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[原著]Amino Acid Sequence of the α Chain of Chicken AI Hemoglobin : Amino Acid Sequenc of the Chymotryptic Peptides of the α Chain

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Amino Acid Sequence of the α Chain of Chicken AI Hemoglobin

——Amino Acid Sequenc of the Chymotryptic Peptides of the α Chain

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SUMMARY

As presented in the preceding paper,[1] α polypeptide chain of AI component of chicken hemoglobins was purified and the amino acid sequence of the tryptic peptides from the α polypeptide chain was determined. Details on the study of the amino acid sequence of the chymotryptic peptides from the α polypeptide chain are described and the amino acid sequence of the whole α polypeptide chain is presented and compared with that of the whole α polypeptide chain of AII component of chicken homoglobin in the present paper. An unexpectedly large difference of amino acids is indicated between α chains of both components.

INTRODUCTION

It has been shown by many authors so far that avian adult hemoglobin is heterogeneous.[(2)～(9)] Present authors are interested in the relationship between the molecular heterogeneity and structural and functional differences, molecular evolution and regulatory mechanism of synthesis of these molecular species. In this paper, amino acid sequences of the chymotryptic peptides from the α chain were determined. The whole amino acid sequence of the α chain was assembled from the amino acid sequence of the tryptic peptides presented in the preceding paper and that of the chymotryptic peptides determined here.

MATERIALS AND METHODS

(1) Preparation of α polypeptide chain of AI component.

Preparation of hemolyzates and purification of hemoglobin were achieved according to the method described in the preceding paper.[1]

Cleavage of the AI hemoglobin into α and β polypeptide chain was also achieved according to the method described in the preceding paper. 2.0g of lyophilized AI hemoglobin were dissolved in 200 ml of 4 M urea-HCl buffer (pH2.3), which was allowed to stand stirring for 30 min at room temperature while Amberlite CG-50(Type 2) column of 4.8×28 cm was prepared after the resin was washed with acetone and water, activated with 1 N NaOH, washed with water, activated with 1 N HCl and washed with 0.05 N HCl successively. The 4 M urea-HCl buffer (pH2.3) was also used as a starting buffer of the chromatography. A mixing bottle was filled with 1,800 ml of the starting buffer, stirred with a magnetic stirrer, and an upper chamber was filled with 10 M urea-HCl buffer (pH 2.3). The two bottles were connected together with a tube. The hemoglobin solution, after treated for 30 min as above, was applied onto the column to which the mixing bottle was connected and an exponential gradient of urea concentration was obtained. The effluent from the column was collected by a fraction collector (Toyo Scientific Product Co.), fraction volume of which was 18 ml each. Each fraction was measured photometrically at 280 nm. Fractions identified as the ones containing α polypeptide chain were combined, dialyzed against deionized water and lyophilized.

(2) Digestion of α polypeptide chain with chymotrypsin

1,500 mg of α polypeptide chain was dissolved in 150 ml of deionized water and 30 mg of α chymotrypsin (SIGMA Co. Ltd.), dissolved in 3 ml of 0.001 N HCl solution, was added to the substrate solution. The pH of the reaction mixture was adjusted to 8.0 with 0.1 N NaOH and the digestion was continued for 19 hours at 37℃. The pH of the reaction mixture was kept at the same level during the digestion by adding 0.1 N NaOH and the digests were lyophilized.

(3) Column chromatography for separation of the chymotryptic peptides of α polypeptide chain

Column chromatography for separation of the chymotryptic peptides was performed according to the method as described in the preceding paper.[1] Activated AG 1×2 (200-400 mesh, Dow Chemical Co.) was packed into a column of 0.9×50 cm and equilibrated with 1 % pyridine, 1 % collidine acetate buffer (pH 9.6). About 300 mg of the chymotryptic peptides of the α polypeptide chain, dissolved in the same volatile buffer (pH 9.6), was applied onto the column. The other buffers of lower pH were substituted for the starting buffer until the pH of the effluent reached 3.8. The peptides thus fractionated were detected with ninhydrin reaction and recorded on the recorder by using an automatic peptide analyzer (Technicon Co.). Effluent corresponding to each peak was pooled and lyophilized for further analysis.

