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Amino Acid Sequence of the α Chain of Chicken AI Hemoglobin
I. Amino Acid Sequence of the Tryptic Peptides of the α Chain

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Adult chicken hemoglobin is heterogeneous containing two major components, A Ⅰ and A Ⅱ[9]. α polypeptide chain of A Ⅰ component was purified and subjected to tryptic digestion for 3 hr. Each tryptic peptide was purified by AG 1×2 column chromatography, Chromo Beads P column chromatography and paper chromatography in sequence and the amino acid sequence of each peptide was studied. A brief report on the results of this study was already presented as a preliminary communication[11]. This communication deals with the details on the structural studies of α polypeptide chain of A Ⅰ component from chicken adult hemoglobin.

INTRODUCTION

It has been shown by many authors so far that avian adult hemoglobin is of heterogeneous nature. Chicken and duck hemoglobins consist of two major components, those from *Pelecaniformes* and *Gruiformes*, of three components and those from *Piciformes* and *Passeriformes*, of two or three. But hemoglobins from *Ciconiiformes*, *Cuculiformes* and some other orders consist of one major component, respectively[3].

This fact draws our attention to the structural and functional difference, evolutional origin and regulatory mechanism of synthesis of these molecular species.

In this paper, amino acid sequence of the tryptic peptides from polypeptide chain of AI hemoglobin was determined and compared with the α polypeptide chain of AII hemoglobin from the same species.

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MATERIALS AND METHODS

(1) Preparation of α Polypeptide Chain of AI Component

Jugular vein of white leghorns was cut and blood was spurted out into a beaker containing 3.8% sodium citrate solution, the volume of which was approximately one forth of the blood volume. After the blood was centrifuged at 3,000 r.p.m. for 5 min, the supernatant was removed with a pipette and the sedimented red blood cells were washed with excess amount of saline solution by mixing the suspension with a pipette. The mixture was centrifuged under the same conditions as described above and the supernatant was removed with a pipette. This washing and centrifugation procedure was repeated three times and the packed red blood cells were hemolyzed according to Drabkin's method[2].

The hemolyzates were centrifuged at 12,000 r.p.m. for 1 hr. at 3°C and the supernatant hemoglobin solution was separated from the precipitates. AI component was purified by CM−cellulose column chromatography according to the method described previously[9].

Cleavage of the AI hemoglobin into α and β polypeptide chain was performed by a modification of the column chromatography method by Matsuda et al.[8] 2.0 g of lyophilized AI hemoglobin were dissolved in 200 ml of 4 M urea−HCl buffer (pH 2.3), 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 HCl and washed with 0.05 N HCl successively. The 4 M urea−HCl buffer (pH 2.3) 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 on to 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 Scienctific 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, dialized against deionized water and lyophilized.

(2) Determination of N−terminal Amino Acid

15 mg of the α polypeptide chain was dinitrophenylated and DNP−derivative α polypeptide chain was obtained, which was hydrolized with twice distilled hydrochloric acid at 105°C for 15 hr. and extracted with ether. The extracts were analyzed by two dimensional paper chromatography. Developer solvent for the first dimension was n−butanol saturated with 2 N NH_4OH and for the second dimension, 1.5 M phosphate buffer (pH 6.0). The yellow spot on the filter paper was compared with that of standard DNP−amino acids and identified.

(3) Determination of C-terminal Amino Acid of the α Polypeptide Chain

2 μM of the α polypeptide chain was dissolved in a 1.5 ml of 0.01 M borate buffer (pH 8.1), to which 0.1 ml of carboxypeptidase B solution was added and the digestion was performed at 37°C for 30 min. 0.2 ml was taken out from the reaction mixture and subjected to amino acid analysis. To the residual solution 0.1 ml of carboxypeptidase A solution was added and the digestion was continued for additional 5, 10, 15, 30, 60, 120 and 210 min. At each interval, 0.2 ml was taken out from the reaction mixture and subjected to amino acid analysis.

(4) Digestion of the Polypeptide Chain with Trypsin

1,500 mg of the α polypeptide chain was dissolved in 150 ml of deionized water and digested with 30 mg of trypsin at pH 9.0, 37°C for 3 hr. The trypsin had been treated beforehand by incubating it in 1/16 HCl overnight at 37°C to minimize chymotryptic activity in the trypsin.

A fraction of peptides was precipitated from the digest solution by adjusting the pH to 6.4 with 5% acetic acid. The precipitates, so called core and the supernatant, soluble tryptic peptides were separated from each other by centrifugation at 5,000 r.p.m. for 20 min and the both fractions were lyophilized.

(5) Digestion of the Longer Tryptic Peptides with Pepsin

Approximately 5~20 μmole of a longer tryptic peptide was dissolved in 0.01 N HCl and 0.5~20 ml of 1 % pepsin solution in 0.05 N HCl was added and pH of the mixture solution was adjusted to 2.0 by adding 0.1 N HCl. The solution was incubated at 37°C for 2~16 hr., to which several drops of 0.1 N NaOH was added and the digest solution lyophilized.

(6) Column Chromatography for Separation of the Tryptic or Peptic Peptides

Column chromatography for separation of the tryptic or peptic peptides was performed according to the method as described previously[5].

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). Soluble tryptic or peptic peptides dissolved in the same volatile buffer (pH 9.6) was applied on to the column.

Other buffers of lower pH were substituted for the starting buffer until the pH of the effluent reached 3.8. Fractionated peptides were detected by 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 (0.9×20 cm) was used for rechromatography of the peptide mixtures and pyridine acetate buffers were used as developing buffers according to the method described previously[5,6,7].

Gel filtration on a Sephadex G−50 column (5.0×150 cm), previously equilibrated with 0.05 M NH_4HCO_3, was used for purification of the core peptide. Amino acid analysis and subtractive Edman degradation were also carried out as described previously[5,6,7].

(7) High Voltage Electrophoresis for Examining the Electric Charge of the Peptides

Electrophoresis were run at 2 k volt for 1.5～2 hr. with pyridine acetate buffer (pH 6.4). Distinction was made between aspartic and glutamic acids and their amides, asparagine and glutamine, by examining electric charge of the peptide before and after Edman degradation of the amino acid in question after electrophoresis.

