

Studies on PTH-Amino Acids by High Voltage Filter Paper Electrophoresis*

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PTH-amino acids were synthesized by EDMAN's method. PTH-amino acids were studied by high voltage filter paper electrophoresis under various conditions. The results of the electrophoresis were investigated by the ultra-violet ray. By the electrophoresis at 2000 V with pH 6 buffer, acid PTH-amino acids and basic PTH-amino acids were distinctly detected, and PTH-asparatic acid and PTH-asparagine, PTH-glutamic acid and PTH-glutamine were respectively confirmed. The neutral PTH-amino acids were detected by the electrophoresis with pH 4 or pH 2 or pH 1 buffer. The results of these experiments are discussed.

In order to determine the primary structure of protein, PTC method is very important. In many cases, however, the subtract method is used, and so it is impossible to perform this method when the material is little. Moreover, amino acid analyzer is necessary for this method. As it is very troublesome, the present authors studied on the method of detecting PTH-amino acids which were separated by EDMAN's method, by means of the high voltage filter paper electrophoresis.

METHODS

First, PTH-amino acids were synthesized by EDMAN's method as shown in Table 1.

By using these PTH-amino acids, the high voltage filter paper electrophoresis was performed under various conditions. The apparatus of the electrophoresis is illustrated in Fig. 1.

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Table 1

PTH-Amino Acid	m. p.
PTH-Glycine	248 ~ 250°C
// Alanine	185 ~ 186°C
// Histidine	200 ~ 205°C
// Aparatic acid	232°C
// Methionine	132°C
// Proline	181°C
// Lysine	158°C
// Threonine	203 ~ 205°C
// Asparagine	233 ~ 234°C
// Arginine	190 ~ 193°C
// Leucine	181°C
// Isoleucine	179°C
// Glutamic acid	170°C
// Glutamine	205°C
// Valine	206 ~ 208°C
// Phenylalanine	188°C
// Tyrosine	215°C
// Tryptophan	177°C
// Serine	176°C
// Cysteic acid	193 ~ 195°C

The buffer solutions were prepared as follows ;

Buffer ;

pH 6 0.25M triethylamine was adjusted to pH 6 by dropping acetic acid and was diluted with water ten times.

pH 4 0.25M triethylamine was adjusted to pH 4 by dropping acetic acid and was diluted with water ten times.

pH 2 Formic acid : Acetic acid : water = 5 : 15 : 80

pH 1 Formic acid : Acetic acid : water = 1 : 3 : 4.5

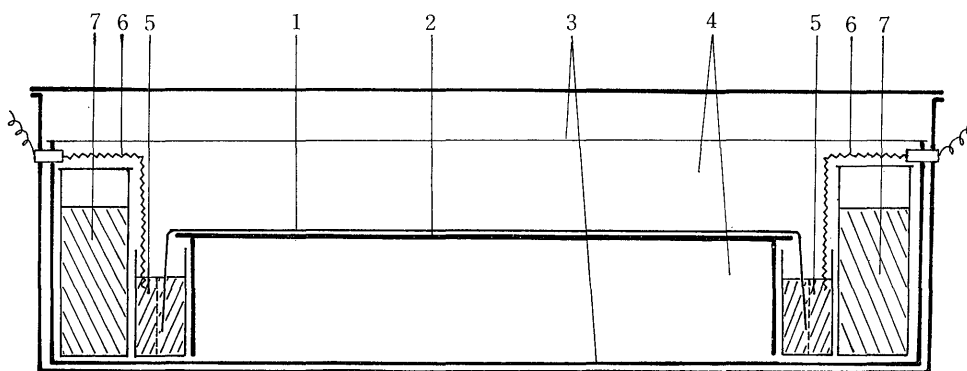


Fig. 1 High-Voltage Electrophoresis Apparatus

- | | | |
|-------------------------|-------------------|--------------|
| 1 Filter paper | 2 Support frame | 3 Tank |
| 4 Solvent | 5 Buffer solution | 6 Electrodes |
| 7 Ice water for cooling | | |

