A new hemoglobin variant, Hb Mito [β144(HC 1) Lys → Glu], with increased oxygen affinity

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A new abnormal hemoglobin, Hb Mito [β144(HC 1) Lys → Glu], with an amino acid substitution in the 2,3-DPG pocket, was discovered in a Japanese female. This hemoglobin showed increased oxygen affinity, and decreased organic phosphate and Bohr effects, while the Hill constant *n* was normal.

Hb Mito (β144 Lys → Glu) *Oxygen affinity*

1. INTRODUCTION

We detected an abnormal fast-moving hemoglobin variant in a blood sample collected from a Japanese female with hypertension (but without diabetes mellitus) living in Mito, Ibaragi Prefecture. The variant constituted 36% of total hemoglobins and was eluted at the same position as glycosylated hemoglobin, Hb A_{Ic}, on HPLC (HLC-723GHb, Toyo Soda, Japan). Structural analysis showed it to have an amino acid substitution of the β-chain, Glu for Lys at position β144(HC 1). It also showed a high oxygen affinity. This variant has not been previously recorded [1]. Therefore, we named the hemoglobin Hb Mito after the name of the city where the patient lived.

2. MATERIALS AND METHODS

A routine hematological examination was performed using standard methods. Studies of the structural and functional properties of the abnormal hemoglobin component purified by isoelectric focusing were then carried out [2]. The polypeptide chain abnormality was detected by urea-dissociation cellulose acetate membrane electrophoresis [3]. A β-chain anomaly was disclosed. Isolation of the abnormal β-chain (β^X) was performed by CM-52 column chromatography using 8 M urea-sodium phosphate buffer solution (pH 6.8, Na^+ gradient 5–35 mM) [4]. The β^X-chain was aminoethylated and digested with TPCK-trypsin, and the digest was subjected to fingerprinting on a cellulose thin layer [5]. The amino acid composition of the hydrolysate of the abnormal peptide was analyzed in an automatic analyzer. The oxygen equilibrium curve was examined according to the method of Imai [6].

3. RESULTS

The carrier was a 63-year-old Japanese female who had suffered from hypertension for 17 years. A hematological examination of her peripheral blood disclosed a tendency toward questionable polycythemia (WBC 7500/μl, RBC 509 × 10^4/μl, Hb 15.1 g/dl, Ht 46.1%, MCV 91 fl, MCH 29.7 pg, MCHC 32.8%, retic. 1.1%). Chemical examinations were within the normal range. The values of serum iron and TIBC were 100 and 288 μg/dl, respectively. Isoelectric focusing of the hemolysate revealed an abnormal hemoglobin

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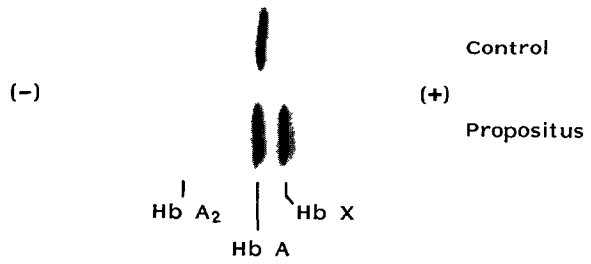

Fig.1. Isoelectric focusing of the hemolysates.

band which had migrated more anodaly than the Hb A band, as shown in fig.1. The hemoglobin composition was Hb F 2.0%, Hb A_2 3.03%, abnormal hemoglobin (Hb X) 42.2% and Hb A 52.5%. An isopropanol precipitation test of the hemolysate was negative. 2,3-DPG content (1.86 μmol/ml whole blood, 12.3 μmol/g Hb, and 4.04 μmol/ml packed cells) was normal.

The oxygen equilibrium curves of the Hb X revealed an increased oxygen affinity at acidic pH (table 1, fig.2) in comparison with those of Hb A. The Bohr and the DPG effects were decreased, while the Hill constant n was normal.

