N-terminal Sequencing of 10 pmols of Human Serum Albumin using Routine 3.1 Method on the HP G1005A N-terminal Protein Sequencing System

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HP G1005A
N-terminal Protein
Sequencing System

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Application Brief

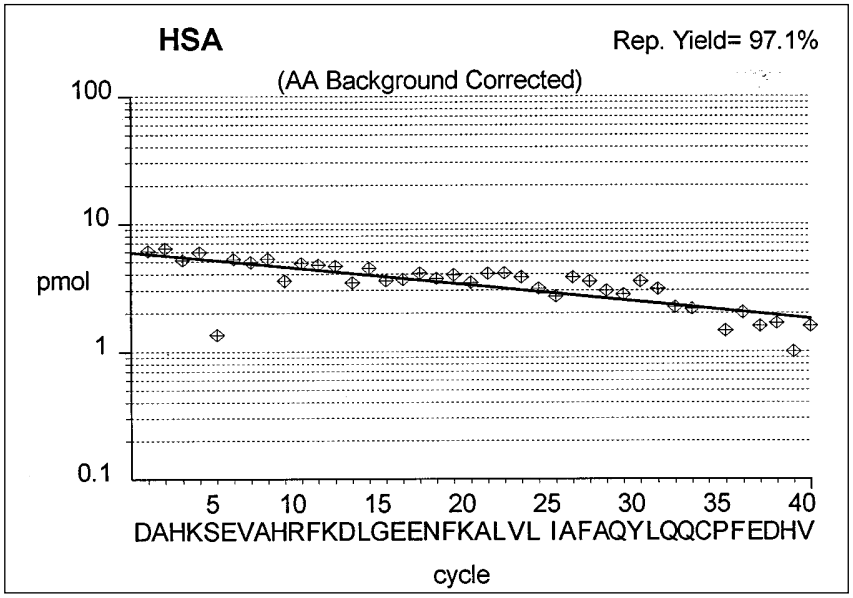

Fig 1. Repetitive yield plot for the sequence analysis of 10 pmols HSA.

Abstract

This Application Brief describes the N-terminal sequence analysis of a 10 pmol of human serum albumin using the HP G1005A N-terminal protein sequence with Routine 3.1 method

Keywords

Sequencing, protein, G1005A

Introduction

The HP G1005A N-terminal Protein Sequencing System performs automated Edman chemistry of peptides and proteins loaded in solution and immobilized on miniature biphasic columns consisting of reverse- phase (RP) sample column mated to a strong anion exchange (SAX) column.[1, 2] The biphasic support facilitates efficient, simple and rapid sample clean-up and concentration as part of the sample loading procedure. Samples can be directly loaded and retained on the RP column in a variety of buffer matrices (salts, denaturants, detergents), washed free of the contaminants, coupled to the SAX column, and sequenced.

Several performance characteristics of the HP G1005A N-terminal Protein Sequencing System and Routine 3.1 chemistry contribute to the routine sequence analysis of proteins at low picomole level.

Features of the N-terminal Routine 3.1 chemistry are:

- **Extremely efficient Edman-based N-terminal sequencing chemistry.** Typical initial yields for pmol amounts are approximately 50%. Repetitive yields can be greater than 95%, permitting the confident assignment of 40 or more amino acid residues.

- **Reproducible PTH-amino acid retention times and gradient chromatography.** Stable retention times enable chromatogram subtraction or overlay for accurate cycle-to-cycle assignment.
- **Stable and low chemical background.** Confident assignment of cycles yielding less than 1 pmol of PTH-amino acid requires this sufficiently low background.

Methods

The sequence analysis presented here was performed human serum albumin (HSA, 68kDa) protein. A sample aliquot of 10 pmols was diluted in 1 mL of aqueous TFA and directly loaded on a reverse-phase sample column (HP G1073A), then mated to a strong anion exchange (SAX) column (HP G1448A). Prior to sample loading this sequencing column was prepared using Column Prep 3.1/3.5 method. Analysis was carried out using the N-terminal Routine 3.1 sequencer method and PTH_4.M HPLC method on an HP G1005A N-terminal Protein Sequencing System with standard Routine 3.1 reagents and solvents.