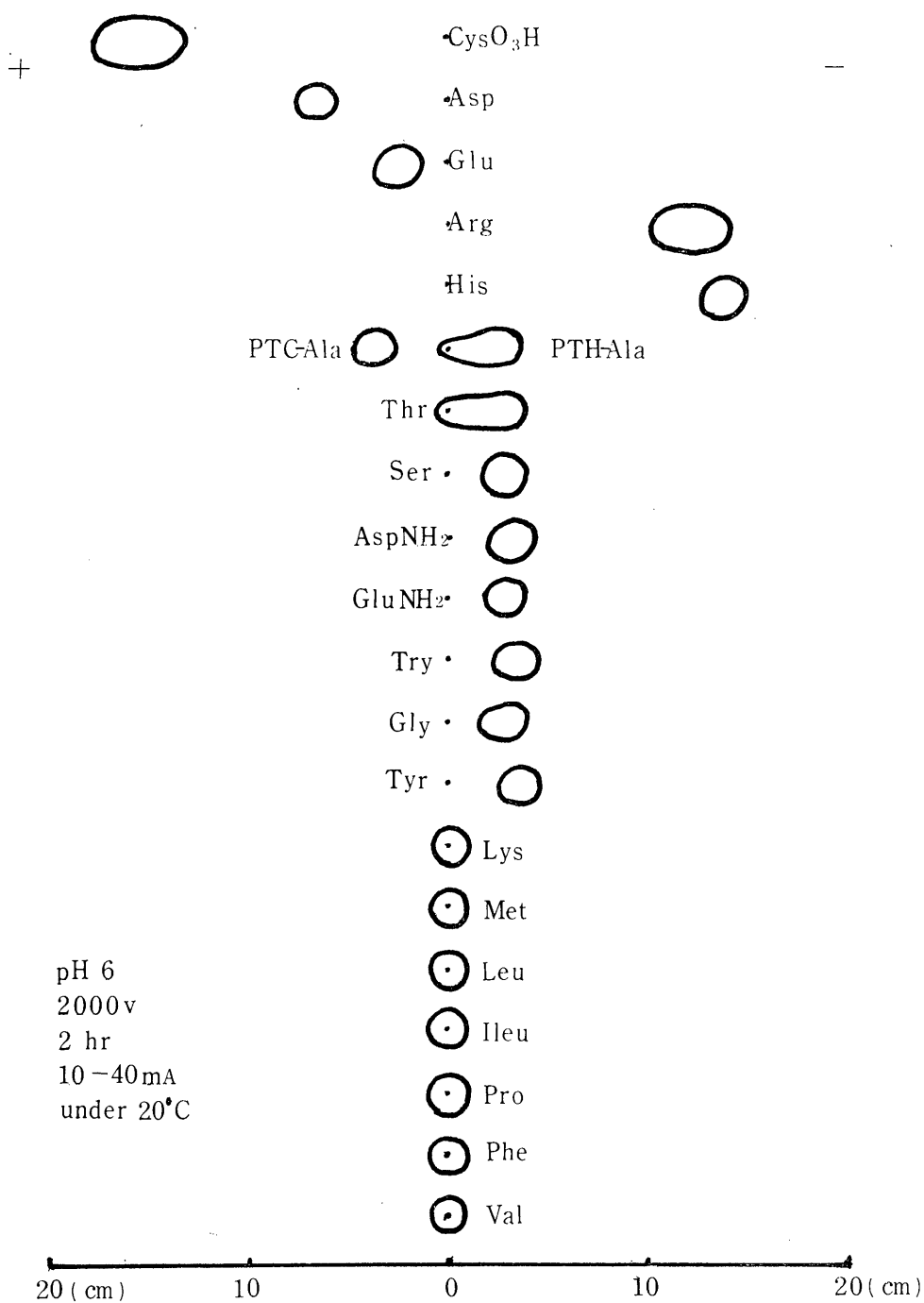


Fig. 2 Migration Distances of FTH-Amino Acids at pH 6

Solvent ; Isopar L

The solvent was isopar L cooled with ice in order to keep it under 20°C during the experiment. The electrophoresis was performed at 2000V or 3000V for 2 or 3 hrs. The filter paper was dried after the electrophoresis, and PTH-amino acids were detected by the ultraviolet ray. PTH-amino acids were recognized as the dark purple spots by the ultraviolet ray, but PTH-tryptophan and PTH-tyrosine were recognized as the pale yellow fluorescent spots by the ultraviolet ray.

