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## Comparative Biochemistry of Hemoglobins

### IV. A Study on the $\alpha$ - and $\beta$ -Polypeptide Chains from Components A<sub>I</sub> and A<sub>II</sub> of Chicken Hemoglobin by the Fingerprinting Method

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Chicken hemoglobin contains two kinds of main components, A<sub>I</sub> and A<sub>II</sub>, and their genetic control mechanism in the red blood cells interests us much. Each of these two components consists of two  $\alpha$ - and two  $\beta$ -polypeptide chains. As a basic experiment for the study of the primary structure of these  $\alpha$ - and  $\beta$ -chains in chicken hemoglobin, the author identified the tryptic peptides obtained from these components and from the chains composing these components by the so-called fingerprinting method. First, component A<sub>I</sub> and A<sub>II</sub> were purified by column chromatography with the salt concentration gradient of phosphate buffer using carboxymethyl cellulose as the adsorbent, and subsequently, the  $\alpha$ - and  $\beta$ -polypeptide chains were isolated from these components by column chromatography by the elution with urea-formic acid solution using CG-50 (type II) as the adsorbent. The obtained components A<sub>I</sub> and A<sub>II</sub>, and the  $\alpha$ - and  $\beta$ -polypeptide chains from these components were hydrolyzed with trypsin, and every variety of tryptic digests thus obtained was identified by the fingerprinting method.

## INTRODUCTION

Out of the interests in the relationship between the structure and the function in hemoglobin or in the problem of the evolution in the hemoglobin molecule, comparative studies are recently being made on the structures of the hemoglobins from various species of animals. Concerning chicken hemoglobin, SAHA et al.<sup>10), 12)</sup>, conducting filter-paper electrophoresis with barbital buffer, reported that it had two kinds of main components and that the rate of their contents varied according to the age of the chickens. VAN DER HELM and HUISMAN<sup>16)</sup> separated two main components of chicken hemoglobin by the method

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of column chromatography using Amberlite IRC-50 as the adsorbent and citrate buffer as the developer, and recognized a clear difference between the amino acids compositions of these two components, a remarkable difference especially in the contents of the basic and acidic amino acids contained in them. Later, SAHA<sup>11)</sup> also separated two components from chicken hemoglobin, examined the amino acid compositions of the two components A<sub>I</sub> and A<sub>II</sub> and found a difference between them. The contents of the individual amino acids, however, are not necessarily consistent in the experimental results of VAN DER HELM et al. and of SAHA. Furthermore, MANWELL et al.<sup>4)</sup> compared the tryptic digests from components A<sub>I</sub> and A<sub>II</sub> by the fingerprinting method and suggested that, though components A<sub>I</sub> and A<sub>II</sub> are quite different from each other, the structures of certain parts of these proteins are common to the two components. Again, SAHA<sup>11)</sup>, also comparing the tryptic digests from components A<sub>I</sub> and A<sub>II</sub> using column chromatography, recognized partial commonness as well as difference in the structure between these two components in agreement with the report of MANWELL et al.<sup>4)</sup>. Judging from the above-mentioned various experimental results, it may be doubtless that the structures of components A<sub>I</sub> and A<sub>II</sub> are different from each other.

Next, relating to the subunits which compose chicken hemoglobin, MASRI and SINGER<sup>5)</sup> reported that one molecule of chicken hemoglobin had only two N-termini, and OZAWA and SATAKE<sup>9)</sup> also recognized only Val-Leu as an N-terminal peptide. And, TAKE<sup>15)</sup> considered, from the fact that the subunits  $\alpha$  and  $\beta$  could not be dissociated by filter-paper electrophoresis in the presence of urea or the fractional precipitation method with trichloroacetic acid, and from the results of other experiments, that chicken hemoglobin might be combined in series in order of  $\alpha$ -polypeptide chain  $\beta$ -polypeptide chain. On the other hand, SAHA and GHOSH<sup>13)</sup>, who assumed that component A<sub>I</sub> consists of the  $\alpha$ <sub>I</sub>- and  $\beta$ <sub>I</sub>-chains, and component A<sub>II</sub> of the  $\alpha$ <sub>II</sub>- and  $\beta$ <sub>II</sub>-chains, conducted a hybridization experiment between these components A<sub>I</sub> and A<sub>II</sub>, and reported that these two components had one each polypeptide chain common to them. SCHNEK et al.<sup>14)</sup>, examining them by the ultracentrifuging method, electrophoresis and N-terminal amino acid analysis, supposed that one component consisted of two kinds of subunits and the other component consisted of four subunits of either the same kind or two kinds which could not be distinguished by electrophoresis or chromatography. MATSUDA and TAKEI<sup>8)</sup> separated components A<sub>I</sub> and A<sub>II</sub> by column chromatography in salt concentration gradient of phosphate buffer using CM-cellulose as the adsorbent. And MATSUDA et al.<sup>7)</sup>, performing N-terminal analysis of components A<sub>I</sub> and A<sub>II</sub> by the DNP-method, recognized 2 moles of Val-His per one mole of hemoglobin from component A<sub>I</sub> and 2 moles of Val-Leu and 2 moles of Val-His per

one mole of hemoglobin from component A<sub>II</sub>, and therefore assumed that component A<sub>I</sub> consisted of two polypeptide chains with N-termini masked, and two polypeptide chains with Val-His N-termini and that component A<sub>II</sub> consisted of two polypeptide chains with Val-Leu N-termini and two polypeptide chains with Val-His N-termini. Later, MATSUDA et al.<sup>6)</sup> isolated each of the  $\alpha$ - and  $\beta$ -subunits contained in components A<sub>I</sub> and A<sub>II</sub> by column chromatography using Amberlite CG-50 (type II) as the adsorbent and urea-formic acid solution as the developer. SAHA<sup>11)</sup> also separated these subunits and compared their amino acid compositions.

