Kawasaki Med. J. Vol. 4: No. 4, 271-277, 1978

HB UBE-4 (α 116 GLU → ALA): A SECOND INDEPENDENT INSTANCE OF A KOREAN FAMILY DISCOVERED BY HEMOGLOBIN SCREENINGS IN OKAYAMA DISTRICT

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Accepted for Publication on December 5, 1978

Abstract

An electrophoretically G-like abnormal hemoglobin was found in a Korean patient with liver cirrhosis eight years ago in the Kawasaki Hospital in Okayama and an α chain anomaly was presumed for this hemoglobin at the time of its discovery. However, establishment of its amino acid substitution has not been feasible on account of shortage of test material for further analysis. Recently it happened to us to see the same patient (a 48 year old man) again in the Saiseikai Hospital in Okayama and could get blood samples from him. Conventional chemical analysis of the purified α chain was successfully performed, this time. It was dislosed that the abnormal hemoglobin was identical with Hb Ube-4 (α 116 Glu → Ala), which had been detected from a Korean family residing in Ube by Ohba and his associates in 1978. The kinship between the family of Ohba and our family was carefully investigated, but no blood relatioship was demonstrable between them. Therefore it is thought that this hemoglobin variant is a second independent instance of Hb Ube-4. This abnormal hemoglobin was heat-stable and entirely normal in function.

INTRODUCTION

In 1968 an electrophoretically slow-moving abnormal hemoglobin was detected in our laboratory by agar gel electrophoresis (pH 8.6) of the hemolysate obtained from a 40yr-old Korean patient with liver cirrhosis.

At that time, this hemoglobin was proved to have an amino acid replacement in the tryptic core fraction of α chain, but the analysis could not be completed on account of its limited supply of the sample[1].

Eight years afterwards, the same abnormal hemoglobin happened to be encountered in our laboratory when the patient was readmitted to the Okayama

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Saiseikai Hospital because of aggravation. This paper aims to describe the results of our studies on structural abnormality, functional properties and ethnic characters of this abnormal hemoglobin.

MATERIALS AND METHODS

Routine hematological and blood chemistry tests were carried out by standard methods. Conventional techniques were employed for the preparation of the hemolysate[2], cellulose acetate membrane electrophoresis for demonstration of abnormal hemoglobin[2,3], heat instability test[4], isopropanol precipitation test[5] and HbF content of the hemolysate[6]. The detection of chain anomaly was performed by starch gel electrophoresis of the PCMB treated hemoglobin[7].

The purified solution of the abnormal hemoglobin for structural and functional studies was prepared as follows: The abnormal hemoglobin was separated by cellulose acetate membrane electrophoresis of the hemolysate, cut out, and eluted into the intended buffer solutions.

For the purpose of examination of oxygen equilibrium curve, aliquotes of about 10 μM solution were prepared after dialysis in a Visking tube against 0.1 M NaCl in 0.05 M bis-Tris buffer solution of desired pH range (6.8-7.8). The oxygen equilibrium was then measured by the method of Imai *et al.*[8].

The abnormal hemoglobin was converted into globin by Anson-Mirsky's method[9]. The abnormal α chain was then isolated by the chromatographic method of Clegg, Naughton and Weatherall[10]. The abnormal chain was digested with trypsin (pH 8.0)[11] and the pH of the digest was adjusted to 6.4 with acetic acid to separate soluble fraction from the insoluble (the so called α core). The precipitated core was then washed four times with large amounts of 0.2 M NH_4HCO_3-acetic acid buffer solution (pH 6.4) to remove the soluble fraction completely.

The abnormal α core fraction was digested with chymotrypsin (pH 8.4, 37°C, 4 hrs)[12,13], centrifuged and the supernate was lyophillized for fingerprinting. The fingerprinting was performed by the method of Baglioni[14].

Amino acid residues of the acid hydrolysate of the peptide was determined by use of automatic amino acid analyzer (Yanaco Type LC-7)[2].

