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Protein Sequencer PPSQ-50A

Application News

Application of Glatiramer Acetate Analysis with Protein Sequencer PPSQ-50A

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User Benefits

- Protein sequencers can be used to reproducibly measure and evaluate the molar ratio of the constituent amino acid residues in the N-terminal portion of a polypeptide mixture.
- Useful for quality control of glatiramer acetate.

■ Introduction

Biopharmaceuticals, including antibody drugs, have emerged to replace small molecule drugs, but they are expensive to produce and their large molecular weight makes it difficult for them to be taken up by cells. Middle molecule drugs are attracting attentions to solve these problems. Peptide drugs produced by peptide synthesis are one of these middle molecule drugs. In this paper, we report an example of the use of a protein sequencer PPSQ-50A systems for the analysis of glatiramer acetate (GA), a complex mixture of polypeptides.

■ Glatiramer acetate

Glatiramer acetate (GA) is comprised of a mixture of synthetic copolymers of four amino acids (Glu, Ala, Tyr, and Lys) at a specific molar ratio with a molecular weight range of 5000 to 9000 Da. The total amino acid compositions have been confirmed by amino acid analysis. Amino acid sequence analysis of GA using a protein sequencer will detect all of the constituent PTH-amino acids in each cycle because GA is a randomly sequenced peptide. Although the protein sequencer cannot quantify each amino acid, the relative amino acid levels at the N-termini can be confirmed by calculating the ratio of the PTH-amino acid yield corresponding to each constituent amino acid to the total PTH-amino acid yield obtained.

■ Chromatogram of PTH-amino acids (phenylthiohydantoin derivatives of amino acids)

The analytical conditions of the PTH-amino acid standard by the isocratic system are shown in Table 1 and its chromatogram in Fig. 1. The analysis conditions using the gradient system are shown in Table 2, and the chromatogram is shown in Fig. 2. Amino acids constituting proteins other than cysteine can be easily separated and identified.

Table 1 Analysis conditions (Isocratic system)

Column	: Wakopak Wakosil PTH-II (S-PSQ) (250 mm x 4.6 mm I.D.)
Mobile phase	: PTH-amino Acids Mobile Phase
Flow Rate	: 1.0 mL/min
Time program	: T. Flow 1.0 mL/min(0 – 21.25 min)- 0.3 mL/min(21.5 - 45.25 min)- 1.0 mL/min(45.5 – 45.51 min)
Column temp.	: 40 ℃
Detection	: UV 269 nm (SPD-M30A) High Sensitivity Flow Cell

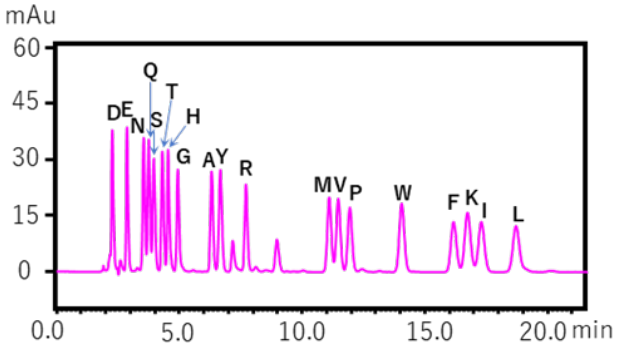

Fig. 1 Chromatogram of PTH-amino acid mixture standards (25 pmol) (Isocratic system)

Table 2 Analysis conditions (Gradient system)

Column	: Wakopak Wakosil PTH-GR (S-PSQ) (250 mm x 2.0 mm I.D.)
Mobile phase A	: PTH-amino Acids Mobile Phase A (for Gradient Elution)
Mobile phase B	: PTH-amino Acids Mobile Phase B (for Gradient Elution)
Flow Rate	: 0.3 mL/min
Time program	: B Conc. 0 %(0 min)- 0%(4 min)- 100%(17 - 30 min)- 0%(30.01 – 45 min)
Column temp.	: 35 ℃
Detection	: UV 269 nm (SPD-M30A) High Sensitivity Flow Cell