The present author, for a basic study for the investigation of the primary structures of these four kinds of polypeptide chains, identified tryptic peptides obtained from the  $\alpha$ - and  $\beta$ -polypeptide chains contained in components A<sub>I</sub> and A<sub>II</sub> of chicken hemoglobin by the fingerprinting method.

## MATERIALS AND METHODS

### 1) Preparation of Chicken Hemoglobin Solution

Hemoglobin solution was prepared from the blood obtained from adult white leghorns according to DRABKIN's method<sup>2)</sup>. That is, the red blood cells were separated from the plasma by centrifuging the blood at 1,000–2000 r.p.m. for 5 min, and washed by 5 volumes of 0.9% physiological saline solution three times. These red blood cells were hemolyzed by adding 2 volumes of distilled water and 0.4 volume of toluene and centrifuged at 15,000 r.p.m. for 1 hr. The middle layer in the centrifuge tube was the hemoglobin solution, which was taken out and used for experiments after being dialyzed against distilled water for 48 hr.

### 2) Separation and Purification of Components A<sub>I</sub> and A<sub>II</sub> from Chicken Hemoglobin.

Components A<sub>I</sub> and A<sub>II</sub> were separated and purified according to the method of MATSUDA and TAKEI<sup>8)</sup>. That is, 200g of carboxymethyl cellulose (Brown, 0.8 meq/g) were first washed with 5 L of acetone, and after addition of 1 N HCl and stirring for 30 min, it was filtered in vacuum and washed with about 9 L of distilled water. Subsequently, 3 L of 1 N NH<sub>4</sub>OH were added to the carboxymethyl cellulose, and filtered off after 30 minutes' stirring, and the carboxymethyl cellulose was washed with about 9 L of distilled water. Again, 1 N HCl was added to it and filtered off in vacuum after 30 minutes' stirring, the carboxymethyl cellulose was washed with about 9 L of distilled water. After that, the carboxymethyl cellulose was adjusted to pH 6.9 with 1 N NH<sub>4</sub>OH and washed with 20 L of M/100 phosphate buffer (pH 6.9). The carboxymethyl cellulose thus activated was packed into a 2×30 cm column and equilibrated with M/100 phosphate buffer (pH 6.9). On

the other hand, 4 cc of hemoglobin solution (containing approximately 60 mg of hemoglobin) were put on to the column, and after the hemoglobin was adsorbed in the column, the column was washed with three 1 cc portions of M/100 phosphate buffer. Then, 15 cc of M/100 phosphate buffer were added on to the column and the elution was made with the salt concentration gradient from M/100 phosphate buffer (pH 6.9) to M/5 phosphate buffer (pH 6.9). The amount of the M/100 phosphate buffer which was placed in the mixing bottle was 250 cc. The elution was carried out in a cold room at 0 - 5°C. The flow rate was set approximately 20 cc per hr, and the eluate was collected in 20 cc fractions. The concentration of the hemoglobin solution in every fraction was measured by the HITACHI spectrophotometer EPU-2 at 542m $\mu$ . The three peaks obtained were designated A<sub>I</sub>, A<sub>II</sub> and A<sub>III</sub>, respectively, and fractions A<sub>I</sub> and A<sub>II</sub> were collected separately and after they were dialyzed against distilled water for 48 hr, they were lyophilized. Fractions A<sub>I</sub> and A<sub>II</sub> obtained in this way were repurified in all the same method and used for the below experiments.

### 3) Preparation of Globin from Components A<sub>I</sub> and A<sub>II</sub>

The hemes were removed from the separated components A<sub>I</sub> and A<sub>II</sub> by the method of ANSON and MIRIK<sup>11</sup>. That is an acetone solution containing 2% HCl was cooled at -15°C and after addition of 1/10 volume of 10% hemoglobin solution and stirring for 20 min, the precipitated globin was separated by centrifuging. The globin obtained was washed with cooled acetone and dissolved in distilled water and after one more repetition of the same treatment, it was lyophilized.

### 4) Separation of Components A<sub>I</sub> and A<sub>II</sub> into their Subunits

The separation into the  $\alpha$ - and  $\beta$ -polypeptide chains which compose components A<sub>I</sub> and A<sub>II</sub> were carried out according to the method of MATSUDA et al<sup>13</sup>. That is, first, Amberlite CG-50 (type II) was washed with distilled water and acetone subsequently. Then, it was washed with water, 1 N NaOH, water, 1 N HCl and water subsequently, packed in a 3 $\times$ 40cm column in a state of H<sup>+</sup> from at last, and equilibrated with 4 N formic acid solution. While, 1 g of globin was dissolved in 100 cc of 10 M urea-4 N formic acid solution, and dissociated into subunits by stirring at 80°C for 30 min. To this, suspension of 30 g of Amberlite CG-50 (type II) in the H<sup>+</sup> from in 200 cc of 4 N formic acid was added drop by drop stirring thoroughly.

