

Comparative Biochemistry of Hemoglobins

X. Amino Acid Sequences in Soluble Tryptic Peptides, β T 9, β T 13, β T 14, and β T 15 from β -polypeptide Chain in *Macaca mulatta* Monkey Hemoglobin

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From the part soluble at pH 6.4 of tryptic hydrolyzate of β -chain in *Macaca mulatta* monkey hemoglobin, the so-called 'soluble tryptic peptides' were isolated and purified by column and paper chromatography. Four of these peptides, which were called β T9, β T13, β T14, and β T15, were hydrolyzed with pepsin and isolated by column and paper chromatography. Then, amino acid compositions of the four peptides were analyzed and sequences were determined by the DNP and PTC methods.

The results were compared respectively with the amino acid sequences of the corresponding peptides in human hemoglobin. Differences between the two hemoglobins were found in β T9 and β T13, namely, human hemoglobin has alanine at the tenth position from the N-terminus of β T9, whereas *macaca mulatta* monkey hemoglobin has asparagine. Similarly, the former has proline at the fifth position from the N-terminus of β T13, whereas the latter, glutamine. The sequence of the remaining forty amino acid residues were all the same with each other.

INTRODUCTION

It is said that amino acid sequences of proteins which are produced in living things are under the control of gene, presumably DNA. However, at the present stage that the structure of DNA has not been wholly elucidated, investigations on specificities in the primary structures of proteins will give some valuable information to evolutionary problems from the viewpoint of molecular level. In this respect, hemoglobin is one of good materials owing to its wide distribution and comparatively easy isolation and purification.

The primary structure of human adult hemoglobin has been already

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determined by BRAUNITZER et al.²⁾ and KONIGSBERG et al.¹¹⁾ It seems very interesting to inquire into differences in the primary structure between human adult and monkey adult hemoglobins from the evolutionary point of view.

It was reported that *macaca mulatta* monkey hemoglobin closely resembled human hemoglobin immunologically,¹⁹⁾ chromatographically,¹⁸⁾ electrophoretically,⁸⁾ and in alkali-denaturation.³⁾ However, ZUCKERKANDDEL et al.²⁵⁾ compared the tryptic peptides of these two hemoglobins by the fingerprint method and pointed out the slight differences between them.

In our laboratory, various kinds of investigations have been carried out in order to discover differences in the primary structure between human and *macaca mulatta* monkey hemoglobin.⁹⁾¹⁴⁾¹⁵⁾¹⁶⁾²⁰⁾²²⁾ According to the results obtained so far, these two adult hemoglobins differ in their amino acid compositions by having five differences in the three tryptic peptides soluble at pH 6.4 in α -chain, and six differences in β -chain. In order to demonstrate these differences clearer, amino acid sequence of each tryptic peptides must be determined.

The present paper reports the determination of amino acid sequences of β T9, β T13, β T14, and β T15 among tryptic peptides from β -chain in *macaca mulatta* monkey hemoglobin.

MATERIALS AND METHODS

1) Preparation of Tryptic Peptides

Isolation and purification of the tryptic peptides from β -chain in *macaca mulatta* monkey hemoglobin were performed by the methods reported previously by TAKEI²²⁾

According to DRABKIN'S method,⁴⁾ hemoglobin was obtained from blood of *macaca mulatta* monkeys. It was dehemed to obtain globin by TEALE'S method.²³⁾ The globin was separated into α - and β -chains by countercurrent distribution method¹⁷⁾ by using a system of sec.-butanol containing 0.08% trichloroacetic acid, propionic acid, and water (8.7:1.8:11.0). β -chain was denatured in 8M urea at 60°C for 45 minutes. After it was dialysed against water to remove urea, it was hydrolyzed with trypsin at 37°C at pH 8.0 for four hours. This hydrolyzate was adjusted to pH 6.4. Consequently, the so-called 'core' was precipitated, and then removed by centrifugation. From the remaining soluble part, the so-called 'soluble tryptic peptides' were isolated and purified by column and paper chromatography. The column chromatography was developed on Dowex 1 \times 2 with acetate buffer containing some organic bases such as pyridine, collidine, and lutidine. Descending paper chromatography was employed with the system of n-butanol, acetic acid, and water (4 : 1 : 5). A portion of each tryptic peptide

thus isolated was subjected to amino acid analysis to assess its quantity and purity.

2) *Digestion of the Peptides with Pepsin*

A sample of each tryptic peptide (15–20 μ mole) was dissolved in 15 ml of deionized water and adjusted to pH 2.0 with 1.0 N HCl. To this solution was added 4 mg of pepsin (Thrice-crystallized, Sigma) which was dissolved in 2 ml of 1/16 N HCl. Digestion was performed at 37 °C for the appropriate time and stopped by adding 1.0 N NaOH to the reaction mixture which was consequently brought to pH 9.0.

