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Sensitization of Edman Amino Acid Derivatives Using the Electron Capture Sensitive Reagent, 1-Trifluoro-2-amino-ethane

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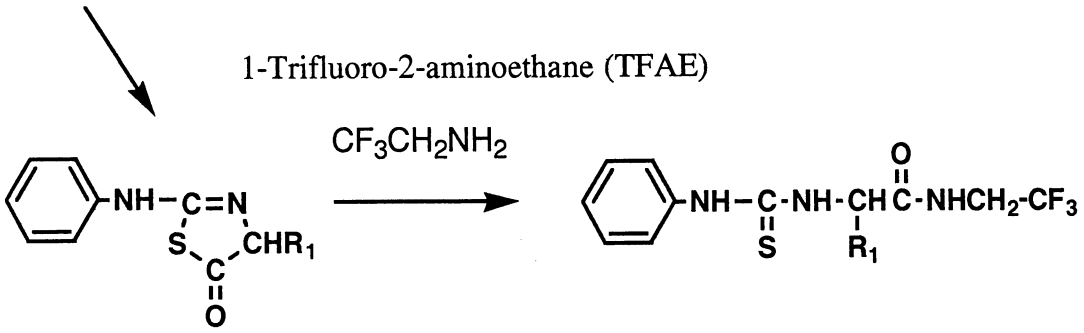
2-Anilino-5-thiazolinone amino acids, intermediates of Edman degradation, were reacted with1-trifluoro-2-amino-ethane in vapor phase. The products were analysed by gas chromatography using an electron capture detector. The reaction was quantitative and capable of being used for micro amount of protein because the large excess of gas phase reagent was easily removed after the reaction. Sensitivity of detection of the reaction product was about 50 femtomole.

Reducing the lower limit of protein required for sequencing is a challenging and important project for biotechnology. Edman degradation established N-terminal step-wise sequencing[1] and its initial automation achieved protein analysis at the low nanomole level.[2] Next, a sensitive and easy-to-use machine was invented resulting in low picomole level sequencing.[3] Recently, we proposed a new femtomole level sequencing technique involving the reaction of the Edman intermediate, 2-anilino-5-thiazolinone (ATZ) compounds with ^{125}I-histamine[4] and 4-aminofluorescein.[5,6] This technique made accessible 100 femtomole sequencing. The reactions were essentially two molecule reactions in a liquid phase, where maintaining high concentrations of the reagents against extremely low concentrations of samples were required to achieve high reaction yields. However, because high reagent concentrations are used, it is troublesome to remove the excess reagents and by-products from the reaction mixture.

The present method consists of reacting solid phase sample ATZ-amino acids with a gas phase reagent, 1-trifluoro-2-amino-ethane (TFAE), thereby achieving large excess of reagents which are also easily removed after the reaction. In addition, reasonable yields are obtained even when using low amounts of sample.

In order to determine the reaction yields various amounts of ATZ-phenylalanine,

Edman degradation



2-Anilino-5-thiazolinone (ATZ) amino acid

Phenylthiocarbamyl-trifluoroaminoethane (PTCTFA) amino acid

synthesized and purified from Phe-Gly,[7] were placed in small tubes (4 mm x 40 mm) and dried. The tubes were placed in large tubes (13 mm x 100 mm), which contained 100 μl (micro liter=$10^{-6}dm^3$) of TFAE, and were then flame-sealed under vacuum. The tubes were incubated at 80 °C for 20 min. The small tubes were taken out, and the samples applied to a microbore HPLC column Spheri-5RP-18 (ϕ1.0 mm x 50 mm, Applied Biosystems) isocratically eluted with 32% acetonitrile 0.1% trifluoroacetic acid (TFA) at 55 °C. The reaction yields of the reaction were estimated photometrically at 245 nm. Table 1 shows the yields of the products, the TFAE derivatives of phenylthiocarbamyl amino acid (PTCTFA), indicating that good yields were maintained even when using low amounts of sample.

Table 1. Yield of the PTCTFA-Phe with gas phase reaction

Starting amount	PTCTFA-Phe	
(mol)	Amount (mol)	Yield /%
100p	85p	85
10p	8.5p	85
1p	810f	81
150f	78f	78
50f	35f	70

PTCTFA-phenylalanine was synthesized and purified. ATZ-phenylalanine (10 mg) in 1ml (milli liter=$10^{-3}dm^3$) of acetonitrile was reacted with TFAE (100 mg)

at 50 °C for 30 min. After dried in vacuo, the product was dissolved in acetonitrile and chromatographed on an HPLC column, YMC-Pack ODS-AP (ϕ4.6 mm x 150 mm, YMC, Kyoto) using a gradient of 0 to 50% acetonitrile in 0.1% trifluoroacetic acid. The purified PTCTFA-Phe was then analyzed by gas chromatography (Hewlett Packard 5896 series II +7675A) eqquiped with an electron capture detector using a capillary column, crosslinked 5% phenyl methyl silicone. Figure 1 shows that the sensitivity of detection is as low as 10 femtomole of the product. Although several ATZ-amino acids have been preliminary studied, for the sake of the simplicity only the data for ATZ-Phe has been presented.

