Hemoglobin Kariya [α40 (C5) Lys → Glu]: A new hemoglobin variant with an increased oxygen affinity

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A new abnormal hemoglobin, Hb Kariya [α40 (C5) Lys → Glu], with an amino acid substitution at the $\alpha_1\beta_2$ contact was discovered in a young Japanese man. This variant migrated to the anode faster than Hb A, being nearly the same as Hb I in electrophoretic mobility. It amounted to about 6% of the total hemoglobin of the hemolysate. This hemoglobin showed an increased oxygen affinity, decreased heme–heme interaction and a lowered 2,3-DPG effect.

Hemoglobin Kariya [α40 (C5) Lys → Glu] *$\alpha_1\beta_2$ Contact* *High oxygen affinity*

1. INTRODUCTION

In 1982, 14 carriers of abnormal hemoglobins were detected amongst 24 000 inhabitants living in a southern sea-coast area of Aichi Prefecture situated in the central part of Japanese main island (Honshu) in the course of a mass screening hemoglobinopathy survey using isoelectric focusing (IEF) [1]. Among them there was an apparently healthy 19-year-old Japanese male who possessed a fast-moving hemoglobin reminiscent of Hb I. Structural analysis of the hemoglobin disclosed a variant with an amino acid substitution of Lys → Glu at the 40th site of the α chain, which had not been reported previously. We called it Hb Kariya after the name of the city where its carrier lived. The results of our study on structure and function of this hemoglobin are presented.

2. MATERIALS AND METHODS

Hematological studies were done by the conventional procedures. IEF was done on thin-layer ampholine–polyacrylamide gel (pH 6–9) as in [1]. The detection of the abnormal polypeptide chain was made by 8 M urea-dissociation–cellulose acetate electrophoresis [2]. The erythrocyte 2,3-DPG content was measured by use of 2,3-DPG measurement kit (Sigma Lab.). Instability tests were done as in [3]. The Heinz body preparation test was done as in [4]. The hemoglobin composition of the hemolysate was evaluated by spectrometry at 415 nm of eluates of isoelectrically focused hemoglobins. The Hb F content was determined by alkali denaturation [5]. The purification of the Hb fraction was done as in [6].

Oxygen equilibrium curves of the purified Hb's were automatically determined in 0.05 M bis-Tris buffer (pH 7.45 and 6.95 containing 0.1 M Cl^-) with or without 2,3-DPG (2 mM) at 25°C [7,8].

After deheminization with HCl–acetone, the globins were chromatographed on a CMC-column to isolate the abnormal α chain [9]. The soluble fraction of the TPCK-tryptic digest of the α-chain was fingerprinted on cellulose thin-layer (Chromagram Sheet, Eastman-Kodak) [10], eluted and the

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peptides subjected to hydrolysis in constant-boiling HCl. The amino acid composition was analyzed in an automatic amino acid analyzer (Yanaco L-7).

3. RESULTS

Hematological features of the propositus were normal (RBC $477 \times 10^4/\mu l$, Hb 15.8 g/dl, Ht 45.3%, retic. count 1.0%, Bil (T) 0.8 mg/dl). The erythrocyte 2,3-DPG level was almost normal: 15.4 μmol/g Hb (normal range: 10.1–15.1). By IEF the abnormal Hb component focused more anodally than Hb A (fig. 1), and constituted 6.1% of the total hemoglobins of the hemolysate. The Hb A_2 content was 2.3% (normal range: 1.8–3.2) and the Hb F content was slightly increased: 1.6% (normal range: <1.1). An instability test of whole hemolysate was questionably positive, but the purified sample of Hb Kariya showed an unambiguously positive result. The Heinz body test was positive: 35% (normal range: 20 ± 5).

Oxygen binding properties of the purified Hb Kariya were compared with those of Hb A (fig. 2).

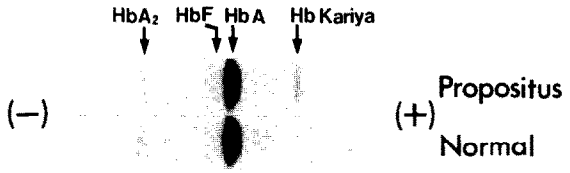

Fig. 1. IEF of the hemolysates.