RESULTS

(1) Separation and Purification of the Tryptic Peptides

The pH of the tryptic digest solution was lowered to 6.4 with 5% acetic acid. The solution, after being allowed to stand overnight in a cold room, was subjected to centrifugation at 5,000 r.p.m. for 20 min. The supernatant, the soluble fraction, was separated from the precipitate, the core, with a pipette and both fractions were lyophilized. 300～400 mg of the soluble fraction, the soluble tryptic peptide mixture was

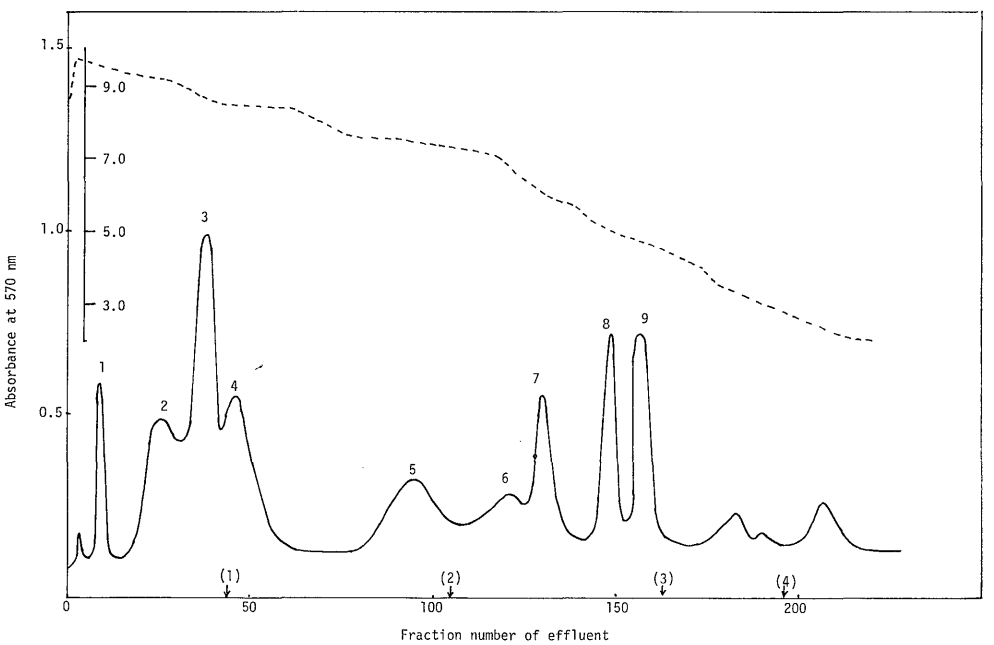


Fig. 1 Column chromatogram of the soluble tryptic peptides from α chain of chicken A I hemoglobin by using AG 1×2 (0.9×50 cm).
The starting buffer, 1 % pyridine collidine acetate (pH 9.5) was substituted by 1 % pyridine collidine acetate buffer (pH 7.0) at (1) on the chart, which was substituted by 0.1 N acetic acid at (2), by 1 N aceticacid at (3) and by 2 N acetic acid at (4).

applied on to an AG 1×2 column (0.9×50 cm) and the column chromatography was run with a starting buffer, 1 % pyridine collidine acetate buffer (pH 9.5) after the column was equilibrated with the same buffer. The buffer was substituted by 1 % pyridine collidine acetate buffer (pH 7.0), 0.1 N acetic acid and 1 N acetic acid solution sequencially until the pH of effluent reached 3.8. The elution pattern of the chromatography was recorded on a recorder by using an automatic peptide analyzer (Technicon Co.). The effluent corresponding to each peak on the chart was pooled, lyophilized and rechromatographed on a Chromo Beads P column with the pyridine

Table 1 Pyridine acetate buffer system for the Chromo Beads P column (0.9×20 cm)
The chamber numbers 1, 2, 3, 4 and 5 are shown on the left column of the table. Pyridine mole concentration and pH are shown on the upper column.

	Pyridine acetate buffer			
	0.05 M (pH 3.1)	0.2 M (pH 3.1)	2.0 M (pH 5.1)	2.0 M (pH 6.5)
1	150 ml	0	0	0
2		150 ml		
3		75 ml	75 ml	
4			150 ml	
5				150 ml