RESULTS

1. In the case of the electrophoresis with pH 6 buffer, PTH-amino acids were put on the center of the filter paper. The result of the electrophoresis at 2000V for 2 hrs is shown in Fig.2. Acid PTH-amino acids moved to the anode and basic PTH-amino acids moved considerably to the cathode, but the neutral PTH-amino acids moved a little or did not move. The effect of the voltage on the electrophoresis is shown in Fig. 3. In the case of the electrophoresis at 3000 V, the migration distance of PTH-amino acid increased considerably. But the difference of the migration distance between PTH-amino acids was not so large.

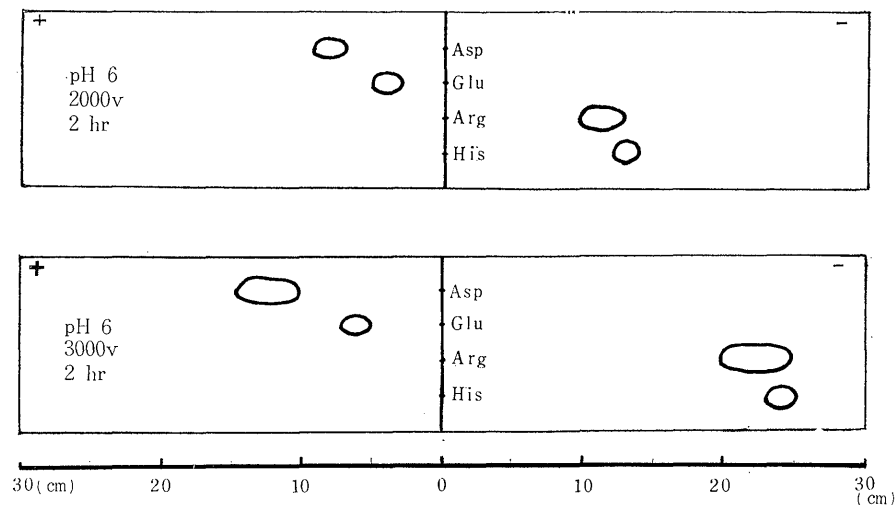


Fig. 3 The Comparison of the Electrophoretic Mobilities at Two Kinds of Voltage.

2. In the case of the electrophoresis with pH 4 buffer, PTH-amino acids were put on 10 cm from the anode of the filter paper. The electrophoresis was performed at 2000V for 2hrs. As shown in Fig. 4, PTH-asparagine, PTH-glutamine, PTH-glycine moved fairly to the cathode. PTH-tryptophan and PTH-tyrosine were recognized as the pale yellow fluorescent spots.