3) *Resolution of the Peptic Peptides by Column Chromatography*

The resin (Dowex 1 \times 2, 200–400 mesh) was washed with 1N NH_4OH , water, acetic acid, and water. It was suspended with the starting buffer, evacuated thoroughly, and filled up in a column (1.0cm \times 60cm). The column was fully equilibrated again with the starting buffer. The peptic digest of each tryptic peptide was put on the column. The acetate buffer containing some organic bases such as pyridine, collidine, and picoline was used as the starting buffer of the developer. Next, pH gradient was carried out with acetic acid. (see Fig 1, 3, 5) The development was performed at 37°C by keeping a flow rate of 100 ml/h. The effluent was collected into 8 ml fractions by a fraction-collector. A portion of each fraction (0.3 ml) was subjected to ninhydrin reaction after alkali-hydrolysis, according to YEMM and COCKING's method.²⁴⁾ The fractions positive to this reaction were combined together, evaporated to dryness under reduced pressure below 30 C. It was dissolved in deionized water.

4) *Identification and Purification of the Peptides by Paper Chromatography*

Descending paper chromatography was employed by using Toyo filter paper No. 50. The upper phase of the system of n-butanol, acetic acid, and water (4 : 1 : 5) was used as a developer. For the detection of the peptides on the paper chromatogram, 0.2 % ninhydrin-n-butanol solution was sprayed on the paper and heated with an iron to color. In addition, PAULI'S reaction²¹⁾ and α -nitrosonaphtol reaction¹⁾ were employed in order to locate the peptides containing histidine and tyrosine. In case of purification of the peptides with the above-mentioned system, elution of the peptide from the paper was done by using 5 % acetic acid.

5) *Paper Electrophoresis of the Peptides*

Electrophoresis was carried out at 2 KV for two hours on an electrophoretic apparatus (Ishidai-type) by using Toyo filter paper No. 51 (10 cm \times 60cm) and pyridine acetate buffer, pH 6.4 (pyridine : acetic acid : water = 100 : 4 : 900). The peptides were detected by ninhydrin reaction as in case of the paper chromatography described previously.

6) *DNP-Method for the N-Terminal Amino Acid Analysis of the Peptides*

Each peptide (0.2–0.5 μ mole) was dissolved in 3 ml of 1 % NaHCO_3 , and to this was added 0.05 ml of dinitrofluorobenzene (DNFB). After it was dinitrophenylated at 40°C for two hours, the excess of DNFB was extracted with ether. The DNP-peptide in the aqueous phase was concentrated to dryness under reduced pressure and redissolved in 4 ml of constant boiling point HCl and hydrolyzed in sealed tubes at 105°C for 24 hours. The N-terminal DNP-amino acid was extracted three times from the hydrolyzate with 10 ml of ether. This ether extract was transferred into a heart-shaped flask (MILLS' apparatus)¹⁰⁾ in order to evaporate to dryness under reduced pressure. Then the flask was connected to the coldfinger, and the content was evacuated by a vacuum pump in order to remove as much dinitrophenol as possible.

Identification of the DNP-amino acids was carried out by ascending paper chromatography on Toyo filter paper No. 51 (40cm \times 40cm). For the first dimension, the developer was the upper phase of an n-butanol-1 N NH_4OH (1:1) mixture; the second dimension, 0.5 M phosphate buffer. Yellow spots on the paper were cut out and eluted with 5 ml of warm water. The quantitative determination was performed by measurement of the optical density at 360 m μ of this eluate.

7) *PTC Method for Amino Acid Sequence Analysis of the Peptides*

EDMAN'S original method^{6,7)} was modified for phenylisothiocyanation of the peptides. The ring-formation was performed with trifluoroacetic acid.¹²⁾ Analysis was carried out by the elimination method⁵⁾ by comparing the amino acid compositions of the PTC-peptides with the original peptides.

A sample of each peptide (0.5–2.0 μ mole) was dissolved in 2.5 ml of 66 % pyridine in a heart-shaped flask and to this was added 0.05 ml of phenylisothiocyanate (PTC). The PTC reaction was performed at 37°C for three hours after adjusting the reaction mixture to pH 7.5. The reaction mixture was concentrated to dryness under reduced pressure. The excess PTC was removed by 30 minute aspiration with a vacuum pump by connecting the flask to the cold finger. To this was added 1 ml of trifluoroacetic acid in order to occur the ring-formation. This was allowed to stand at room temperature for four hours. After the reaction, trifluoroacetic acid was removed under reduced pressure. The dry residue was dissolved in 3 ml of deionized water and extracted with 3 ml of benzene three times. Consequently, the PTH-amino acids were extracted in the benzene phase.

