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Some Clinical and Molecular Aspects of Hemoglobinopathy Detected by Our Screening Test for the Past 11 Years

Kazuo HIDAKA and Iwao IUCHI

Department of Biochemistry, Kawasaki Medical School, Kurashiki 701-01, Japan

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Abstract

A hemoglobinopathy survey totaling 170,000 specimens was carried out for the past eleven years in four cities lain on the coast of Seto Inland Sea from 1979 to 1989. Nineteen variants of abnormal hemoglobin were detected and four of these were noteworthy to depict because of the instances of a homozygogous Hb Takamatsu, the notable change physico-chemical nature of Hb Nunobiki and high oxygen affinity of Hb Syracuse and Hb Fukutomi.

Introduction

A hemoglobinopathy survey have been enforcely conducted by us in four cities lain on the coast of Seto Inland Sea, namely, Takamatsu, Kobe, Fukuyama and Okayama for the past eleven years from 1979 to 1989. In total of 170,000 specimens, 19 kinds of abnormal hemoglobin (abn Hb) were detected. The present paper aims to describe four abn Hbs which are characteristic clinically and functionally.

Materials and Methods

An aliquot (2.0 ml) of whole blood for hematological examination was stored in a refrigerator (4℃) in the clinical laboratory department in the the Kagawa Central Hospital (Takamatsu), the Kobe Central City Hospital (Kobe), the Fukuyama National Hospital (Fukuyama) and the Kawasaki Hospital (Okayama).

They were then transported once a week in an ice cooled container to our laboratory. Then the specimens to be screened were picked up by employing the criteria of age, sex and full name of individual by use of a computer.

When an abn Hb was detected by our conventional isoelectric focusing (IEF) on polyacrylamide slab gels (1), hematological and physical examination of the carrier and family studies were informed us from the laboratory.

Then the following tests were carried out to characterize the abn Hb, namely, electrophoretic nature, instability (2), auto-oxidation (3) and oxygen equilibrium curve (4). For structural analysis of all the abn Hbs, determination of the amino acid substitution in the aberrant chain and the amino acid sequencing of peptide by Edman's stepwise method were performed (5).

Result and discussion

Nineteen variants of abn Hb were identified for the eleven years. These were classified according to their nature as shown in table 1. Of these abn Hbs, four specific Hb variants, namely, Hb Takamatsu (Hb Ta), Hb Syracuse (Hb Sy), Hb Nunobiki (Hb Nu) and Hb Fukutomi (Hb Fu) are described as the representatives.

Table. 1. Classification of abnormal Hb according to characteristics.

1 abn Hb detected in the restricted area
Hb Takamatsu, Hb Yusa, Hb Mizushi, Hb Fukuyama
2 rare but widely distributed abn Hb in Japan
Hb Ube-2
3 rare and functionally abn Hb
Hb Syracuse, Hb Nunobiki, Hb Fukutomi
4 rare and stable abn Hb
Hb J Bangkok, Hb G Szuhu, Hb St. Lukes, Hb Umi
Hb Riyadh, Hb G Coushatta, Hb Ankara, Hb Handa
Hb J Habana, Hb Albany-Suma, Hb Hoshida,

1) Hb Takamatsu (β120 Lys→Gln).

The first case of this Hb variant in the Takamatsu area was found in 1979 (6, 7). Since then eighteen additional Hb Takamatsu families without apparent consanguinity in each family have been detected in this area and they were distributed over mountainous area rather than in the sea coast of Kagawa prefecture. One individual of family 1 of heterozygous Hb Takamatsu was the common person by marrige with that of family 6. Accordingly, one of his two daughters happened to be born as a homozygote for this Hb variant by a family study. She was the first homozygote for a Hb variant found in the Japanese. She was a 12 year-old who was well-developed, well-nourished and had no particular abnormalities in her physical examinations at the time of discovery. She showed neither hematological abnormality nor hemolytic tendencies (RBC 4.04×10^{12}/l, Hb 11.8 g/dl, PCV 0.35 l/l, MCV 87 fl, MCHC 33.4 g/dl, WBC 4.3×10^{9}/l, serum bilirubin within normal limits, serum iron 84μg/dl and serum TIBC 331μg/dl). Isoelectric focusing of her hemolysate showed only two bands of Hb Takamatsu and Hb A_2 and their proportion was 97.8 and 2.2%, respectively. Hb F was 0.8% by alkaline denaturation. The oxygen equilibrium curve for the purified Hb Takamatsu was the same as that measured for Hb A. Structural analysis of Hb Takamatsu showed that a lysine at 120 position of β chain was replaced by a glutamine.

Hb Takamatsu is harmless to the carrier and is extremely stable abnormal Hb, as shown by the presence of the healthy homozygote. Therefore, it is considered that Hb Takamatsu might be originated from a single common ancestor and distributed in a high frequency without a natural selection in the same area.