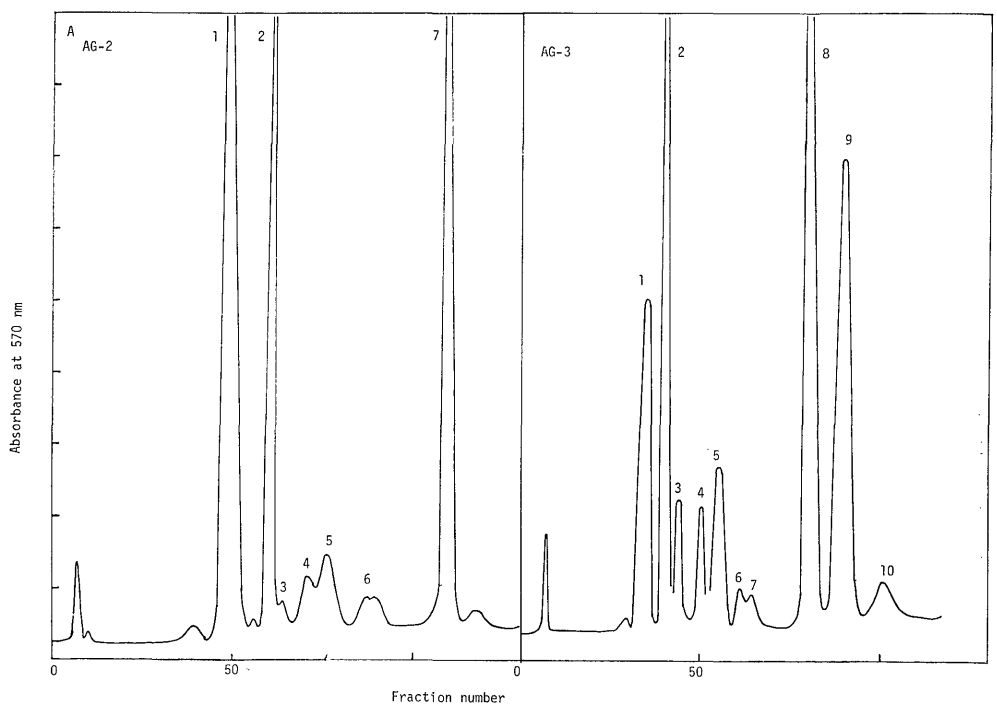
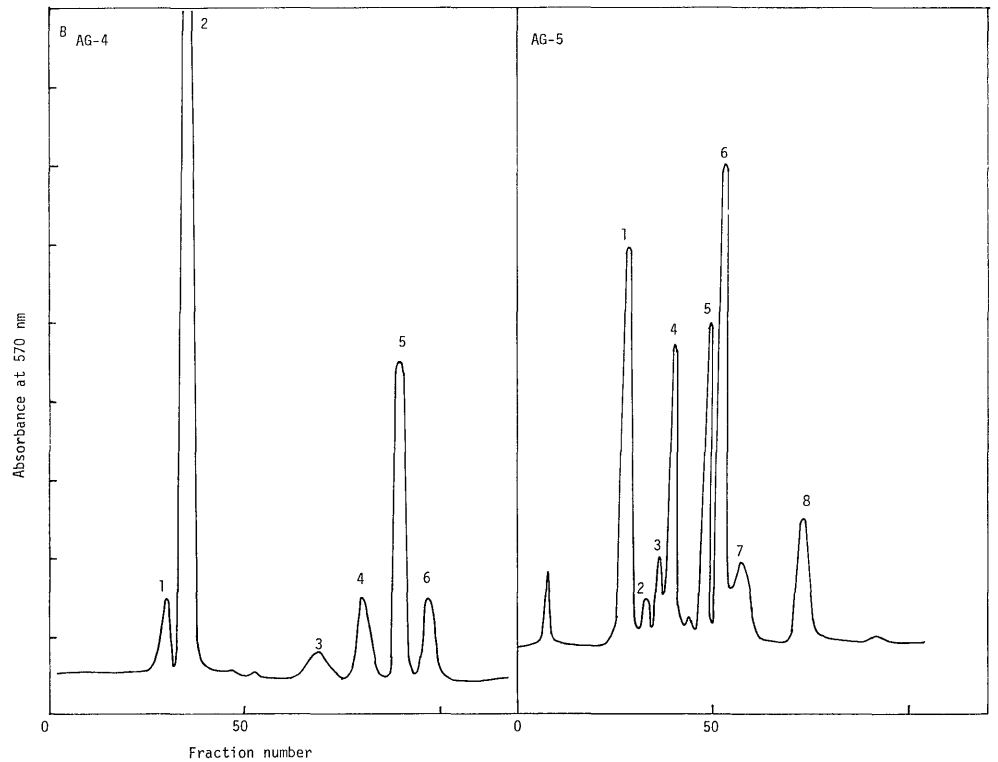
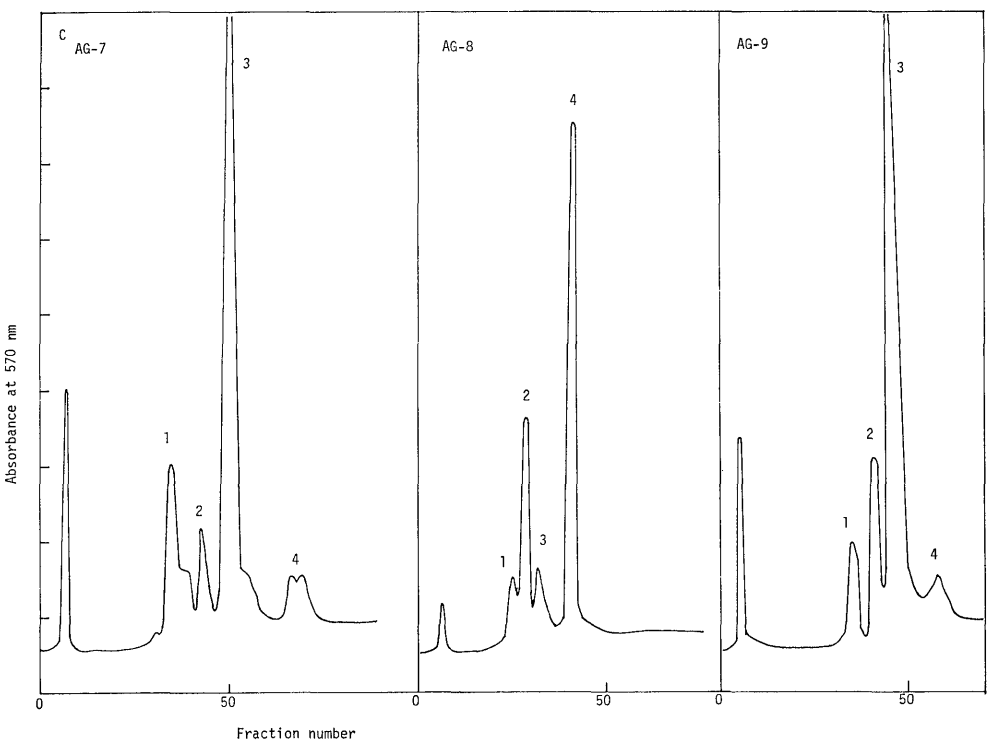


Fig. 2 Rechromatography of the peptides fractionated on a Chromo Beads P (0.9×20 cm) by using automatic peptide analyzer.
The buffer system for developing these columns was shown in Table 1. AG−2, 3, ……, 9 show the peak numbers on the chromatogram in Fig. 1.


B
AG-4
AG-5
Absorbance at 570 nm
Fraction number
0
50
1
2
3
4
5
6
7
8


C
AG-7
AG-8
AG-9
Absorbance at 570 nm
Fraction number
0
50
1
2
3
4

acetate buffer system shown in Table 1. The chromatographic patterns of the fractionated peptides were shown in Figs. 2A, 2B and 2C. All of the efficient peptides purified, together with the core, were analyzed with an automatic amino acid analyzer as shown in Table 2A and 2B.

(2) Amino Acid Sequence of the Shorter Peptides

Amino acid sequence of the shorter peptides was studied by the use of the subtractive Edman degradation as described elsewhere[5,6,7] and carboxypeptidase digestion method described above for determination of C-terminal amino acid of α chain.

(3) Amino Acid Sequence of the Longer Peptides

The longer peptides AG-7-4, AG-8-2 and AG-9-3 were further digested with pepsin and each peptic peptide mixture was chromatographed on a Chromo Beads P

Table 2 Amino Acid Composition of Tryptic Peptides from 3 hour Digests of the α Chain from Chicken Hemoglobin

In the designation like AG−2−2, AG−2−7, ····· on the top of the table, the first number following 'AG−', represents the peak number on the chromatogram of the AG 1×2 column in Fig. 1 and the second number, the peak number on the chromatogram of the Chromo Beads P column in Fig. 2. The integral number in parentheses are supposed integral numbers of the amino acids. Total number on the bottom represents the number of amino acid contained in the peptide. Molar yield (%) is the yield of each peptide in moles against the starting amount of the protein in moles.

