3.1 A Second Generation Solid-phase Protein Sequencer: The Prosequencer™

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INTRODUCTION

The solid-phase version of the Edman degradation, first described over 20 years ago (Laursen, 1966) and later automated (Laursen, 1971), takes advantage of the principle that proteins which are covalently linked to an insoluble matrix can readily be separated, without losses, from reagents and reaction products. The first commercial solid-phase sequencers were introduced in the early 1970's and improvements in the technique, primarily in methods of protein immobilization, continued for several years more (Laursen and Machleidt, 1980). With the introduction (Hewick et al 1981) of the gas-phase protein sequencer, interest in the solid-phase technique declined, primarily because of the microsequencing capabilities of the newer instrument and because the protein immobilization steps were not required. Recently, however, there has been a resurgence of interest in solid-phase chemistry because of its advantages as a method for immobilizing proteins electroeluted from polyacrylamide gels and for preventing losses of material with ultramicrosequencing techniques (Aebersold et al 1988).

Although a solid-phase microsequencer has been described recently (Walker et al, 1986), existing commercial machines are not engineered for subnanomole sequencing. Our aim is to remedy this situation.

THE PROSEQUENCER

In developing a new solid-phase sequencer, the ProSequencer, we have had several design goals:
* Sensitivity down to 10 picomoles or less
* Short cycle time
* Flexible programming for extended runs
* Simplified immobilization techniques
* Ability to handle any kind of sample
* On-line HPLC PTH analysis
* Automatic data analysis
* Simplified, fail-safe operation

Achievement of these goals has required complete redesign of the instrument. However the most important design factors are miniaturization, which results in lower background, short cycle times, reagent economy and simplified components; and computerization, which permits accurate control of reaction conditions and is needed for data analysis. A prototype ProSequencer is shown in figure 1. Not shown is the NEC APCIV computer, which, after it has loaded the operating program into a 265K RAM XT board in the sequencer, is free for HPLC data acquisition and