Amino Acid Sequence of the Insoluble Tryptic Peptide, αT12,13 from the α-Polypeptide Chain in Macaca mulatta Monkey Hemoglobin

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Amino acid sequence analysis was carried out on the so-called 'insoluble tryptic peptide (αT12,13)' which was precipitated when the tryptic hydrolysate of the α-polypeptide chain in Macaca mulatta monkey hemoglobin was adjusted to pH 6.4. This peptide was oxidized with performic acid and then was hydrolyzed with chymotrypsin. The chymotryptic hydrolysate was isolated by column chromatography, purified by paper chromatography, and amino acid compositions of the peptides in the hydrolysate were analyzed. Amino acid sequences of these chymotryptic peptides were determined by the DNP and PTC methods and the partial hydrolysis. In addition, the oxidized insoluble tryptic peptide was rehydrolyzed in 1N NH₄OH at 0°C with trypsin for 7 hours. Amino acid compositions and N-terminal amino acid residues of the peptides isolated and purified by column and paper chromatographies were examined. By considering these results together, the amino acid sequence of the insoluble tryptic peptide (αT12, 13) was determined. It was compared with the known sequence of human hemoglobin.

INTRODUCTION

A protein from a certain living organism has its own peculiar structure, and therefore it is a matter of course that not only its amino acid composition but its sequence is strictly definite. It is because biosynthesis of proteins is under the strict genetic control, and so a difference in a gene results in a difference in a protein molecule. It is natural that the structures and the properties of proteins are slightly different among the species whose origins were different, even if the proteins have the same name and similar functions. It is, what is called, species specificities of proteins, and investigations on this matter will surely give much valuable information to evolutional problems of living organisms.
Since hemoglobin has the structure similar to all animals, comparative biochemical studies on how the molecular structures of hemoglobins from various species have changed in the evolutionary process are of interest as much as the elucidation of the correlation between their structures and functions. In addition, hemoglobins are one of good materials because they widely distribute in the animal kingdom and because they are isolated and purified relatively more easily than other proteins. The whole primary structure of human hemoglobin was first determined and successively horse hemoglobin was studied. Besides, the primary structures of hemoglobins of bovine, sheep, llama, pig, rabbit, carp, gorilla, lemur fulvus, camel, and so forth are being investigated.

According to Matsuda et al., the α-polypeptide chain of horse hemoglobin is composed of 141 amino acid residues just like that of human hemoglobin, and between both there are 17 amino acid exchanges. Similarly, there are 12 exchanges between the primary structures of human cytochrome C and horse cytochrome C, both of which have already been determined to be composed of 104 amino acid residues. The above examples on hemoglobin and cytochrome C appear to show that the molecular evolution rate of these proteins are nearly the same as each other. However, further investigation will be required in order to confirm the relation between the evolution of living organisms and the molecular evolution of proteins which compose the organisms themselves. At this stage, however, it can be readily reduced that the proteins, for instance, the primary structures of hemoglobins, from the two species of organisms which are more closely related in the evolutionary process have greater similarity than those from the species which are remote. In fact, according to reports so far published, these assumptions are fully expected. A lot of investigations concerning the differences between human and macaca mulatta monkey hemoglobins have been performed immunologically, or by using as paper chromatography and agar gel electrophoresis. Most reports demonstrated the resemblance between them. In this laboratory, Shikaya pointed out the similarity by the fingerprinting and Matsuda and Maita, by the determination of the N-terminal amino acid residue. Concerning the primary structure Takei and Maita reported that the amino acid compositions of the tryptic peptides soluble at pH 6.4 have four differences in the three peptides from the α-polypeptide chain and six differences in the five peptides from the β-polypeptide chain. The present author determined the amino acid composition and sequence of the tryptic peptide insoluble at pH 6.4 in the α-polypeptide chain of macaca mulatta monkey hemoglobin.

MATERIALS AND METHODS

1) Preparation of the Insoluble Tryptic Peptide (αT12, 13)
Hemoglobin was prepared by Drabkin's method\textsuperscript{10} from blood obtained from the abdominal aorta of an adult \textit{macaca mulatta} monkey. By Teal's method\textsuperscript{28}, the heme was removed to get globin, which was separated into $\alpha$ and $\beta$-polypeptide chains by the countercurrent distribution method\textsuperscript{21} with the solvent system of sec.-butanol containing 0.08\% trichloroacetic acid, propionic acid, and water (8.7:1.5:11.0). The $\alpha$-polypeptide chain (3g) was hydrolyzed at pH 8.0 for 4 hours at 37\(^\circ\)C with 30mg of trypsin which was prepared by inactivating chymotrypsin. When this hydrolysate was adjusted to pH 6.4, the so-called insoluble tryptic peptide was precipitated. The precipitate was obtained by centrifugation (3,000 r.p.m, 20 min.).