A	AG−2−2	AG−2−7	AG−3−1	AG−3−9	AG−4−2	AG−5−4	AG−5−5	AG−5−6
Trp ·····								
$CysO_3H$ ···								
Lys ·····	1	0.97(1)	1.88(2)		1.20(1)	1.25(1)	1.57(2)	1.25(1)
His ·····		1.08(1)						
Arg ·····				1.07(1)				
Asp ·····			1.12(1)			1.93(2)	1.00(1)	1.12(1)
Thr ·····					2.90(3)		0.97(1)	
Ser ·····								
Glu ·····					0.97(1)		1.07(1)	
Pro ·····					1.13(1)	0.95(1)		
Gly ·····		1.94(2)	2.23(2)					2.00(2)
Ala ·····			1.88(2)				0.97(1)	2.07(2)
Val ·····			1.63(2)			1.71(2)		1.67(2)
Met·····					0.71(1)		0.68(1)	
Ile ·····								
Leu ·····			1.86(2)				1.05(1)	1.93(2)
Tyr ·····				0.93(1)	0.87(1)			
Phe ·····					0.93(1)	1.15(1)		
Total Number	1	4	11	2	9	7	8	10
Molar Yield(%)	16	29	4	17	11	14	15	5

B	AG−7−4	AG−8−1	AG−8−2	AG−9−2	AG−9−3	Core
Trp ·····		(+)				
$CysO_3H$ ···						(+)
Lys ·····		0.93(1)		1.03(1)		2.93(3)
His ·····	1.04(1)		1.24(1)		1.17(1)	1.79(2)
Arg ·····	0.97(1)		1.11(1)		1.05(1)	0.96(1)
Asp ·····	2.07(2)		5.36(5)	1.00(1)		3.40(3)
Thr ·····	1.03(1)			1.03(1)	1.04(1)	1.64(2)
Ser ·····	1.83(2)		1.88(2)		0.98(1)	2.37(3)
Glu ·····	1.04(1)	3.12(3)	2.18(2)	1.06(1)	3.42(3)	3.64(4)
Pro ·····	1.97(2)					2.05(2)
Gly ·····	1.07(1)				1.56(2)	1.74(2)
Ala ·····		1.11(1)	2.88(3)	0.91(1)	3.61(4)	4.92(5)
Val ·····	0.89(1)		1.06(1)			3.48(4)
Met ·····			0.73(1)	0.64(1)		0.84(1)
Ile ·····		0.89(1)				0.83(1)
Leu ·····	0.99(1)	0.98(1)	4.55(5)	1.00(1)	0.96(1)	4.96(5)
Tyr ·····	0.94(1)		0.82(1)			1.33(1)
Phe ·····	2.04(2)				1.00(1)	2.26(2)
Total Number	16	8	21	7	15	42
Molar Yield(%)	1	48	57	18	30	60

column using pyridine acetate buffer as described above. Effluent corresponding to each peak was lyophilized, further purified by paper chromatography. The developer solvent for the paper chromatography was a mixture of n−butanol, acetic acid and water (4 : 1 : 1). Peptide spots developed on the filterpaper were sprayed with ninhydrin and stained at 100°C. The paper chromatograms were shown on the upper right

Table 3 Edman degradation and carboxypeptidase digestion of the shorter peptides
Underlined part shows the amino acid subtracted. The arrow [⟶] represents Edman degradation and the arrow [⟵], carboxypeptidase digestion. α T−1, 1+2, 3,····· in parentheses represent tryptic peptide order from the N−terminus supposed when compared to the amino acid sequence of the whole α chain of All hemoglobin.

AG−9−2	Composition	Step 1	2	3	4	5
	Lys 1.03					
	Asp 1.00	1.10	1.06	1.03	0.98	1.00
	Thr 1.03	0.90	0.95	trace	0	0
	Glu 1.06	1.11	1.07	1.03	1.01	0.71
	Ala 0.91	0.98	0.89	0.95	trace	0
	Met 0.64	0	0	0	0	0
	Leu 1.00	1.01	trace	0	0	0

Sequence : Met−Leu−Thr−Ala−Glx−Asx−Lys (αT−1)

AG−5−5

Composition	Step	1	2	3	4	5
Lys 1.57						
Asp 1.00		1.02	1.05	1.00	0.97	1.00
Thr 0.97		0.98	0.95	0	0	0
Glu 1.07		1.08	1.17	1.08	1.03	0.62
Ala 0.97		0.99	0.83	0.92	trace	0
Met 0.68		0	0	0	0	0
Leu 1.05		0.93	trace	0	0	0

Sequence : Met−Leu−Thr−Ala−Glx−Asx−Lys−Lys (αT−1+2)

AG−8−1

Composition	Step	1	2	3	4	5
Lys 1.13		0.98	1.00	1.00	1.00	1.00
Glu 3.06		3.18	3.09	2.42	1.72	1.00
Ala 1.02		0.97	0.91	1.00	1.00	0.35
Ile 0.82		0.86	0	0	0	0
Leu 0.96		0.42	0	0	0	0

Sequence : Leu−Ile−Glx−Glx−Ala, Trp, Glx, Lys (αT−3)

AG−4−2

Composition	Step	1	2	3	4	5
Lys 1.20						
Thr 2.90		2.97	2.89	2.06	1.69	1.50
Glu 0.97		1.15	1.16	1.03	1.05	1.10
Pro 1.13		0.92	0.97	0.94	0.95	0.93
Met 0.71		0	0	0	0	0
Tyr 0.87		1.01	1.01	0.97	0.87	0.61
phe 0.93		0.95	0.17	0.14	0.18	0

Sequence : Met−Phe−Thr−Thr−Tyr−(Pro, Glx) −Thr−Lys (αT−5)

AG−2−7

Composition	Step	1	2	3
Lys 0.97		0.80	0.78	1.00
His 1.08		1.20	0.14	0.24
Gly 1.94		1.48	1.22	0.62

Sequence : Gly−His−Gly−Lys (αT−7)

AG−3−1

Composition	Step	1	2	3	4	5	6	7
Lys 1.88		0.64	1.00	1.00	1.00	1.00	1.00	1.00
Asp 1.12		1.04	0.97	1.03	1.00	0.96	0.94	0.92
Gly 2.00		1.95	1.96	1.89	1.07	1.05	0.99	0.53
Ala 2.07		2.13	2.08	2.10	1.96	1.14	1.08	1.19
Val 1.67		2.00	1.10	0.95	0.87	0.74	0.77	0.90
Leu 1.93		1.87	1.87	1.07	1.07	0.99	0.26	0

Sequence : Lys−Val−Leu−Gly−Ala−Leu−Gly, Asn, Ala, Val, Lys (αT−8+9)