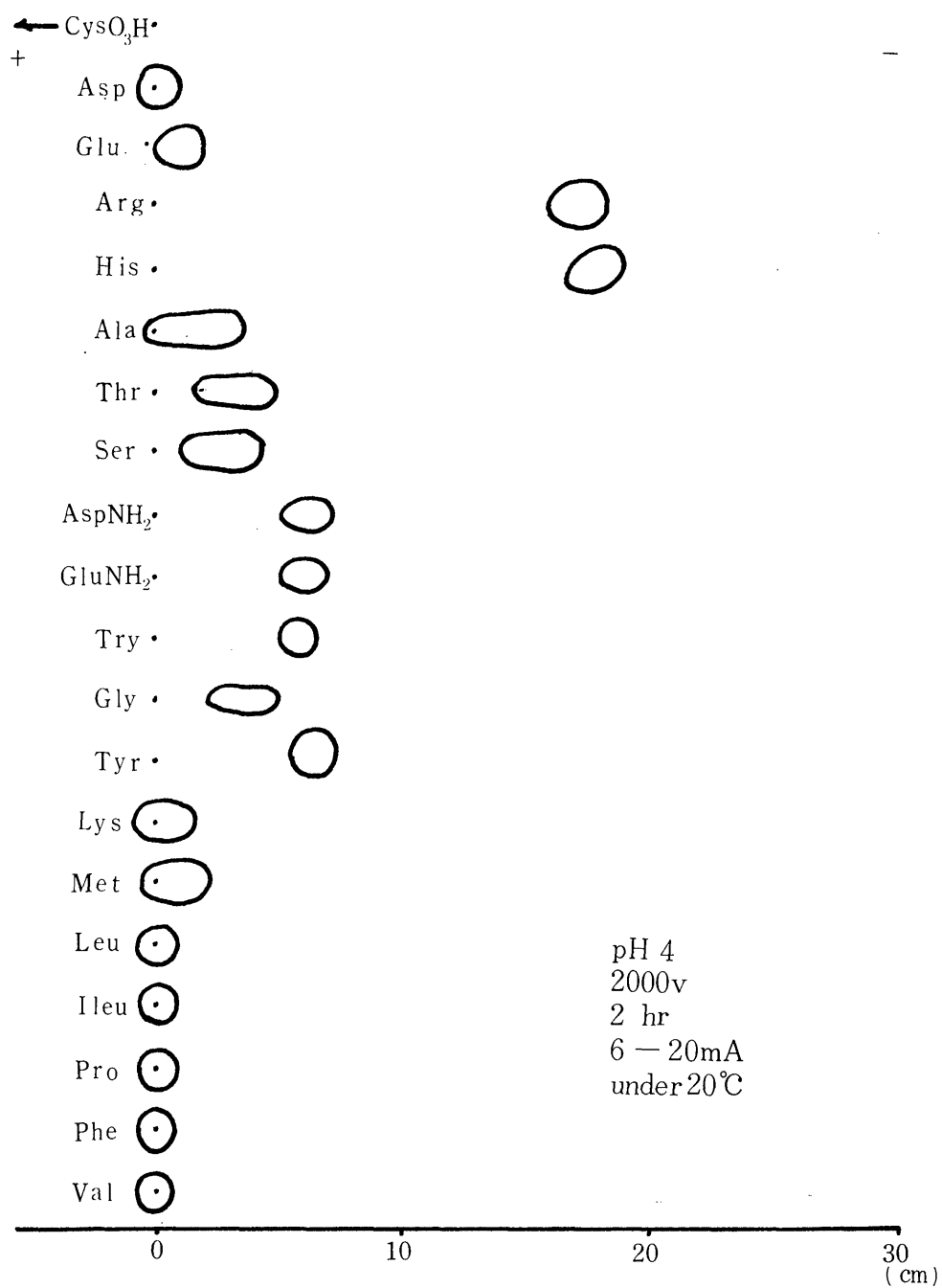


Fig. 4 Migration Distances of PTH-Amino Acids at pH 4.

