

Fluorescence detection of amino acids in the postcleavage conversions for manual sequencing of a peptide

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Abstract

A modified Edman degradation method where fluorescent derivatives of amino acids were generated from the postcleavage products of a peptide is described. In the method, the target peptide was applied onto double glass fiber membranes in a small filter disk (4 mm i.d.) and then treated with small amounts of reagents for the manual sequencing of the peptide. The anilinothiazolinone (ATZ) of N-terminus amino acid residue after the isolation from the solid-phase membranes was reacted with a primary amine, 4-(1'-cyanoisindolyl)aniline (CIA), to form a more stable and sensitive fluorescent derivative, phenylthiocarbamoyl-CIA. An average yield of 85% was obtained in neutral pH conditions for the CIA reaction. The ATZ-CIA-amino acids were separated by reversed-phase liquid chromatography and detected by fluorometry. The lower limits of the detection for amino acids after the Edman degradation were 0.16 to 0.52 pmol (signal/noise ratio = 3) on the column. The sensitivity was approximately 10 times higher than ultraviolet absorbance detection of phenylthiohydantoin products in the conventional Edman degradation. The suitability of the method was demonstrated by the sensitive manual sequencing of insulin chain B composed of 30 amino acids.