Then on to the column previously equilibrated, this Amberlite CG-50 containing globin was added and the globin was eluted in the urea concentration gradient with 2 L of 2 M urea-4 N formic acid solution in the mixing bottle being supplied with 10 M urea-4 N formic acid solution. The parts of the three peaks thus obtained were collected separately and dialyzed against distilled water for 24 hr to remove urea

and formic acid and lyophilized. The  $\alpha$ - and  $\beta$ -polypeptide chains obtained in this way were purified by rechromatography in the same method, and after lyophilization, they were used for the below experiments.

#### 5) Tryptic Digestion

Tryptic digestion of globin and the  $\alpha$ - and  $\beta$ -polypeptide chains was performed by INGRAM's method<sup>3)</sup>. That is, a 50 cc portion of 20% solution of the sample, first adjusted to pH 8.0 with 0.5 N  $\text{NH}_4\text{OH}$  and denatured by heating in a water bath at 90°C for 4 min, was digested by the addition of 0.5 cc of a trypsin solution (0.001 M HCl containing 0.5% of trypsin) and digested at 38°C adding 0.5 N  $\text{NH}_4\text{OH}$  so as to keep pH 8.0. After the digestion, the digest was adjusted to pH 6.5 with 0.5 N HCl to precipitate so-called "cores". The precipitation was removed by centrifuging and the supernatant was lyophilized. This was dissolved in distilled water before use for the below experiments.

#### 6) Fingerprinting Method

Filter paper (TOYO filter paper No. 21 A, 60×60 cm) was cut in the shape as illustrated in Fig.1 and the sample was applied to the origin. First, high-voltage filterpaper electrophoresis was carried out by the high-voltage filter-paper electrophoretic apparatus of ISHIDAI type FE 105 B at 1.5 KV for 2 hr using a mixture of pyridine, acetic acid and water (100 : 4 : 900) at pH 6.4 as the buffer, and cooling with ice

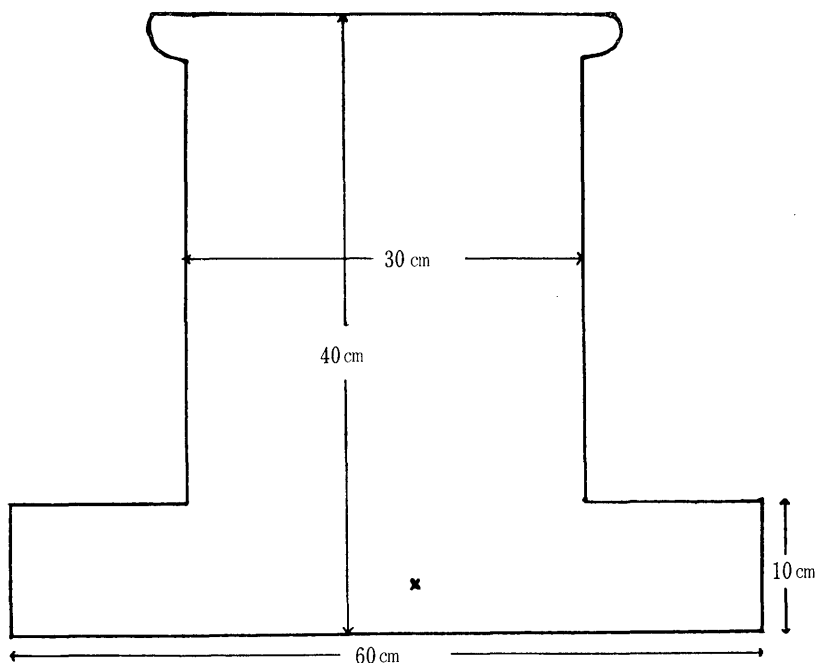


Fig. 1. Filter paper for Fingerprinting technique.

and hexane. After the filter paper was dried completely, it was developed to the second dimension by ascending paper chromatography. As the developer, a mixture of butanol, pyridine, acetic acid and water (45:30:9:36) was used. After developing for 20 hr, the filter paper was dried completely and tested with various coloring agents.

#### 7) Various color Reactions on Filter Paper

##### a) Ninhydrin reaction

An acetone solution containing 2% ninhydrin was prepared, and the fingerprinted filter paper was dipped in the solution and heated with an iron to give colored spots.

##### b) SAKAGUCHI's reaction

For the detection of arginine residues, 0.1% 8-oxyquinoline acetone solution was sprayed on the filter paper. After drying, a solution composed of 100 cc of 5 N NaOH and 0.3 cc of bromine was sprayed on the filter paper to present red spots.

##### c) PAULY's reaction

For the detection of histidine residues, three agents, a mixture of 9 g of sulfanilic acid, 90 cc of concentrated HCl and 910 cc of water, 5% (w/v)  $\text{NaNO}_2$  aqueous solution and 10% (w/v)  $\text{Na}_2\text{CO}_3$  aqueous solution were prepared separately. Immediately before use, the first two agents were mixed at the rate of 1:1, and after standing for 4-5 min, the mixture was sprayed on the paper. Then the  $\text{Na}_2\text{CO}_3$  solution was sprayed and a brownish red color was presented.