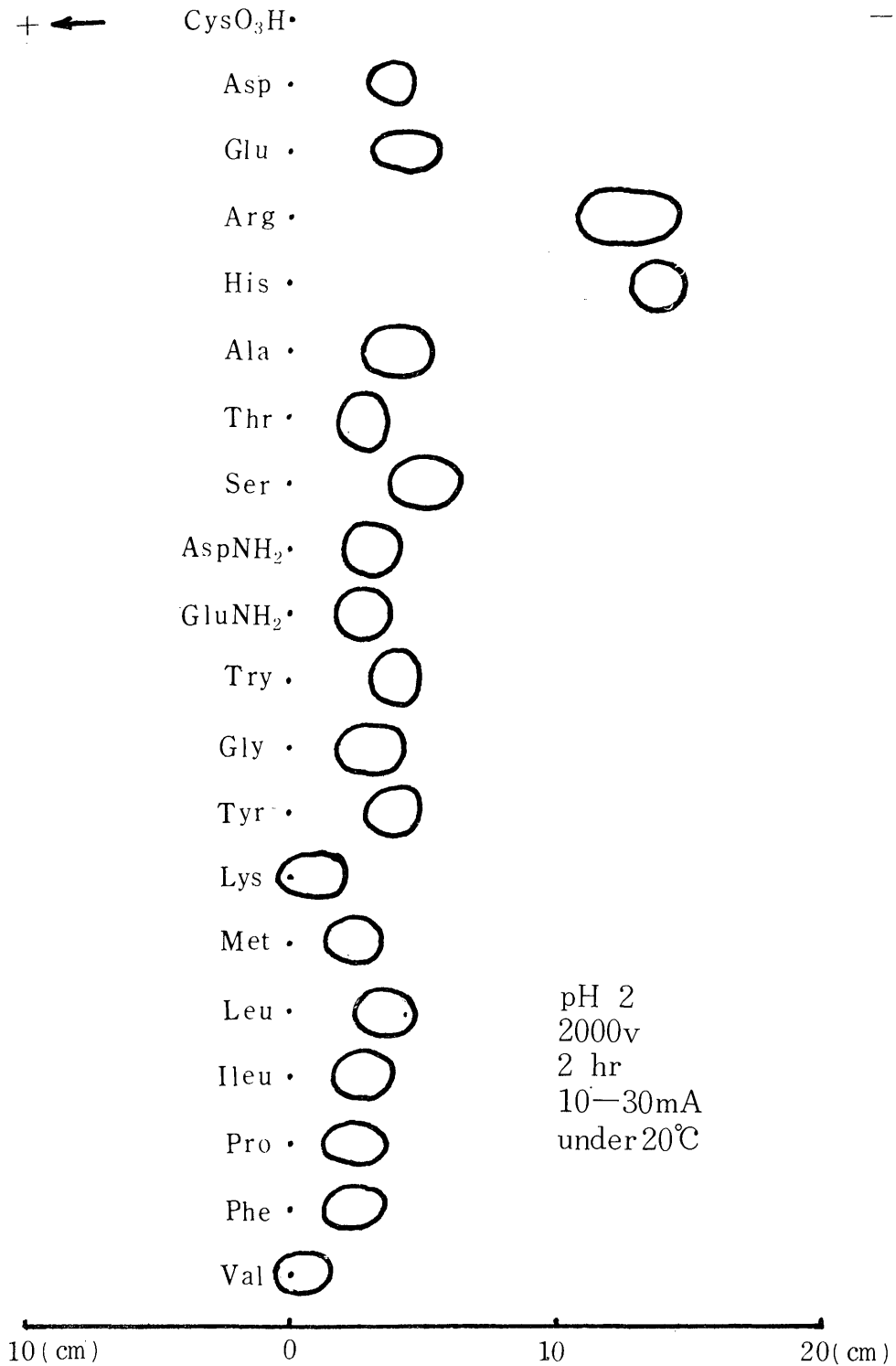


Fig. 5 Migration Distances of PTH-Amino Acids at pH 2

3. The electrophoresis with pH 2 buffer was performed under the same conditons as the experiment with pH 4 buffer. The result is shown in Fig. 5. PTH-alanine, PTH-serine, PTH-leucine, PTH-isoleucine, and PTH-methionine which did not move with pH 4 buffer moved fairly to the cathode. If the mobilities of PTH-amino acids are near and are not identified each other, it is necessary to perform electrophoresis longer. The effect of time on the electrophoresis is shown in Fig. 6.

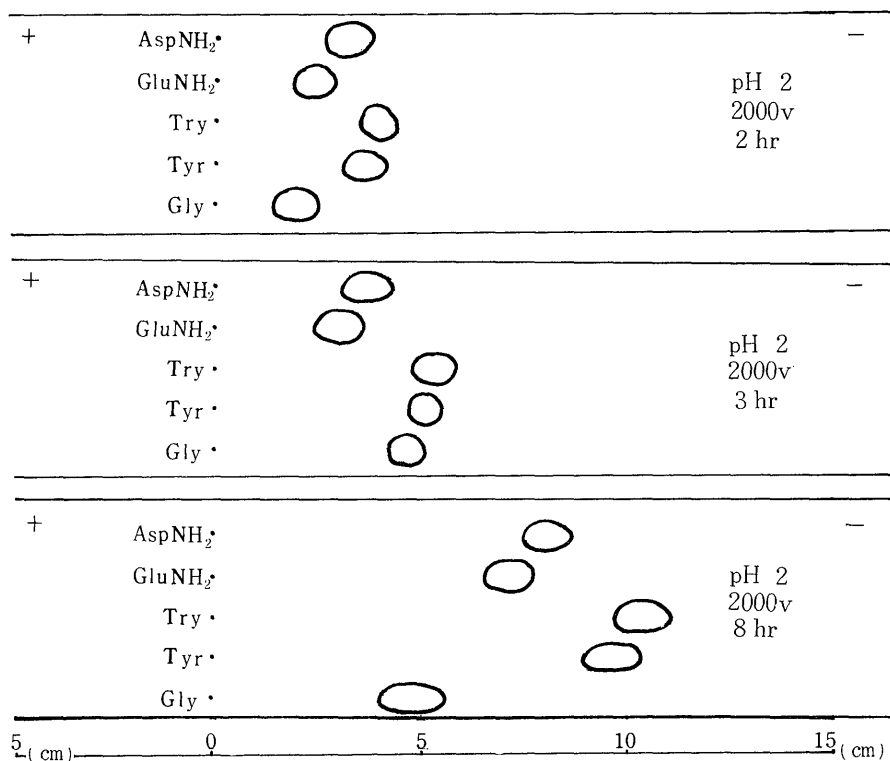


Fig. 6 The Comparison of the Electrophoretic Mobilities at Various Electrophoretic Times

4. The electrophoresis with pH 1 buffer was performed under the same conditions as the experiment with pH 4 buffer. The result is shown in Fig. 7. PTH-phenylalanine, PTH-proline, PTH-valine, and PTH-lysine which didn't move with pH 2 buffer moved fairly to the cathode. when the mobilities of PTH-amino acids are not distinguished, it is necessary to perform electrophoresis longer.