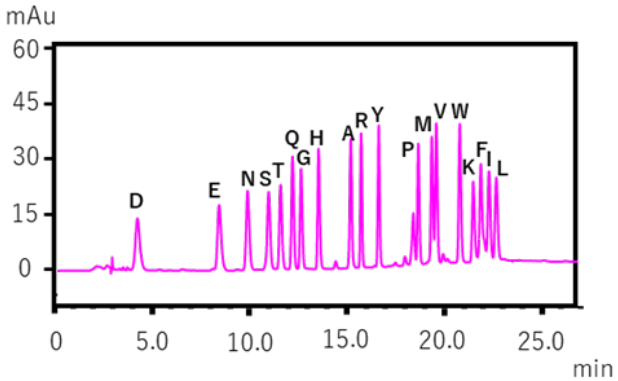

Fig. 2 Chromatogram of PTH-amino acid mixture standards (10 pmol) (Gradient system)

■ Sample preparation and Amino acid sequence analysis (Isocratic system

GA used as the sample was manufactured by Tront Research Chemicals Inc, (code : G406800). The prepared GA was dissolved in a 1:1 (vol/vol) mixture of acetonitrile/0.1% trifluoroacetic acid solution to prepare a sample solution with a concentration of 2 µg/µL.

Three µL of the prepared sample solution was subjected to amino acid sequence analysis on a Protein Sequencer PPSQ-50A Isocratic System. A polyvinylidene fluoride (PVDF) membrane and a polybrene-treated glass fiber disk were used as the sample carrier. The analysis results (chromatograms) are shown in Fig. 3.