2) Oxidation of the Insoluble Tryptic Peptide ($\alpha$T12, 13) with Performic Acid

The insoluble tryptic peptide ($\alpha$T12,13) was oxidized with performic acid according to Hirs' method.\textsuperscript{15} To 9ml of 99\% formic acid, 1ml of 30\% H\textsubscript{2} O\textsubscript{2} was mixed, immediately sealed perfectly, and allowed to stand for 2 hours at room temperature (25\(^\circ\)C). After that, the solution was kept at 0\(^\circ\)C for 30 minutes. On the other hand, 300mg of the insoluble tryptic peptide ($\alpha$T12, 13) was dissolved in 5ml of 99\% performic acid and was mixed with the above-described solution. The mixture was allowed to stand at 0\(^\circ\)C for 3 hours. After the reaction was stopped, the reaction mixture was added deionized water so that its volume became 100ml. It was dried under reduced pressure by a rotary evaporator in order to remove performic acid.

3) Digestion of the Oxidized Insoluble Tryptic Peptide ($\alpha$T12, 13) with Chymotrypsin

300mg of the insoluble tryptic peptide ($\alpha$T12, 13) oxidized with performic acid was dissolved in 15ml of deionized water and denatured in 95\(^\circ\)C hot water for 5 minutes. After cooling, the pH was brought to 10.0 with 0.1N NaOH and the solution was shaken for 30 minutes. To this was added 9mg of chymotrypsin, and the mixture was hydrolyzed at 37\(^\circ\)C for 4 hours. The pH of the hydrolysate was measured every hour and kept to be 9.0 by the addition of 0.1N NaOH. During the hydrolysis 37\(^\circ\)C was maintained by means of a circulating constant temperature bath. After the digestion, the hydrolysate was dried under reduced pressure below pH 6.0.

4) Redigestion of the Oxidized Insoluble Tryptic Peptide ($\alpha$T12, 13) with Trypsin

In 5ml of 1N NH\textsubscript{4}OH, 100mg of the performic acid-oxidized insoluble tryptic peptide ($\alpha$T12, 13) was dissolved and adjusted to pH 8.0
with 0.1N acetic acid. To this was added 3mg of trypsin prepared by the inactivation of chymotrypsin. Hydrolysis was carried out at 0°C for 7 hours. The pH of the hydrolysate was measured every hour and maintained at 8.0 by adding 0.1N NH₄OH. After the digestion, the hydrolysate was dried under reduced pressure below pH 6.0 in order to remove ammonia.

5) Digestion of the Chymotryptic Peptide with Pepsin

Long peptides in the chymotryptic digest were necessary to be further hydrolyzed with pepsin into shorter peptides. About 2μmoles of a peptide was dissolved in 2ml of 0.01N HCl and to this was added 2mg of pepsin which was dissolved in 0.05N HCl. After shaken well, the solution was adjusted to pH 2.0 with 0.1N HCl and then hydrolyzed at 37°C for 3 hours. The hydrolysate dissolved in deionized water was dried under reduced pressure.

6) Column Chromatography of Peptides on Dowex 1×2

The resin (Dowex 1×2), after washed with 1N NH₄OH, water, glacial acetic acid, and water successively to be acetic acid type and evacuated, was placed in a column (2×150cm) which was warmed at 37°C. The column was thoroughly equilibrated with the starting buffer, pH 8.5, the mixture of 1% picoline, 1% pyridine, and 1% 2.4 lutidine. The chymotryptic hydrolysate of the insoluble tryptic peptide (αT12, 13) dissolved in 10ml of deionized water and adjusted to pH 9.0 was put on the column. The elution was carried out at a flow rate of 200 ml per hour until the fraction No. 35 with the above-described starting buffer, and after that the gradient elution was employed; Gradient I was performed from the fraction No. 36 by supplying 0.05N acetic acid solution in the upper chamber to 1 L of the starting buffer in the mixing chamber; similarly, Gradient II was done from the fraction No. 151 by supplying 1N acetic acid solution. The eluate was collected into 15 ml-fractions by a fraction collector. A 0.3 ml portion of each fraction, after alkali hydrolysis, was subjected to ninhydrin color reaction according to Yemm and Cocking's method. The ninhydrin-positive fractions were fractionally combined and dried under reduced pressure below 37°C.