Chromo Beads P column (0.9×20cm) was used for rechromatography of the peptide mixtures and pyridine acetate buffers were used as developing buffers according to the method described previously.[10]~[12]

(4) Determination of the C-terminal amino acids of the peptides

Determination of the C-terminal amino acids of the peptides were performed according to the method as described in the preceding paper.[1]

(5) Amino acid composition and sequence of the chymotryptic peptides

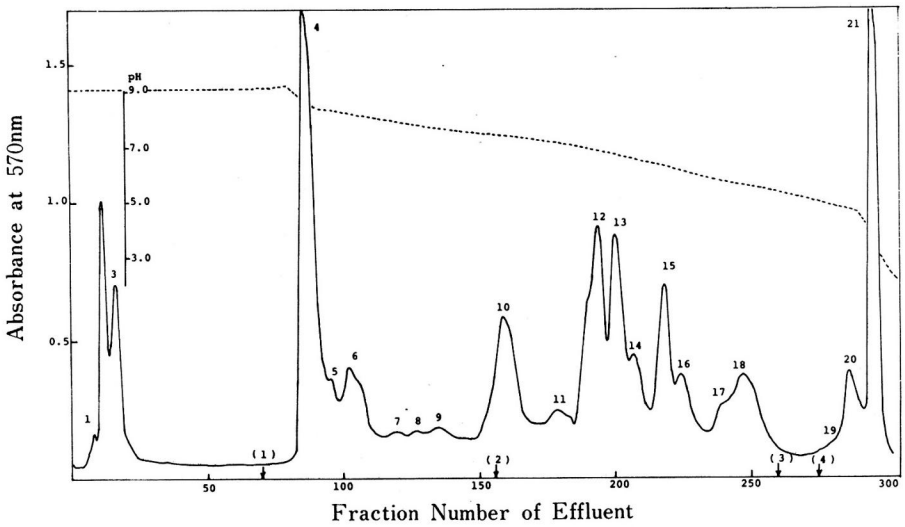
Amino acid analysis and subtractive Edman degradation for sequence study of the chymotryptic peptides were also carried out according to the method as described previously.[10]~[12]

RESULTS

(1) Fractionation and purification of the chymotryptic peptides

After the undigested precipitates were removed by centrifugation at 3,000 r.p.m. for 10 min, the chymotryptic digest solution was lyophilized. About 300 mg of the chymotryptic digests was dissolved in 5 ml of 1% pyridine collidine acetate buffer (pH 9.6). An AG 1×2 column (0.9×50 cm) was equilibrated with the same buffer and the above digest solution was applied onto the column. A mixing chamber cotaining about 400 ml of the same buffer was connected with a supply bottle filled with 1% pyridine collidine acetate buffer (pH 8.0) on the upper side with a Teflon tube and the mixing chamber was connected with the column on the lower side. The column was run and the buffer in the supply bottle was substituted by 1% pyridine collidine acetate buffer (pH7.0), 0.1 N acetic acid and 1 N acetic acid solution one after another until the pH of the effluent reached 3.8. The chromatography was recorded on a recorder by using an automatic peptide analyzer as described in the preceding paper. The chromatography pattern was shown in Fig. 1. The effluent corresponding to each peak on the chromatogram

Fig. 1 Column chromatogram of the chymotryptic peptides from α chain of chicken AI hemoglobin by using AG 1 x 2 (column size: 0.9×50cm)
The buffer system used for the chromatography was the same as described for separation of the the tryptic peptides in the preceding paper. pH of the effluent was checked during the chromatography.



was pooled, lyophilized and rechromatographed on a Chromo Beads P column with the pyridine acetate buffer system as described in the preceding paper. The rechromatographic pattern of each fraction was shown in Figs. 2A, B, C, D and E. Purity of each peptide thus obtained was confirmed by paper chromatography and several peptides which were not pure were rechromatographed by using a filter paper (Toyo Scientific Co., No. 51 filter paper).