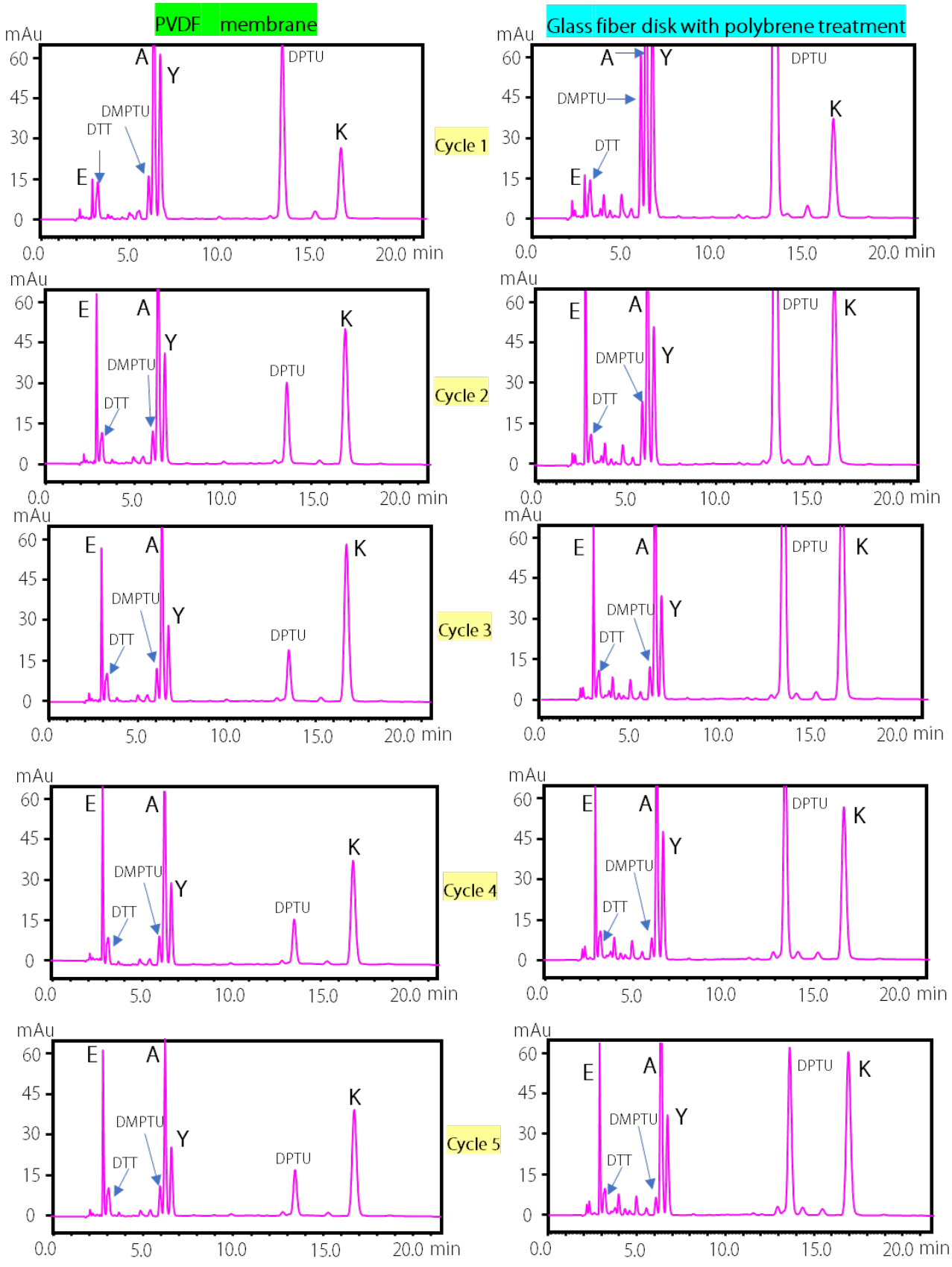

Fig. 3 Amino acid sequence analysis of glatiramer acetate (Isocratic system)

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Glu	1st run	0.03	0.13	0.13	0.16	0.16
	2nd run	0.03	0.13	0.13	0.16	0.16
	3rd run	0.03	0.13	0.13	0.17	0.16

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Ala	1st run	0.42	0.35	0.33	0.36	0.37
	2nd run	0.49	0.38	0.36	0.38	0.37
	3rd run	0.47	0.35	0.33	0.36	0.37

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Tyr	1st run	0.36	0.20	0.15	0.18	0.15
	2nd run	0.32	0.21	0.15	0.17	0.16
	3rd run	0.32	0.20	0.15	0.18	0.15

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Lys	1st run	0.19	0.31	0.40	0.30	0.31
	2nd run	0.16	0.28	0.36	0.29	0.31
	3rd run	0.17	0.32	0.39	0.30	0.32

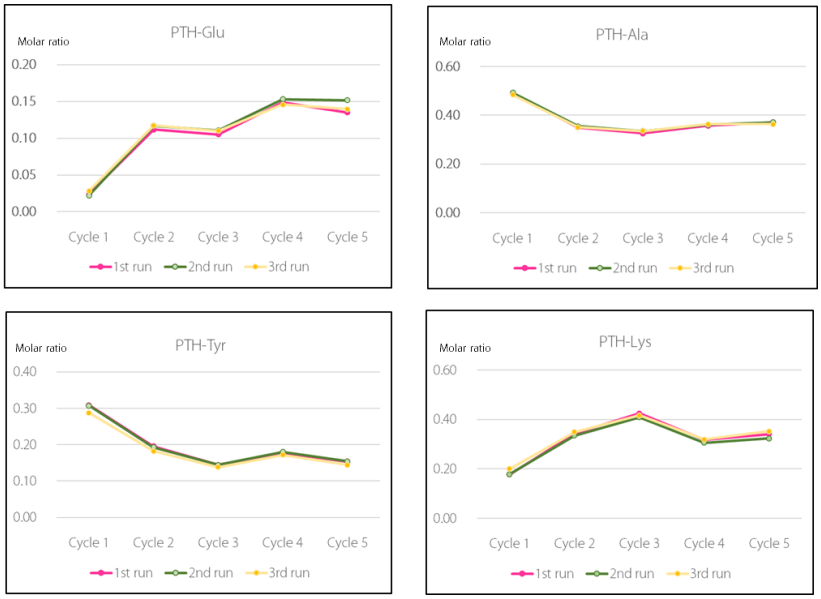

Fig. 4 Relative amino acid levels at the N-termini of GA for the first 5 cycles of N-terminal analysis by PPSQ-50A isocratic system using PVDF membrane
Left：Table of molar ratios, Right：Graph of molar ratios

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Glu	1st run	0.03	0.12	0.11	0.14	0.13
	2nd run	0.02	0.08	0.08	0.10	0.11
	3rd run	0.02	0.09	0.10	0.13	0.13

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Ala	1st run	0.46	0.34	0.33	0.37	0.38
	2nd run	0.54	0.38	0.36	0.42	0.40
	3rd run	0.49	0.37	0.34	0.38	0.38

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Tyr	1st run	0.32	0.19	0.15	0.19	0.16
	2nd run	0.21	0.16	0.13	0.17	0.15
	3rd run	0.28	0.17	0.14	0.19	0.16