The tryptic hydrolysate in 1N NH₄OH was also isolated by column chromatography. The resin (Dowex 1×2), the acetic acid type, was placed in a column (0.9×60cm) after evacuation. First 20 fractions were eluted with starting buffer pH 8.5, acetate buffer, the mixture of 1% picoline and 4% pyridine. From the fraction No. 21 Gradient I was employed with the acetate buffer pH 5.0 of 4% pyridine; from the fraction No. 45, Gradient II, with 1N acetic acid. The flow rate was 150ml per hour. The eluate was colored by ninhydrin reagent, and
the positive fractions were collected and dried under reduced pressure.

7) Identification of Peptides by Paper Chromatography
Each dried peptide was dissolved in 3ml of deionized water and a small portion of this solution was applied on a sheet of Toyo filter paper, No.50. Development was carried out in the constant temperature room at 24–25°C by using the descending method with the upper phase of the solvent composed of n-butanol, acetic acid, and water (4:1:5). Peptides on the paper were located by spraying with 0.2% n-butanol-ninhydrin and heating to color with an iron. PAULY'S reaction\textsuperscript{26} and chloroplatinic acid reaction\textsuperscript{29} were also employed as specific reactions.

8) Purification of Peptides by Paper Chromatography
Purification of peptides was performed by paper chromatography in the same way as above. Peptides on the paper was colored by spraying with 0.02% n-butanol-ninhydrin solution. The spots were cut out, washed out ninhydrin with acetone, eluted with 5% acetic acid for 24 hours, and finally dried under reduced pressure.

9) Analyses of Amino Acid compositions of Peptides
Peptides isolated and purified were dissolved in 5ml of constant boiling point HCl and then hydrolyzed at 110°C for 24 hours. The hydrolysates, after dried under reduced pressure to remove HCl, were subjected to an automatic amino acid analyzer (HITACHI KLA 2) for their amino acid compositions. Loss of amino acids during hydrolysis was not corrected.

10) High Voltage Paper Electrophoresis
In order to discriminate asparagine from aspartic acid and glutamine from glutamic acid, high voltage paper electrophoresis was carried out. The apparatus used was Ishidai type and filter paper was Toyo Filter paper No. 51 (60×10cm). The mixture of pyridine, acetic acid, and water (400:1:5), pH 6.4 was, used as a buffer. The paper applied a peptide near the center was dried, sprayed with the buffer, and put in an electrophoretic tank. Electrophoresis was performed at 2 KV for 2 hours. The peptide was detected in the same way as in the paper chromatography.

11) The DNP Method for the N-Terminal Amino Acid Analyses of Peptides
To 0.2–0.5 $\mu$mole of individual peptide dissolved in 3ml of 1% NaHCO\textsubscript{3} was added 0.05ml of dinitrofluorobenzene (DNFB). The mixture
was allowed to react at 40°C for 2 hours. After that, the excess of DNFB was extracted with ether. The DNP-peptide in the aqueous phase was dried under reduced pressure and hydrolyzed with 4ml of constant boiling point HCl in a sealed tube at 105°C for 20 hours. The N-terminal amino acid was extracted with three 10ml portions of ether. The ether extract was transferred in a heart-shaped flask, the modification of MILLS’ apparatus, and dried under reduced pressure. The flask was connected to the cold finger and evacuated by a vacuum pump in order to remove as much dinitrophenol as possible. The DNP-amino acids were identified by ascending paper chromatography; The developer used for the first dimension was the upper phase of the mixture of 1N NH₄OH and n-butanol (1:1) and for the second dimension, 0.5M phosphate buffer was used.

