[症例報告]Abnormal Hemogrobins in Ryukyu Islands : I. A Case of Hemoglobin E Trait

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Abnormal Hemogrobins in Ryukyu Islands

I. A Case of Hemoglobin E Trait

Yukinori Eguchi, Hiroshi Takei, Fusako Maehira
Fukuichi Nakada* and Fumihide Inoue*

Department of Biochemistry, School of Health Science, Faculty of Medicine
University of the Ryukyus
*Department of Biochemistry, School of Medicine, Faculty of Medicine
University of the Ryukyus

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Abstract

A case of hemoglobin E trait was detected from screening the population, 813 residents, of Henza Island, Okinawa Prefecture. The subject was an eight year old female and the abnormality has not yet been detected in other members of the family so far examined.

Electrophoretic mobility of the abnormal hemoglobin component by cellulose acetate membrane electrophoresis was almost identical to that of hemoglobin A_2 and the amount of the abnormal component was 36.1 % of that of total hemoglobin.

Beta chain abnormality was examined by peptide map technique and the substitution of glutamic acid at the position β-26 by lysine was confirmed by the amino acid analyses of the purified tryptic peptides.

Introduction

Hemoglobin E was first described in terms of chemical structure in 1961,[1] which is a frequently occurring inherited disease largely localized in south-east Asia[2] and in the southern part of Mainland China,[3] but not in Mainland Japan.[4] So far hemoglobin E has not been investigated extensively in the Ryukyu Islands. Significantly higher frequencies of hemoglobin E have been observed in highly malarious parts of Thailand than in neighboring regions with a low incidence of malaria.[5] The purpose of this paper is to investigate the occurrence of hemoglobin E in the Ryukyu Islands. It is worth investigating from the viewpoint of epidemiology and advantageouness in selection against malaria.

Materials and Methods

I. Preparation of hemolysates

Venous blood was drawn from an eight year old female and hemolysates were prepared

according to Drabkin's method. [6]

II. Cellulose acetate membrane electrophoresis

Electrophoresis of the hemolysates was run in a Tris-glycin buffer (pH 8.6) by using the membrane Separax (Jōkō, Japan). The membrane was stained with 0.5 % amino black solution after the run and washed with acetic acid-methanol solution.

III. Preparation of globin

Heme was removed from hemoglobin according to the method of Anson and Mirsky. [7]

IV. Separation of the abnormal polypeptide chain

CM-52 (Whatman) column chromatography was carried out in principle according to the method of Clegg et al[8]. Column chromatography was started with 0.005 M Na_2HPO_4-8 M urea-0.5 % 2-mercaptoethanol solution (pH 7.0). Linear gradient elution was performed in sodium ion concentration from 0.01 M to 0.06 M. The size of the column was 2.5 × 20 cm and 15 ml fractions were collected by a fraction collector (MS Instruments, Inc). Fractions corresponding to each peak were pooled separately, dialyzed against deionized water and lyophilized.

V. Carboxymethylation of polypeptide chains

100 mg of a polypeptide chain was dissolved in 12 ml of 8 M urea-Tris-HCl solution (pH 8.3), to which 0.1 ml of 2-mercaptoethanol was added. The mixture solution was stirred continuously at room temperature for 4 hr under N_2 gas. One ml of 30 % monoiodo acetic acid-2 N NaOH solution was added to the reaction mixture, which was then allowed to stand for another hour. The reaction mixture was applied to a Sephadex G-25 column (3.0 × 95 cm) for removal of urea and salts and the effluent corresponding to the protein was pooled and lyophilized.

VI. Tryptic digestion of the CM-polypeptide chain

Tryptic digestion of the CM-polypeptide chain was carried out at 37°C for 2 hr , during which process the pH of the solution was kept at 9.0. Weight ratio of the enzyme to the substrate was 1 : 100 . After digestion, pH of the digest solution was adjusted to 6.4. The "core" was precipitated by centrifuge at 5 000 rpm for 20 min and the supernatant was lyophilized.

