amino-ethane. Thereby a large excess of reagent is achieved, which is easily removed after the reaction. The reasonable reaction yields (70–80%) are obtained even when using low sample amounts, tested with ATZ-Phe (10 fmol level). The product, trifluoroethyl amide of phenylthiocarbamyl-Phe, was analyzed by gas chromatography equipped with an electron capture detector using a capillary column. The sensitivity of detection is as low as 10 fmol of the product. The combination of the reasonable reaction yield and the high detectability of the stable fluorinated product suggest a possible new sensitive protein sequencing method.

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Koji Muramoto,[1] Kiyoshi Nokihara,[2] Akira Ueda,[2] and Hisao Kamiya.[1] Sensitization of Gas-Phase Protein Sequencer Using Fluorescein Isothiocyanate FITC. ([1] School of Fisheries Sciences, Kitasato University, Sanriku, Iwate, 022-01 Japan; [2] Biotechnology Instruments Department, Shimadzu Corp., Kyoto, 604 Japan)

Since gas-phase sequencers were introduced into the market, it has been possible to analyze several 10–100 pmol samples. Further sensitization of sequence analysis has become one of the most important targets, together with the development of micropurification methods and blotting technology with two-dimensional gel electrophoresis. For sensitive detection, several fluorescent reagents have been tried; however, it is still not possible to use these routinely for Edman-type automated sequencers.

FITC was used for manual sequencing to open the possibility for sensitization (Maeda and Kawauchi, 1968). On the other hand, 50 fmol of FTH-amino acid (FTH-AA) analysis using HPLC has been performed (Muramoto *et al.*, 1978). However, the FITC-method showed several difficulties in adapting this for automated Edman-type sequencing. First, the purification of FITC itself. We have established an optimized protocol for synthesis and purification for obtaining isomer-free FITC crystalline. Second, we have improved the RP-HPLC system for FTH-AA, which is carried out at higher *p*H to gain maximum fluorescent intensity. Modified Edman reagents suffer from low reactivity against N-terminal residues and problems in removing excess reagents and their by-products.

We have modified a protein/peptide sequencer (Shimadzu Model PSQ-1) with detection for FTH-AA by RP-HPLC to give a more sensitive automated sequencer. Several HPLC Systems, including columns, were investigated in order to give elongated column-life and excellent resolution of each FTH-AA with by-products. The reaction chamber has been miniaturized. A gradient system with the Shimadzu Model, LC-6A and a monitor, Shimadzu Model RF-500 (Ex: 490 nm, Em: 520 nm) were adapted for the on-line detection of FTH-AA. Combination with the use of PITC, which has no fluorescent grouping, allowed sequence analysis below the picomole level to be realized. Separation was performed on the specially designed reversed-phase column (3μ, 4.6×50 mm), using a gradient of acetone in 10 mM sodium phosphate buffer (*p*H 7.0). FITC and standard FTH-AA were prepared in our laboratory. The detection limit of the FTH-AA was approximately 10 fmol. The optimized protocol was developed for FITC followed by PITC coupling. To optimized coupling conditions, FITC/PITC double coupling was adopted. The excess FITC and its by-products were removed by successive washing with ethyl acetate/heptane, ethyl acetate, and acetone.

Myoglobin (5 pmol) and lysozyme (5 pmol) were analyzed on the modified PSQ-1 to obtain 35–51% of initial yield (IY) and 82–88% of repetitive yield (RY) for the former, and 51–66% of IY and 91–92% of RY for the latter. These figures permitted the identification of 20–25 amino acid residues from the N-terminus of 1–5 pmol samples applied on the sequencer. Samples spotted or electroblotted onto PVDF membranes can also be performed to give similar results with less background on HPLC.

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Frank Reimann and Stefan Fischer. The Latest KNAUER Sequencer with the New "Loop Reactor" for Higher Reproducibility of Reagent Delivery. (Dr. Ing. Herbert Knauer, Dept. Biochemie, Am Schlangengraben 16, W-1000 Berlin 20, Germany)

The KNAUER Modular Protein Sequencer was introduced with a new kind of reactor, using a combination of liquid