12) The PTC Method for Amino Acid Sequence Analyses of Peptides

EDMAN’S original method (12), (13) was modified here for phenylisothiocyanation of peptides. Trifluoroacetic acid was used for the ring-formation. Analyses were performed by the elimination method (11), that is, by comparing amino acid compositions of the original peptides with those of the peptides after each step of the PTC method. In 2.5ml of 66% pyridine contained in a heart-shaped flask, 0.5—2.0 µmoles of a peptide was dissolved and to this was added 0.05ml of phenylisothiocyanate (PTC). The solution was adjusted to pH 7.5 by adding 0.1N NH₄OH and then allowed to react for 3 hours at 37°C. After that, it was dried under reduced pressure and evacuated for 30 minutes by a vacuum pump with a cold finger in order to remove the excess of PTC. To this was added 1ml of trifluoroacetic acid and it was allowed to stand at room temperature for 4 hours to produce the ring-formation. Trifluoroacetic acid was removed under reduced pressure. The residue, after dissolved in 3ml of deionized water, was extracted with three 3ml portions of benzene. By this extraction, the PTH-amino acid was taken in the benzene phase and the peptide which had one N-terminal residue less than the original peptide was in the aqueous phase. A portion of the aqueous phase after dried was hydrolyzed with constant boiling point HCl for 24 hours and the amino acid composition of the hydrolysate was analyzed. The remainder of the aqueous phase was subjected to the next step of the PTC method.

RESULTS AND DISCUSSION

1) The Amino Acid Sequences of Chymotryptic Peptides from the α-Insoluble Tryptic Peptide (αT12, 13)

The insoluble tryptic peptide after oxidation was hydrolyzed with chymotrypsin for 4 hours and the hydrolyzed peptides were isolated and
Fig. 1 Column chromatography of the chymotryptic peptides from the oxidized insoluble tryptic peptide, αT12,13 of the α-polypeptide chain in *macaca mulatta* monkey hemoglobin.

Fig. 2 Paper chromatography of the chymotryptic peptides from the oxidized insoluble tryptic peptide, αT12,13 of the α-polypeptide chain in *macaca mulatta* monkey hemoglobin.
purified by column chromatography (Fig. 1) and paper chromatography (Fig. 2). The amino acid sequences of these peptides were determined by the following procedure. Rf Leu values in the explanation indicate the relative values of the spots against leucine (=1.00) on the paper chromatogram.

2) Amino Acid Sequence of Chy I-a
The amino acid composition of this peptide was Lys 0.99, Thr 0.98, Ser 1.02 as shown in Table I. Its Rf Leu value was 0.08. Two stages of the PTC method were performed.
Stage 1 Thr 0.00 ; Ser 0.97 ; Lys 1.02
Stage 2 Thr 0.00 ; Ser 0.18 ; Lys 1.00
Therefore, the sequence was determined to be Thr-Ser-Lys.

3) Amino Acid Sequence of Chy V-b
The composition was His 0.94, Thr 0.96, Pro 0.93, Ala 1.14, and

Table I  Amino acid compositions of the chymotryptic peptides from the insoluble tryptic peptide, aT12,13 of the a-chain in macaca mulatta monkey hemoglobin

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<th>Chy Vc</th>
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<th>Chy VIIa</th>
<th>Chy VIIc</th>
<th>Chy VIIIb</th>
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Val 0.98 as shown in Table I, and the Rf value was 0.32. Four stages of the PTC method were performed.
Stage 1 His+Thr 0.00  Pro 0.93  Ala 1.05  Val 0.98
Stage 2 His+Thr 0.00  Pro 0.20  Ala 1.06  Val 0.94
Stage 3 His+Thr 0.00  Pro 0.12  Ala 0.22  Val 0.96
Stage 4  His 1.00  Thr 0.00  Pro 0.00  Ala 0.11  Val 0.25  
As the result, the sequence was Thr-Pro-Ala-Val-His.

4) Amino Acid Sequence of Chy V-c
   As shown in Table I this peptide consisted of eight amino acid residues such as Thr 0.93, Ser 1.94, Ala 0.99, Val 2.10, and Leu 2.08. The Rf Leu value was 1.17. The N-terminal residue of this peptide was known to be leucine by the DNP method. This peptide was further hydrolyzed with pepsin at 37°C for 3 hours and subjected to paper chromatography for isolation and purification. Two peptides were obtained.
   a) Chy V-c pep I
      The amino acid composition was Thr 0.98, Val 0.94, and Leu 1.06. The Rf Leu value was 1.14. Two stages of the PTC method were carried out.
      Stage 1  Thr 0.00  Val 0.98  Leu 1.02
      Stage 2  Thr 0.00  Val 0.20  Leu 1.00
      The sequence was, therefore, Thr-Val-Leu.
   b) Chy V-c pep II
      The other peptic peptide was composed of Leu 1.03, Ala 1.06, Ser 1.95, and Val 0.96. The Rf Leu value was 0.80. Four stages of the PTC method were performed.
      Stage 1  Leu 0.00  Ala 1.02  Ser 1.98  Val 1.00
      Stage 2  Leu 0.00  Ala 0.12  Ser 2.01  Val 0.98
      Stage 3  Leu 0.00  Ala 0.00  Ser 0.99  Val 1.00
      Stage 4  Leu 0.00  Ala 0.00  Ser 1.00  Val 0.21
      As the result, the sequence was determined to be Leu-Ala-Ser-Val-Ser. Since the N-terminal residue had been known to be leucine, the sequence of Chy V-c was determined as follows;
      Leu-Ala-Ser-Val-Ser-Thr-Val-Leu.