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Lys	1st run	0.19	0.34	0.41	0.30	0.34
	2nd run	0.23	0.37	0.43	0.32	0.35
	3rd run	0.21	0.37	0.42	0.30	0.34

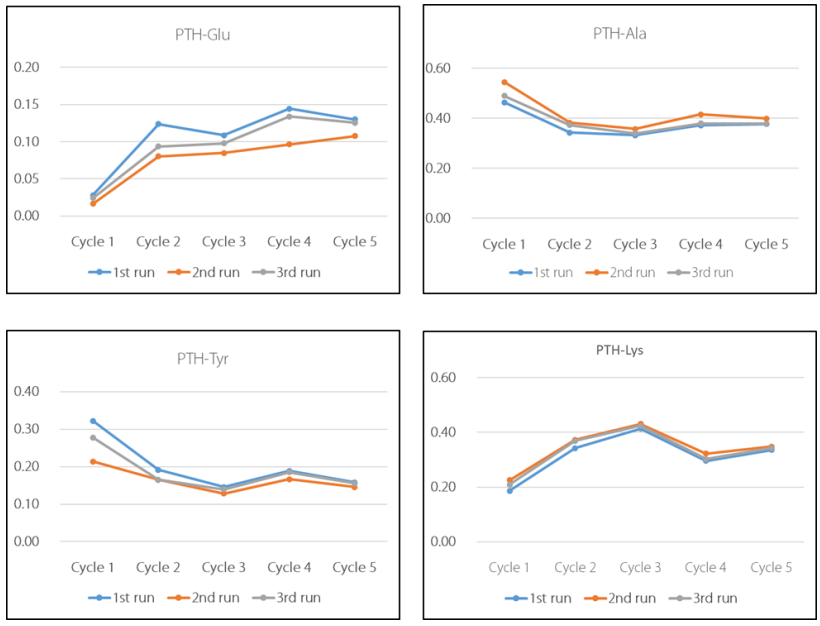

Fig. 5 Relative amino acid levels at the N-termini of GA for the first 5 cycles of N-terminal analysis by PPSQ-50A isocratic system using glass fiber disk
Left：Table of molar ratios, Right：Graph of molar ratios

The same analysis was repeated three times with the prepared sample solutions. Figure 4 shows the results of the analysis using the PVDF membrane, and Fig. 5 shows the results of the analysis using the glass fiber disk. The table on the left side of the figure shows the value (molar ratio) obtained by dividing the respective amino acid yield from the chromatogram by the sum of the four amino acid amounts (see formula below).

$$\text{Molar ratio} = \frac{\text{pmol of PTH-Glu, PTH-Ala, PTH-Tyr, or PTH-Lys}}{\text{pmol of (PTH-Glu + PTH-Ala + PTH-Tyr + PTH-Lys)}}$$

Three analyses of molar ratios using PVDF membranes yielded results with good reproducibility (Fig. 4). On the other hand, the use of polybrene-treated glass fiber disks yielded results with a large variation in the molar ratio of Glu (Fig. 5).

■ Amino acid sequence analysis (Gradient system

A 3 µL of the prepared sample solution was analyzed using a PVDF membrane and a polybrene-treated glass fiber disk, as in the isocratic system. The analysis results (chromatogram) are shown in Fig. 6.

Some of the reagents for Edman degradation used are different from those used in the isocratic system and the separation conditions are different, resulting in fewer by-products being detected and reducing the effect on the PTH-amino acid peak shape.