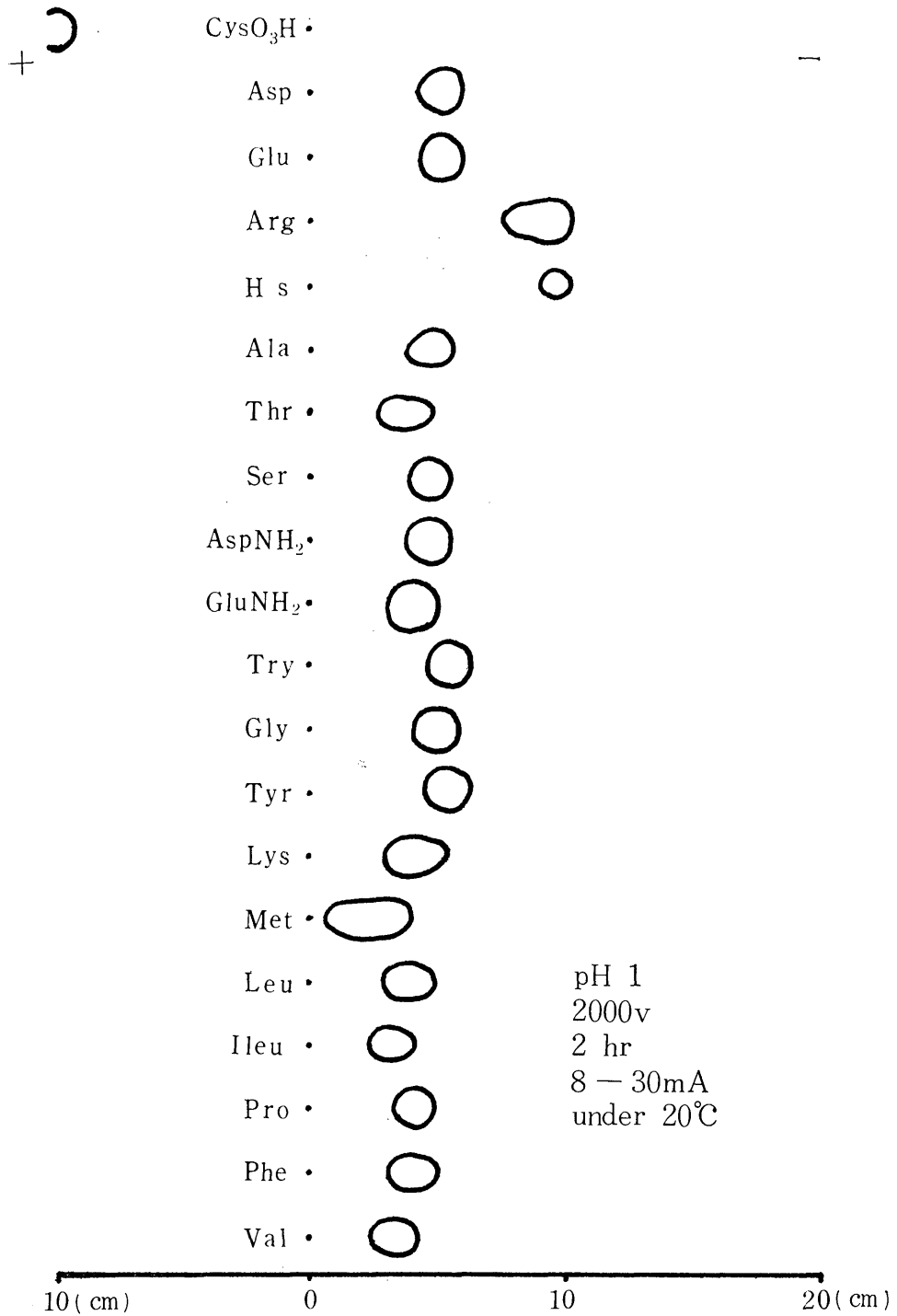


Fig. 7 Migration Distances of PTH-Amino Acids at pH 1

DISCUSSION

According to the results of this experiment, the amino acids which were identified at each pH are as follows ;

pH 6 CysO₃H, Asp, Glu, Arg, His.

pH 4 AspNH₂, GluNH₂, Try, Tyr, Gly, Thr.

pH 2 Ala, Ser, Gly, Thr, Leu, Ileu, Met.

pH 1 Leu, Pro, Phe, Val, Lys.

