THE IDENTICAL STRUCTURAL ANOMALIES OF HEMOGLOBINS $J_{Meinung}$ AND J_{Korat}*

R. Quentin Blackwell and Chen-Sheng Liu
Biochemistry Department
U. S. Naval Medical Research Unit No.2 (NAMRU-2)
Taipei, Taiwan, Republic of China

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In 1962, fast hemoglobins with the anodal mobility of J were found in a Hakkanese Chinese family in Meinung, Taiwan (Blackwell et al, 1965a) and in a Thai family near Korat, Thailand (Thephusdin et al, 1965), during population surveys for abnormal hemoglobins among normal subjects. Subsequent clinical and hematological studies in both families revealed no abnormalities ascribable to the J hemoglobins (Blackwell and Huang, 1963; Blackwell et al, 1965b). Recent studies in Thailand (Blackwell et al, 1965c; NaNakorn, 1965) and in Taiwan have revealed additional cases of the fast hemoglobins in both countries.

Electrophoretic comparison of the two hemoglobins with J hemoglobin from Dr. Oscar Thorup's original patient (Thorup et al, 1956) at several pH's between 6.5 and 9.0 in both starch gel and paper showed all three to have essentially identical mobilities. A sample of the J hemoglobin from one of the Thai subjects also was identified as a J by Dr. H. Lehmann.

Preliminary chemical studies on the two hemoglobins, provisionally named $J_{Meinung}$ and J_{Korat}, revealed that their βT5 peptides which normally constitute the residues 41 to 59 in the beta chain contained one additional aspartyl residue (Blackwell et al, 1965c). The present work has established that both hemoglobins have the same structural anomaly in which aspartyl moieties replace those of glycyl at position

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56 in the beta chains. Accordingly, hemoglobins $J_{Meinung}$ and J_{Korat} can be properly designated as $\alpha_2^A \beta_2^{56\ Asp}$.

While the present study was in progress it was learned from Dr. Supa NaNakorn, Hematology Department, Siriraj Hospital, Bangkok, that a fast hemoglobin found by her in a Thai family has been studied by Dr. D. J. Weatherall and associates and found to have the same structural anomaly as that reported in this paper. Details of that study are to be published.

MATERIALS AND METHODS

Heparinized blood samples were obtained from male adults in the two kindreds; the Thai subject had both hemoglobins E and J_{Korat} and the Hakkanese Chinese subject had hemoglobins A and $J_{Meinung}$. In the Thai blood, the relative amount of J_{Korat} hemoglobin was approximately 3 times that of the hemoglobin E; the $J_{Meinung}$ hemoglobin was present in slightly larger amounts than the A in the blood of the Chinese subject. Hemolysates were prepared according to the method of Drabkin (1946) and treated as described by Ingram (1958). After dialysis at 5°C against pH 8,6 veronal buffer for 3 days the hemolysate containing hemoglobins E and J_{Korat} was subjected to starch block electrophoresis (Masri et al, 1958) to separate the E and J components. The separate bands were eluted with 0,05 M pH 8,1 phosphate buffer, concentrated by adding approximately one-third volume of Sephadex G-25, and dialysed against the same phosphate buffer for 3 days at 5°C. Aliquots of the separated E and J_{Korat} components were used for studies of peptide maps and amino acid composition. Hemolysate containing the hemoglobins A + $J_{Meinung}$ was not subjected to starch block electrophoresis; instead, the purified mixture of the two hemoglobins was used directly for structure studies.

Aliquots of the hemoglobin solutions were subjected to tryptic digestion by the method of Ingram (1958) using crystalline trypsin (Worthington 2x, salt free) which had been dissolved in 0,063 M HCl to a concentration of 1 per cent and incubated at 37°C for 18 to 24 hours to reduce the reactivity of any contaminating chymotrypsin (Redfield and Anfinsen, 1956; Liebold and Braunitzer, 1959). After the tryptic digestion of the hemoglobin for 20 hours at 37°C, the mixture was adjusted to pH 6.5 and centrifuged at 12,000 g for 20 minutes; the resulting clear supernate was divided into 0.4 ml portions and vacuum dried over sulfuric acid.

Peptide maps of the tryptic digests were made by the method of Baglioni (1961); the solvent system used in the ascending chromatography procedure was pyridine-isoamyl alcohol-water solution in the volume proportions of 35:35:27.

Larger quantities of the peptides resulting from tryptic digestion of the hemoglobins were obtained for further study by high voltage electrophoresis with the pH 6.4 pyridine-acetate buffer (Baglioni, 1962). For amino acid composition studies the peptides were eluted directly with double distilled 5.7 M HCl and the resulting 1 to 2 ml volumes sealed in glass tubes under vacuum and hydrolysis carried out at 110° for 16 hours.