AG−5−6 Composition	Step	1	2	3	4	5	6	7
Lys 1.25		1.00	1.00	1.00	1.00	1.00	1.00	1.00
Asp 1.12		1.17	1.11	1.07	1.01	1.06	1.03	0.37
Gly 2.23		2.19	2.08	1.55	1.18	1.12	0.60	0.37
Ala 1.88		1.81	1.86	1.90	1.08	1.00	0.97	1.00
Val 1.63		0.88	0.96	0.99	0.84	0.81	0.71	0.99
Leu 1.86		1.99	1.06	1.05	0.90	trace	0	0

Sequence : Val−Leu−Gly−Ala−Leu−Gly−Asn−Ala−Val−Lys (αT−9)

AG−5−4 Composition	Step	1	2	3	4	5
Lys 1.25						
Asp 1.93		2.09	1.12	1.03	1.00	0.72
Pro 0.95		0.83	0.77	0.32	0	0
Val 1.71		1.01	0.94	0.96	0.26	0.27
Phe 1.15		1.07	1.06	1.01	0.99	1.00

Sequence : Val−Asx−Pro−Val−Asp−Phe−Lys (αT−11)

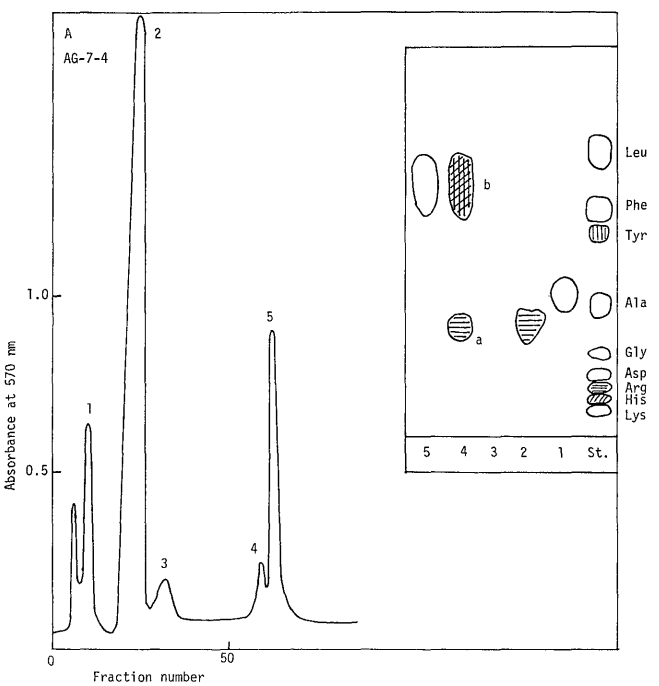
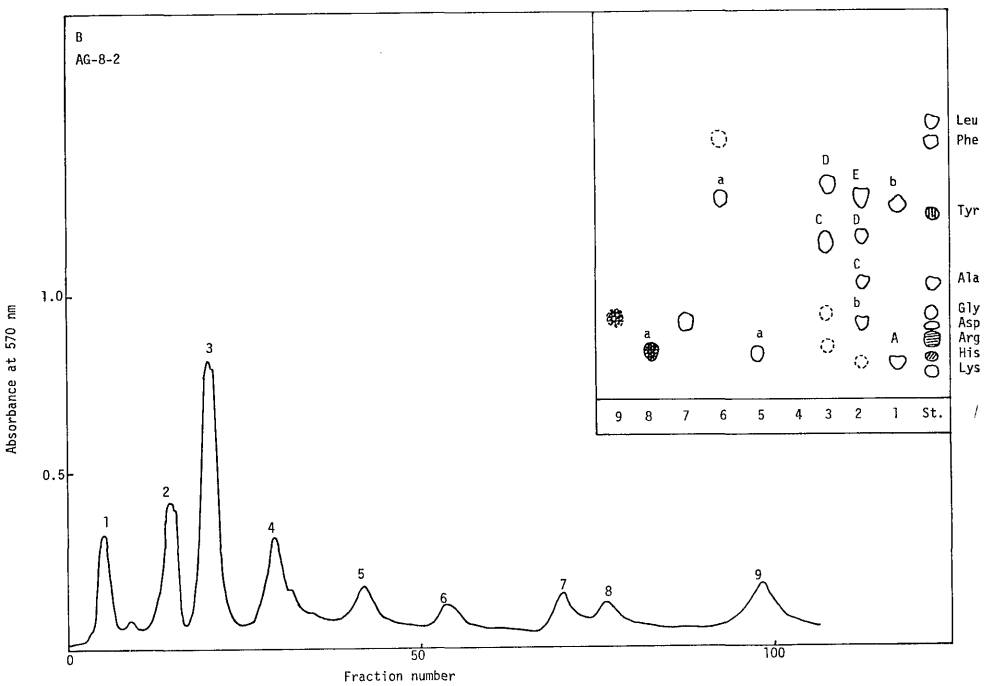
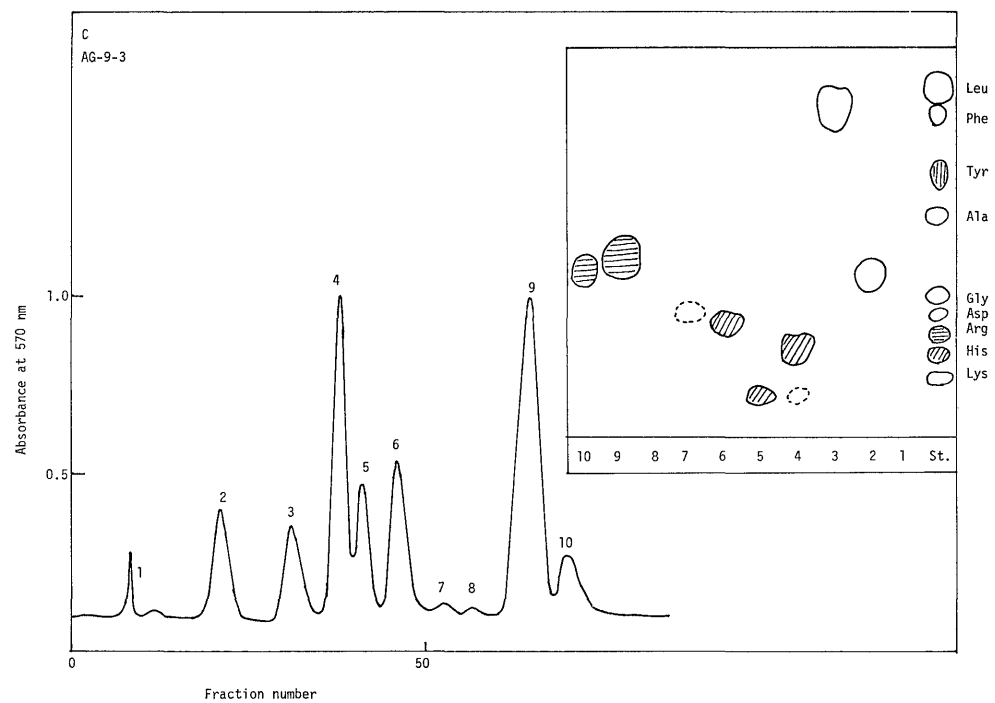