VII. Peptide mapping of the tryptic digests of the polypeptide chain

Peptide map technique was employed to detect the abnormal tryptic peptide[9] . Filter paper Tōyō No. 50 (60 × 60 cm) was used and for the first dimension paper chromatography was carried out with the upper phase of the mixture of n-butanol-acetic acid-water (4 : 1 : 5) as a developing solvent, followed by high voltage electrophoresis for the second dimension with a pyridine-acetic acid buffer (pH 3.3) at 3000 volts for 90 min. Peptides on the map were detected with ninhydrin reaction.

VIII. Preparation of the abnormal tryptic peptide

After the abnormal tryptic peptide was identified on peptide map using the filter paper, the filter paper was cut out, eluted with 5 % acetic and then lyophilized.

IX. Amino acid analysis

The purified peptide was hydrolysed with 6 N HCl at 110°C for 20 hr and the hydrolysates were subjected to amino acid analysis by using an amino acid analyzer

(JEOL JLC-6AH).

Results

As shown in Fig. 1, electrophoretic mobility of the abnormal hemoglobin component was almost equal to that of A_2 component. The isoelectric point of the abnormal component was also equal to that of A_2 by isoelectric focusing (Fig. 2). The ratio of the abnormal component to A_1 was 1 : 1.8; i.e. the abnormal component was about 36 % of the total hemoglobin when analyzed by the disc electrophoresis (Fig. 3).

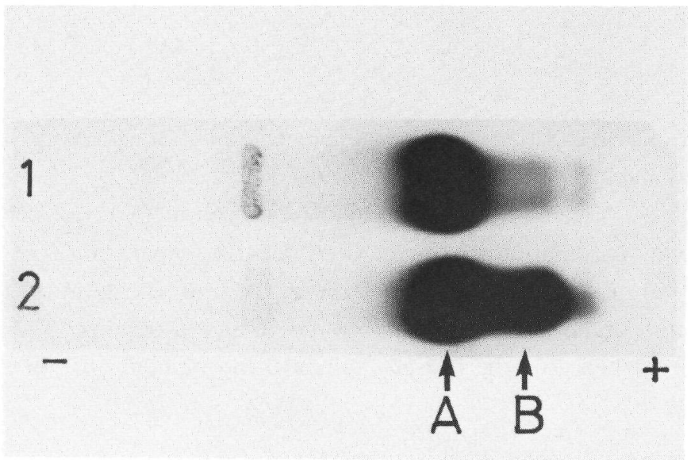

Fig. 1. Cellulose acetate membrane electrophoretic pattern of normal and abnormal human hemoglobins. 1 : normal, 2 : abnormal, A : A_1 component, B : abnormal component.

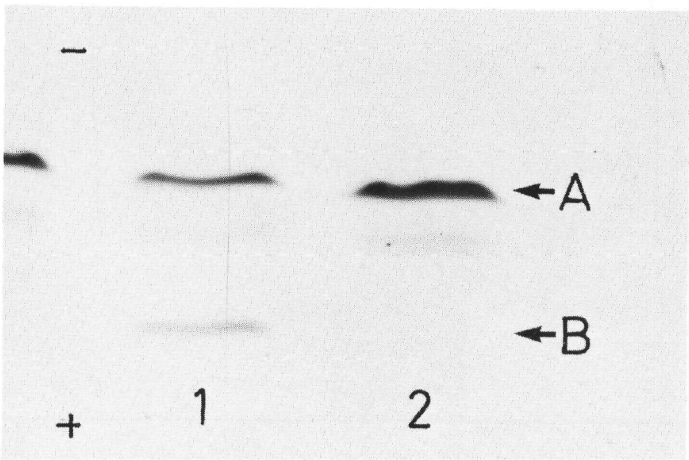

Fig. 2. Isoelectric focusing of normal and abnormal hemoglobins. 1 : abnormal hemoglobin, 2 : normal hemoglobin, A : A_1 component, B : abnormal component.

