Hb Okazaki [β93(F8) Cys⟶Arg], a new hemoglobin variant with increased oxygen affinity and instability

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A new abnormal hemoglobin, Hb Okazaki [β93(F9) Cys⟶Arg], with an amino acid substitution at the tyrosine pocket of the β chain as well as at the $\alpha_2\beta_1$ contact of the quaternary structure of molecule, was discovered in a Japanese man. This hemoglobin showed increased oxygen affinity and molecular instability.

Hb Okazaki [β93(F9) Cys⟶Arg] *Tyrosine pocket* *$\alpha_2\beta_1$ contact*

1. INTRODUCTION

In the course of a hemoglobinopathy survey by isoelectric focusing we encountered a slow-moving abnormal hemoglobin which was isoelectrofocused close to the Hb F band. The carrier was an apparently healthy 45-year-old man. Structural analysis demonstrated that the Cys residue at the 93rd position of its β chain was substituted by Arg. Since this amino acid substitution had not yet been recorded [1], we designated this hemoglobin Hb Okazaki after the name of the city where the carrier lived. This Hb showed high oxygen affinity and instability. We report the characteristics of this new hemoglobin variant.

2. MATERIALS AND METHODS

Hematological and clinical data were obtained by standard methods [2]. Examinations for structural and functional properties were done by use of the hemoglobin purified by isoelectric focusing [3]. The contents of Hb F and Hb A_2 were measured by standard methods [4,5]. The abnormal hemoglobin content was determined by spectrophotometry of the eluates of individual hemoglobins after isoelectrofocusing. The abnormal polypeptide chain was detected by urea-dissociation cellulose acetate electrophoresis [6]. Isolation of the abnormal β chain (β^X) was performed on CM-52 column chromatography by use of 8 M urea–sodium phosphate buffer solution (pH 6.8; $[Na^+]$ 8 mM ⟶ 45 mM) [7]. The β^X chain was digested overnight with TPCK-trypsin (Worthington) at room temperature. The soluble fraction of the digest was subjected to fingerprinting on cellulose thin layer (Eastman Kodak, chromagram sheet, 20 × 20 cm) [8]. The amino acid composition of the hydrolyzate of the abnormal peptide was analyzed in an automatic amino acid analyzer.

The oxygen equilibrium curves were examined as in [9]. Hemoglobin instability test was carried out as in [10] with slight modification [17–21% isopropanol in Na-K phosphate buffer (pH 7.4), incubation at 37°C for 1 h].

3. RESULTS

Hematological and chemical examinations of the peripheral blood of the propositus were within the normal ranges, except for a slight increase of 2,3-DPG content (WBC 3.3 × 10^9/l, RBC 4.67 × 10^{12}/l, Hb 13.5 g/dl, PCV 0.41 l/l, MCV 88 fl, MCH 28.9 pg, MCHC 32.9 g/dl, serum iron 114 μg/dl, total bilirubin 0.6 mg/dl, reticulocyte

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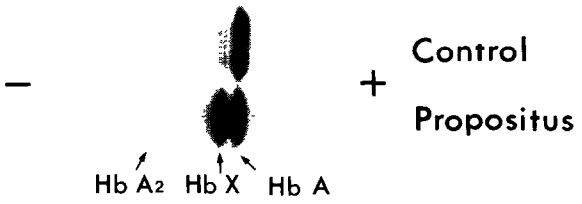

Fig.1. Isoelectric focusing of the hemolysates.

count 1.4%; 2,3-DPG content· 2.46 μmol/ml whole blood, 18.2 μmol/g Hb, 6.0 μmol/ml packed cells). Isoelectric focusing of the hemolysate yielded hemoglobin bands as shown in fig.1. The composition of the hemoglobins was: Hb F, 1.1%; Hb A_2, 3.3%; Hb X (abnormal Hb), 40.2%. The isopropanol precipitation test of the purified abnormal hemoglobin in comparison with Hb A gave a positive result (fig.2). The oxygen equilibrium curves of the abnormal hemoglobin revealed slightly increased oxygen affinity at various pH values in comparison with Hb A. Hill's n constant and Bohr effect were slightly decreased (table 1).

