INTRODUCTION

The recent availability of the Applied Biosystems 470A gas phase sequencer has greatly facilitated the structural analysis of proteins and peptides. The most important aspect of the gas phase sequencer is its dramatically increased sensitivity; in particular, its ability to obtain sequence information in the low picomol range (Hewick et al. 1981). However, its major drawback is that the glass filter holding the samples must be preconditioned with polybrene before peptide analysis can be performed. Recently, Matsudaira showed that protein can be spotted or electroblotted onto polyvinylidene difluoride (PVDF) membranes, detected by staining with Comassie Blue, and then sequenced directly (Matsudaira 1987). This method is very simple and requires no special chemistry for PVDF membranes, but the repetitive yield of PVDF membranes is lower than that of glass fiber filters. To obtain a higher repetitive yield for short peptide sequence analysis, a polybrene-coated support is recommended (Matsudaira 1987). In this paper, we describe a procedure for preconditioning the glass filter before its insertion into the sequencer. This simple additional step drastically saves sequencer time and makes sequencing more efficient.

MATERIALS AND METHODS

Whatman GF/C glass 12.5 cm microfiber filter was obtained from Whatman, Maidstone, England. Polybrene, triethylamine, phenylisothiocyanate (PITC), and trifluoroacetic acid (TFA) were from Pierce Chemical Co. (Rockford, IL, U.S.A.). Ethanol, heptane, ethylacetate, and butylchloride were purchased from Burdick and Jackson (Muskegon, MI, U.S.A.).
Parafilm (roll type), 4-liter beakers, and glass beads were ordered from Fisher Scientific Co. (Pittsburgh, PA, U.S.A.). Cork borer (cutting tube) size no. 6 was purchased from Sargent-Welch Scientific Co. (Skokie, IL, U.S.A.).

**Manual Preparation of Polybrene Precycled Filter** Place one sheet of Whatman GF/C glass microfiber filter on top of a layer of glass beads completely covering the bottom of a 4-liter beaker. Next, prepare approximately 5 ml of a 66 mg/ml solution of polybrene containing 6.6 mg of NaCl per ml and apply it to the filter paper just to the point of saturation. This should be done on top of a light box for better observation of filter saturation. Place the beaker in an oven and dry the filter paper at 45°C. Meanwhile, prepare 5 mls of derivatization solution (3.55 ml ethanol, 0.5 ml H2O, 1.0 ml triethylamine, 0.5 ml phenylisothiocyanate). Using the derivatization solution, bring the filter just to the point of saturation as before and then quickly cover with Parafilm or Saran Wrap. After allowing the beaker to sit at room temperature for 15 min, dry the filter paper in a hood by directing a gentle stream of nitrogen or argon into the beaker.

Following this, wash the filter consecutively, first with heptane, then with ethyl acetate, and finally with butyl chloride by adding enough solvent to the beaker to completely cover the filter paper and then removing the wash solvent with an aspirator. It is not necessary to dry the filter paper between solvent washes. After repeating this three-step wash procedure twice, dry the filter paper, first with a stream of nitrogen or argon as before and then for a short time in an oven set at 45°C.

After drying, quickly take the beaker out of the oven, saturate the filter with TFA, and cover with Parafilm. Allow the filter to remain at room temperature for 10 min and then dry as previously described. A final drying is then done at 45°C for 30 min. Using a 1.2 cm cork borer, filters can be cut to appropriate size on a clean manila folder.

**Sequencing** The Applied Biosystems Model 470A gas-phase sequencer with the Model 120A PTH analyzer was used for sequence analysis. One hundred pm of myoglobin was applied to a manually precycled polybrene-loaded filter (our process), or to a filter precycled in the 470A Sequencer according to the procedure specified by Applied Biosystems,