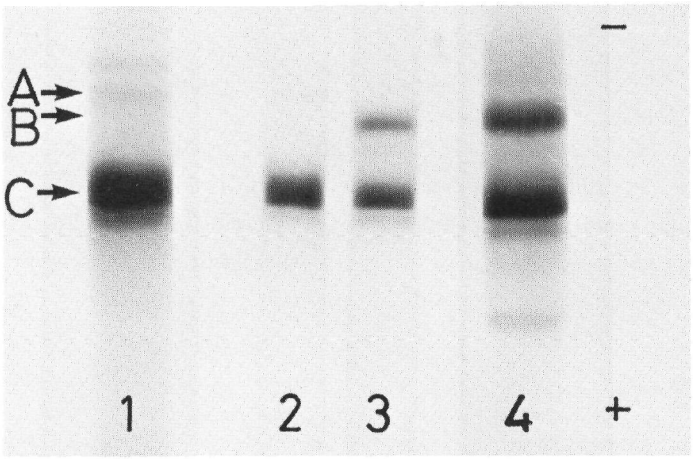

Fig. 3. Disc electrophoresis pattern of normal and abnormal hemoglobins.
1 and 4 : normal and abnormal, stained with amino black, 2 and 3 : normal and abnormal, without staining, A : A_2 component, B : abnormal component, C : A_1 component.

CM-52 column chromatography was used for the separation of the abnormal polypeptide chain in principle according to the method of Clegg et al[7] (Fig. 4). The abnormal polypeptide chain was eluted between the β and α chains. After 100 mg of the whole globin was applied to the CM-52 column, the amount of the abnormal chain recovered was 20 mg.

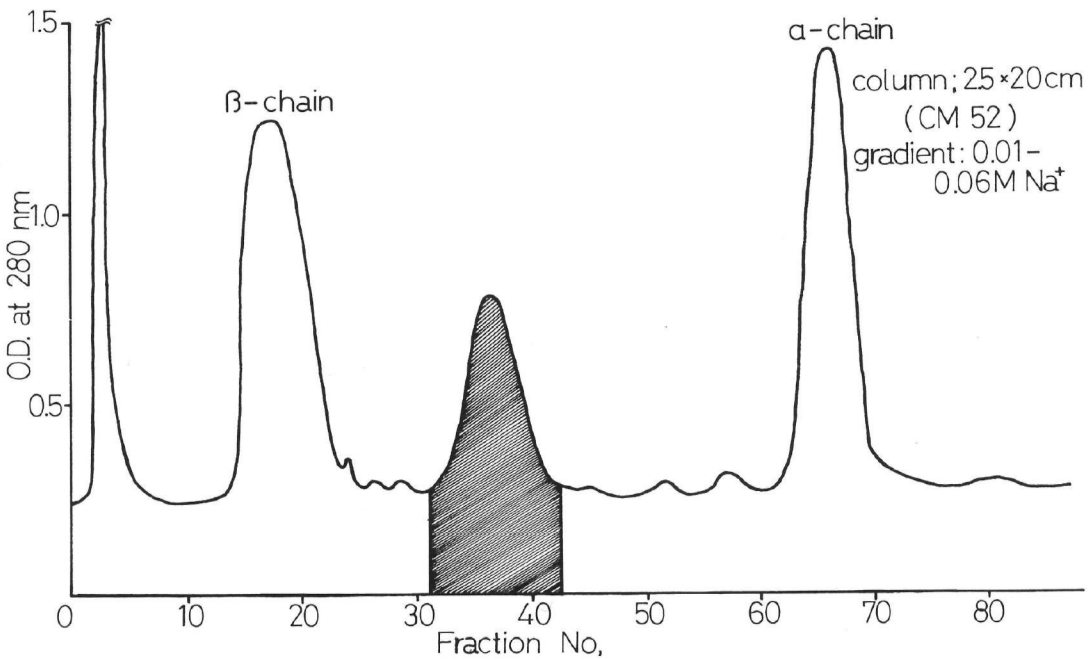

Fig. 4. Purification of the abnormal chain on CM-cellulose

The abnormal polypeptide chain was subjected to tryptic digestion and the tryptic peptides were subjected to peptide map technique[9]. The peptide map of the abnormal polypeptide chain was compared with that of the β-chain and the abnormal peptides A-I and A-II were obtained on the peptide map (Fig. 5).