Urea-dissociation cellulose acetate electrophoresis of the hemolysate revealed the β-chain anomaly (fig.3). Deviation of 2 electron charges was conceivable on the basis of a comparison of the electrophoretic migration of the abnormal β-chain with that of the normal one. The fingerprint of the tryptic digest showed the absence of βT-14 and βT-15 and the presence of a new abnormal spot close to the βT-8–9 spot (fig.3). The amino acid composition of the abnormal peptide (βT-X) was Asp 0.96 (theoretical number: 1), Glx 1.36 (0), Gly 1.31 (1), Ala 4.21 (4), Val 1.97 (3), Leu 0.83 (1), Tyr 1.03 (1), Lys 0.00 (1), and His 2.19 (2). Because of the presence of a Val-Val sequence in βT-14, the estimated value of the Val residue might have been lower than the expected value in the abnormal peptide. This finding suggests that the Lys residue was replaced by Glu or Gln at the β144(HC 1) position. From the results of chromatography and electrophoresis (fig.3), it is presumed that the charge difference between the

Table 1

Oxygen-binding properties of Hb Mito and Hb A

Conditions[a]	K_T[b]	K_R[c]	L_0[d]	P_{50}[e]	n_{max}[f]	P_{50}^{A}/P_{50}^{Mito}[g]	$P_{50}^{phos}/P_{50}^{none}$[h]
Hb Mito							
pH 5.85	0.056	6.5	1.8×10^6	6.4	2.83	2.0	–
pH 7.4	0.083	6.6	2.9×10^5	3.9	2.94	1.2	–
pH 8.8	0.18	4.5	7.1×10^3	2.3	2.78	0.83	–
pH 7.4, 1 mM DPG	0.048	5.0	7.3×10^5	6.5	3.01	1.6	1.7
pH 7.4, 1 mM IHP	0.015	2.4	8.2×10^6	25.6	2.67	1.8	6.6
Hb A							
pH 5.85	0.030	1.6	9.6×10^4	12.6	2.77	–	–
pH 7.4	0.067	5.1	1.9×10^5	4.5	3.03	–	–
pH 8.8	0.13	3.7	2.3×10^3	1.9	2.87	–	–
pH 7.4, 1 mM DPG	0.029	3.3	9.9×10^5	10.6	3.03	–	2.4
pH 7.4, 1 mM IHP	0.010	0.62	4.1×10^5	47.3	2.39	–	10.5

[a] Other conditions: 25°C; [Hb] 60 μM on heme basis; in 0.05 M Bistris (pH < 7.4) or 0.05 M Tris (pH > 7.4) containing 0.1 M Cl^-

[b] Oxygen association equilibrium constant for the T state (in $mmHg^{-1}$)

[c] Oxygen association equilibrium constant for the R state (in $mmHg^{-1}$)

[d] Equilibrium constant for $T_0 \rightleftarrows R_0$ (T_0 and R_0 denote the deoxy T and R states, respectively)

[e] Oxygen pressure at half saturation (in mmHg)

[f] Maximal slope of the Hill plot

[g] Ratio of P_{50} for Hb A to P_{50} for Hb Mito

[h] Ratio of P_{50} in the presence of DPG or IHP to P_{50} in its absence

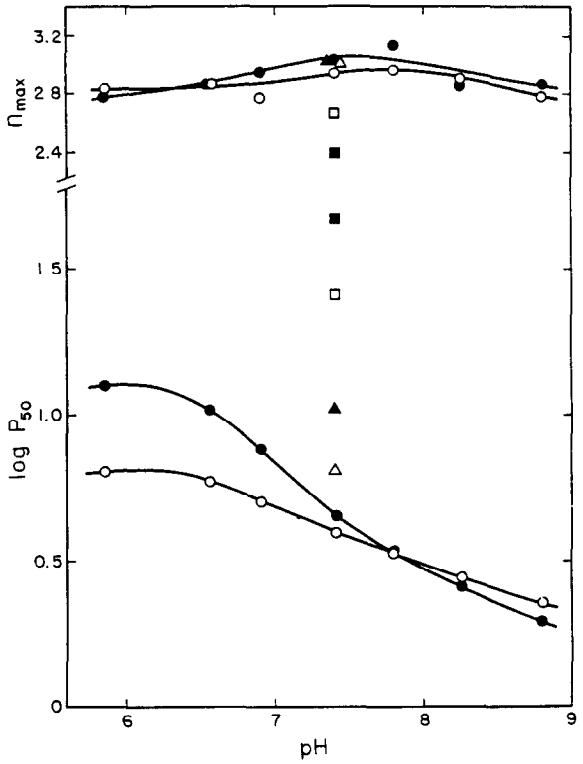

Fig.2. Oxygen affinity (log p_{50}) and cooperativity (n_{max}) of Hb Mito and Hb A. Open symbols, Hb Mito; closed symbols, Hb A. Circles, phosphate-free; triangles, + 1 mM DPG; squares, + 1 mM IHP. Other conditions as in table 1.