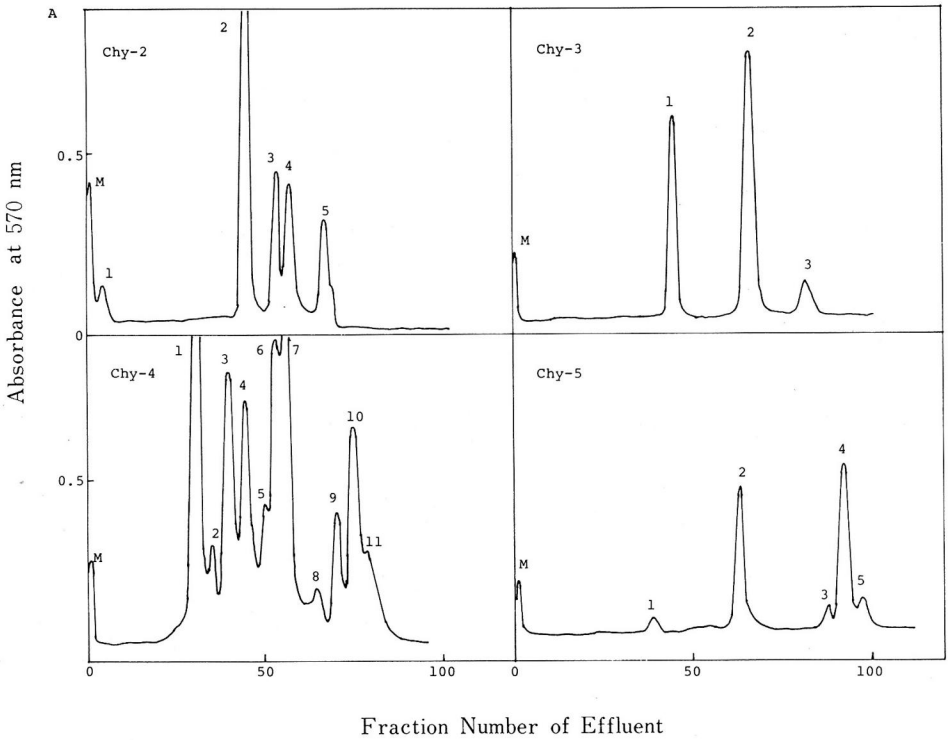
(2) Amino acid analysis of the chymotryptic peptides

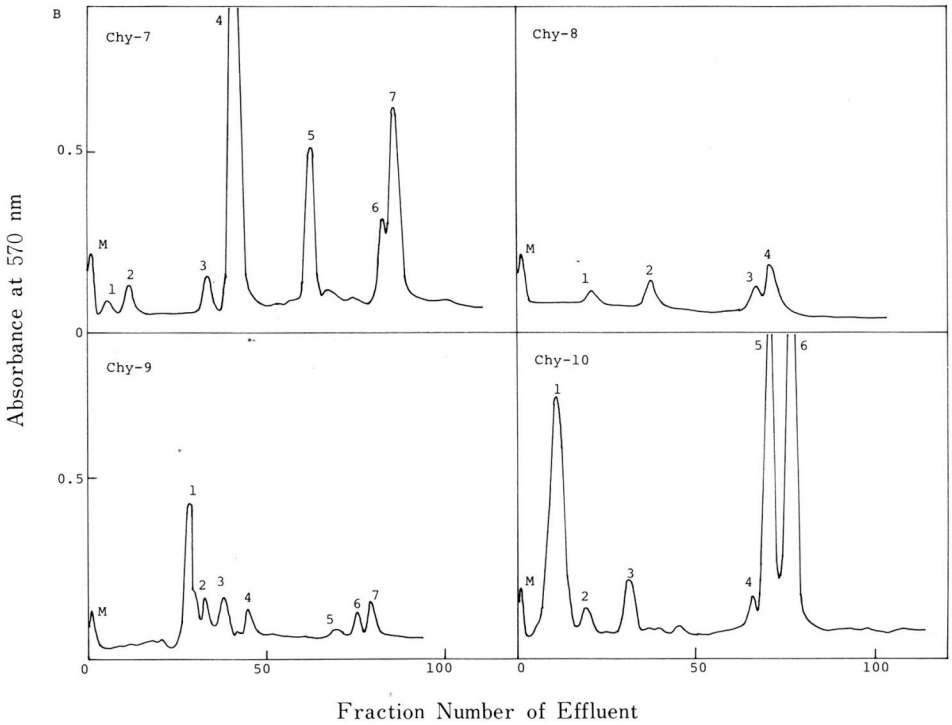
All of the efficient peptides thus purified were hydrolyzed with a twice distilled hydrochloric acid and subjected to amino acid analysis with an automatic amino acid analyzer as shown on the first column in Table 1.