##### d) EHRLICH's reaction

For the detection of tryptophan residues, 10% (w/v) p-dimethylaminobenzaldehyde-concentrated HCl solution was prepared and immediately before use, four volumes of acetone were added to the solution. The mixture was sprayed on the filter paper to give a blue-purple color.

##### e) $\alpha$ -Nitroso- $\beta$ -naphthol reaction

For the detection of tyrosine residues, 0.1% (w/v)  $\alpha$ -nitroso- $\beta$ -naphthol-90% ethanol solution and concentrated  $\text{HNO}_3$  were prepared. Immediately before use, they were mixed at the rate of 9:1 and the mixture was sprayed on the filter paper. After drying in air for 2-3 min, the paper was heated at 100°C for 2-3 min. A red color was presented.

## RESULTS AND DISCUSSION

The column chromatography for the separation of components A<sub>I</sub> and A<sub>II</sub> from chicken hemoglobin was performed according to the method of MATSUDA and TAKEI<sup>8)</sup> on the adsorbent of CM-cellulose and by the elution with the salt concentration gradient of phosphate buffer. Fig.2 gives a chromatogram obtained in this method. Besides the main components A<sub>I</sub> and A<sub>II</sub>, a minor component A<sub>III</sub> was observed, but component A<sub>III</sub> was so little that it was not used for the later experiments.

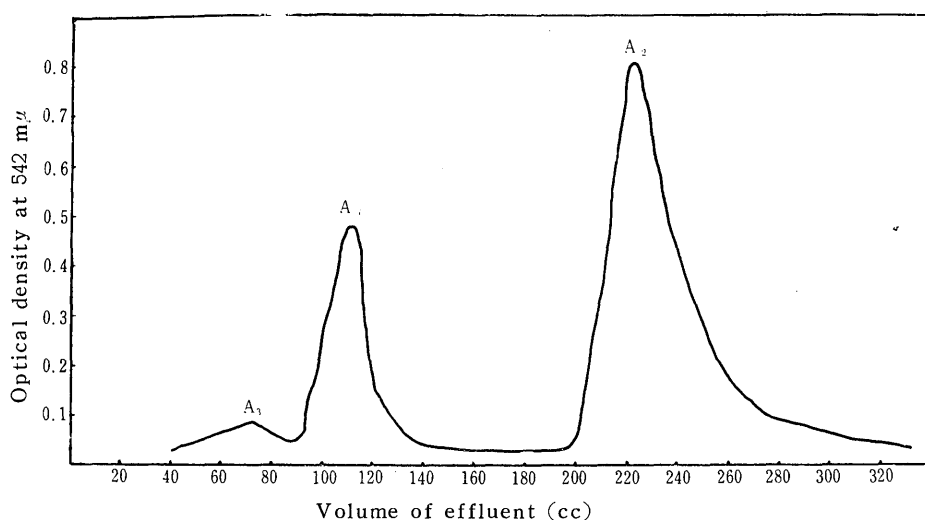


Fig. 2. Chromatogram of chicken adult carboxyhemoglobin on a 2×30cm column of CM-cellulose.

Components A<sub>I</sub> and A<sub>II</sub> thus obtained were purified more perfectly by rechromatography. These treatments by column chromatography were carried out in a large scale using a column 3–4cm in diameter according to circumstances. From components A<sub>I</sub> and A<sub>II</sub> separated and purified in the above-mentioned way, their respective globins were prepared by removing hemes. Then from globins A<sub>I</sub> and A<sub>II</sub>, their respective subunits  $\alpha$  and  $\beta$  were separated. The results are shown in Fig. 3.

Although MATSUDA et al<sup>6)</sup> had eluted with 3 N formic acid-urea solution, the present author substituted 4 N formic acid-urea solution for it. Moreover, for preincubation, MATSUDA et al<sup>6)</sup> allowed the sample to stand at a room temperature for 2–3 days, but the author at 80°C for 30 min, which seems to give better results. The part eluted earlier was designated as  $\alpha$  and the part eluted later as  $\beta$ . Before these peaks of  $\alpha$  and  $\beta$ , two little peaks were found, which are thought either because they failed to be adsorbed on the resin or because



