute of Technology, Nine Cambridge Center, Cambridge, MA. 02142

SUMMARY; N-terminal sequence analysis from proteins electroblotted onto PVDF membrane is influenced by the efficiency of protein transfer to the membrane. Comparisons of the blotting efficiency of Immobilon P, Westran, and ProBlott, showed that ProBlott consistently retained larger amounts of small proteins and peptides than the other PVDF membranes. As a consequence, the initial yields of proteins blotted onto ProBlott are higher than proteins blotted onto Westran or Immobilon P. However, in the gas phase sequencer identical repetitive yields were obtained from soybean trypsin inhibitor and alpha-lactalbumin blotted onto all three membranes. The data suggests that all three membranes equally retain the sample during sequence analysis.
PVDF (polyvinylidene fluoride) membranes have proven an excellent support for sequence analysis of electroblotted proteins because protein can be easily visualized with commonly used stains and the membranes do not require pretreatment or derivitization (Matsudaira, 1987). In addition, unlike polybrene-coated glass fiber filters, the repetitive yields of the sequences from samples are independent of the amount of sample analyzed (Matsudaira, 1989). However, to permit maximal transfer of sample to the membrane, the transfer time from the gel to the membrane must be optimized. Long transfer times cause sample to "blow through" the membrane. Blow through is detected by stained protein on the backside of the membrane and from stained protein on the backup membrane. Short transfer times results in incomplete transfer. Optimal transfer times are sometimes difficult to achieve when large proteins and small peptides are blotted from the same gel because large proteins require longer transfer times than small peptides. To improve the blotting efficiency of the membrane it is desireable to increase the binding capacity of the membrane. This can be achieved by increasing the surface area of the membrane or by derivitizing the membrane to permit covalent attachment or entrapment. Recently two PVDF membranes (Westran, Schleicher and Schull; ProBlott, Applied Biosystems) with improved binding capacity and sequencing efficiency have been introduced. Comparison with Immobilon P (Millipore) or Westran shows that ProBlott has improved binding capacity for small proteins that results in higher initial yields but not repetitive yields. The identical repetitive yields suggest that in the gas phase sequencer the sample retention properties of the different membranes are identical.