The original peptide lost its N-terminal residue and remained in the aqueous phase. An aliquot of the aqueous phase was concentrated to dryness after HCl-hydrolysis and subjected to amino acid analysis. The remainder of the aqueous phase after the concentration to dryness was for the following stage of the PTC method.

8) *Amino Acid Analysis of the Peptides*

Each peptide was dissolved in 4 ml of constant boiling point HCl (twice distilled) and was hydrolyzed in sealed tubes at 105°C for 24 hours. The HCl was removed from the hydrolyzate by repeating dissolution in water and concentration to dryness. The amino acid analysis was carried out on Hitachi KLA-2 amino acid analyzer. The losses of the amino acids during the hydrolysis were not corrected and the compositions were indicated by molecular ratios of the amino acids.

RESULTS AND DISCUSSION

1) *Preparation of the tryptic peptides β T9, β T13, β T14, and β T15*

Table I shows the amino acid compositions and the yields of β T9, β T13, β T14, and β T15 which were obtained by paper and column chromatographies from the tryptic hydrolyzate of 1 g of β -chain in macaca mulatta monkey hemoglobin.

Table I Amino acid compositions of the soluble tryptic peptides, β T9, β T13, β T14, and β T15 from β -chain in *macaca mulatta* monkey hemoglobin

Peptide	Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Leu	Tyr	Phe	Try	Yield*
β T 9	1.05	0.97		3.89		0.89			2.13	1.08		0.90		4.08		0.96		29 μ mole
β T 13	1.00				0.85		3.94	1.14		1.98		0.96			0.76	0.97		31
β T 14	1.02	0.98		1.04					0.86	3.76		2.54			1.13			38
β T 15		1.13													0.86			42

* This row shows the μ mole numbers of the peptides obtained from 1g of β -chain.

These peptides seem to be nearly pure since the molecular ratios of their amino acids are near the integral values except valine in β T14. It is considered that three valine residues are in β T14. The low value of valine may result from the strong resistance of Val-Val bond against HCl hydrolysis.

2) *Amino acid sequence of β T9*

β T9 consists of 16 amino acid residues. The composition is shown in Table I. As reported by TAKEI,²²⁾ this peptide has aspartic acid one mole more and alanine one mole less than β T9 of human hemoglobin. By DNP method the N-terminal amino acid of this peptide turned out to be valine. The peptide was hydrolyzed with pepsin for 20 hours. The peptic peptides were first subjected to column chromatography and

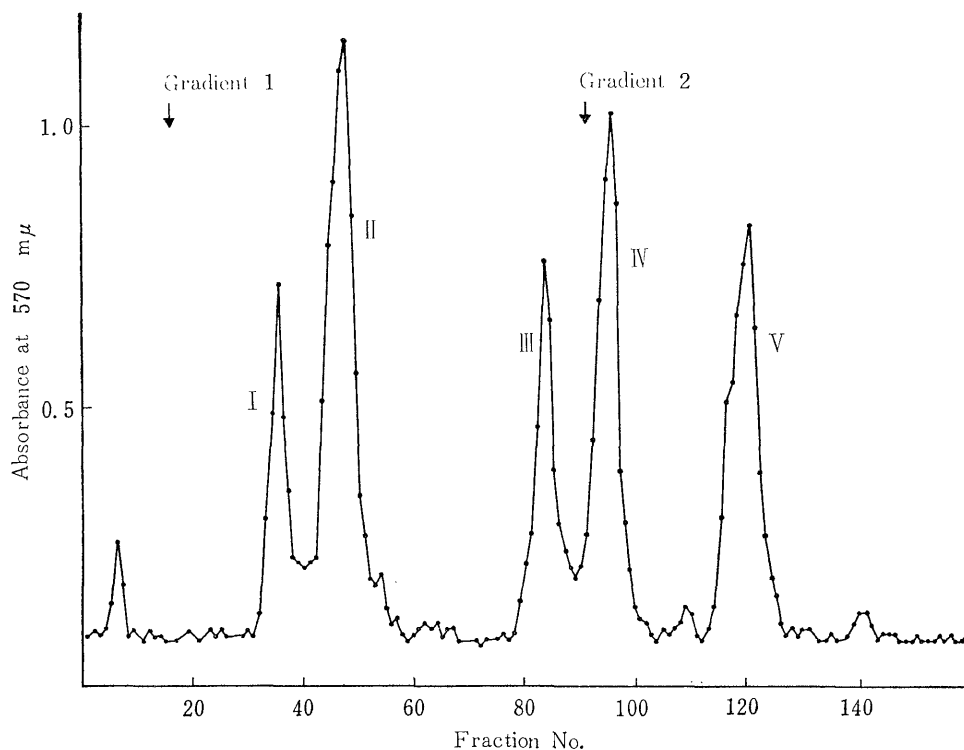


Fig. 1 Chromatography of peptic peptides of β T9 on Dowex 1 \times 2 Column: 1.0 \times 60cm, Developer: Starting, 1 % Pyridine 1% collidine acetate buffer pH 8.5 Gradient 1, Mixing chamber 550 ml of starting buffer, upper chamber 0.1N acetic acid, Gradient 2, upper chamber 1.0 N acetic acid

then to paper chromatography for the isolation and purification. The results are shown in Fig. 1 and Fig. 2.