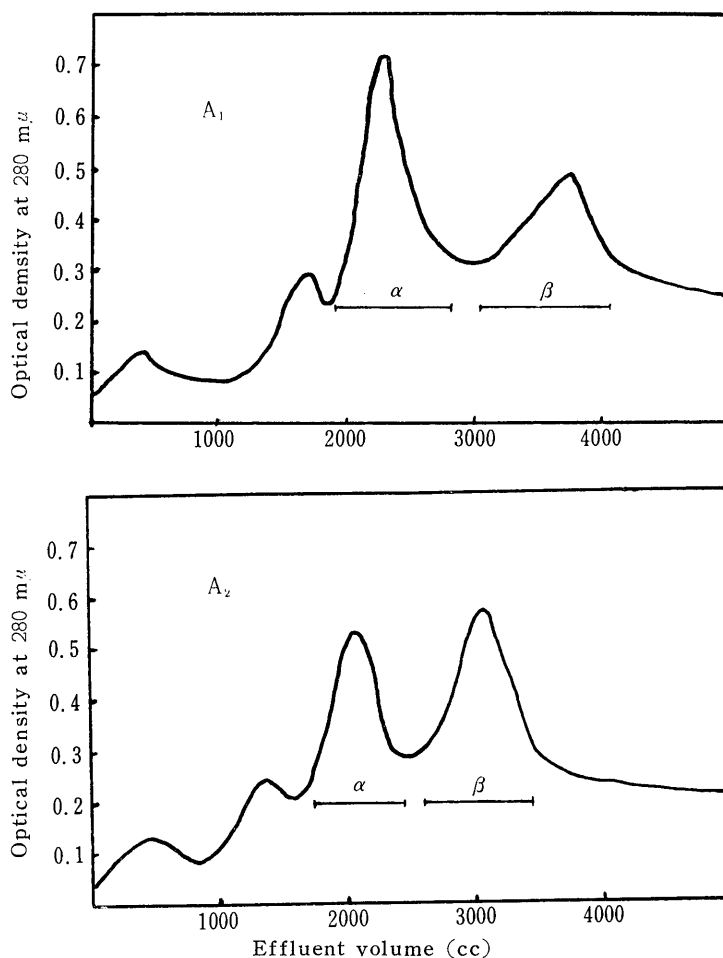


Fig. 3. Chromatographic resolution of components  $A_1$  and  $A_2$  into the  $\alpha$ - and  $\beta$  chains. Column; Amberlite CG-50,  $H^+$ -phase,  $3 \times 50$ .cm

the dissociation into subunits were incomplete. Moreover, in order to examine whether or not the separation into subunits  $\alpha$  and  $\beta$  were perfectly achieved, the N-terminal amino acids of the  $\alpha$ - and  $\beta$ -polypeptide chains obtained in the above mentioned method were studied. In consequence, the  $\beta$ -chains were observed to be contaminated with 20% of  $\alpha$ -chains, and therefore the  $\beta$ -chains were purified by rechromatography. Subsequently, globins  $A_I$  and  $A_{II}$ , and  $\alpha_I$ -,  $\beta_I$ -,  $\alpha_{II}$ - and  $\beta_{II}$ -chains were digested with trypsin, and the courses of the tryptic digestion of only globins  $A_I$  and  $A_{II}$  among them are shown in Fig. 4. In this figure in which the time was set on the abscissa and the uptake (cc) of the 0.5 N  $NH_4OH$  on the ordinate, the same results were obtained for all the cases of  $A_I$  globin and  $A_{II}$  globin, the  $\alpha_I$ -chain, the  $\beta_I$ -chain, the  $\alpha_{II}$ -chain and the  $\beta_{II}$ -chain. That is, most proteins underwent digestion within 1 hr, and completed digestion in 2 hr. The digestion