Quantitative studies of the amino acid composition of peptides were made both by the method of Levy (1954) and a combination of the procedures described by Naughton and Hagopian (1962) and Rothman and Higa (1962).

Partial fragmentation of the tryptic digest peptides of special interest, particularly the β^{A}T5 and β^{J}T5 peptides, was carried out by the mild acid hydrolysis procedure (Schultz et al, 1962; Tsung and Fraenkel-Conrat, 1965) which provides a moderately specific splitting of peptide bonds involving aspartyl moieties. One to 2 micromolar aliquots of peptide were dissolved in 0.03 M HCl, sealed in tubes under vacuum, and hydrolysed for 6 hours and for 16 hours at 105°C. The resulting mixtures of free aspartic acid and smaller peptides were vacuum dried over NaOH pellets, dissolved in 0.03 ml of water, applied to paper, and subjected to separation by electrophoresis and chromatography. Electrophoresis was done with the pH 6.4 pyridine-acetic acid buffer at 2200 V (40 V/cm) for 60 minutes; descending chromatography was accomplished overnight with n-butanol-acetic acid-water (4:1:5). The peptide spots and free aspartic acid spot were located with 0.025 per cent ninhydrin stain at 60°C for 20 minutes (Anfinsen et al, 1959). The spots were cut out, washed with acetone twice to remove excess ninhydrin, dried, eluted with 5.7 M HCl, hydrolysed in evacuated sealed tubes at 110° for 16 hours and vacuum dried over NaOH pellets. Constituent amino acids were identified by two-step electrophoresis at pH 6.4 and 1.9. Proline was located by isatin staining, 0.2 per cent in acetone, prior to general staining with 0.2 per cent ninhydrin in absolute alcohol (Smith, 1953).

RESULTS AND DISCUSSION

Comparison of the separate peptide maps of the hemoglobins A and ^{J}Korat revealed in the latter a displacement of the peptides 24 and 25 (Ingram, 1958) or βT5 oxidized and βT5 (Baglioni, 1961). The β^{J}T5

spots were displaced toward the anodal side to a position above peptide 26 or βT3. This increased anodal mobility and the absence of other new spots on the map suggested the presence of an additional negatively charged residue in the β^{J}T5 peptides in agreement with the increased anodal mobility of the hemoglobin J_{Korat}. The difference between the β^{A}T5 and β^{J}T5 peptides was readily apparent after the one-dimensional high-voltage electrophoretic separation and, although carried out, the subsequent chromatography step was not required to show the difference. Accordingly, the β^{J}T-5 peptides for further analysis were obtained by electrophoresis alone. Likewise, since the β^{J}T-5 peptide from $J_{Meinung}$ could be separated from the adjacent peptides no separation of A and $J_{Meinung}$ was required prior to tryptic digestion.

The molar ratios found for the amino acid constituents of the βT5 peptides are summarized in Table 1.

Table 1. Amino Acid Constituents of βT-5 Peptides.

Amino Acid	Peptide β^{A}T5				Peptide β^{J}T5			
	Method 1*		Method 2*		Method 1		Method 2	
	Molar Ratio	Nearest Integer	Molar Ratio	Nearest Integer	Molar Ratio	Nearest Integer	Molar Ratio	Nearest Integer
Asp	3.7	4	2.6	3	4.6	5	4.1	4
Glu			0.9	1			1.0	1
Gly	1.9	2	1.5	2	1.5	1	0.8	1
Ala	1.3	1	1.0	1	1.1	1	1.0	1
Val	1.0	1	0.8	1	1.3	1	0.8	1
Leu	1.1	1	2.8	3	1.1	1	2.8	3
Ser	1.7	2			1.6	2		
Thr	1.1	1	1.1	1	0.8	1	0.7	1
Met	0.8	1	0.7	1	0.4	1	0.3	1
Phe	2.4	3	1.5	3	2.2	3	1.3	3
Pro	1.9	2	+		1.7	2	+	
Lys	0.9	1	0.2	1	0.6	1	0.5	1

*Method 1: Levy (1954). Levy's correction factors were employed. Values given are means of 6 analyses. Asp and Glu were not separated.

*Method 2: Combination of methods described by Naughton and Hagopian (1962) and Rothman and Higa (1962). Values given are means of 4 analyses. Leu and Ser were not separated.

Except for the low recovery of phenylalanine, our results for β^{A}T5 agreed with the established results for this peptide. Low results for phenylalanine in βT5 peptides were reported previously by Bowman et al (1964) in hemoglobin G studies and by Allan et al (1965) in hemoglobin K studies. Results for the β^{J}T5 peptides from both J hemoglobins were

the same as those for A except for one additional aspartic acid and one less glycine. These results indicated that J_{Korat} and $J_{Meinung}$ had an aspartyl moiety replacing the usual glycyl moiety at either the β46 or β56 position. Such replacement of glycyl by aspartyl has been reported in several other fast hemoglobins (Beale and Lehmann, 1965).