Seven segmentary peptides were obtained as shown in Fig. 2. The analyses of the amino acid sequences of these peptides were carried out by the undermentioned procedure. Rf Leu in the explanation means the Rf value of each peptide against leucine. The value given in per cent (%) is the yield of the peptide through all the procedures.

a) β T9 P-I Rf Leu 0.43 23%

Lys : 1.04, His : 0.98, Asp : 1.96, Leu : 2.02

The PTC method was carried out in two steps.

Step 1 : His : 0.02, Leu : 2.05, Asp : 1.95, Lys : 1.03

Step 2 : His : 0.00, Leu : 1.12, Asp : 2.00, Lys : 1.05

From this result, the sequence of this peptide is considered to be His-Leu-(Asp 2, Leu 1, Lys 1), that is, His-Leu- β T9PIIa.

b) β T9 P-IIa Rf Leu 0.38 29%

Lys : 1.00, Asp : 1.93, Leu : 1.07

The PTC method was carried out in two steps.

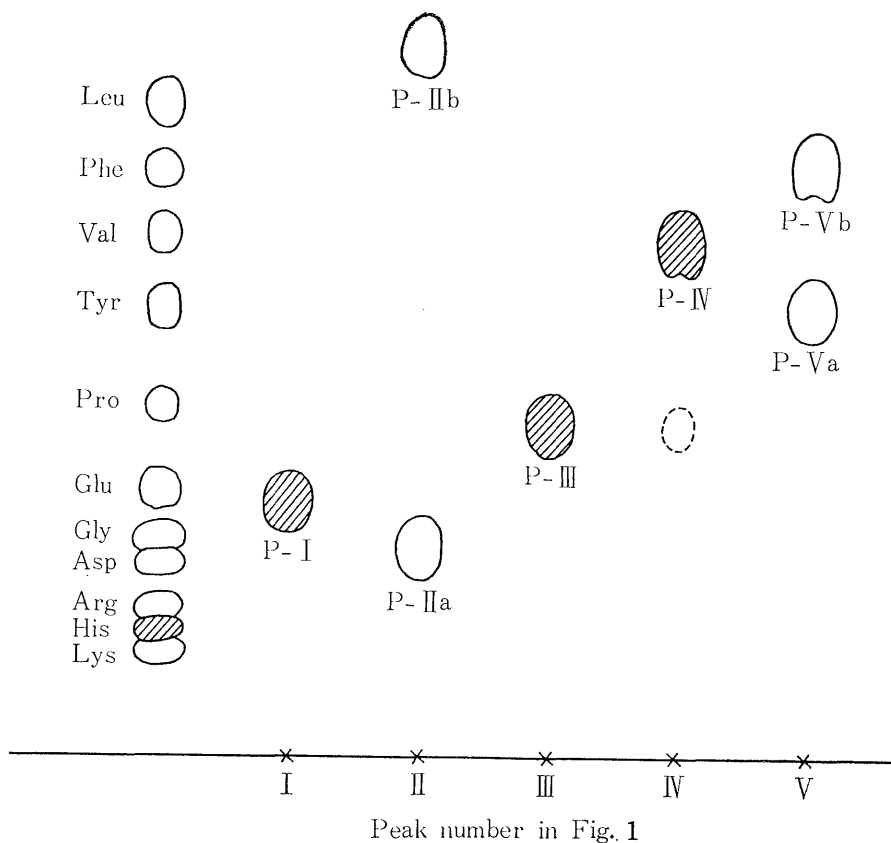


Fig. 2 Paper chromatography of peptic peptides of β T9 n-butanol: acetic acid: water (4:1:5).

Step 1 : *Asp* : 1.00, *Leu* : 1.00, *Lys*, not determined

Step 2 : *Asp* : 0.26, *Leu* : 1.00, *Lys*, not determined.

This peptide had been known neutral by the paper electrophoresis. This suggests that one of the two residues which are present as aspartic acid is in fact asparagine. The peptide after the first step of the PTC method moved further to the cathode in the electrophoresis than the original peptide, suggesting that it was basic and that asparagine is not the N-terminal amino acid, but the second from the N-terminus of this peptide. As lysine is likely to be the C-terminal amino acid of β T9, the sequence of β T9 PIIa was determined as follows;

Asp-Asp (NH₂)-Leu-Lys

This peptide appears to be located in the C-terminus of β T9.

c) β T9 P-IIb Rf *Leu* 1.10 80%

Gly : 1.00, *Ala* : 1.06, *Val* : 0.95, *Leu* : 0.99

The PTC method was carried out in three steps.