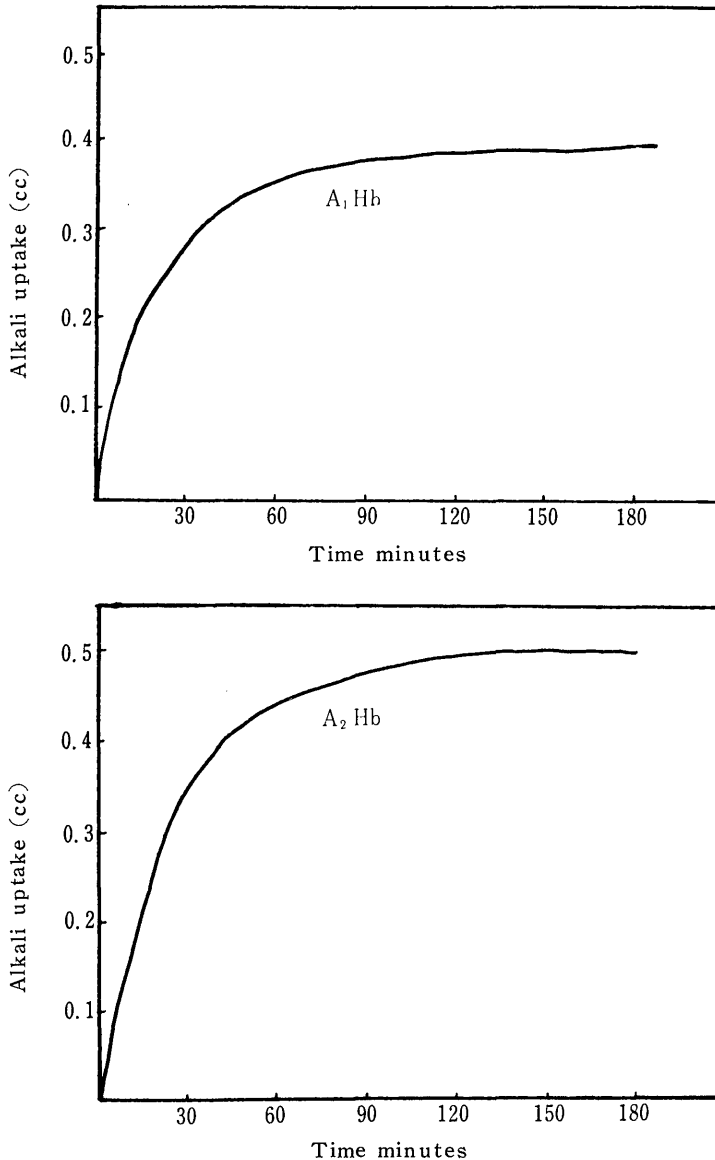
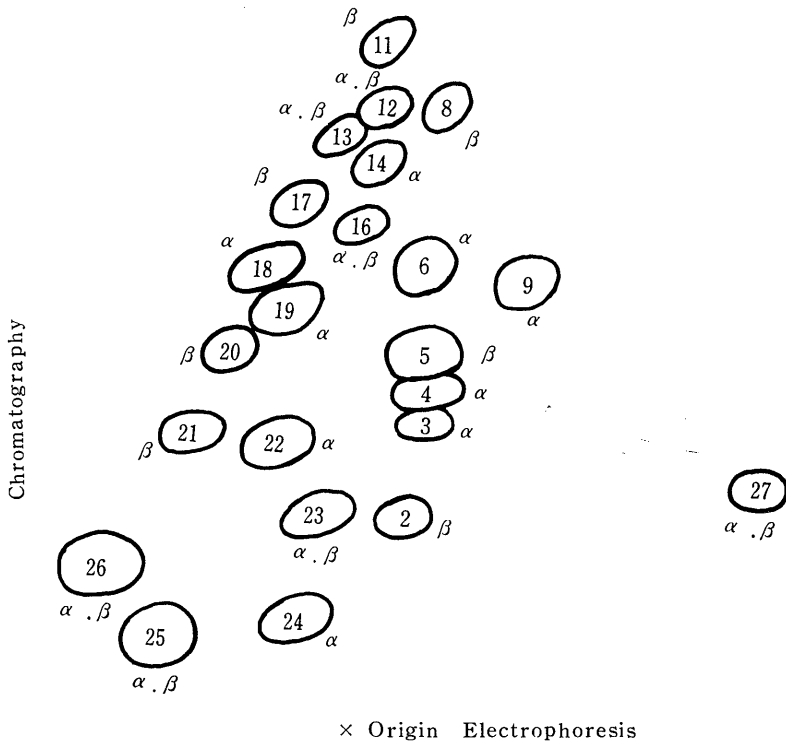
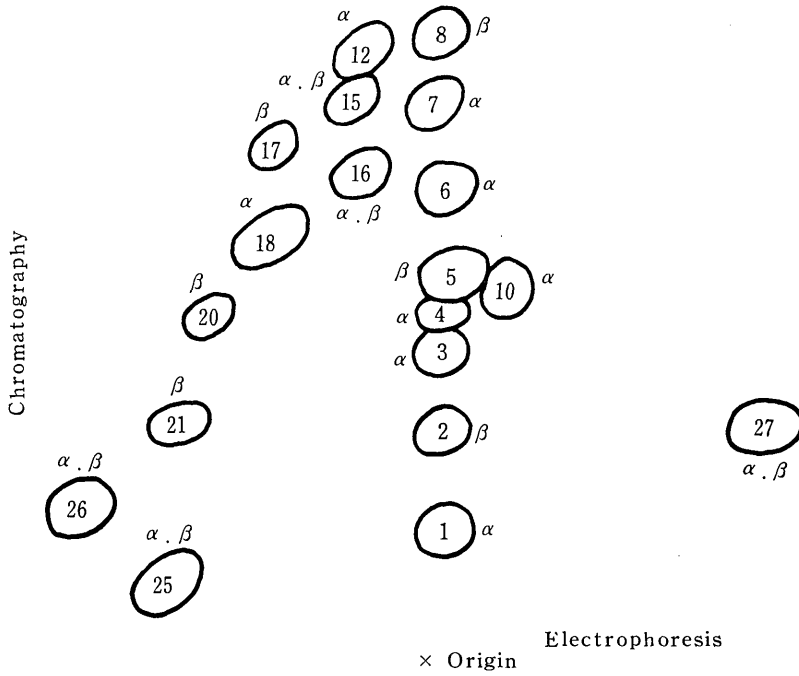


Fig. 4. Course of the tryptic digestion of components A<sub>1</sub> and A<sub>2</sub> from chicken hemoglobin.

was performed for 3hr.

Next, Figs. 5, 6, 7 and 8 are given to show the position of various tryptic peptides from A<sub>I</sub> globin and A<sub>II</sub> globin identified by the fingerprinting method. Fig.5 summarized the tryptic peptides from A<sub>I</sub> globin,  $\alpha$ <sub>I</sub>-chain and  $\beta$ <sub>I</sub>-chain, and Fig.6 those from A<sub>II</sub> globin,  $\alpha$ <sub>II</sub>-chain and  $\beta$ <sub>II</sub>-chain. Figs.7 and 8 show the reaction of the specific amino acid residues on the fingerprints of the tryptic peptides from A<sub>I</sub> globin and A<sub>II</sub> globin, respectively.