2) Hb Syracuse (β143 His→Pro) (8)

The propositus was a 22 year-old female who was admitted to the hospital for rhinitis in Sep., 1981. Her hematologic examination revealed polycythemia without cyanosis (RBC 6.29×10^{12}/l, Hb 15.8g/dl, PCV 0.52 l/l, MCV 83 fl, MCH 24.2 pg, MCHC 29.6g/dl, reticulocyte 1.0%, serum bilirubin 0.6mg/dl). She was hospitalized again, by complaining of pyelitis in Dec., 1981. Her polycythemia was still evident. In IEF of her hemolysate, Hb Syracuse demostrated fast-moving but very close to Hb A.

Successful separation of these two bands was, therefore, achieved only by IEF. The other electrophoretic methods, such as cellulose acetate membrane, agar gel, agarose gel and starch gel, were performed in vain. Hb Syracuse constituted 41.4% of the total Hb in IEF. The result of the oxygen equilibrium curve of Hb Syracuse at 25℃ showed a high oxygen affinity, showing the following value of log $P_{50} = -0.01$ (Hb A=1.02) and -0.14 (Hb A=0.85) at pH 7.0 and 7.4, respectively. The heme-heme interaction, alkaline Bohr effect and 2,3-DPG effect of Hb Syracuse were also markedly decreased as indicating: Hill's n=1.23 (Hb A=2.54) and 1.24 (Hb A=2.77) at pH 7.0 and 7.4, respectively, the alkaline Bohr effect $= -0.33$ (Hb A$= -0.44$) and the 2,3-DPG effect=0.09 (Hb A=0.14). Sequence analysis of Hb Syracuse revealed that the Pro residue was substituted for the His residue at position 143 of the β chain. In deoxyHb structure (T structure), The C-terminal His (β146) forms two salt bridges; namely, carboxyl group is linked to the ε-amino group of α40 Lys, while its imidazole group is linked to the carboxyl group of β94 Asp. In addition, β143 His plays an important role for the binding of 2,3-DPG and also the formation of salt bridge between β146 His and β94 Asp contributes about 50 per cent to the normal alkaline Bohr effect (9, 10). In Hb Syracuse, the substituted Pro disrupts the H helix at position 143 of the C-terminal end of the β chain and these salt bridges described above are impaired by the conformational alteration and the stability of the T structure would be weakened, making the R structure favored over the T structure, resulting in increased oxygen affinity, decreased both Bohr effect and 2,3-DPG effect. The auto-oxidation velocity of Hb Syracuse was slightly but significantly slower than that of Hb A. According to Wallance (11), the formation of metHb by auto-oxidation begins with the dissociation of the oxygen molecule from fully liganded Hb (oxy Hb), followed by the oxidation of deoxyHb by free oxygen at low pH that leads to the formation of metHb and superoxide anion as shown below.

$$\text{Hb } Fe^{2+}(O_2) \longrightarrow \text{Hb } Fe^{2+} + O_2 \qquad (1)$$

$$\text{Hb } Fe^{2+} + O_2 \longrightarrow \text{metHb } Fe^{3+} + O_2^- \qquad (2)$$

Because of the difference in oxygen affinity, reaction (1) may proceed more slowly in Hb Syracuse than in Hb A. Consequently, the rate of auto-oxidation of Hb Syracuse may be less than that of Hb A. Likewise, it may be possible to explain the rate of auto-oxidation of other Hb variants, Hb Nunobiki and Hb Fukutomi, because of having high oxygen affinity.

3) Hb Nunobiki (141 Arg→Cys) (12)

The propositus was a 44 year-old male who was admitted to the hospital for surgery for acute ulcerative colitis. His hematological examination before surgical operation indicated an increased leukocyte count and slight anemia, but no tendency toward hemolytic anemia. After the operation, he showed a tendency to polycythemia: RBC 5.31×10^{12}/l, Hb 16.1 g/dl, PCV 46.5 l/l, MCV 88 fl, MCH 30.3 pg, MCHC 34.6 g/dl, WBC 7.7×10^{9}/l, platelet 21.1×10^{10}/l, Retic. 0.9%. IEF of his hemolysate revealed discrete bands of Hb Nunobiki, Hb A and Hb A_2 in that order from the anode to the cathode.