Step 1 : *Val*: 0.00, *Leu* : 0.97, *Gly* : 1.01, *Ala* : 1.02

Step 2 : Val : 0.00, *Leu* : 0.06, Gly : 1.00, Ala : 1.00

Step 3 : Val : 0.00, *Leu* : 0.00, *Gly* : 0.16, Ala : 1.00

This appears to be the N-terminal peptide of β T9 since it has the only one valine that β T9 has, and this valine has been already known to be the N-terminal amino acid of β T9. The amino acid sequence was determined as follows;

Val-Leu-Gly-Ala

d) β T9 P-III Rf *Leu* 0.52 16%

Lys : 1.04, His : 1.02, Asp : 3.92, Ser : 0.93, Gly : 0.98, *Leu* : 3.12, Phe : 0.99

By the DNP method, the N-terminal amino acid of this peptide was phenylalanine. Judging from its amino acid composition, this peptide is deduced to be a combination of β T9 P-IV and β T9 P-IIa, and that of β T9 P-Va and β T9 P-I.

e) β T9 P-IV Rf *Leu* 0.80 20%

His : 0.98, Asp : 1.98, Ser : 0.84, Gly : 1.01, *Leu* : 2.23, Phe : 0.89

f) β T9 P-Va Rf *Leu* 0.71 25%

Asp : 2.05, Ser : 0.96, Gly : 1.01, *Leu* : 1.04, Phe : 0.92

The PTC method was carried out in five steps, and the peptide after every step was examined by electrophoresis.

Step 1 : *Phe* : 0.00, Ser : 0.90, Asp : 2.02, Gly : 1.02, *Leu* : 1.05

Step 2 : *Phe* : 0.00, *Ser* : 0.06 Asp : 1.91, Gly : 1.07, *Leu* : 1.02

Step 3 : *Phe* : 0.00, Ser : 0.00, *Asp* : 1.18, Gly : 0.98, *Leu* : 0.94

Step 4 : *Phe* : 0.00, Ser : 0.00, Asp : 0.99, *Gly* : 0.26, *Leu* : 1.01

Step 5 : *Phe*. 0.00, Ser : 0.00, Asp : 1.00, Gly : 0.08, *Leu* : 0.38

This peptide was originally acidic, but became neutral after the third step. In addition, by the C-terminal analysis with carboxypeptidase A, asparagine was detected earliest. It was known that the peptide β T9 P-Vb, which had aspartic acid one mole less than this peptide, moved in the same manner as this peptide in the electrophoresis. From the above-mentioned results, the amino acid sequence of this peptide was determined as follows;

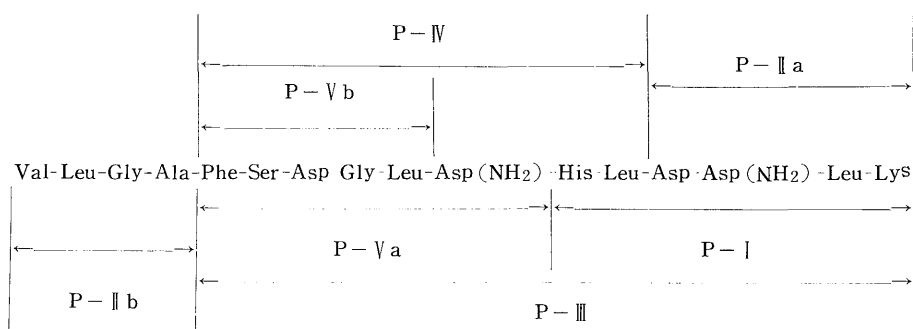
Phe-Ser-Asp-Gly-Leu-Asp(NH₂)

g) β T9 P-Vb Rf *Leu* 0.92 5%

Asp : 1.03, Ser : 0.09, Gly : 0.08, *Leu* : 1.03, Phe : 1.06

As mentioned above, this is a peptide which has aspartic acid one residue less than β T9 P-Va.

Putting all the above-mentioned results together, the amino acid sequence of β T9 was determined as follows;



This was compared with the sequence of the corresponding peptide of human hemoglobin. One difference was recognized at the tenth from the N-terminus, that is, it was alanine in case of human hemoglobin, but asparagine in case of *macaca mulatta* monkey hemoglobin. Except this difference, their amino acid sequences were quite similar to each other.

