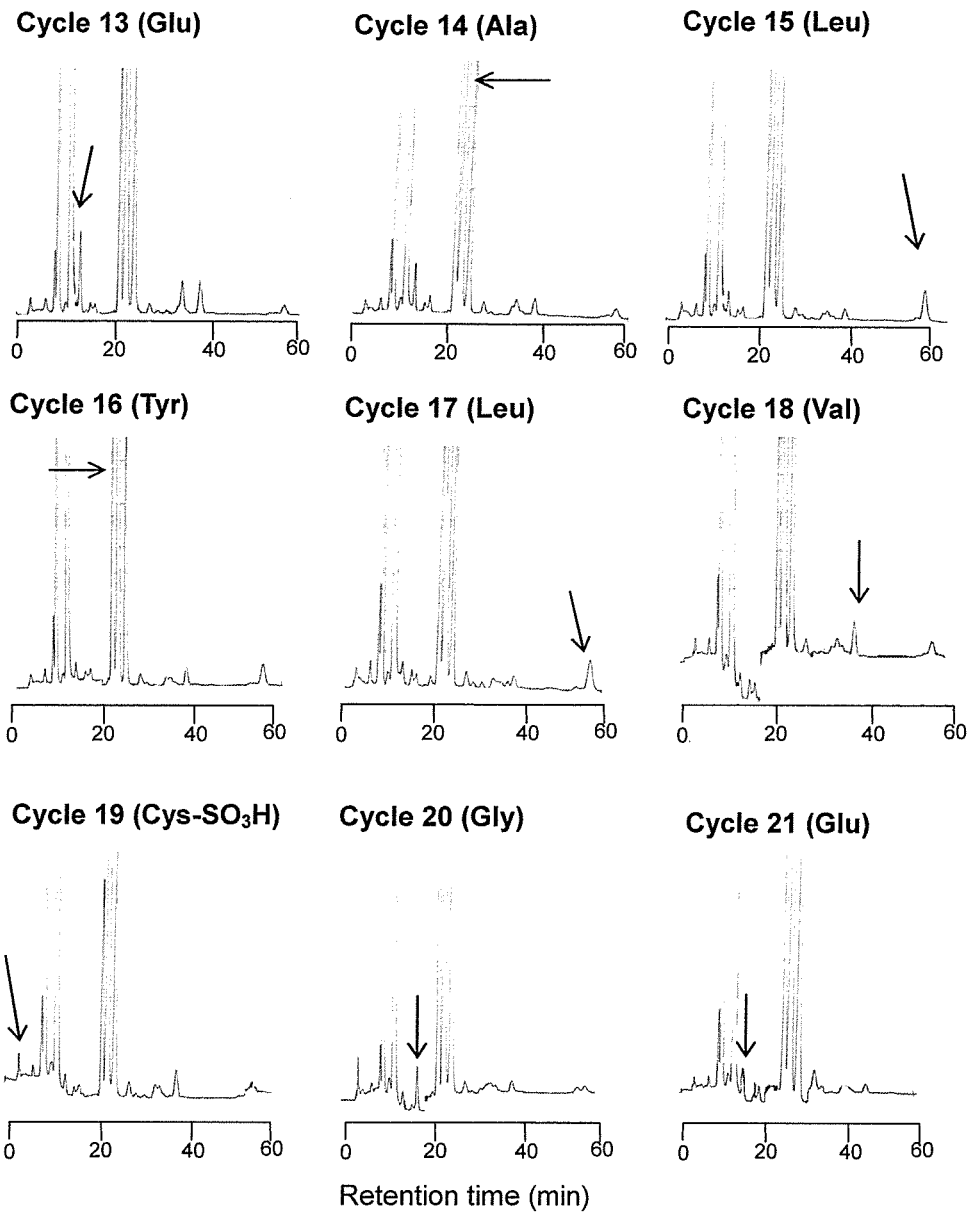

Fig. 4: Chromatograms of manual sequencing of insulin chain-B on GF membranes in a filter disk. RPLC column was a Wakosil PTH column (250 x 4.6 mm ID) at 40 °C. Mobile phase consisted of CH_3CN, 0.12 M CH_3COOH with 0.14 % SDS (w/v), and H_2O (55:10:35). Other sequencing procedures and conditions are described in the experimental section.

Precision and sensitivity

Precision and calibration studies were investigated with independent reactions of amino-acid amides with the amount ranging of 25-500 pmol on the membranes. The calibration curves for Gly-NH_2, Val-NH_2, Glu-NH_2 and Ser-NH_2 showed each linear relationship between FL intensities of their PTC-CIA-amino acids and their amounts on membrane. The linear correlation coefficient was 0.9883-0.9958 for the amino acid tested (n=3). The RSD for three replicates did not exceed 2.6% for within-day analyses and this demonstrates high precision. The lower limits of detection at a signal-to-noise ratio of 3 (S/N=3) for the PTC-CIA-amino acids were 0.16, 0.32, 0.32, 0.52 pmol for Gly, Val, Glu and Ser, respectively. The sensitivity of the method was 7-12 times higher than conventional UV detection of their PTH-amino acids in the Edman degradation.

For the sequencing analysis, the reproducibility of the peak heights of the PTC-CIA-amino acids was examined for the first three cycles. The peak heights corresponding to Phe, Val and Gln at the 1^{st}, 2^{nd}, and 4^{th} cycles showed RSD of 3.3-4.1% for three repeated analyses.

Conclusions

A manual method with modified Edman-degradation procedures has been developed for peptide sequencing on solid-phase GF membranes in a small filter disk. The reaction of ATZ-amino acids with CIA gave highly fluorescent and stable products for all amino acid residues, and approximately 10-time higher sensitivity of detection than that of conventional UV detection. The FL detection also reduced high background level that is usually observed with UV detection of PTH-amino acids. The high yield of conversion of

ATZ to PTC-CIA for all amino acids indicates that our method is suitable for micro-sequential analysis of peptides, allowing use of small amounts of sample and reagents. By the current method, we could identify amino acid residues of a peptide analyzed up to 20 cycles of which the sequencing is comparable to that of conventional automatic sequencer. However, it is noted that the repeated use of the filter disk made of polypropylene may reduce its strength and thus may contribute to generation of hindering contaminants. We are investigating the use of Teflon or glass type disks which are stronger and resistant to heat and most acidic and basic organic solvents.

Acknowledgements

This work was supported by Shimadzu foundation (Japan) and partly by grants-in-aid for scientific researches from the Ministry of Education, Culture, Sports, Science and Technology and from the Ministry of Health, Labor and Welfare, Japan, which are gratefully acknowledged.

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