RESULTS

Routine hematological examination and blood chemistry revealed. Hb 7.2g/dl; Ht 0.22 l/l; RBC 2.18×10^{12}/l; MCV 100 fl; MCH 33.5 pg; MCHC 33.1 g/dl; WBC 6.9×10^{9}/l; total bilirubin 1.7 mg/dl; SGOT 32 IU/l; SGPT 47 IU/l. The clinical findings were consistent with advanced liver cirrhosis. He died one year after readmission.

The mother and three children of the patient possessed the same abnormal hemoglobin together with normal adult hemoglobin. The carriers except the patient were apparently healthy and their hematological examinations were within the normal limits.

On cellulose acetate membrane electrophoresis (pH 8.6), the abnormal hemoglobin (Hb X) moved as a discrete band with a mobility similar to that of Hb G, and a minor Hb A_2 component was seen as a faint stripe to the cathodic side of Hb A_2.

The proportion of the abnormal hemoglobin to total hemoglobin was about 12 (10–15) percent in all the carriers (Hb A 86.0, Hb X 11.6, Hb A_2 2.0, and abnormal Hb A_2 0.4 percent, respectively, on the average). Hb F content was 1.08%. Heat denaturation and Carrell's isopropanol precipitation tests of the hemolysate were negative.

Examinations of oxygen equilibrium curve[7] of the purified abnormal hemoglobin solution (pH 7.4, 37°C) were log $P_{50}=0.93$ (Hb A $=0.87$) mmHg, Hill's $n=2.64$ (Hb A $=2.87$). and alkaline Bohr effects $\Delta \log P_{50}/\Delta pH=-0.43$ (Hb A $=-0.50$), indicating virtually normal functional abilities.

Alpha chain anomaly of the abnormal hemoglobin was reconfirmed by starch gel electrophoresis of the preliminary PCMB treated hemoglobin solution.

The fingerprint of the soluble fraction of tryptic digest of abnormal α chain was perfectly identical with that of control α^A chain in the scattering of the spots.

Accordingly, the abnormal α core fraction was digested with chymotrypsin (pH 8.4, 37°C, 4 hrs), centrifuged and the supernate was lyophillized.

The fingerprint of the soluble fraction of chymotryptic digest gave nine ninhydrin positive spots as shown by A, B, ..., J in Figure 1. The spot F′ was not seen in its usual position, but was shifted upward and towards the cathode as compared with the corresponding normal spot F. Both F and F′ gave positive His reaction[15]. The other spots visualized on the map were the same as those of the control α^A core.

The amino acid analysis of the acid hydrolysate of spot F′ demonstrated that it consisted of eight amino acid residues coinciding with α110–117, and the results showed one glutamyl residue was missing and one more alanyl residue was inserted in comparison with the composition of the control spot F (Table 1). This led us to conceive the Glu residue normally occupying the 116 position in the α chain was replaced by Ala.