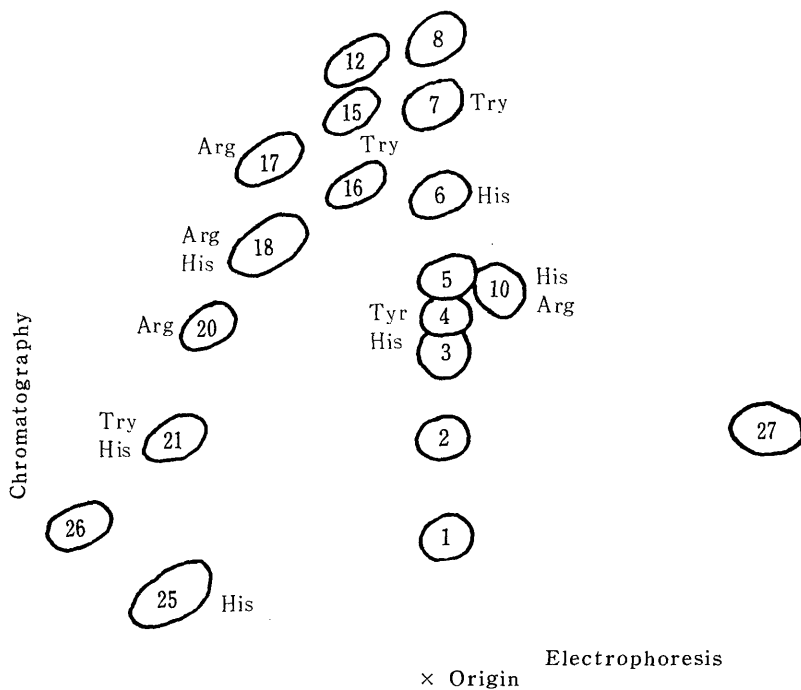


Fig. 7. Specific amino acid reaction on fingerprint of component A1.

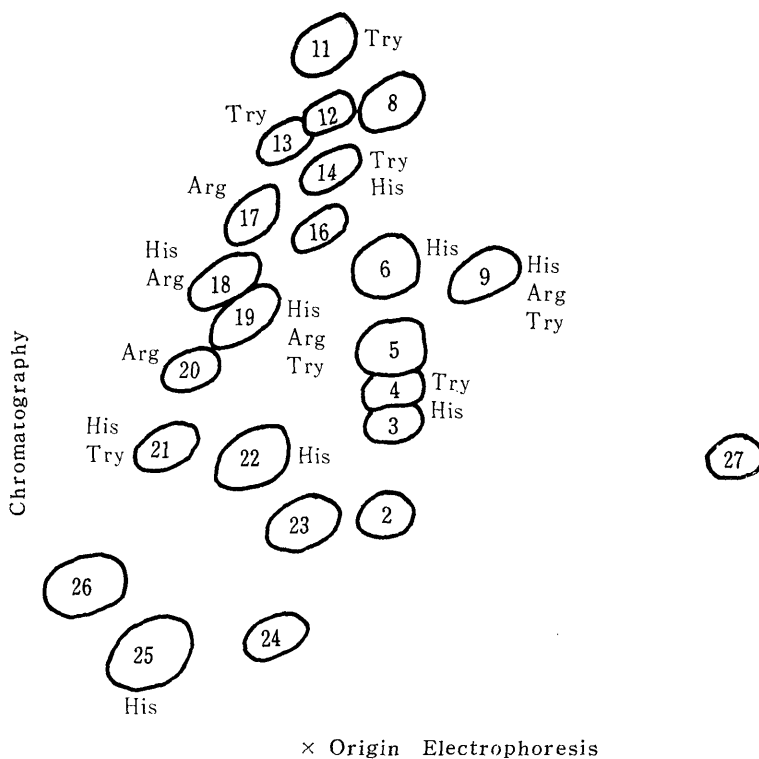


Fig. 8. Specific amino acid reaction on fingerprint of component A2.

As shown in Figs.5 and 6, 19 spots in total, namely, Nos. 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 16, 17, 18, 20, 21, 25, 26 and 27, were obtained on the fingerprint of the tryptic peptides from A<sub>I</sub> globin, and 23 spots in total, namely, Nos. 2, 3, 4, 5, 6, 8, 9, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 and 27 were obtained on the fingerprint of the tryptic peptides from A<sub>II</sub> globin. As for the reaction of the specific amino acid residues of the tryptic peptides from A<sub>I</sub> and A<sub>II</sub> globins shown in Figs.7 and 8, the fingerprint of A<sub>I</sub> globin, as Fig.7 shows, gave 4 arginine-positive spots, Nos. 10, 17, 18 and 20, 6 histidine-positive spots, Nos. 4, 6, 10, 18, 21 and 25, 2 tryptophan-positive spots, Nos. 4 and 7, and 2 tyrosine-positive spots, Nos. 15 and 21, while the fingerprint of A<sub>II</sub> globin, as Fig.8. shows, gave 5 arginine-positive spots, Nos. 9, 17, 18, 19 and 20, 9 histidine-positive spots, Nos. 4, 6, 9, 14, 18, 19, 21, 22 and 25, 2 tryptophan-positive spots, Nos. 4 and 11, and 5 tyrosine-positive spots, Nos. 9, 13, 14, 19 and 21. The comparison of the fingerprints of A<sub>I</sub> and A<sub>II</sub> globins gave 4 spots which were found in A<sub>I</sub> globin and not in A<sub>II</sub> globin, namely, Nos. 1, 7, 10 and 15, and 8 spots which were found only in A<sub>II</sub> globin and not in A<sub>I</sub> globin, namely, Nos. 9, 11, 13, 14, 19, 22, 23, and 24. The other 15 spots were observed commonly in both the globins. This fact, consistent with the reports of MANWELL et al.<sup>4)</sup> or of SAHA<sup>11)</sup>, suggests that components A<sub>I</sub> and A<sub>II</sub> are quite different from each other, but that some parts of the structures are common to the two components. Therefore, it may possibly be considered that two of the four chains of each of components A<sub>I</sub> and A<sub>II</sub> of chicken hemoglobin are common to them just like the relationship between Hb A and Hb F of human hemoglobin. In the present experiment, therefore, the components A<sub>I</sub> and A<sub>II</sub> were separated into their respective subunits,  $\alpha_I$  and  $\beta_I$  and  $\alpha_{II}$  and  $\beta_{II}$ , and their tryptic digests were subjected to the fingerprinting method.