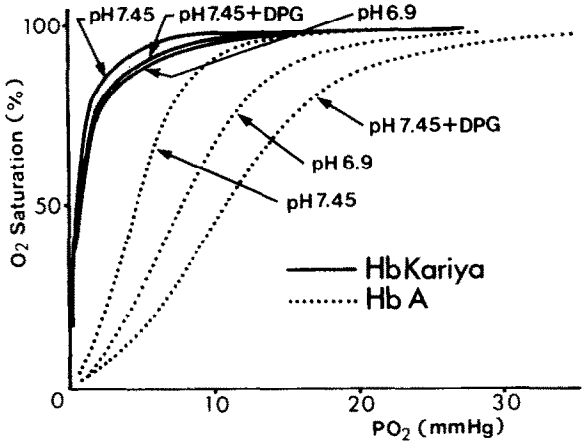

Fig. 2. Oxygen dissociation curves of Hb Kariya and Hb A.

Hb Kariya shows an increase in oxygen affinity, decreased heme–heme interaction and diminution of 2,3-DPG effect. These experiments indicated that Hb Kariya has a normal alkaline Bohr effect. However, the most recent experiments indicate that the Bohr effect is somewhat decreased in Hb Kariya (not shown).

Urea-dissociation electrophoresis of globin chains from Hb Kariya unravelled the presence of an abnormal α chain (α^{Ka}). The fingerprint of the soluble fraction of the tryptic digest of the purely isolated α^{Ka} chain disclosed the absence of αT-5, αT-5ox and αT-6 peptides at the proper sites and the presence of a new abnormal spot at the neutral zone on the map (fig. 3), suggesting a substitution of the C-terminal Lys (α40) of αT-5 peptide. The amino acid composition of this peptide (table 1) was consistent with substitution of Lys → Glx in the α40 position. The fact that Glx at α40 is Glu will be deduced by the electrophoretic property of this abnormal hemoglobin. Accordingly, Hb Kariya is an abnormal hemoglobin [α40 (C5) Lys → Glu], which has not yet been recorded.

4. DISCUSSION

The Lys at the 40th position of the α-chain is in the $\alpha_1\beta_2$ contact. Its ϵ-amino group forms a salt bridge with the α-carboxyl group of the C-terminal His residue of the β chain, and plays a very impor-

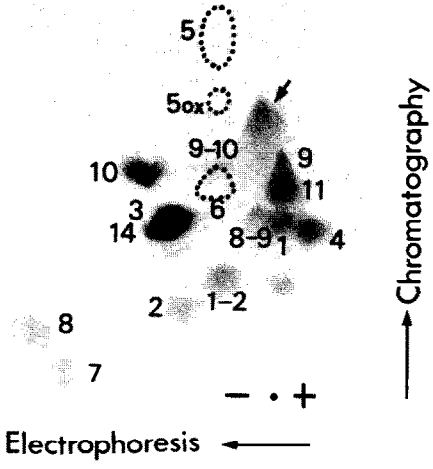

Fig. 3. Fingerprint of the soluble fraction of the tryptic digest of the α^{Ka} chain: (⋱) missing spots; (→) abnormal spot.

Table 1

Amino acid composition of abnormal peptide

	Found (mol. ratio)	α^{A}T 5–6
Lys	0.84	2
His	1.88	2
Asp	1.17	1
Thr	3.02	3
Ser	3.09	3
Glu	2.01	1
Pro	1.88	2
Gly	1.07	1
Ala	1.12	1
Val	1.06	1
Met[a]	0.60	1
Leu	2.23	2
Tyr	0.82	1
Phe	3.72	4

[a] N-terminal residue

tant role in controlling the oxygen affinity by stabilizing the T quaternary structure [11]. In Hb Kariya this salt bridge will be absent due to the replacement of Lys → Glu, resulting in the destabilization of the T structure and displacement of the T ⇄ R equilibrium toward the R state. The increased oxygen affinity and decreased heme–heme interaction of Hb Kariya can be understood on this basis. A similar mechamism causing high oxygen affinity is seen in the abnormal hemoglobins possessing amino acid substitution at the C-terminus of the β chain (β146), such as Hb Hiroshima (His → Asp) [12], Hb York (His → Pro) [13], and Hb Cowtown (His → Leu) [14]. The reduced 2,3-DPG effect observed in Hb Kariya may be explained by assuming partial inhibition of the T–R transition upon oxygenation since the DPG binding depends on the quaternary structure [11].

Notwithstanding the high oxygen affinity of Hb Kariya, its carrier did not show any sign of polycythemia. The low percentage of the variant in the circulating blood (some of it possibly due to its instability) may not be adequate to cause a polycythemic response.

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