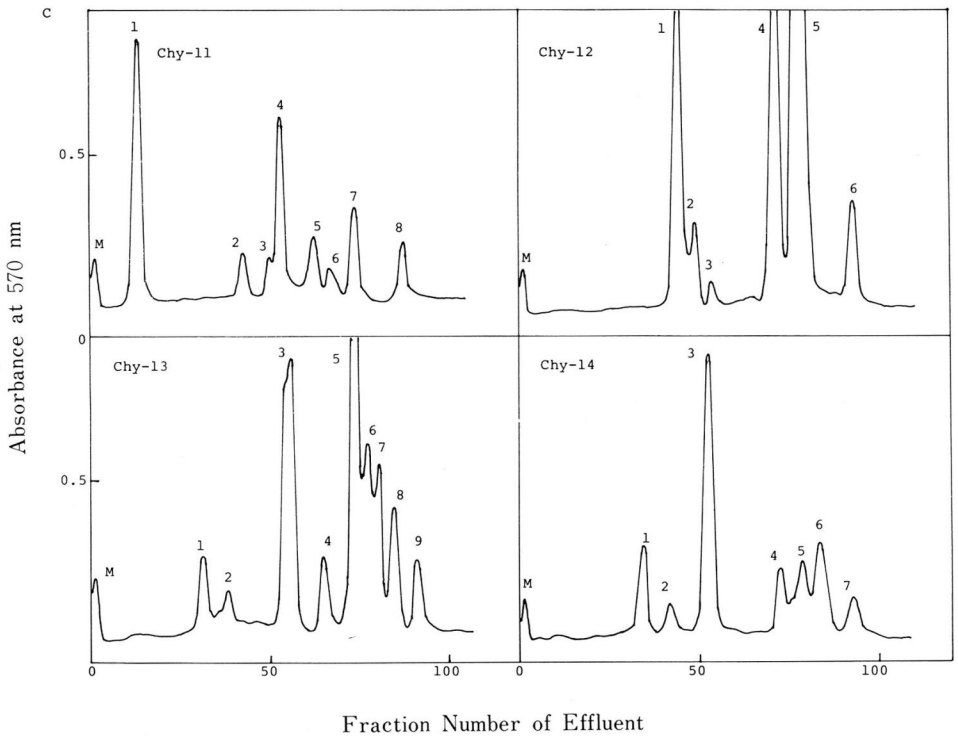
(3) Amino acid sequence of the chymotryptic peptides

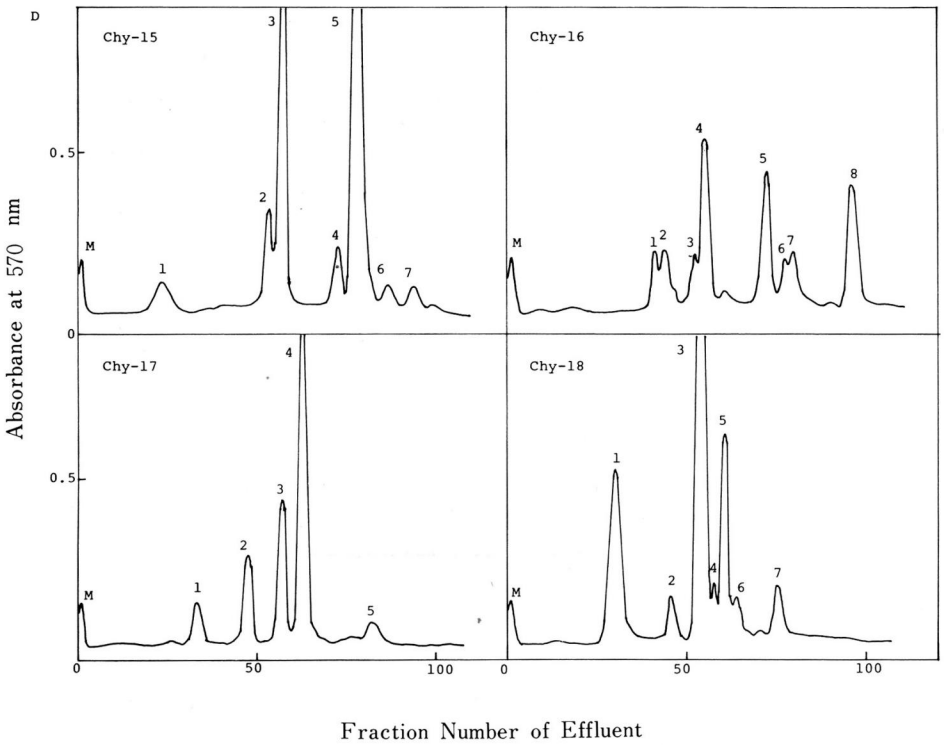
Amino acid sequence of the chymotryptic peptides was studied by the use of the subtractive Edman degradation as described elswhere and carboxypeptidase digestion method as described in the preceding paper. The results were shown in Table 1 A, B, C, D and E.