Comparison of results from the partial fragmentation at the aspartyl linkages of the βT5 peptides from A hemoglobin and from both J hemoglobins provided the definitive information concerning the location of the glycyl residue replacement. Table 2 provides a summary of the principal peptides obtained from β^{A}T5 and both β^{J}T5 peptides.

Table 2. Results of Selective Hydrolysis of βT5 Peptides.

a. Established Structure[1] of β^{A}T5:

Phe-Phe-Glu-Ser-Phe-Gly-Asp-Leu-Ser-Thr-Pro-Asp-Ala-Val-Met-Gly-Asn-Pro-Lys
41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59

▲[2] ▲ ▲ ▲ ▲ ▲

b. Amino Acids[3] in Principal Peptides from β^{A}T5 Cleavage:

(Phe,Glu,Ser,Gly) (Leu,Ser,Thr,Pro) (Ala,Val,Met,Gly) (Pro,Lys)
(Asp[4],Leu,Ser,Thr,Pro) ((Ala,Val,Met,Gly,Asn,Pro,Lys))[5]

c. Amino Acids[3] in Principal Peptides from β^{J}T5 Cleavage:

(Phe,Glu,Ser,Gly) (Leu,Ser,Thr,Pro) (Ala,Val,Met) (Pro,Lys)
(Asp[4],Leu,Ser,Thr,Pro) ((Ala,Val,Met,Asp))[4,5]

1. Braunitzer, G. et al (1961). 2. Principal cleavage points expected with 0.03M HCl. 3. Amino acid compositions of peptides were determined but no sequence studies were made; sequences are assumed to remain normal. 4. Asp could be either at the amino or carboxyl ends of the peptides. 5. Peptide ((Ala,Val,Met,Gly,Asn,Pro,Lys)) was present after 6 hrs. hydrolysis of β^{A}T5 but not after 16 hrs. hydrolysis. Peptide ((Ala, Val,Met,Asp)) was present after 6 hrs. hydrolysis of β^{J}T5 but not after 16 hrs. hydrolysis.

A peptide containing (Phe,Glu,Ser,Gly), presumably the hexapeptide Phe-Phe-Glu-Ser-Phe-Gly from positions 41-46 of the β-chain and located at the amino end of the βT5 peptide, was found in the peptide mixtures resulting from partial hydrolysis of the β^{A}T5 peptide and also from both of the β^{J}T5 peptides. Its constant presence renders unlikely any possibility that one of the phenylalanine moieties is replaced by aspartyl in the J hemoglobins. That possibility also is theoretically unlikely

due to the nature of the codons for phenylalanine and aspartic acid. As discussed by Beale and Lehmann (1965) the interchange of Gly and Asp is one of a number of possible one-step mutations; replacement of Phe by Asp would not be as simple.

The constant presence of the hexapeptide Phe-Phe-Glu-Ser-Phe-Gly in all of the peptide mixtures is very important evidence that the glycine residue remains intact in position 46 of the two J hemoglobins. Furthermore, the peptide (Ala,Val,Met,Gly), representing the tetrapeptide Ala-Val-Met-Gly in positions 53-56, was found in the peptide mixture from β^{A}T5 peptide whereas only (Ala,Val,Met), representing the tripeptide, Ala-Val-Met, was found in the β^{J}T5 peptides. This would be expected if the glycyl residue at position 56 is replaced by an aspartyl group. The presence of the peptide (Ala,Val,Met,Gly,Asn,Pro,Lys), representing the heptapeptide from positions 53-59, in the 6 hr hydrolysis mixture from β^{A}T5 peptide indicated the slower hydrolysis of bonds involving asparagine; after 16 hrs the asparaginyl group had been converted to aspartyl, and the peptide bonds of that aspartyl group cleaved to leave only the smaller peptides. No such heptapeptide was found in the 6 hr hydrolysis mixture from the β^{J}T5 peptides and none would be expected if aspartyl replaces glycyl at the 56 position in the J hemoglobin.

It is considered that the above results provide adequate proof that of the two glycine residues in βT5, the one at position $\beta 56$ is replaced by aspartyl in both $J_{Meinung}$ and J_{Korat}.

SUMMARY

Hemoglobin $J_{Meinung}$ from a Hakkanese Chinese family in Taiwan and hemoglobin J_{Korat} from a Thai family have the same structural anomaly. In both hemoglobins the glycyl residue normally present at position 56 in the beta chain is replaced by aspartyl; therefore both can be designated as $\alpha_2^A \beta_2^{56\ Asp}$.

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