Fig. 3 Column chromatogram of the peptic peptides AG−7−4, AG−8−2 and AG−9−3 on a Chromo Beads P column (0.9×20 cm, on the lower left) and paper chromatogram of the effluent corresponding to each peak on the chart (on the upper right). The diagonally lined spots show positive Pauli reaction, transversely lined ones, positive Sakaguchi reaction and vertically lined ones, positive reaction for tyrosine (α-nitroso-β-naphthol reaction), respectively.


B
AG-8-2
Absorbance at 570 nm
1.0
0.5
0
50
100
Fraction number
1
2
3
4
5
6
7
8
9
Leu
Phe
Tyr
Ala
Gly
Asp
Arg
His
Lys
9 8 7 6 5 4 3 2 1 St.


C
AG-9-3
Absorbance at 570 nm
1.0
0.5
0
50
Fraction number
1
2
3
4
5
6
7
8
9
10
Leu
Phe
Tyr
Ala
Gly
Asp
Arg
His
Lys
10 9 8 7 6 5 4 3 2 1 St.

of the charts. Each peptic peptide was analyzed with an automatic amino acid analyzer and amino acid sequence study performed according to the method described above, was shown in Fig. 4, 5, 6, and Table 4, 5.

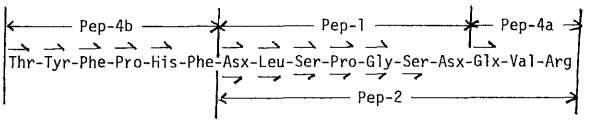


Fig. 4 Sequence of a longer tryptic peptide, AG−7−4 (α T−6)
Sequence of the peptic peptides was determined and they were assembled to a longer peptide.

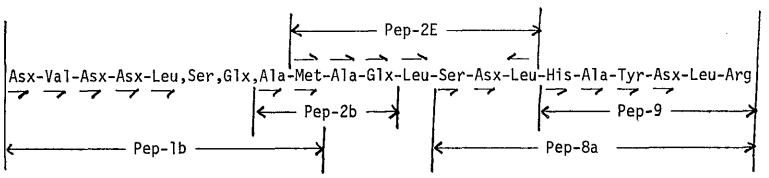


Fig. 5 Sequence of a longer tryptic peptide, AG−8−2 (α T−10)
Sequence of the peptic peptides was determined and they were assembled to a longer peptide.

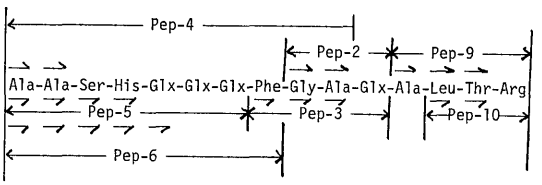


Fig. 6 Sequence of a longer tryptic peptide, AG−9−3 (α T−4)
Sequence of the peptic peptides was determined and they were assembled to a longer peptide.

Table 4 Edman degradation and carboxypeptidase digestion of the peptic peptides from AG−8−2 (α T−10)

Pep−1b	Composition	Step 1	2	3	4	5
	Asp 3.03 (3)	2.08	2.19	1.08	0.54	0.48
	Ser 0.91 (1)	0.94	0.89	0.81	0.92	1.08
	Glu 1.09 (1)	1.15	1.02	1.10	1.10	1.07
	Ala 0.94 (1)	0.86	0.79	0.97	1.04	0.85
	Val 0.97 (1)	0.93	0.18	0	0	
	Met 0.55 (1)	0.51	+	+	+	
	Leu 1.04 (1)	1.04	1.06	1.15	0.90	0.56

Sequence : Asx−Val−Asx−Asx−Leu, Glx, Ala, Ser, Met

Pep−2b

Composition	Step 1	2
Glu 1.02 (1)	1.06	0.99
Ala 1.98 (2)	0.94	1.01
Met 0.91 (1)	0.70	0.49

Sequence : Ala−Met, Ala, Glx

Pep−2E

Composition	Step 1	2	3	4
Asp 1.00 (1)	1.03	1.09	1.02	1.15
Ser 0.83 (1)	0.91	0.93	0.81	0.85
Glu 1.00 (1)	1.07	1.13	0.33	0.21
Ala 0.73 (1)	0.84	0.14	0	0
Met 0.66 (1)	0	0	0	0
Leu 2.16 (2)	2.09	1.85	1.91	1.00

Sequence : Met−Ala−Glx−Leu, Ser, Asx, Leu

Pep−8a

Composition	Step 1	2
His 1.02 (1)	1.10	0.90
Arg 1.11 (1)	1.18	1.06
Asp 1.93 (2)	1.73	1.09
Ser 0.96 (1)	0	0
Ala 0.98 (1)	0.90	1.00
Leu 2.12 (2)	2.12	1.94
Tyr 0.65 (1)	0.91	+

Sequence : Ser−Asx, Leu, His, Ala, Tyr, Asx, Leu, Arg

Pep−9

Composition	Step 1	2	3	4
His 1.00 (1)	0			
Arg 1.00 (1)	1.00	0.99	1.12	1.12
Asp 0.95 (1)	0.99	1.03	0.86	0.18
Ala 0.93 (1)	0.83	0	0	0
Leu 1.09 (1)	1.09	1.08	1.02	0.88
Tyr 1.03 (1)	1.08	0.90	0.12	0

Sequence : His−Ala−Tyr−Asx, Leu, Arg

Table 5 Edman Degradation of Peptic Peptides from AG−9−3(α T−4)

Pep−2

Composition		Step 1	2
Glu	0.81	0.98	1.00
Gly	1.12	0.31	0
Ala	1.07	1.02	0.57

Sequence : Gly−Ala−Glx

Pep−3

Composition		Step 1	2	3
Glu	0.71	1.04	1.06	1.00
Gly	1.15	1.02	0	0
Ala	1.05	0.95	0.94	0.44
Phe	1.03	0	0	0