3) Amino acid sequence of $\beta T13$

The amino acid composition of $\beta T13$ is given in Table I. This peptide has glutamic acid one mole more and proline one mole less

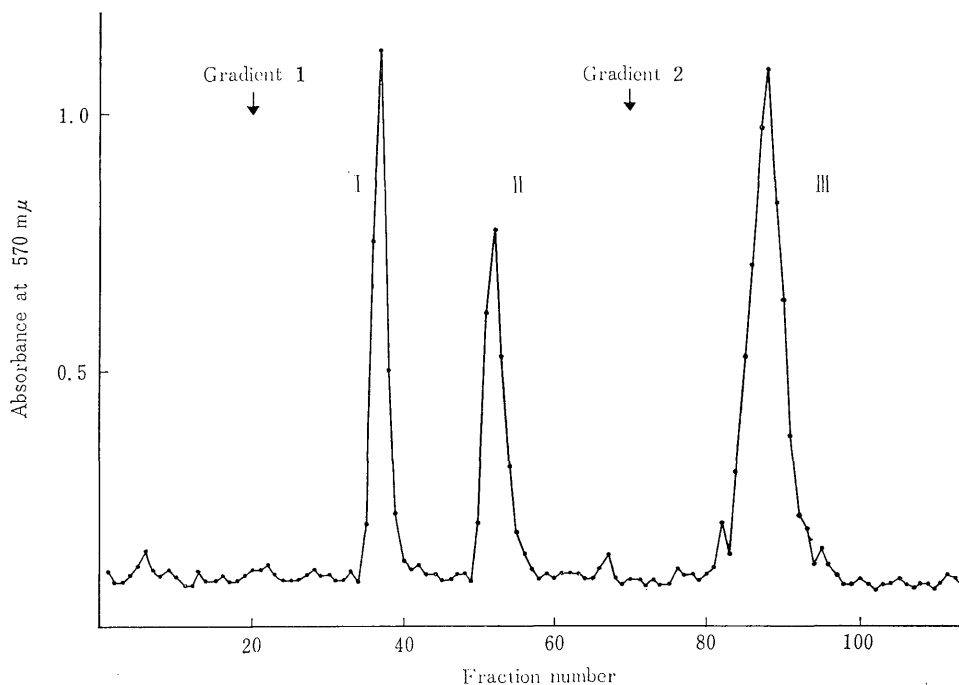


Fig. 3 Chromatography of peptic peptides of $\beta T13$ on Dowex 1 \times 2
 Column : 1.0 \times 60cm. Developer : Starting, 1% pyridine 1% Picoline acetate buffer pH 8.5; Gradient 1, Mixing chamber 750 ml of starting buffer upper chamber 0.075N acetic acid: Gradient 2, upper chamber 1.0 N acetic acid.

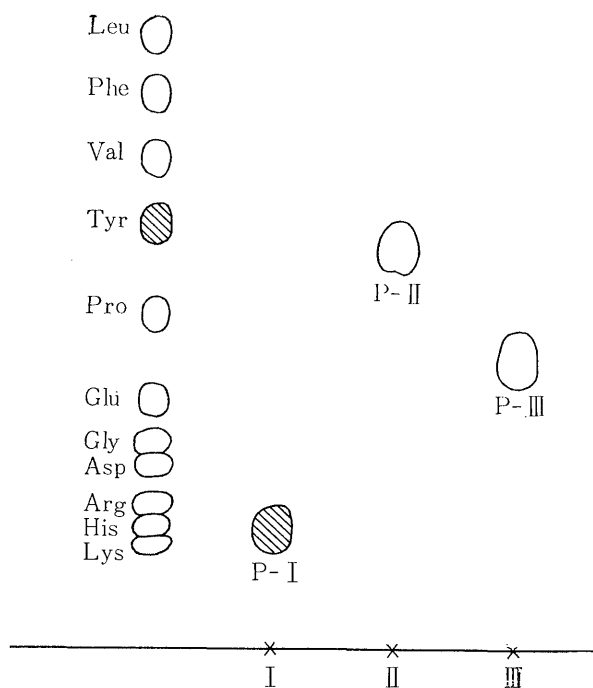


Fig. 4 Paper chromatography of peptic peptides of β T13
n-butanol : acetic acid : water (4 : 1 : 5)

than β T13 of human hemoglobin. The N-terminal amino acid of this peptide was first determined to be glutamic acid by the DNP method. The fragmentary peptides were obtained by the hydrolysis of β T13 with pepsin for 16 hours. They were isolated by column chromatography. As shown in Fig. 3, three peaks were recognized. Each peak was subjected to paper chromatography.

On the paper chromatogram, all the peaks presented only one spot, respectively. Therefore, the peptide contained in each peak had not to be purified further. The amino acid sequence of each peptide was determined.

a) β T13 P-I Rf Leu 0.20 82%

Lys : 0.96, Glu : 1.04, Tyr : 0.98

The PTC method was carried out in one step.

