N-Terminal Sequence Analysis of Peptides Using Routine 3.5 Method on the HP G1005A N-Terminal Protein Sequencing System

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HP G1005A N-Terminal Sequencing System

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Application Brief

Abstract

This Application Brief describes the N-terminal sequence analysis of peptides at low pmol levels.

Keywords

Sequence, peptide, G1005A

Introduction

Many peptides of biological significance, recovered from isolation techniques in limited sample amounts, require highly efficient sequencing methodologies enabling as much of the complete amino acid sequence determination as possible. The results of sequence analysis on low-level amounts of representative peptides using the HP G1005A N-terminal Protein Sequencing System are discussed below.

Methods

20-pmol samples of human α-endorphin (Sigma Chemical Co., E6136) and diazepam binding inhibitor fragment 51-70 (Sigma Chemical Co., G9898) peptides, reconstituted in water and diluted in 100 μl of 2% aqueous TFA , were directly loaded on reverse-phase (RP) N-terminal sequencing columns (HP G1073A). The samples were sequenced using the "Routine 3.5" sequencing method[1] and "PTH_4.M" HPLC method.

Results and Discussion

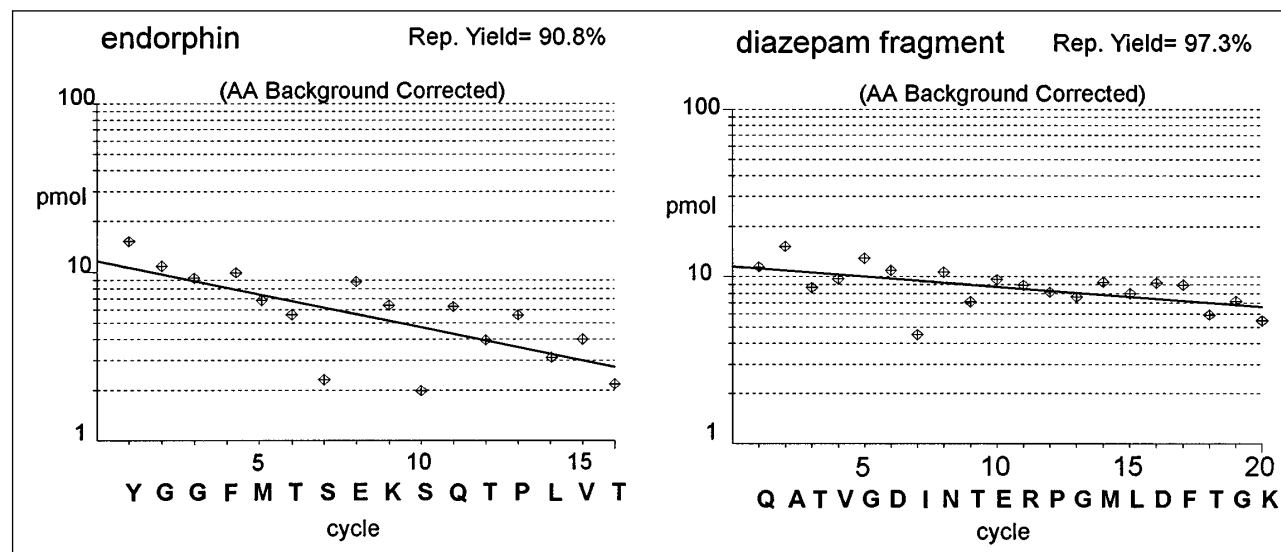As shown in Figure 1, sequence analysis of the 16-amino acid

Figure 1. Repetitive yield plot for the sequence analysis of 20 pmols of Human α-endorphin and diazepam binding factor fragment 51-70 peptides.

endorphin resulted in an overall repetitive yield of 90.8% and an initial yield of 16 pmol. The C-terminal amino acid residue was clearly identified as PTH-Thr. The 20-amino acid diazepam fragment resulted in an overall repetitive yield of 97.3% and an initial yield of 12 pmol. The C-terminal amino acid residue was unambiguously identified as PTH-Lys.

Sequencer cycle chromatograms of representative cycles are shown in Figure 2. These results demonstrate the high-efficiency sequence analysis of peptides at low pmol levels using the N-terminal sequence method "Routine 3.5" on the HP G1005A N-terminal Protein Sequencing System.

References

1. Hewlett-Packard Technical Note. "N-terminal Routine 3.1/3.5 Sequencer Methods," TN 96-1.
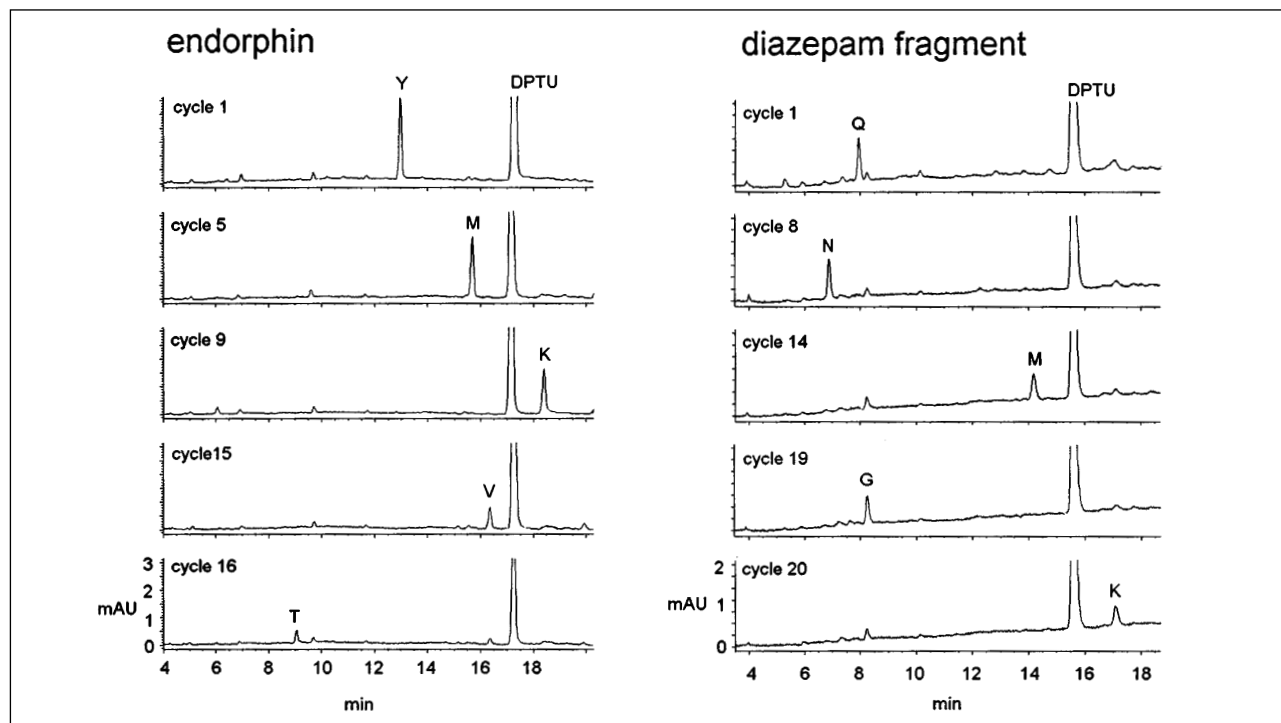
Acknowledgment

Figure 2. Results of sequence analysis of 20 pmols of Human α-endorphin and diazepam binding factor fragment 51-70 using "Routine 3.5" sequencing method.