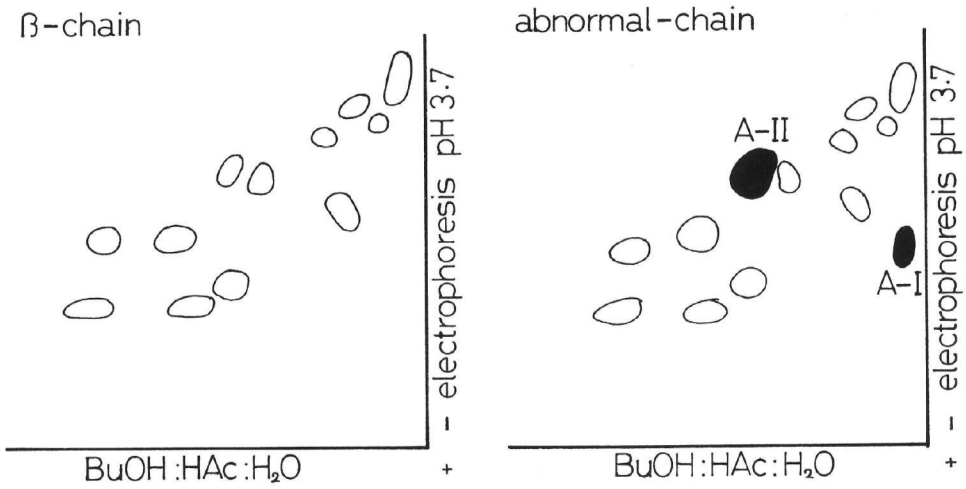

Fig. 5. Peptide map of tryptic digest of abnormal chain

									20											
ß-chain	Val	-	Asn	-	Val	-	Asp	-	Glu	-	Val	-	Gly	-						
A-I	Val	-	Asn	-	Val	-	Asp	-	Glu	-	Val	-	Gly	-						
A-II																				
																		30		
ß-chain	Gly	-	Glu	-	Ala	-	Leu	-	Gly	-	Arg									
A-I	Gly	-	Lys																	
A-II							Ala	-	Leu	-	Gly	-	Arg							

Fig. 6. Amino acid seqence of the abnormal chain

These abnormal peptides were eluted with 5 % acetic acid and the amino acid compositions of the peptides were examined. A-I peptide was composed of 9 amino acids, Lys (1), Asp (2), Glu (1) Gly (2) and Val (3) and A-II peptide was composed of 4 amino acids, Arg (1), Gly (1), Ala (1) and Leu (1) (Table 1).

Table. 1. Amino acid composition of the abnormal peptides

	β 18-30	A-I	A-II
Lys	—	1.00 (1)	—
Arg	1	—	1.00 (1)
Asp	2	1.70 (2)	—
Glu	2	1.56 (1)	—
Gly	3	1.83 (2)	1.00 (1)
Ala	1	—	1.22 (1)
Val	3	2.82 (3)	—
Leu	1	—	0.78 (1)

Discussion

According to these compositions, amino acid sequences of A-I and A-II are considered to correspond to the sequences from the position β 18 to β 26 and from β 27 to β 30 respectively. The 13 amino acid peptide from the position β 18 to β 30 found in the β polypeptide chain is considered to be split into 2 fragments A-I and A-II by tryptic digestion because the amino acid residue 26 Glu is substituted by Lys.

No symptoms or laboratory findings were observed in this subject. The hemoglobin of the grandfather, the grandmother and the aunt of the subject were examined by electrophoresis but they were normal. The hemoglobin of her mother has not been examined yet.

It was concluded from these findings that this was a case of hemoglobin E (26 Glu → Lys) trait. This is the first case of hemoglobin E found among residents in Okinawa Prefecture. Hemoglobin E has been found among Siamese (13.6 %)[10], Burmese (15.3 %)[11] and Cambodian (35 %)[12]. Hemoglobin E is characteristic of the people of South-east Asia. High incidence for hemoglobin E in the southern part of Mainland China was also reported[3]. Therefore it is very interesting to investigate the occurrence of hemoglobin E in the Ryukyu islands from the viewpoint of its geographical distribution and advantageousness in selection against malaria.

The complete nucleotide sequence of a β^E globin gene was determined recently and a change in codon 26 GAG → AAG was revealed[13]. There are two codons, GAA and GAG for Glu and AAA and AAG for Lys, respectively. Therefore it is the first position base change from G to A in this case, so that the amino acid substitution of Glu by Lys takes place.

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