Results and Discussion

Shown in Figure 1, the initial yield (cycle 1) of HSA was 6.1 pmol of PTH-Asp , corresponding to an overall repetitive yield of 97.1%.

Representative chromatograms of 10 pmols HSA show the unambiguous assignment of the PTH-amino acid residues and the low chemical background. A PTH-amino acids residues are well-resolved and separated from the Edman chemistry by-products of DPTU (as identified in the figure) and DPU (which elutes after PTH-Leu).

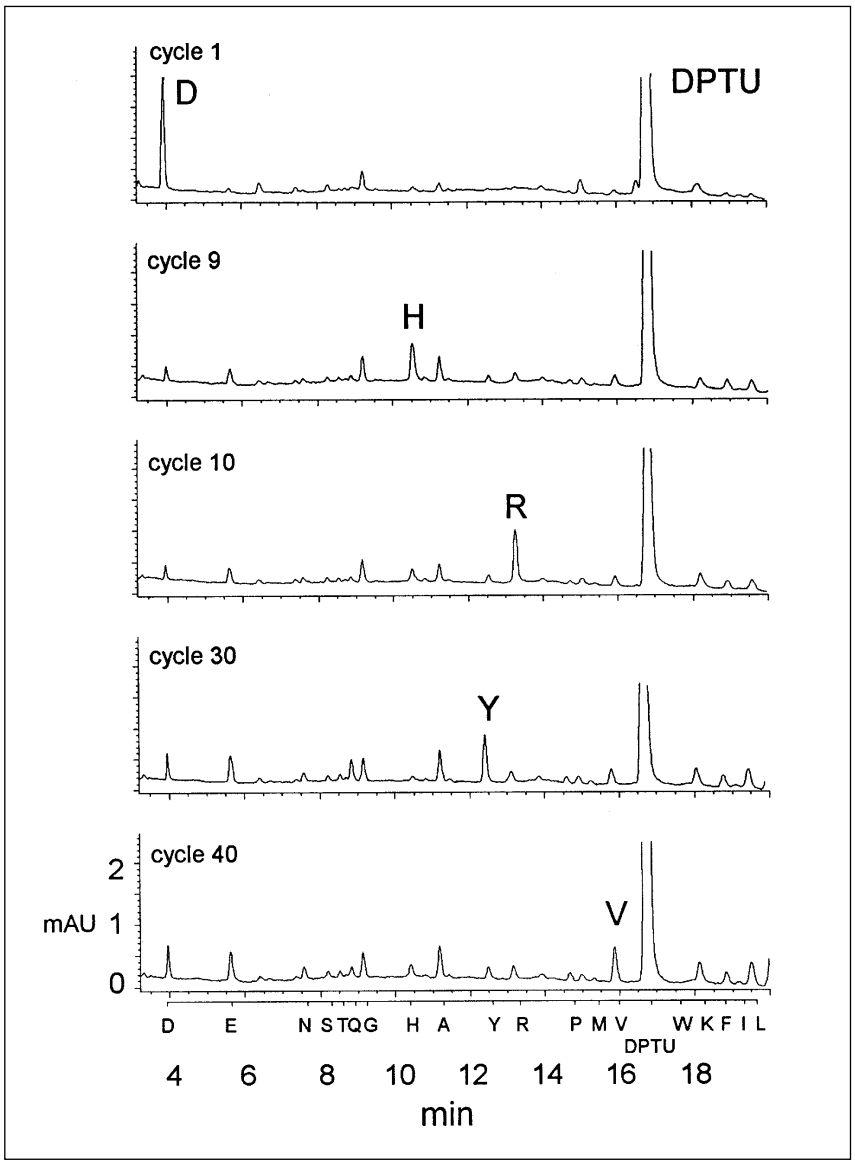

Fig 2. Results of sequence analysis of 10 pmols of HSA using "Routine 3.1" sequencing method.

References

1. Hewlett-Packard Technical Note. "*Routine 3.0 Sequencer Methods,*" TN 95-1.
2. Chad G. Miller, "*Adsorptive Biphasic Column technology for Protein Sequence Analysis and Protein Chemical Modification,*" METHODS: A Companion to Methods in Enzymology, **6**, 315–333 (1994).

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