5) Amino Acid Sequence of Chy VI-d
   The amino acid composition of this peptide was Thr 0.95, Val 1.12, and 1.98. The Rf Leu value was 1.29. Three stages of the PTC method were carried out.
   Stage 1  Leu 0.99  Val 1.00  Thr 0.99
   Stage 2  Leu 1.00  Val 0.20  Thr 0.98
   Stage 3  Leu 1.00  Val 0.15  Thr 0.23
   The sequence was Leu-Val-Thr-Leu.

6) Amino Acid Sequence of Chy VII-a and Chy VIII-b
   The amino acid composition of Chy VII-a is as follows; Lys 0.98, His 0.96, Asp 0.99, Thr 0.92, Ser 0.95, Pro 1.06, Ala 2.09, Val 1.02, Leu 1.11, Phe 0.91. The Rf Leu value was 0.34. The N-terminal residue was known to be threonine by the DNP method. It appeared from the amino acid composition that Chy VII-a was the combination
of Chy V-b and Chy VIII-b, being the former at the N-terminal side. Now, the sequence of Chy VIII-b had to be determined. The composition of Chy VIII-b was Ser 0.98, Ala 1.00, Lys 1.02, Asp 1.07, Leu 1.08, and Phe 0.95. The Rf Leu value was 0.73. Five stages of the PTC method were carried out.

Stage 1  Ala 0.00  Ser 0.96  Leu 1.04  Asp 1.07  Lys + Phe 0.96
Stage 2  Ala 0.00  Ser 0.08  Leu 1.02  Asp 1.04  Lys + Phe 0.94
Stage 3  Ala 0.00  Ser 0.00  Leu 1.15  Asp 1.02  Lys + Phe 1.00
Stage 4  Ala 0.00  Ser 0.00  Leu 0.11  Asp 0.23  Lys 1.02  Phe 1.00
Stage 5  Ala 0.00  Ser 0.00  Leu 0.09  Asp 0.10  Lys 0.29  Phe 1.00

According to the above result, the sequence of Chy VIII-b was Ala-Ser-Leu-Asp-Lys-Phe. The result from the high voltage paper electrophoresis showed the residue presented as Asp in this sequence was aspartic acid.

The sequence of Chy VII-a was, therefore, Thr-Pro-Ala-Val-His-Ala-Ser-Leu-Asp-Lys-Phe.

7) Amino Acid Sequence of Chy IX-a

The amino acid composition of this peptide was as follows; His 0.90, Glu 1.02, Pro 0.93, Ala 3.01, Leu 1.05, Phe 0.93. The Rf Leu value was 0.60. Four stages of the PTC method were first carried out.

Stage 1  Ala 1.97  His + Leu 1.03  Pro 1.00  Glu 0.98  Phe 0.98
Stage 2  Ala 1.01  His 0.98  Leu 1.02  Pro 0.98  Glu 1.03  Phe 0.97
Stage 3  Ala 1.00  His 0.21  Leu 0.99  Pro 0.97  Glu 1.04  Phe 0.96
Stage 4  Ala 1.01  His 0.00  Leu 0.30  Pro 0.98  Glu 1.02  Phe 0.98

After the fourth stage, the remaining peptide was purified by paper chromatography. This purified peptide was composed of Pro 0.98, Ala 1.02, Glu 1.05, and Phe 0.95. The Rf Leu value was 0.83. Three stages of the PTC method were further performed.

Stage 1  Pro 0.00  Ala 1.04  Glu 1.02  Phe 0.94
Stage 2  Pro 0.00  Ala 0.12  Glu 1.05  Phe 0.95
Stage 3  Pro 0.00  Ala 0.08  Glu 0.20  Phe 1.00

The sequence of Chy IX-a was, therefore, Ala-Ala-His-Leu-Pro-Ala-Glu-Phe. The result from the high voltage paper electrophoresis showed that the residue presented as Glu in the sequence was glutamic acid.