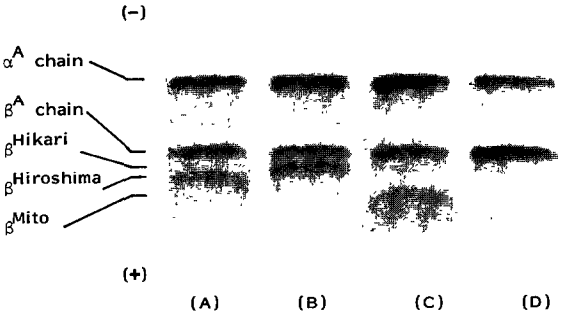

Fig.3. Comparison of the electrophoretic migrations of fast-moving abnormal hemoglobins on cellulose acetate electrophoresis in Tris-EDTA-borate buffer (pH 8.3). Whole hemolysate: (A) Hb Hiroshima (β146 His $\longrightarrow$ Asp), 2 negative charges in excess of the β^A chain. (B) Hb Hikari (β61 Lys $\longrightarrow$ Asn), one negative charge in excess of the β^A chain. (C) Hb Mito (β144 Lys $\longrightarrow$ Glu). (D) Normal control hemolysate.

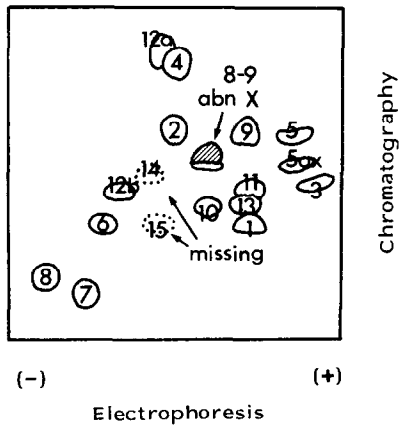

Fig.4. Fingerprint of the tryptic digest of the AE-β^X chain on a cellulose thin layer.

abnormal β chain and the normal one is 2. Accordingly, it has been concluded that the amino acid substitution of Hb Mito is β144(HC 1) Lys $\rightarrow$ Glu.

4. DISCUSSION

This abnormal hemoglobin, Hb Mito, is a new Hb variant which has substitution of Lys $\longrightarrow$ Glu at position 144(HC 1) of the β-chain.

Lys 144β of Hb A is free in the deoxy form and salt-bridged to the α-carboxyl group of the same β-chain in the oxy form [7], so that one might expect Hb Mito to have a lowered oxygen affinity. Also, one might expect that Glu 144β of Hb Mito points toward the DPG-binding site and neutralizes part of the positive charges inside the binding site, resulting in lowering of oxygen affinity in the absence of DPG and IHP [8]. Thus, the altered function of Hb Mito cannot be explained easily on the basis of the 3-dimensional structure. However, amino acid substitution on the atomic model indicates that Glu 144β of oxy Hb Mito could form a hydrogen bond with the imidazole group of His 146 of the same β-chain which was broken in the deoxy form. This mechanism involving perturbation of His 146β would explain the increased oxygen affinity at neutral and acid pH values and the reduced Bohr effect of Hb Mito. The perturbation does not seem to be so intense that oxygen affinity is profoundly increased and cooperativity influenced. A similar structural interpretation was pro-

posed for Hb Andrew-Minneapolis that is another variant having an amino acid substitution at 144β (Lys $\longrightarrow$ Asn) and oxygen-binding properties similar to those of Hb Mito [9].

Values of the Monod-Wyman-Changeux model parameters [10] in table 1 indicate that the oxygen affinity of Hb Mito is increased both in the T state and in the R state while the allosteric equilibrium (L_0) of Hb Mito shows no consistent variation compared to that of Hb A.

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