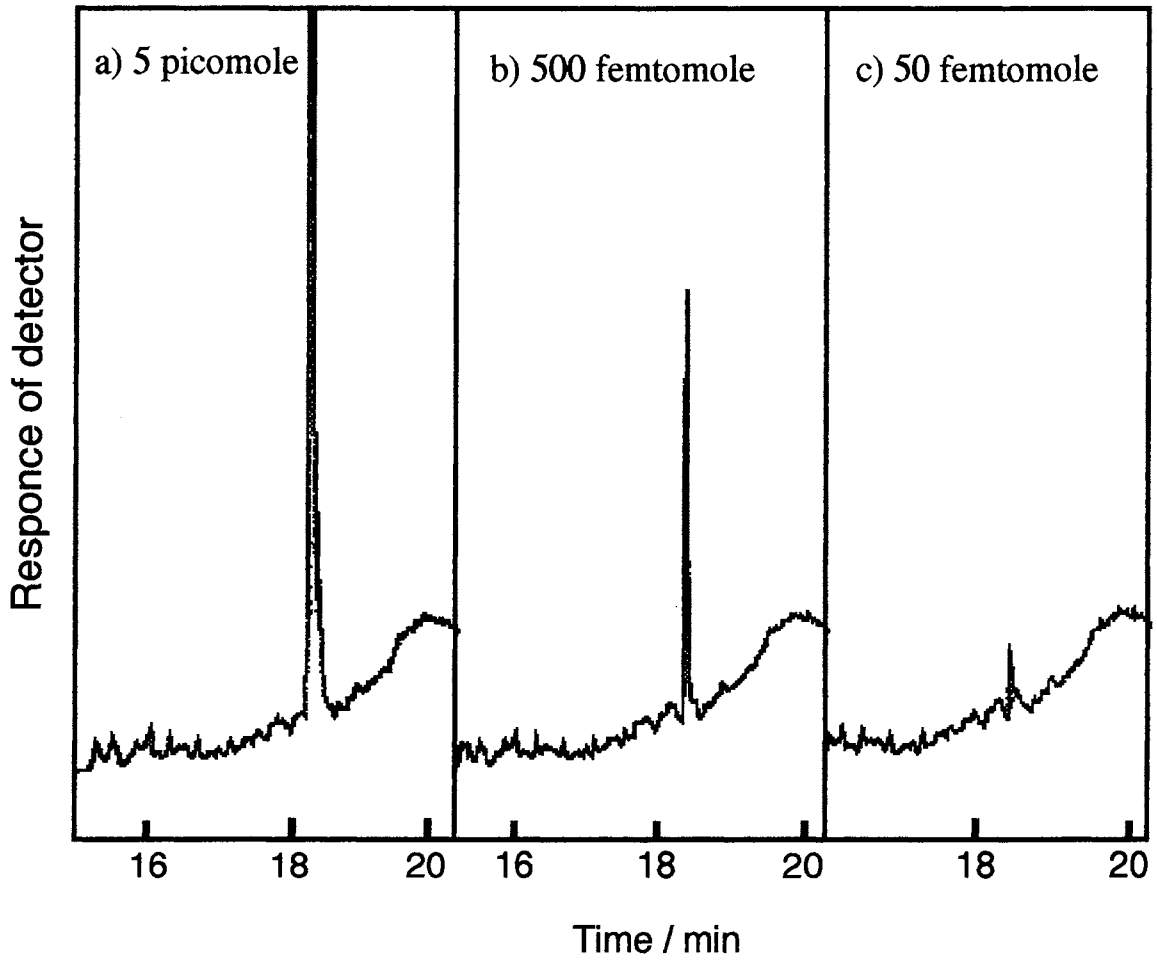


Fig. 1. Gas chromatogram of PTCTFA-phenylalanine. column; ULTRA#2 (crosslinked 5% phenyl methyl silicone, Ø0.32 mm X 25 m, coating width 0.17 μm) , Carrier gas; He 1.5ml/min (8 psi), Oven temperaturewas 50 °C at start. After 1 min, elevation speed of temparature was 10 °C/min, and final temperature was 300 °C and then kept for 15min, Detection; electron capture detector operating at 300 °C. a) 5 picomole , b) 500 femtomole, and c) 50 femtomole.

The combination of reasonable reaction yield and high detectability of the product suggests this approach as a possible ultra high sensitive method of protein sequence analysis.

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