Fig. 2 Rechromatography of the chymotryptic peptides fractionated with AG 1 × 2 column by using Chromo Beads P column (0.9×20cm).
The numbers, Chy-2, -3, ····· in the chart represent the peak numbers shown in Fig. 1.




B
Chy-7
Chy-8
Chy-9
Chy-10
Absorbance at 570 nm
Fraction Number of Effluent


C
Chy-11
Chy-12
Chy-13
Chy-14
Absorbance at 570 nm
Fraction Number of Effluent


D
Chy-15
Chy-16
Chy-17
Chy-18
Absorbance at 570 nm
0.5
0
M
0
50
100
Fraction Number of Effluent

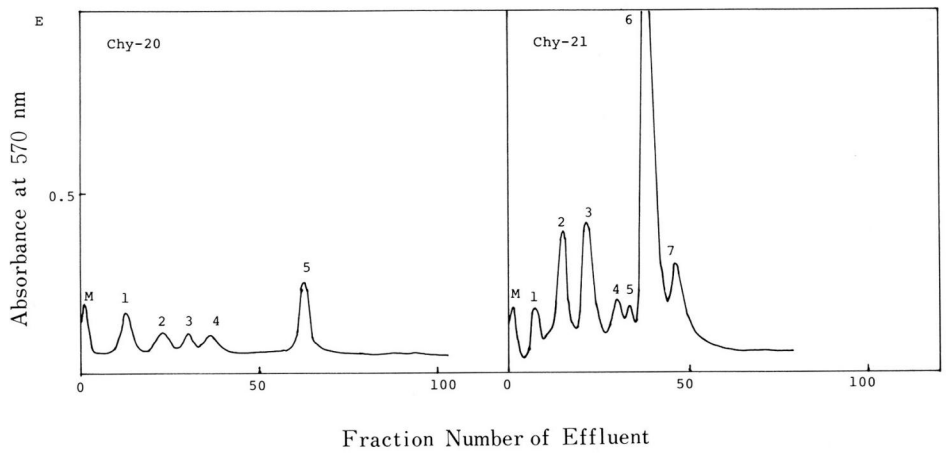

E
Chy-20
Chy-21
Absorbance at 570 nm
0.5
M
0
50
100
Fraction Number of Effluent

Table 1 Edman degradation and carboxypeptidase digestion of the chymotryptic peptides from the α chain of chicken AI hemoglobin

In the designation on the left column like Chy-14-3, Chy-10-6-c, ..., the first number following 'Chy-' represents the peak number in the chromatogram in Fig. 1, the second number, the peak number in the rechromatogram in Fig. 2 and the alphabet, the position of the peptide spot on the paper chromatogram from the bottom (see Fig. 3 in the preceding paper).

Each chymotryptic peptide was identified to be situated on the tryptic peptide or peptides shown in parenthesis. Underlined part shows the amino acid sequence determined. The arrow [→] represents Edman degradation and the arrow [←], carboxypeptidase digestion.

A Chy-14-3 (αT-1)

Composition		Step 1
Met	0.62 (1)	0
Leu	1.00 (1)	1.00

Sequence : Met→-Leu

Chy-10-6-C (αT-1~2~3)

Composition		Step 1	2	3	4	5	6
Lys	1.70 (2)	2.00	2.00	2.00	2.00	2.00	2.00
Asp	1.01 (1)	1.29	1.24	1.12	0.97	1.02	0.56
Thr	1.01 (1)	0.63	0.86	0	0	0	0
Glu	0.90 (1)	1.07	1.09	0.83	1.23	0.62	0
Ala	0.90 (1)	0.71	1.00	1.03	0.36	0	0
Met	0.94 (1)	0	0	0	0	0	0
Leu	2.24 (2)	2.24	1.06	1.25	0.80	0.98	1.00

Sequence : Met→-Leu→-Thr→-Ala→-Glu→-Asp→,Lys,←Lys-←Leu

Chy-13-6-b (αT-1~2~3)