Hb Nunobiki constituted 13.1% of the total Hb and Hb F was 0.95%. The oxygen equilibrium curve of the purified Hb Nunobiki demonstrated a high oxygen affinity: log P_{50}=0.360 (Hb A=1.080) and 0.223 (Hb A=0.900) at pH 7.0 and 7.4, respectively. The heme-heme interaction, alkaline Bohr effect and 2, 3-DPG effect of Hb Nunobiki were also markedly decreased, namely, Hill's n=1.25 (Hb A=2.88) and 1.23 (Hb A=2.77) at pH 7.0 and 7.4, respectively, alkaline Bohr effect=−0.34 (Hb A=−0.45) and 2,3-PG effect=0.060 (Hb A=0.132). The auto-oxidation rate of Hb Nunobiki was remarkably slower than that of Hb A. Structural analysis of Hb Nunobiki was performed as follows: the α^{Nu} chain was oxidized at first with performic acid and digested with pepsin. The fingerprint of the digest was compared with that of the normal control α^{A} chain. One extra spot was clearly seen as compared with the fingerprint from the normal α^{A} chain. Amino acid analysis of the acid hydrolysate of the abnormal spot showed that it consisted of 5 amino acid residues and considered to be $\overset{141}{Cys}$ SO_3H-$\overset{140}{Tyr}$-$\overset{139}{Lys}$-$\overset{138}{Ser}$-$\overset{137}{Thr}$ from C-terminus of α^{Nu} chain, in which Cys had substituted for Arg at α 141. The confirmation of this substitution was provided when the aminoethylated α^{Nu} chain was treated with carboxypeptidase B and the amino acids from the C-terminus of the α^{Nu} chain was detected as aminoethylated $\overset{141}{Cys}$, $\overset{140}{Tyr}$ and $\overset{139}{Lys}$ with amounts in the order production with incubation time course. In IEF, Hb Nunobiki migrated to one more negative position than Hb A because of substitution of Cys for Arg. However, when the separated Hb Nunobiki was stored for one month at 4℃. it moved more anodally than the freshly prepared Hb Nunobiki, meaning it had two more negative charge than Hb A. It was obvious that the S atom of the thiol (-SH) in Cys was oxidized partially to lead to sulfoxide ($-SO^{-}$) or sulfinic acid ($-SOO^{-}$) ion form and that each of these ion have one more negative charge than neutral Cys.

On IEF of PMB (p-mercurybenzoic acid)-treated Hb Nunobiki (data not shown), a single band of PMB modified α chain of Hb Nunobiki moved more anordally as compared with that of the normal PMB-treated Hb A. This supported that the Cys at α 141 did not take part in the formation of a disulfide bond and the polymerization of Hb Nunobiki. But Hb Port Alegre (α9 Ser→Cys) and Hb Ta-li (β83 Gly→Cys) allow polymerization of the molecule by disulfide bonding.

4) Hb Fukutomi (126 Asp→Val) (13)

The propositus was a 53 year-old male with liver cirrhosis and hemorrhagic gastritis. The results of routine analysis suggested a slight erythrocytosis in the presence

of hemorrhage and latent jaundice due to liver dysfunction: RBC 4.3×10^{12}/l, Hb 15.2 g/dl, PCV 0.45 l/l, WBC 6.0×10^{9}/l, MCV 104 fl, MCH 35.5 pg, MCHC 34.1 g/dl, gammaglutamyl transpeptidase 161 IU/l, total bilirubin 1.8 mg/dl.

He has no siblings and his parents are deceased. IEF of his hemolysate demonstrated the presence of Hbs A, Fukutomi, A_2 and Fukutomi-A_2 and these Hbs amounted to 72.7, 24.4, 2.2 and 0.7% of the total pigment in the order described. The reversed phase HPL chromatogram of the tryptic digest of the α^{Fu} chain showed an extra peptide (αT-13), which joined core fraction of the normal chain as shown in figure 1. This finding suggested that the structural anomaly was located between αT-12 and αT-13 in the core region. Two fragments labeled A and B were present in the reversed phase HPL chromatogram of the chymotryptic digest of the oxidized α^{Fu} core obtained by performic acid oxidation.

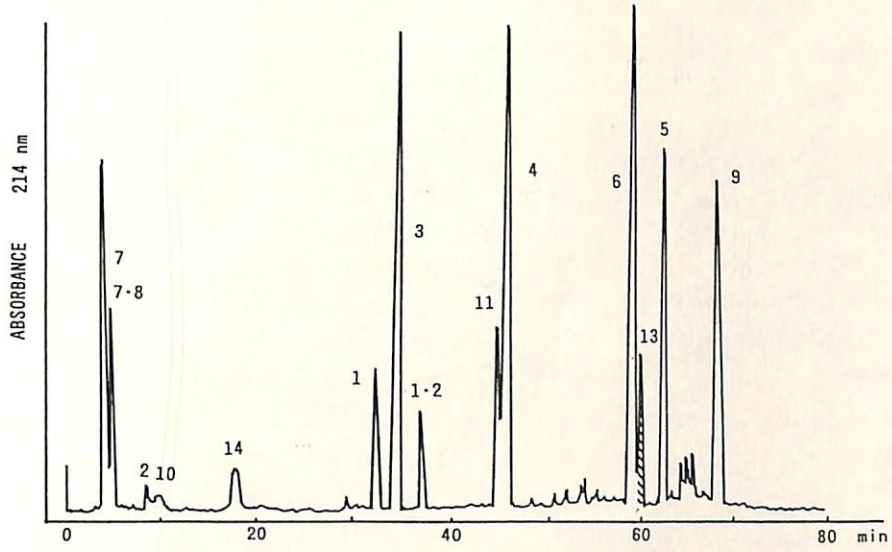

Fig. 1. Elution of tryptic digest of α chain of Hb Fukutomi. Note that the αT-13 peptide appears as an extra peak.