Sequence : Phe−Gly−Ala−Glx

Pep−4

Composition		Step 1	2
His	0.90	0.88	1.16
Ser	0.78	0.75	0.71
Glu	3.01	2.98	3.05
Gly	1.10	1.00	0.99
Ala	2.35	1.50	0.95
Phe	0.88	0.70	0.91

Sequence : Ala−Ala,Ser,His,Glx,Glx,Glx,Phe,Gly,Ala

Pep−5

Composition		Step 1	2	3	4
His	1.10	0.98	0.81	1.00	0
Ser	1.01	0.90	0.83	0	0
Glu	2.93	3.10	3.17	3.50	3.00
Ala	2.07	1.03	0	0	0

Sequence : Ala−Ala−Ser−His,Glx,Glx,Glx

Pep−6

Composition		Step 1	2	3	4	5
His	1.23	1.20	1.25	1.10	0	0
Ser	0.94	0.97	0.81	0	0	0
Glu	2.80	3.04	3.29	3.15	3.12	2.76
Ala	1.87	1.04	0	0	0	0
Phe	1.15	0.99	0.89	0.96	0.86	1.00

Sequence : Ala−Ala−Ser−His−Glx,Glx,Glx,Phe

Pep−9	Composition	Step 1	2	3
	Arg 1.47	1.10	1.15	1.00
	Thr 1.01	1.03	0.98	0.15
	Ala 0.94	0	0	0
	Leu 1.05	0.90	0	0

Sequence : Ala−Leu−Thr−Arg

Pep−10	Composition	Step 1	2
	Arg 0.86	1.00	1.00
	Thr 1.05	1.01	0.25
	Leu 1.08	0	0

Sequence : Leu−Thr−Arg

(4) Amino Acid Sequence of the Core Peptide

Core peptide purified was oxidized with performic acid prior to peptic digestion according to Hirs's method[4]. Peptic digestion was performed for 2 hr. as described above. The peptic digests were chromatographed on an AG 1×2 column by the use of the volatile buffers as described above. The elution pattern of the chromatography is shown in Fig. 7. The effluent corresponding to each peak on the chart was rechroma-

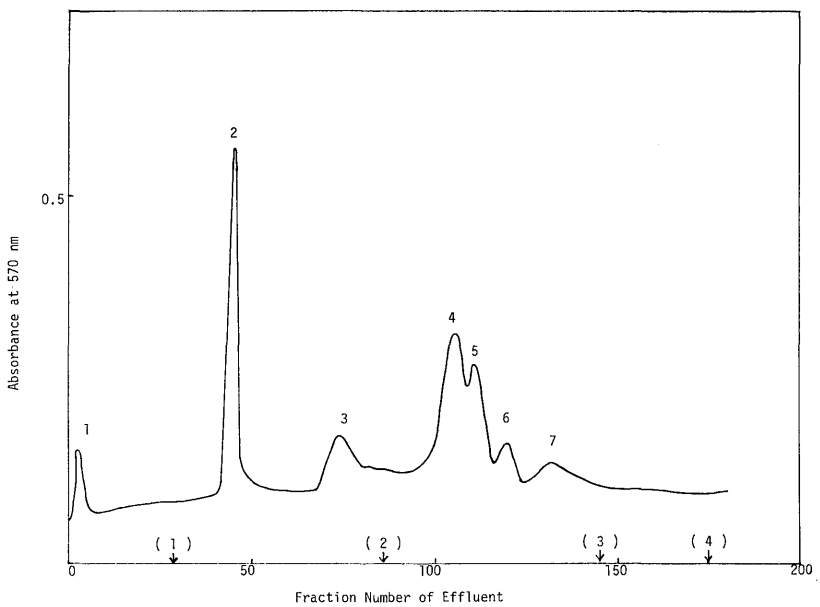


Fig. 7 Separation of the peptic peptides from the core oxidized with performic acid by using AG 1×2 column chromatography
(1), (2), (3) and (4) at the bottom of the chromatogram show the positions of buffer substitution. The buffer system used is the same as described in Fig. 1.

tographed on a Chromo Beads P column as described above. The peptic peptides thus purified were analyzed by using the automatic amino acid analyzer as described above and the amino acid sequence of each peptide was studied.

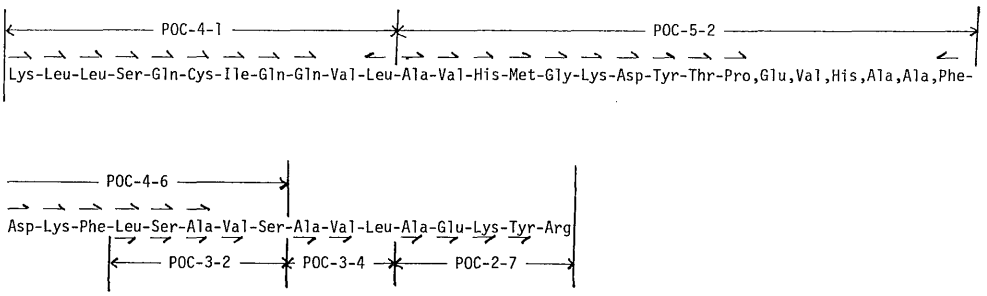


Fig. 8 Whole sequence of so called core from the α chain
Sequence of the peptic peptides from the core was determined and they were assembled to a long peptide, core.

Table 6 Edman degradation and carboxypeptidase degestion of the purified peptic peptides from so called core

POC−4−1	Composition	Step 1	2	3	4	5	6	7	3	9
$CysO_3H$	0.94	1.15	0.93	1.04	1.14	1.00	0.62	0	0	0
Lys	1.09	0	0	0	0	0	0	0	0	0
Ser	1.00	1.02	1.05	1.03	0	0	0	0	0	0
Glu	2.45	2.57	2.20	2.65	2.22	1.98	2.08	1.84	1.35	0.99
Val	0.91	0.66	0.82	0.95	0.88	1.09	0.94	0.91	0.97	0.91
Ile	0.90	0.75	0.72	0.77	0.83	0.85	0.82	0.70	0	0
Leu	2.93	2.83	1.37	0.97	0.92	0.94	1.18	1.26	1.03	1.10