Composition		Step 1	2
Trp	(+)		
Lys	1.97 (2)	2.00	2.00
Asp	1.17 (1)	1.21	1.14
Thr	1.01 (1)	0.22	0
Glu	2.90 (3)	2.92	3.25
Ala	1.96 (2)	2.14	1.69
Ile	0.98 (1)	0.80	0.78
Leu	1.00 (1)	0.93	0.93

Sequence : Thr→-Ala→-Glu-Asp,Lys,Lys,Leu,Ile,Gln,Gln,←Ala,Trp

B Chy-16-3 (αT-3)

Composition		Step 1	2	3	4	5
Trp	(+)					
Glu	2.02 (2)	2.32	2.00	1.65	1.34	
Ala	1.10 (1)	1.18	1.00	1.00	1.00	
Ile	0.67 (1)	0.65	0.19	0	0	
Leu	0.88 (1)	0.34	0	0	0	

Sequence : Leu-Ile-Glu-Glu,Ala,Trp

Chy-21-6-A (αT-3~4)

Composition		Step 1	2	3	4	5	6
Lys	1.10 (1)	0.60	0	0	0	0	0
His	1.05 (1)	1.00	0.95	0.97	0.93	0.95	0.57
Ser	0.88 (1)	0.83	0.90	0.93	0.92	0.59	0
Glu	5.33 (5)	4.96	4.90	4.45	4.75	4.95	4.54
Gly	0.96 (1)	1.07	1.06	1.02	1.05	1.09	1.05
Ala	3.83 (4)	4.08	4.03	3.70	2.90	2.73	2.58
Leu	0.93 (1)	0.98	0.98	1.03	1.02	1.09	0.99
Phe	0.93 (1)	0.98	0.98	0.98	1.00	1.07	0.96

Sequence : Glx-Lys-Ala-Ala-Ser-His,Glx,Glx,Glx,Phe,Gly,Ala,Glx,Ala,Leu

Chy-3-2 (αT-4~5)

Composition		Step 1	2	3
Arg	1.00 (1)	1.03	0.12	0
Thr	0.97 (1)	0.38	0	0
Met	0.58 (1)	0.70	0.71	trace
Phe	1.03 (1)	0.97	1.00	1.00

Sequence : Thr-Arg-Met-Phe

Chy-6-4-A (αT-5~6)

Composition		Step 1	2	3	4
Lys	0.95 (1)	1.00	1.00	1.00	0.20
Thr	1.98 (2)	1.96	1.52	1.14	1.25
Glu	1.21 (1)	0.85	0.35	0	0
Pro	1.09 (1)	0.67	0.58	0	0
Tyr	0.98 (1)	1.19	1.00	0.76	0.75

Sequence : Pro-Glx-Thr-Lys-Thr-Tyr

C Chy-9-5 (αT-6~7)

Composition		Step	1	2	3	4
Lys	0.94 (1)		0.77	0.65	0.63	1.00
His	2.01 (2)		2.24	1.84	1.00	1.11
Arg	0.76 (1)		0.93	0.65	0.77	0.88
Asp	2.24 (2)		1.89	1.98	1.93	1.93
Ser	1.80 (2)		1.64	1.74	1.77	1.71
Glu	1.01 (1)		1.04	1.20	1.12	1.13
Pro	1.39 (2)		1.36	1.04	0.80	0.90
Gly	2.72 (3)		3.26	2.98	3.18	3.34
Val	0.69 (1)		0.67	0.68	0.82	0.92
Leu	1.04 (1)		0.98	1.12	1.17	0.93
Phe	2.25 (2)		0.83	1.11	1.22	0

Sequence : Phe-Pro-His-Phe,Asx,Leu,Ser,Pro,Gly,Ser,Asx,Glx,Val,
Arg,Gly,His,Gly,Lys

Chy 6-6-C (αT-7~8~9)

Composition		Step	1	2
Lys	2.04 (2)		1.98	1.00
Gly	1.11 (1)		0	0
Val	0.69 (1)		0.71	0.65
Leu	0.86 (1)		1.02	1.00

Sequence : Gly-Lys-Lys-Val-Leu

Chy 18-1 (αT-9~10)