By the electrophoresis with pH 6 buffer, acid PTH-amino acids and basic PTH-amino acids except PTH-lysine are detected. PTH-lysine is a basic amino acid but it did not move like the neutral PTH-amino acid. It is considered that ϵ -amino radical of lysine combined with PTC. In the case of PTH-alanine, two spots were recognized. It seems to be the mixture of PTH-alanine and PTC-alanine as shown in Fig. 8. The maximum absorption spectrums of PTH-amino acid and PTC-amino acid were 269 m μ and 240 m μ respectively. By this experiment, PTH-glutamic acid and PTH-glutamine, PTH-asparatic acid and asparagine were respectively confirmed. The neutral PTH-amino acids which were not recognized by the electrophoresis in pH 6 or pH 4 buffer were detected by the electrophoresis with pH 2 or pH 1 buffer.

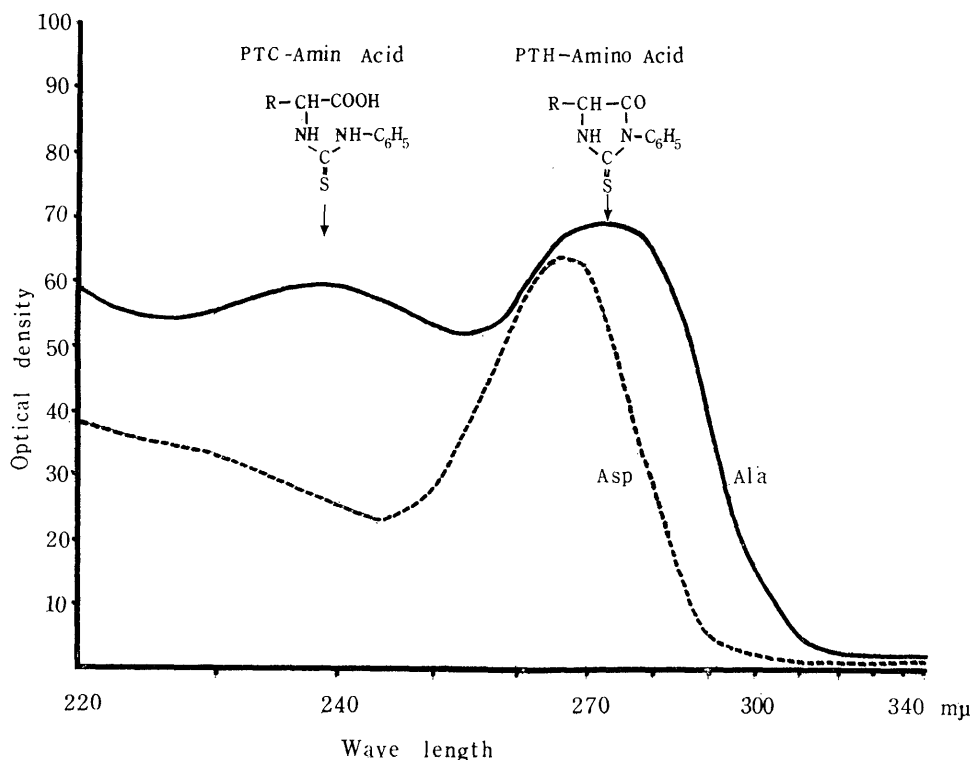


Fig. 8 Adsorption Spectrum of PTC- and PTH- Amino Acids

Namely, PTH-alanine, PTH-serine, PTH-glycine, PTH-threonine, PTH-leucine, PTH-isoleucine, and PTH-methionine moved fairly to the cathode with pH 2 buffer. PTH-leucine, PTH-proline, PTH-phenylalanine, PTH-valine, and PTH-lysine moved fairly to the cathode with pH 1 buffer. As the mobility of the electrophoresis varies more or less according to the various conditions, it is necessary to carry out the electrophoresis by using a standard PTH-amino acid. However, this method is considered to be very useful for the determination of the sequences of the comparatively small peptides whose amino acids are known.

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