Step 1 : Tyr : 0.07, Glu : 1.02, Lys : 0.98

This peptide had been known to be basic by the electrophoresis. This suggests that the amino acid detected as glutamic acid residue in the amino acid analysis is in fact a glutamine residue. Lysine was the C-terminal amino acid of β T13. Therefore, this peptide is located in the C-terminus of β T13. The sequence was determined as follows;

Tyr-Glu(NH₂)-Lys

b) β T13 P-II Rf Leu 0.68 80%

In the analysis of this peptide after HCl hydrolysis, alanine was only detected. The Rf value of this peptide on the paper chromatogram was, however, higher than that of alanine. β T13 had presumably two residues of alanine. Therefore, this peptide may consist of Ala-Ala.

In order to confirm this presumption, the following analysis was carried out by the DNP method. The peptide (0.2 μ mole) was dinitrophenylated in 1% NaHCO₃, and the excess DNFB was removed by the ether extraction. The aqueous phase was acidified by the addition of HCl and was again extracted with ether. Consequently, DNP-Ala-Ala was extracted in the ether phase. If irresponsive Ala-Ala or Ala existed, it must have been left in the aqueous phase by this operation. The ether phase containing DNP-Ala-Ala was dried and then hydrolyzed with constant boiling point HCl at 105°C for 20 hours. From this hydrolyzate, the DNP-Ala which was due to the N-terminus was extracted in the ether phase, and other residue of alanine, in the aqueous phase. The DNP-Ala in the ether phase was isolated, purified, and determined quantitatively by the two-dimensional paper chromatography. On the other hand, the isolated alanine in the aqueous phase was dinitrophenylated in the same way as mentioned above. It was subjected to the paper chromatography for the isolation and the quantitative determination. As the results, 0.136 μ mole of the DNP-Ala due to the N-terminus and 0.126 μ mole of the DNP-Ala which was not due to the N-terminus were obtained. This fact proves that β T13 P-II is a dipeptide Ala-Ala.

c) β T13 P-III Rf Leu 0.47 75%

Thr : 0.90, Glu : 3.01, Pro : 1.07, Val : 0.97, Phe : 1.05

The PTC method was carried out in six steps, and the peptide after every step was examined by electrophoresis in order to know its approximate electric charge.

Step 1 : *Glu* : 2.16, *Phe* : 0.97, *Thr* : 0.95, *Pro* : 1.10, *Val* : 0.99

Step 2 : *Glu* : 2.04, *Phe* : 0.06, *Thr* : 0.94, *Pro* : 1.08, *Val* : 0.98

Step 3 : *Glu* : 1.98, *Phe* : 0.00, *Thr* : 0.05, *Pro* : 1.02, *Val* : 0.97

Step 4 : *Glu* : 2.04, *Phe* : 0.00, *Thr* : 0.05, *Pro* : 0.08, *Val* : 0.96

Step 5 : *Glu* : 1.32, *Phe* : 0.00, *Thr* : 0.00, *Pro* : 0.00, *Val* : 1.00

Step 6 : *Glu* : 1.00, *Phe* : 0.00, *Thr* : 0.00, *Pro* : 0.00, *Val* : 0.20

This peptide was originally acidic, but changed into neutral after the first step. Even after the fifth step, it remained neutral. This indicates that except the N-terminal glutamic acid, the residues detected as glutamic acids in the analysis are all glutamine. Accordingly, the amino acid sequence of this peptide was determined as follows;

Glu-Phe-Thr-Pro-Glu(NH₂)-Val-Glu(NH₂)

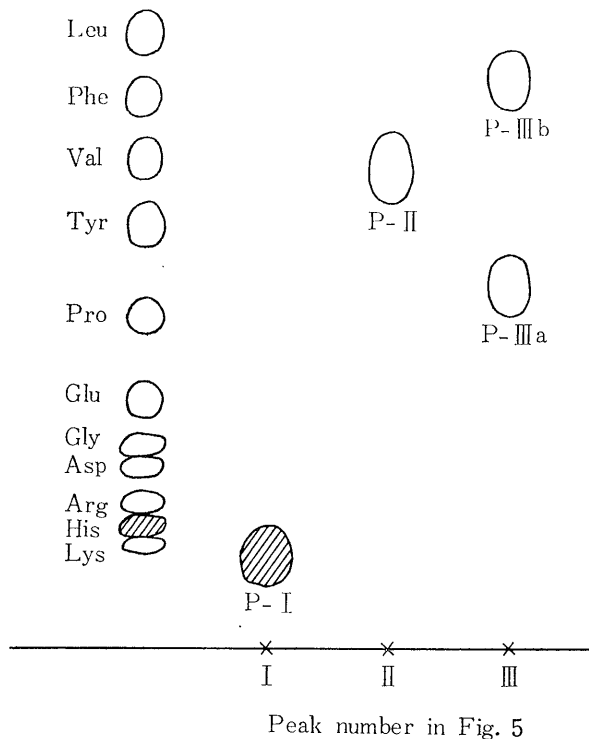


Fig. 6 Paper chromatography of peptic peptides of β T14
n-butanol : acetic acid : water (4 : 1 : 5)

known to be valine. The peptide was first hydrolyzed with pepsin for 20 hours. The peptic peptides were isolated by column and paper chromatographies. The results are shown in Fig. 5 and Fig. 6.