Sequence : Lys−Leu−Leu−Ser−Gln−Cys−Ile−Gln−Gln−Val−Leu

POC−5−2	Composition	Step 1	2	3	4	5	6	7	8	9	10
Lys	1.06	0.77	1.00	0.67	0.59	0.63	0	0	0	0	0
His	1.23	1.21	2.00	1.00	0.91	1.04	1.00	1.00	1.00	0.86	0.96
Asp	1.01	1.01	1.10	1.10	1.14	1.01	1.06	trace	0	0	0
Thr	0.95	0.88	0.95	0.98	0.98	0.92	0.75	0.72	0.80	trace	0
Glu	0.99	1.04	1.13	1.10	1.16	1.06	0.83	0.88	1.01	1.02	1.05
Pro	1.21	+	1.28	1.43	1.14	1.02	0.60	+	1.00	1.08	trace
Gly	0.99	1.12	0.98	0.96	0.92	0.49	0	0	0	0	0
Ala	2.53	1.79	1.99	2.02	2.12	1.97	1.85	1.93	1.76	2.02	1.97
Val	1.26	1.35	0.84	1.05	0.99	0.99	0.79	0.68	0.79	0.68	1.07
Met	1.10	0.84	0.83	0.88	0.35	0	0	0	0	0	0
Tyr	0.73	0.95	0.86	0.77	0.87	0.72	0.66	0.61	0.39	0	0
Phe	0.71	1.01	1.01	0.92	1.06	0.77	0.94	1.09	0.91	0.88	0.91

Sequence : Ala−Val−His−Met−Gly−Lys−Asp−Tyr−Thr−Pro−Glu,Val,His,Ala,Ala,Phe

POC−4−6

Composition		Step 1	2	3	4	5	6
Lys	0.98	1.00	0	0	0	0	0
Asp	0.94	0.40	0	0	0	0	0
Ser	1.56	1.77	1.54	1.72	1.45	1.19	1.10
Ala	1.01	1.10	0.98	1.04	0.91	1.08	0.71
Val	1.02	0.93	0.99	0.95	1.09	0.92	0.96
Leu	1.11	1.20	1.10	1.01	0.44	0	0
Phe	0.94	0.99	0.93	0.31	0	0	0

Sequence : Asp→Lys→Phe→Leu→Ser→Aa, Val, Ser

POC−3−2

Composition		Step 1	2	3	4
Ser	1.26	1.21	0.65	1.00	1.00
Ala	0.93	0.98	1.00	0	0
Val	1.11	1.00	0.99	0.74	0
Leu	0.95	0	0	0	0

Sequence : Leu→Ser→Ala→Val→Ser

POC−3−4

Composition		Step 1	2
Ala	0.99	0	0
Val	0.96	1.00	0
Leu	1.05	0.99	1.00

Sequence : Ala→Val→Leu

POC−2−7

Composition		Step 1	2	3	4
Lys	1.08	0.61	0.45	0	0
Arg	0.91	1.25	1.11	1.09	1.00
Glu	1.00	1.05	0	0	0
Ala	0.90	0	0	0	0
Tyr	1.09	0.95	0.89	0.91	0.15

Sequence : Ala→Glu→Lys→Tyr→Arg

DISCUSSION

Authors were interested in comparison of the two hemoglobin molecules within identical species from the view point of molecular evolution and functional properties of hemoglobin. Homology was already shown between α chains of chicken AII hemoglobin and human hemoglobin with the substitution of 35 amino acid residues[1]. No significant change will be considered in alkaline Bohr effect, although the N−terminal amino acid valine in α chain of AII hemoglobin is substituted by methionine in that of AI hemoglobin because of the similarity of the two amino acids[10].

Since so called core was cysteine containing peptide, difficulty was encountered in purification and amino acid sequence study without any modification of the peptide.

After confirmed that the peptide contained no tryptophan by Ehrlich reaction, oxidation of cysteine residue with performic acid was performed prior to the analysis. One cysteine residue was detected in the core located in No. 104 residue. This cysteine is considered to be nonreactive buried in a crevice between the α_1 and β_1 chains[10].

Tentative whole sequence of α chain of AI hemoglobin in comparison with that of AII hemoglobin is shown in Fig. 9. Complete sequence of the whole α chain will be reported in the following paper.

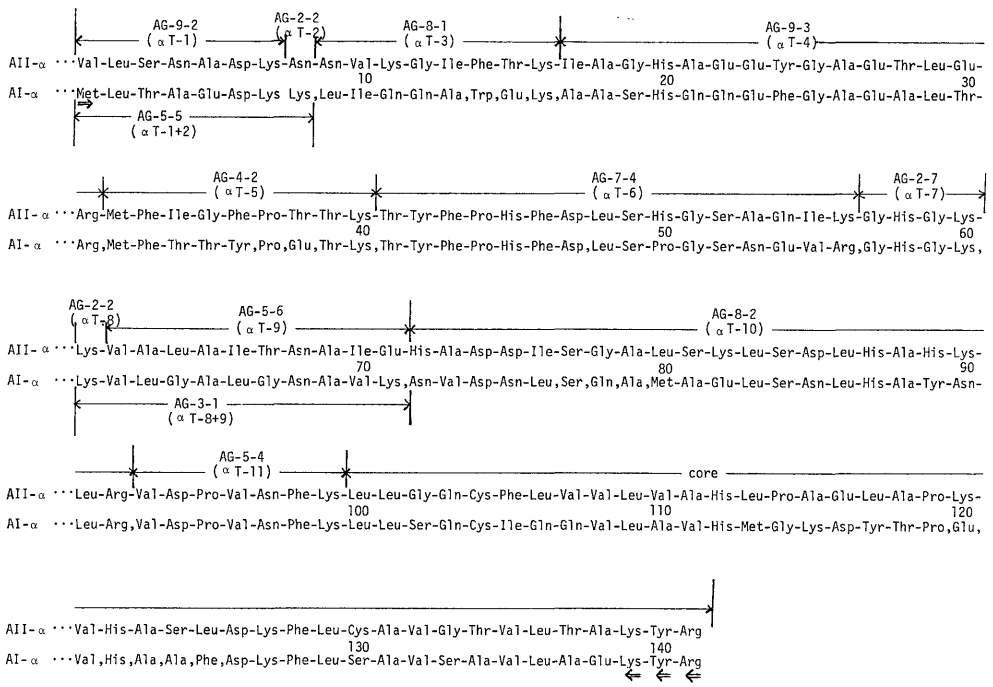


Fig. 9 Tentative order of the tryptic peptides in the whole sequence of α chain of A I hemoglobin in comparison with α chain of A II hemoglobin.
In the figure, dash [—] shows the sequence already determined, comma [,], the sequence not yet determined, arrow [⇨], N—terminal amino acid determined by dinitrophenylation and arrow [⇦], C—terminal amino acid determined by carboxypeptidase digestion.

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