Composition		Step	1	2	3	4	5	6	7
Lys	0.76 (1)		0.65	0.75	1.00	1.00	1.00	1.00	1.00
Asp	3.96 (4)		3.88	3.82	3.80	3.71	3.28	3.26	3.10
Ser	0.98 (1)		0.97	1.05	1.19	0.95	0.98	1.02	0.93
Glu	2.09 (2)		2.00	1.95	1.99	2.03	2.20	1.96	2.00
Gly	2.35 (2)		1.27	1.28	1.32	0.45	0	0	0
Ala	3.43 (4)		3.89	3.12	2.74	2.81	2.84	2.26	2.24
Val	1.58 (2)		1.40	1.65	1.74	1.70	1.76	1.50	1.14
Met	0.61 (1)		0.68	0.63	0.65	+	+	0.51	+
Leu	3.02 (3)		3.24	3.22	2.26	2.22	1.97	1.73	1.73

Sequence : Gly-Ala-Leu-Gly-Asx-Ala-Val,Lys,Asx,Val,Asx,Asx,Leu,Glx,
Ala,Ser,Met,Ala,Glx,Leu

Chy-15-5-A (αT-10)

Composition	Step	1	2	3	4	5
His		1.00	1.00	1.00	0	0
Asp		1.10	0	0	0	0
Ser		0	0	0	0	0
Ala		0.90	1.03	0.83	1.04	0.35
Leu		1.00	1.05	0.15	0	0
Tyr		0.40	0.92	1.17	0.95	1.00

Sequence : Ser-Asx-Leu-His-Ala-Tyr

D Chy-13-4 (αT-10~11)

Composition		Step 1	2	3	4
Arg	0.96 (1)	1.07	0.89	0.09	0
Asp	2.86 (3)	1.84	2.18	1.89	1.92
Pro	0.87 (1)	0.56	0.87	0.66	0.83
Val	1.68 (2)	1.62	1.65	1.33	1.01
Leu	0.77 (1)	0.93	0.12	0	0
Phe	1.20 (1)	1.16	1.10	1.06	1.08

Sequence : Asx-Leu-Arg-Val,Asp,Pro,Val,Asn,Phe

Chy-4-7-C (αT-11~12)

Composition		Step 1
Lys	0.90 (1)	0
Leu	1.10 (1)	1.00

Sequence : Lys-Leu

Chy-16-5-A (αT-12~13)

Composition		Step 1	2	3	4	5
Lys	0.96 (1)	0.67	0.65	0	0	0
His	0.86 (1)	1.00	1.00	1.00	1.00	1.00
Asp	1.18 (1)	1.07	1.03	1.06	0.36	0
Thr	1.04 (1)	0.99	0.79	0.94	0.96	0.71
Glu	1.03 (1)	0.99	1.02	1.05	1.12	1.00
Pro	0.71 (1)	0.81	0.64	0.70	0.96	0.70
Gly	1.07 (1)	0.98	0.20	0	0	0
Val	0.67 (1)	0.69	0.65	0.68	0.96	0.67
Met	0.90 (1)	0	0	0	0	0
Tyr	0.92 (1)	0.96	0.94	0.96	0.78	0.35

Sequence : Met-Gly-Lys-Asx-Tyr-Thr,Pro,Glx,Val-His

Chy-16-5b (αT-13~14)

Composition		Step 1	2	3	4
Lys	0.94 (1)	0.69	0.65	0.71	0.68
His	0.79 (1)	1.00	1.00	1.00	1.00
Asp	1.18 (1)	1.19	1.03	1.08	1.10
Thr	0.88 (1)	0	0	0	0
Glu	1.02 (1)	0.96	1.06	0.43	0
Pro	0.80 (1)	0.89	0	0	0
Ala	2.19 (2)	2.02	1.94	1.03	1.94
Val	0.87 (1)	0.78	0.75	0.87	0.23
Phe	2.27 (2)	1.87	1.98	2.07	1.99

Sequence : Thr-Pro-Glx-Val,His,Ala,Ala,Phe,Asp,Lys-Phe

E Chy-12-5-B (αT-13~14)

Composition		Step	1	2	3	4	5
Lys	1.00 (1)		1.00	1.00	1.00	1.00	0
Asp	0.98 (1)		1.00	1.06	1.03	0.33	0
Ala	1.90 (2)		1.05	0	0	0	0
Phe	2.12 (2)		1.94	1.95	0.98	1.00	1.00

Sequence : Ala-Ala-Phe-Asp-Lys-Phe

Chy-10-1 (αT-14)