Concerning the  $\alpha_I$ - and  $\beta_I$ -chains, Fig.5 presents 13 spots which seem to come from  $\alpha_I$  namely, Nos. 1, 3, 4, 6, 7, 10, 12, 15, 16, 18, 25, 26 and 27, and 11 spots which seem to come from  $\beta_I$ , namely, Nos. 2, 5, 8, 15, 16, 17, 20, 21, 25, 26, and 27 while concerning the  $\alpha_{II}$ - and  $\beta_{II}$ -chains, Fig.6 presents 16 spots which seem to come from  $\alpha_{II}$ , namely, Nos. 3, 4, 6, 9, 12, 13, 14, 16, 18, 19, 22, 23, 24, 25, 26 and 27, and 14 spots which seem to come from  $\beta_{II}$ , namely, Nos. 2, 5, 8, 11, 12, 13, 16, 17, 20, 21, 23, 25, 26 and 27.

The comparison between  $\alpha_I$  and  $\alpha_{II}$  shows that they have 9 spots which seem to be common to both of them, namely, Nos. 3, 4, 6, 12, 16, 18, 25, 26 and 27, and 11 spots which seem to be peculiar to each of them, namely, Nos. 1, 7, 9, 10, 13, 14, 15, 19, 22, 23 and 24. The comparison between  $\beta_I$  and  $\beta_{II}$  shows that they have 10 spots which seem to be common to both of them, namely, Nos. 2, 5, 8, 16, 17, 20,

21, 25, 26 and 27, and 4 spots which seem to be peculiar to each of them, namely, Nos. 12, 13, 15 and 23. When  $\alpha_I$  is compared with  $\beta_I$ , subsequently, there are detected 5 spots common to both of them, namely Nos. 15, 16, 25, 26 and 27, and 14 spots peculiar to each of them, namely, Nos. 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 17, 18, 20 and 21. Then when  $\alpha_{II}$  is compared with  $\beta_{II}$ , there are observed 7 common spots, Nos. 12, 13, 16, 23, 25, 26 and 27 and 16 peculiar spots, Nos. 2, 3, 4, 5, 6, 8, 9, 11, 14, 17, 18, 19, 20, 21, 22 and 24. From these results, the primary structures of  $\alpha_I$ ,  $\beta_I$ ,  $\alpha_{II}$  and  $\beta_{II}$  can be thought to be different from each other, and this fact is consistent with SAHA's result<sup>11)</sup>. The comparison of the fingerprints of  $\alpha_I$ ,  $\beta_I$ ,  $\alpha_{II}$  and  $\beta_{II}$  shows high similarity in order of  $\beta_I$  and  $\beta_{II}$ ,  $\alpha_I$  and  $\alpha_{II}$ ,  $\alpha_{II}$  and  $\beta_{II}$ ,  $\alpha_I$  and  $\beta_I$ . The fact that the similarities between  $\beta_I$  and  $\beta_{II}$  and between  $\alpha_I$  and  $\alpha_{II}$  are by far higher than those between  $\alpha_{II}$  and  $\beta_{II}$  and between  $\alpha_I$  and  $\beta_I$  may suggest that the genes which control the biosynthesis of the subunits  $\alpha_I$  and  $\alpha_{II}$  have close relations between them and that so do those for the subunits  $\beta_I$  and  $\beta_{II}$ .

### CONCLUSION

As a basic study of the primary structure of the protein moiety of components  $A_I$  and  $A_{II}$  from hemoglobin of the white leghorn, the tryptic peptides from the subunits  $\alpha_I$ ,  $\beta_I$ ,  $\alpha_{II}$  and  $\beta_{II}$  were identified by the fingerprinting method. First, components  $A_I$  and  $A_{II}$  were separated and purified by the method of column chromatography in salt concentration gradient using CM-cellulose as the adsorbent, and subsequently the subunits  $\alpha_I$ -,  $\beta_I$ -,  $\alpha_{II}$ - and  $\beta_{II}$ -polypeptide chains were isolated by column chromatography using Amberlite CG-50 (type II) as the adsorbent and a urea-formic solution as the developer. The components  $A_I$  and  $A_{II}$  and the  $\alpha_I$ -,  $\beta_I$ -,  $\alpha_{II}$ - and  $\beta_{II}$ -polypeptide chains thus obtained were hydrolyzed with trypsin and their tryptic peptides were identified by the fingerprinting method. The results of these experiments suggested that the  $\alpha_I$ -,  $\beta_I$ -,  $\alpha_{II}$ - and  $\beta_{II}$ -polypeptide chains have their respective different primary structures and that the similarity is high in order of  $\beta_I$  and  $\beta_{II}$ ,  $\alpha_I$  and  $\alpha_{II}$ ,  $\alpha_{II}$  and  $\beta_{II}$ ,  $\alpha_I$  and  $\beta_I$ .

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