8) Amino Acid Sequence of Chy X-c

The amino acid composition of this peptide was as follows; His 0.98, CysSO₃ 0.90, Thr 0.90, Ser 0.96, Val 1.08, and Leu 5.25. The Rf Leu value was 0.93. Six stages of the PTC method were carried out.

Stage 1  His (+)  Cys (+)  Thr 0.94  Ser 0.98  Val 1.00  Leu 4.04
Stage 2  His (+)  Cys (+)  Thr 0.96  Ser 0.96  Val 0.95  Leu 3.12
Stage 3  His 0.98  Cys (+)  Thr 0.98  Ser 0.12  Val 0.94  Leu 3.10
Stage 4  His 0.18  Cys (+)  Thr 0.98  Ser 0.09  Val 0.98  Leu 3.11
Fig. 3 Column chromatography of rehydrolyzed products with trypsin of the oxidized peptide, αT12,13 in *macaca mulatta* monkey hemoglobin

Fig. 4 Paper chromatography of rehydrolyzed products with trypsin of the oxidized peptide, αT12,13 in *macaca mulatta* monkey hemoglobin
Stage 5 His 0.11  Cys (−)  Thr 0.99  Ser 0.02  Val 0.98  Leu 3.04
Stage 6 His 0.12  Cys (−)  Thr 0.99  Ser 0.03  Val 0.95  Leu 2.06

The above-described result showed that the remaining peptide which was composed of the four amino acid was Chy VI-d. The sequence of Chy X-c was determined as follows;

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<th>Leu</th>
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<td>Chy VI-d</td>
<td>Chy X-c</td>
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</table>

As described above, the amino acid sequences of the chymotryptic peptides from the insoluble tryptic peptide (αT12, 13) were determined.

Next, in order to determine the peptide sequence of these chymotryptic peptides, 100mg of the insoluble tryptic peptide (αT12, 13) was rehydrolyzed with trypsin at 0°C for 7 hours in 1N NH₄ OH. The hydrolysate was isolated by the Dowex 1×2 column chromatography (Fig. 3) and purified by the paper chromatography (Fig. 4). Consequently, the amino acid compositions and the N-terminal residues of the two spots, TTI-a and TTVI-c were examined.

Analysis of TTI-a

The amino acid composition of this peptide was as follows; Lys 1.12, Thr 1.96, Ser 2.93, Ala 1.06, Val 1.92, Leu 2.01, Phe 0.92. The Rf Leu value was 0.71. The N-terminal residue was phenylalanine.

By considering these results together with those on the chymotryptic peptides from the insoluble tryptic peptide (αT12, 13), this TTI-a appeared to be the combination of Chy I-a and Chy V-c plus one residue of phenylalanine. Accordingly, since the N-terminal residue of TTI-a had been known to be phenylalanine and the C-terminal residue was considered to be lysine because it was the tryptic hydrolysate, the amino acid sequence of TTI-a was determined as follows;

D
Phe Leu Ala Ser Val Ser Thr Val Leu Thr Ser Lys
| Chy V-c | Chy I-a |

Analysis of TTVI-c

The amino acid composition of this peptide was as follows; Lys 1.02, His 2.93, Cys 0.90, Asp 1.02, Thr 1.94, Ser 1.92, Glu 1.10, Pro 2.01, Ala 5.11, Val 1.86, Leu 7.25, Phe 0.92.

The Rf Leu value was 0.71. The N-terminal amino acid residue was confirmed to be leucine by the DNP method. By considering the results on the chymotryptic peptides from the insoluble tryptic peptide (αT12,
13), this peptide agreed with the combination of Chy X-c, Chy IX-a, and Chy VII-a minus one residue of phenylalanine. Because this was a trypic hydrolysate, the C-terminal residue was considered to be lysine. Therefore, the sequence of TTVI-c was determined as follows;

![Amino acid sequence diagram]

The N-terminal residue of the insoluble tryptic peptide (αT12, 13) had been known to be leucine, TTVI-c was considered to be located at the N-terminal side and TTI-a at the C-terminal side, that is, TTVI-c accorded with αT12 and TTI-a, with αT13. Accordingly, the amino acid sequence of the insoluble trptic peptide (αT12,13) was determined as illustrated in Fig. 5.

**CONCLUSION**

The amino acid composition and the sequence of the insoluble try-
ptic peptide (αT12,13) in the α-polypeptide chain from *macaca mulatta* monkey hemoglobin were determined. The results were compared with the known composition and sequence of human hemoglobin. Both hemoglobins are quite similar as to this region.

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