Composition		Step	1	2	3	4	5	6	7
Ser	2.26 (2)		2.00	1.17	1.13	1.09	0.29	0	0
Ala	1.74 (2)		2.28	2.23	1.43	1.23	1.28	0.45	0
Val	1.86 (2)		1.86	1.90	1.98	0.90	0.90	1.05	0
Leu	2.14 (2)		0.88	0.87	0.89	0.76	0.82	0.95	1.00

Sequence : Leu-Ser-Ala-Val-Ser-Ala-Val-Leu

Chy-12-5-A (αT-14~15)

Composition		Step	1	2	3
Lys	1.00 (1)		0.65	0.63	0
Glu	1.09 (1)		1.15	0	0
Ala	0.91 (1)		0	0	0
Tyr	0.68 (1)		0.85	1.00	1.00

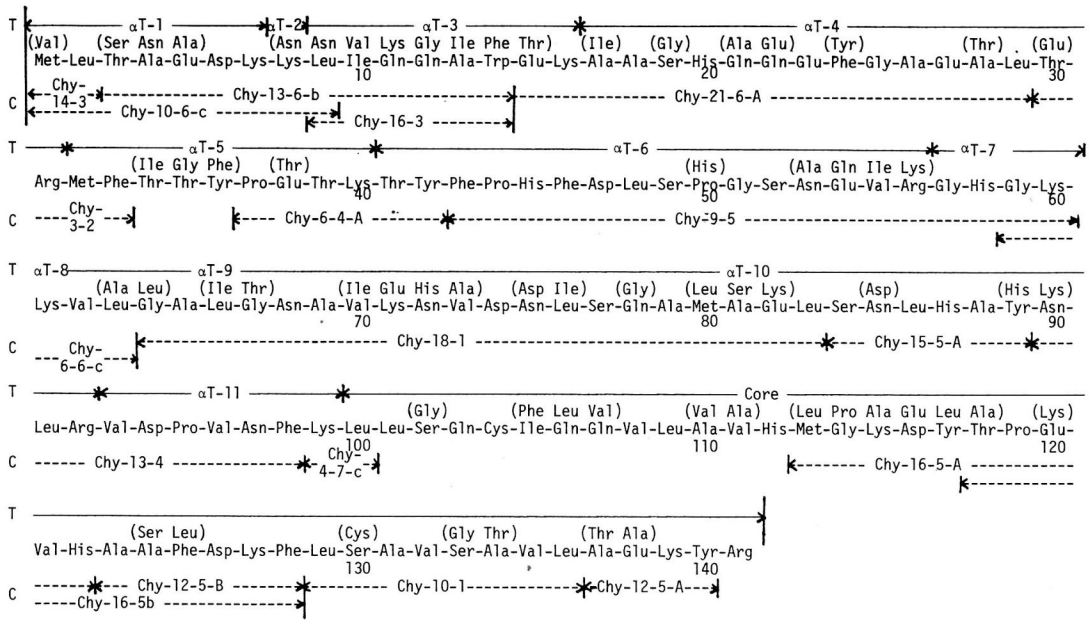
Sequence : Ala-Glx-Lys-Tyr

DISCUSSION

As we already described in the preceding paper,[1] we are interested in comparison of the two hemoglobin molecules within identical species from the view point of molecular evolution and functional properties of hemoglobin.

The amino acid sequence of the tryptic peptide fragments as presented in the preceding paper[1] and that of the chymotryptic peptide fragments as determined here are examined and the whole amino acid sequence of the α chain of the AI component of chicken hemoglobin are constructed as shown in Fig. 3. In this figure the amino acid sequence of the α chain of the AI component is compared with that of AII component and 65 amino acid substitution is indicated. N-terminal amino acid valine of the α chain of AII component involved in alkali Bohr effect, is substituted by methionine in case of α chain of AI component, which is considered to be 'very conservative' substitution according to the classification by Pauling.[13] However a recent study was reported that the hemoglobin AI exhibited a greater Bohr effect than hemoglobin AII.[14] Other sites of amino acid substitution are not considered to be so significantly correlated with the hemoglobin function.

Fig. 3 Amino acid sequence of the α chain of the AI component of chicken hemoglobin.
In this sequence, tryptic and chymotryptic peptides of the α chain are shown respectively as T ⟷ and Chy←--→. Amino acid residues in the α chain of AII hemoglobin which differ from those of AI hemoglobin are shown in parentheses.



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