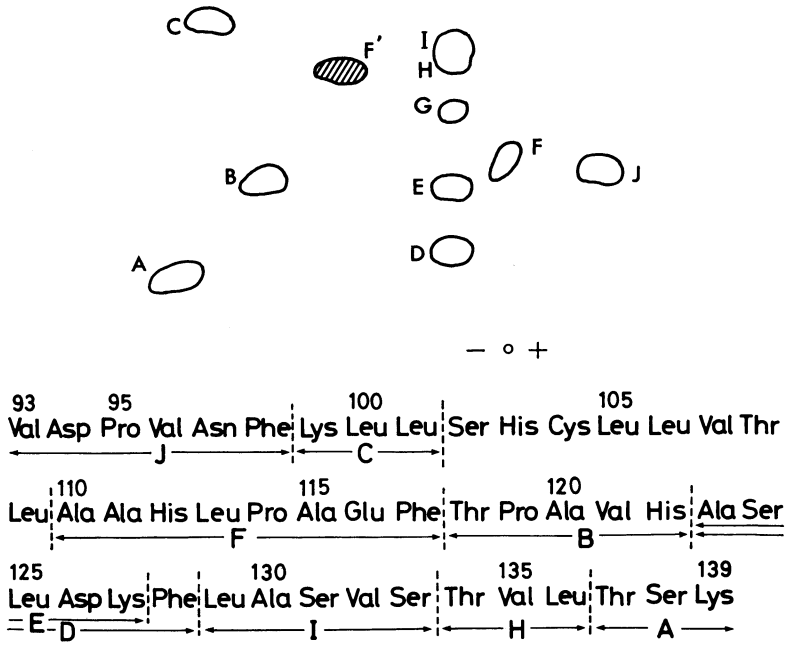

Fig. 1. Composite fingerprint of chymotryptic digest of normal and abnormal tryptic core of α chain. Nine spots (A, B,..., H-I, J) were seen in the fingerprint. The abnormal spot F′ shifted from its usual spot F position is indicated with hatched circle. The amino acid residues of spot G extracted from both α^{Ok} and α^{A} core were the same but were not of the core origin. Spot H contained a mixture two peptides comprising residues 129-139 and residues 134-136.

TABLE 1. The number of amino acid residues in the acid hydrolysate of an abnormal spot F′ in Fig. 1.

	observed	
	abnomal spot F′	normal spot F
His	0.87	0.90
Glu	0.09	1.12
Pro	1.01	0.83
Ala	*3.58	*2.87
Leu	1.02	1.02
Phe	0.97	1.00

* N-terminal amino acid

	110 111 112 113 114 115 116 117
F (α^{A} 110–117)	Ala–Ala–His–Leu–Pro–Ala–Glu–Phe
	↓
	110 111 112 113 114 115 116 117
F′(α^{Ok} 110–117)	Ala–Ala–His–Leu–Pro–Ala–Ala–Phe

The amino acid compositions of all the other peptide spots (A, B, C, D, E, G, H–I, J) were the same as those of corresponding fragments of normal α^{A} core,

However. the peptide α102–109 was not seen, as expected[16], since it did not dissolve in chymotryptic soluble fraction.

DISCUSSION

The abnormal hemoglobin seems to be unrelated to the pathogenesis of the patients disease, because tests revealed neither the functional abnormality of the oxygen transport, nor the increased instability, and furthermore all the carriers of this hemoglobin in the family are apparently healthy.

According to Perutz's three-dimensional model of hemoglobin molecule[17] α116 residue belongs to non helical GH–4 position and it protrudes outside the surface of hemoglobin molecule.

All GH–4 mutants including α, β and γ chain are harmless to the carriers as evidenced by Hb O Indonesia (α116 Glu → Lys)[18,19], Hb D Punjab[20,21] =Hb D Los Angeles[22,23]=Hb D Chicago (β 121 Glu → Gln)[24], Hb O Arab (β 121 Glu → Lys)[25,26], Hb Beograd (β 121 Glu → Val)[27,28] and Hb F Hull (γ 121 Glu → Lys)[29]. It is therefore presumed that the amino acid substitution at GH–4 position does not affect the function of hemoglobin molecule, irrespective of the size, shape and polarities of the amino acid introduced in substitution.

Quite recently, Ohba *et al.* reported an abnormal hemoglobin called Hb Ube–4 (α 116 → Ala)[30]. Shortly after completion of chemical analysis of this Hb variant we recognized that these two abnormal hemoglobin were identical.

According to Ohba *et al.* the carriers of Hb Ube–4 were Korean living in Japan. The kinship between the family of Ohba and our family was therefore inquired carefully. Their native districts in Korea were different and no blood relationship was recalled by any family members, extending over more than five generations backward to the ancestors. It is accordingly concluded that Hb Okayama is the second independent instance of Hb Ube–4.

This investigation was supported by the Research Projects Grants (53–102 and 53–103) of the Kawasaki Medical School.

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