INTRODUCTION

The solid phase method of protein sequence analysis (Laursen, 1971) requires the covalent attachment of a peptide or protein to a solid matrix that is stable to the reaction conditions of the Edman degradation. Although a variety of support matrices and attachment chemistries have been described (for a review see Laursen and Machleidt, 1980) the commonly employed covalent attachment supports have been diisothiocyanate (DITC) derivatized glass beads (Wachter et al., 1973) or DITC functionalized glass fiber sheets (Aebersold et al., 1986). Both types of matrices react efficiently with the ε-amino groups of the lysine side chains of peptides and proteins. For the protein sequencer, the most significant advantage of the latter support is that proteins that have been resolved by gel electrophoresis can be electroblotted directly onto DITC-activated glass fiber sheets with covalent attachment occurring during the transfer. Although employed quite effectively in this application, the DITC glass fiber sheets were mechanically fragile, with rather low protein binding capacity, and it proved difficult to detect the protein molecules on the glass surface.

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Pluskal et al. (1986) demonstrated that complex mixtures of proteins could be resolved by 1-D and 2-D polyacrylamide gel electrophoresis and efficiently electroblotted onto polyvinylidene difluoride (PVDF) membranes. The membrane bound proteins could be visualized with a variety of compounds such as coomassie brilliant blue, ponceau S, amido black and stains-all. Matsudaira (1987) and LeGendre and Matsudaira (1988) further demonstrated that areas of the PVDF membrane containing discrete bands of adsorbed protein could be excised from the membrane sheet and used as supports in gas-phase protein sequencing equipment. A number of important points could be summarized from this work:

1. PVDF membranes are mechanically strong, flexible and easily handled.
2. PVDF membranes bind proteins very tightly and are highly useful for obtaining immobilized proteins after electrophoretic separation.
3. The protein binding capacity of PVDF membrane is large (~170 μg/cm²).
4. PVDF membranes are stable to the chemical conditions of the Edman degradation.
5. Initial and repetitive sequencing yields of proteins adsorbed to PVDF membranes is excellent, even when the amount of protein sample is less than 10 pmol (Matsudaira, P. this volume).

In an effort to develop the first membrane based support specifically designed for protein sequence analysis by solid-phase methods, we chose to combine the excellent physical and chemical properties of PVDF with the well-proven diisothiocyanate attachment chemistry. The synthetic strategy involved "light" modification of the PVDF surface such that the favorable protein adsorbing properties of the membrane were not significantly affected. The membranes have been tested as supports for the solid-phase sequence analysis of proteins.