a) β T14 P-I Rf Leu 0.17 70%

Lys : 0.96, His : 1.04, Ala : 1.01, Leu, : 0.99

The PTC method was carried out in two steps.

Step 1 : Leu : 0.06, Ala : 0.98, His : 1.02, Lys : 1.00

Step 2 : Leu : 0.06, Ala 0.08, His : 0.96, Lys : 1.04

Since lysine appears to be the C-terminal amino acid of β T14, this peptide is located at the C-terminus of β T14. The sequence was determined as follows;

Leu-Ala-His-Lys

b) β T14 P-II Rf Leu 0.78 27%

Asp : 0.99, Gly : 1.08, Ala : 3.01, Val : 2.44

The combination of this peptide and β T14 P-I is identical to β T14 in the amino acid composition. Therefore, it is considered that this peptide is located at the N-terminus of β T14 and that this peptide is the combination of β T14 P-IIIa and β T14 P-IIIb. The PTC method

was carried out in two steps.

Step 1 : Val : 2.01, Ala : 3.00, Gly : 0.96, Asp : 1.04

Step 2 : Val : 0.98, Ala : 2.92, Gly : 1.08, Asp : 1.00

From this result, it is known that the N-terminus of β T14 consists of Val-Val bond.

c) β T14 P-IIIa Rf Leu 0.60 29%

Asp : 1.00, Ala : 2.01, Val : 0.99

The PTC method was carried out in three steps.

Step 1 : Val : 0.00, Ala : 2.04, Asp : 0.96

Step 2 : Val : 0.00, Ala : 0.99, Asp : 1.01

Step 3 : Val : 0.00, Ala : 1.00 Asp : 0.06

By electrophoresis, this peptide turned out to be neutral. This suggests that the residue detected as aspartic acid in the analysis is asparagine. The sequence was determined as follows;

Val-Ala-Asp (NH₂)-Ala

d) β T14 P-IIIb Rf Leu 0.95 31%

Gly : 0.98, Ala : 1.02, Val : 1.44

The PTC method was carried out in three steps.

Step 1 : Val : 0.96, Ala : 1.04, Gly : 1.00

Step 2 : Val : 0.08, Ala : 1.02, Gly : 0.98

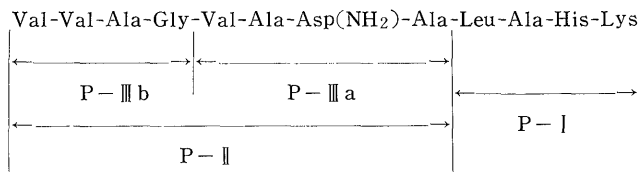
Step 3 : Val : 0.08, Ala : 0.13, Gly : 1.00

Accordingly, the amino acid sequence of this peptide was determined as follows;

Val-Val-Ala-Gly

It is considered that this is the N-terminal peptide of β T14 because Val-Val bond exists at the N-terminus of this peptide.

From the above-mentioned results, the amino acid sequence of β T14 was determined as follows;



This is quite the same as the sequence of β T14 in human hemoglobin.

5) Amino acid sequence of β T15

β T15 is a dipeptide which consists of histidine and tyrosine. By the DNP method di-DNP-tyrosine was detected, and in the amino acid analysis after the first step of the PTC method only histidine was recognized. Therefore the amino acid sequence of this peptide was Tyr-His. This is quite the same as β T15, the C-terminal peptide of

β -chain in human hemoglobin.

CONCLUSION

The present author chose four, i. e. β T9, β T13, β T14, and β T15, among the peptides obtained by the tryptic hydrolysis of β -polypeptide chain of *macaca mulatta* monkey hemoglobin, and determined the amino acid sequences of these four peptides which contain 42 amino acid residues. Moreover, he compared the results with the amino acid sequences of the corresponding peptides of human hemoglobin. Two differences were recognized between the two hemoglobins. Namely, the tenth amino acid from the N-terminus of β T9 was alanine in case of human hemoglobin, but asparagine in case of *macaca mulatta* monkey hemoglobin. The fifth from the N-terminus of β T13 was proline in case of the former, but glutamine in case of the latter.

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