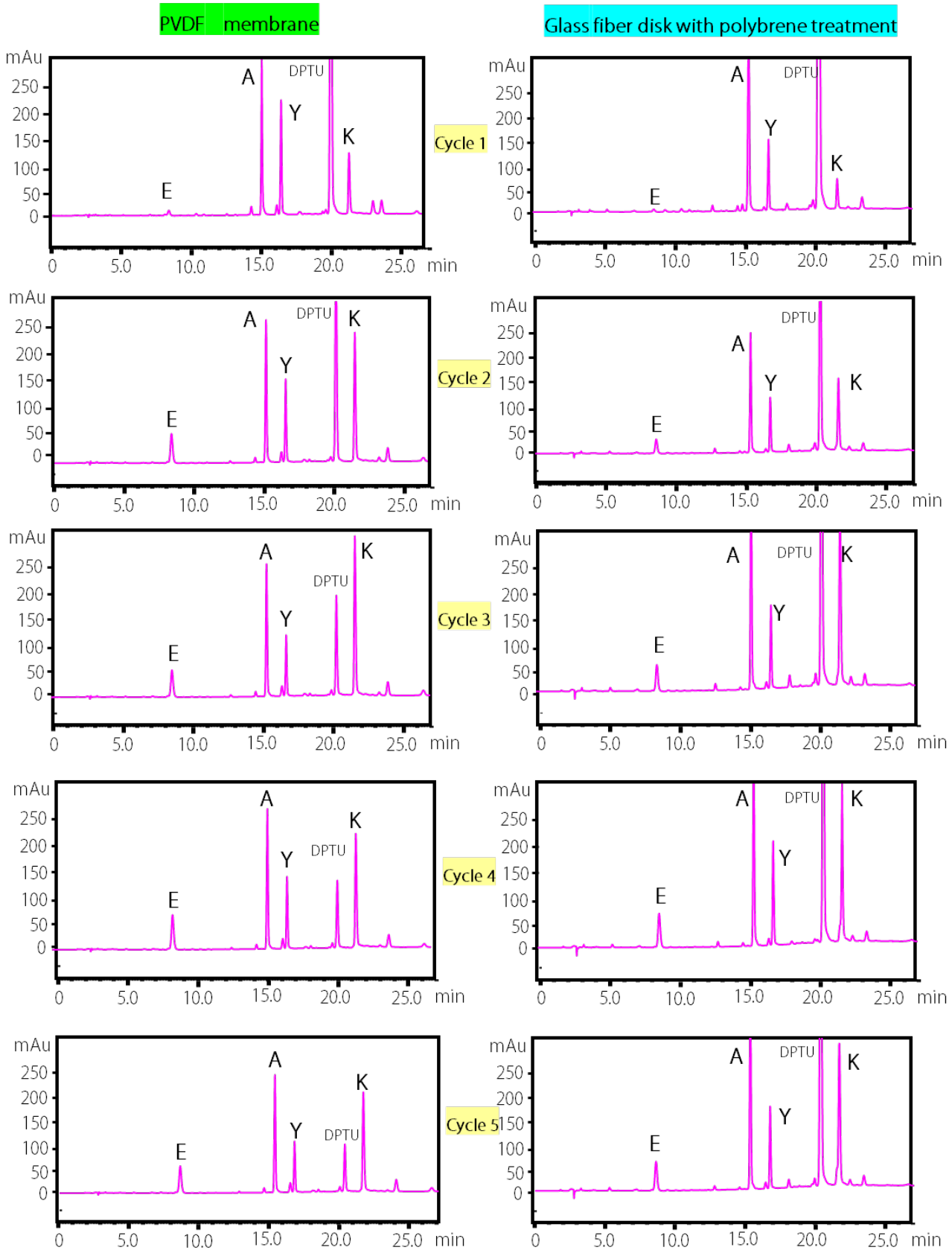

Fig. 6 Amino acid sequence analysis of glatiramer acetate (Gradient system)

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Glu	1st run	0.02	0.11	0.10	0.15	0.14
	2nd run	0.02	0.12	0.11	0.15	0.15
	3rd run	0.03	0.12	0.11	0.15	0.14

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Ala	1st run	0.49	0.35	0.33	0.36	0.37
	2nd run	0.49	0.36	0.34	0.36	0.37
	3rd run	0.48	0.35	0.34	0.36	0.36

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Tyr	1st run	0.31	0.20	0.14	0.18	0.15
	2nd run	0.31	0.19	0.14	0.18	0.15
	3rd run	0.29	0.18	0.14	0.17	0.14

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Lys	1st run	0.18	0.34	0.43	0.32	0.34
	2nd run	0.18	0.33	0.41	0.31	0.32
	3rd run	0.20	0.35	0.42	0.32	0.35

