2.1 Modern Manual Microsequencing Methods

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1 Introduction

Today manual Edman sequencing procedures are frequently taken to be too slow, too insensitive or too labor-intensive to be of significant practical, let alone competitive, value to protein structural studies. Over the last 10 years, George Tarr, the man who developed polybrene as a sequencing reagent [1], has enhanced the performance of manual sequencing [2–5] to the point that his procedures can be important assets to even the best-equipped protein chemistry laboratories. Tarr's methods are not difficult to master and can produce multiple picomole level analyses more rapidly than most automatic sequenators produce a single analysis. Using Tarr's batchwise procedures, HPLC peptide map fractions may be quickly screened for NH₂-terminal purity and identification of peptides. Quality control sequence analyses of synthetic peptides, which are usually abundant, can also be done efficiently by Tarr's manual methods rather than using valuable automatic instrument time for the task. While automatic sequencing is the method of choice for extended degradations, the manual procedures of Tarr provide an attractive alternative for analyzing short peptides. For example, ten peptides, each of about 6 residues, would in total require about 1 week of automatic gas-phase sequencing time provided two peptides are analyzed per day;
all ten could be sequenced in 1 day by using Tarr's batchwise manual strategy. For peptide screening purposes, for quality control analyses, for sequencing short peptides and for investigators on a low budget, the Tarr manual strategies can constitute methods of choice.

2 Methods

2.1 Sensitivity and Cycle Time

The Tarr procedures are similar in sensitivity performance to DABITC manual sequencing [6], and are routinely useful for determining 10 – 30 residues with 500 – 2000 pmol of peptide or protein. Useful sequence information has, however, been obtained with less than 100 pmol of sample [4]. In contrast to the manual DABITC procedure which requires 120 – 140 min per cycle [6], the Tarr strategies are much more rapid, requiring only 15 – 60 min per cycle.

2.2 Tarr Sequencing Strategies

Tarr's established sequencing procedures include two batchwise methods, one for small peptides and the other for large peptides and proteins, and another very rapid method useful for analysis of a single large peptide or protein. The batchwise procedures utilize ethanol or dimethyl formamide coupling mediums, are carried out in 6 x 50 mm glass culture tubes containing polybrene and have recently been described in detail by Tarr [5]. Repetitive yields are typically about 90% for the large peptide/protein batchwise method and about 80 ± 10% for the small peptide batchwise procedure. The rapid large peptide/protein method utilizes an aqueous pyridine coupling medium, is carried out in a 1-ml reactivial, exhibits repetitive yields of about 90% and has been described briefly [4, 7] but with subsequent modifications [8]. Examples of the efficacy of these methods include several NH₂-terminal analyses [8 – 10] some of which revealed homologous relationships [11 – 13] or defined the reading frame of a gene [14] as well as complete primary structured analyses of human hypoxanthine-guanine phosphoribosyltransferase [15], rabbit cytochrome P-450 LM₂ [16] and the cellular retinoic acid-binding protein from bovine retina [17]. An outline of each of the Tarr manual sequencing methods is presented in Tables 1, 2 and 3 along with examples of typical results in Figs. 1 – 3.

3 Equipment and Supplies

3.1 The Manual Sequencing Station

A work area equipped so that all the steps of the Edman degradation may be carried out from a chair or stool, without moving about the laboratory, is a major