The result of amino acid analysis of these fragments demonstrated an Asp→Val substitution at position 126. The sequence Asp-Lys (126-127) in the normal αT-12 peptide is well known to resist to trypsin digestion because the acidic aspartic acid is close to the basic lysine. Therefore, αT-13 is usually belongs to the trypsin-resistant core of the α chain. However, when the sequnce is changed to Val-Lys as in Hb Fukutomi, C-terminal end of the lysine residue is cleaved by trypsin in the usual way. Therefore, the aberrant αT-13 peptide appears together with all other soluble peptides. The oxygen equilibrium curve for the purified Hb Fukutomi revealed a markedly increased oxygen affinity and a decreased heme-heme interaction as compared to the purified Hb A in parentheses: log P_{50} = 0.201 (Hb A = 1.084) and 0.087 (Hb A = 0.970) at pH 7.0 and 7.4, Hill's n = 1.64 (Hb A = 2.58) and 1.92 (Hb A = 2.91) at pH 7.0 and 7.4, respectively. The Hill plot of the oxygen equlibrium of Hb Fukutomi showed the

biphasic nature of the oxygen affinity, but the cause of it could not be elucidated (figure 2).

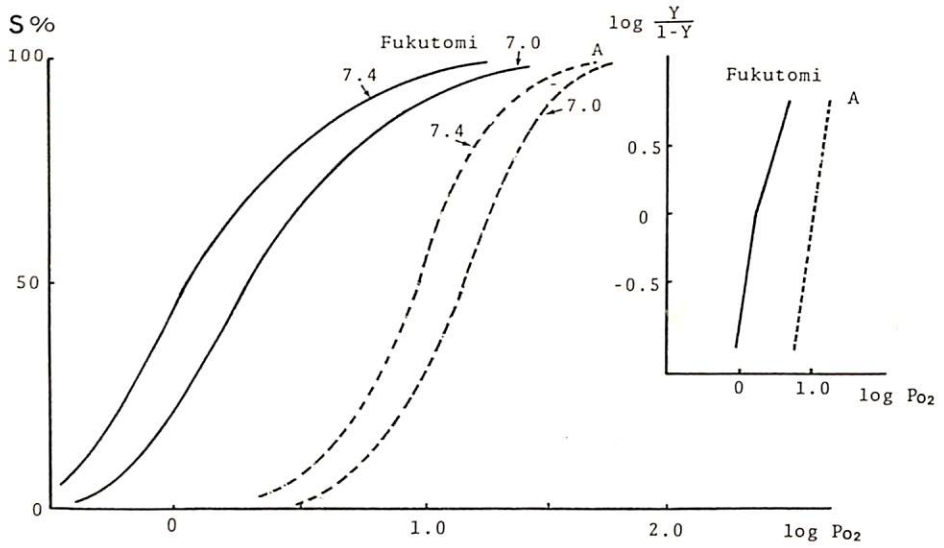

Fig. 2. Oxygen equilibrium curves of purified Hb Fukutomi and Hb A. The right section shows the Hill plot obtained from these curves at pH 7.0

The hemoglobin molecule model of Perutz (14) suggests, in the deoxy hemoglobin form (T structure), the carboxyl group of the C-treminal Arg of the α chain (α1) is linked to the ε- amino group of the 127 Lys of its partner α chain (α2) and the guanidium group of Arg at α1 141 is directed toward the α-carboxyl group of Asp at α2 126 and to the α-amino group of Val at α1 via an intervening chloride ion. The formation of these ionic linkage might be interrupted in the α Nunobiki subunit having C-terminal Cys instead of Arg and in the α Fukutomi subunit having Val instead of Asp at α 126. As a result, the stability of T structure would be weakened and that of the R structure would be strengthened as is characteristic of high oxygen affinity.

Hb J Bangkok, Hb G Szuhu, Hb G Coushatta, Hb Ankara, Hb Habana and Hb Albany-Suma were detected in Takamatsu and Kobe area. These two cities have been prosperous since ancient times. Therefore, it is thought that a variform abnormal Hb which is not racial was found as expected.

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