The urea-dissociation cellulose acetate electrophoresis of the purified hemoglobin showed that the β^X chain moiety of Hb X migrated more slowly than the β^A chain, suggesting a β chain anomaly. The β^X chain obtained by CM-52 column chromatographic separation was aminoethylated [11] and digested with TPCK-trypsin. The fingerprint of the digest showed the absence of βT-10 spot and the presence of a new abnormal spot near the βT-8-9 spot (fig.3). The amino acid composition of the abnormal peptide βT-X was Gly (0.76), Thr (1.84), Phe (1.03), Ala (0.93), Leu (1.75), Ser (0.95), His (0.82), AE-Cys (0), Arg (0.99), while that of βT-10 was theoretically Gly (1), Thr (2), Phe (1), Ala (1), Leu (2), Ser (1), His (1), AE-Cys (1), Arg (0). These data suggested that the Cys residue was substituted by Arg at the β93 position. Thus, the amino acid substitution of this Hb variant is β93 Cys⟶Arg.

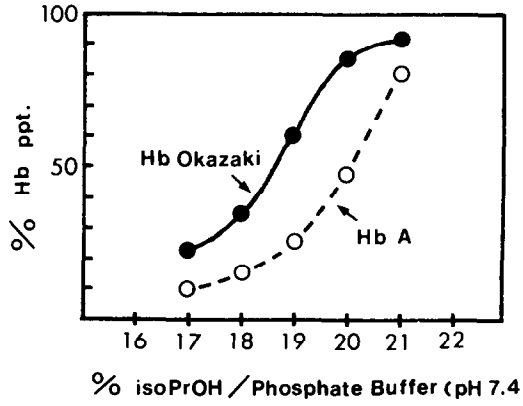

Fig.2. Instability test of the hemoglobins purified by isoelectric focusing.

4. DISCUSSION

Hb Okazaki [β93(F9) Cys⟶Arg] is a new hemoglobin variant which has not yet been recorded in the list of abnormal hemoglobins [1]. It has high oxygen affinity and molecular instability. In

Table 1

Oxygen-binding properties of the purified hemoglobins (at 25°C)

	Hb Okazaki		Hb A	
	P_{50} (mmHg)	$P_{50}^{OP}/P_{50}^{free}$	P_{50} (mmHg)	$P_{50}^{OP}/P_{50}^{free}$
pH 7.9	1.6		2.4	
pH 7.4	2.3		4.0	
pH 6.9	3.9		7.6	
pH 7.4 (1 mM DPG)	5.1	2.2	10.6	2.7
pH 7.4 (1 mM IHP)	19.0	8.3	40.9	10.2
Bohr effect (pH 6.9–7.9)	−0.39		−0.51	
Hill's n (pH 7.4)	1.7		2.8	

P_{50}^{OP}, $P_{50}^{organic\ phosphate}$

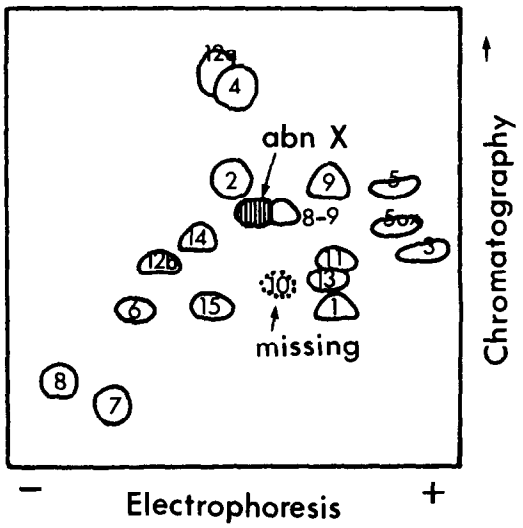

Fig.3. Fingerprint of the tryptic digest of the AE-β^X chain on cellulose thin layer.

deoxyhemoglobin, the C-terminal His β146 forms salt-bridges with Lys α40 and Asp β94. The histidine forms a ring around the SH group of Cys β93. Modification of the SH group with bulky reagents such as *N*-ethylmaleimide prevents closure of the ring, thus inhibiting formation of the salt-bridge and raising the oxygen affinity by destabilizing the deoxy (T) structure [12]. Since an Arg residue possesses a bulky branched side chain, the replacement of Cys by Arg in Hb Okazaki would act in the same way to result in an increase in its oxygen affinity. When the reactive SH group of Cys β93 is blocked by *N*-ethylmaleimide, both the Bohr effect and Hill's *n* value are reduced, because His β146, which contribute 40% of the Bohr effect, changes its position from the normal position in deoxyhemoglobin. As expected the alkaline Bohr effect and Hill's *n* value are reduced in Hb Okazaki as shown in table 1.

In addition, Cys β93 is located at the $\alpha_2\beta_1$ contact site [13]. The Arg residue in Hb Okazaki is too big to be settled in the interchain region of the α and the β chains. This may cause its molecular instability.

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