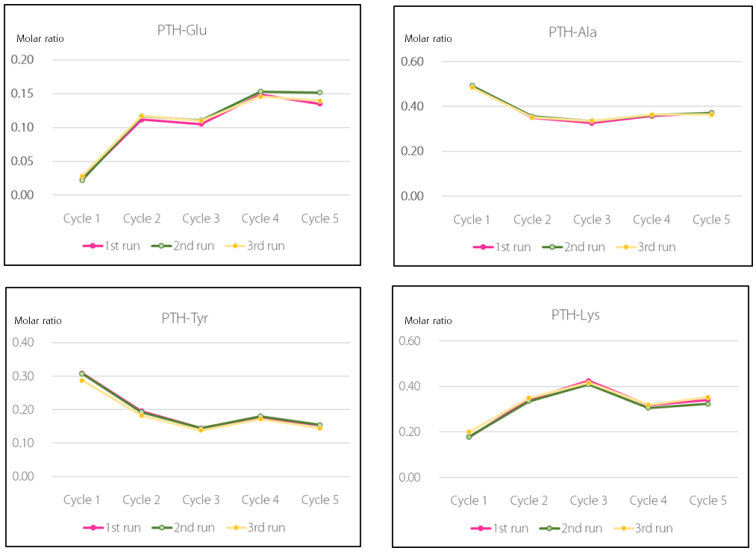

Fig. 7 Relative amino acid levels at the N-termini of GA for the first 5 cycles of N-terminal analysis by PPSQ-50A gradient system using PVDF membrane
Left：Table of molar ratios, Right：Graph of molar ratios

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Glu	1st run	0.02	0.09	0.08	0.10	0.10
	2nd run	0.02	0.11	0.08	0.09	0.10
	3rd run	0.02	0.10	0.09	0.12	0.10

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Ala	1st run	0.63	0.46	0.41	0.41	0.43
	2nd run	0.63	0.48	0.42	0.44	0.41
	3rd run	0.60	0.42	0.40	0.40	0.40

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Tyr	1st run	0.25	0.20	0.14	0.18	0.16
	2nd run	0.23	0.22	0.13	0.17	0.16
	3rd run	0.27	0.18	0.14	0.18	0.15

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
PTH-Lys	1st run	0.10	0.26	0.36	0.30	0.31
	2nd run	0.12	0.20	0.37	0.29	0.33
	3rd run	0.11	0.29	0.37	0.29	0.34

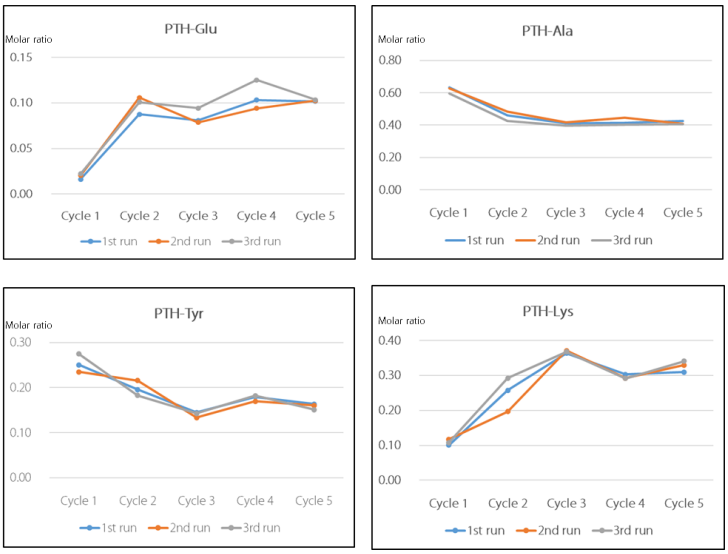

Fig. 8 Relative amino acid levels at the N-termini of GA for the first 5 cycles of N-terminal analysis by PPSQ-50A gradient system using glass fiber disk
Left：Table of molar ratios, Right：Graph of molar ratios

The analysis was repeated three times and the results obtained are shown in Fig. 7 and 8. As with the analysis with the isocratic system, the PVDF membrane produced results with less variation than the glass fiber disks. In particular, both systems produced results with a greater variation in the molar ratio of Glu when glass fiber disks were used.

■ Conclusion

This report describes the analysis by protein sequencing of glatiramer acetate (GA), a complex mixture of polypeptides with a random sequence consisting of four amino acids, Glu, Ala, Tyr, and Lys. The yield of PTH-amino acids obtained was calculated as a ratio, and the relative molar ratio of each amino acid from the N-terminal portion for the first 5 cycles was determined.
Amino acid analysis can determine the total amino acid compositions, but the N-terminal characteristics are unknown. In this sense, the use of protein sequencers can be useful for quality control of glatiramer acetate.

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