# Hemoglobin Okayama [β2 (NA 2) His → Gln]: A new 'silent' hemoglobin variant with substituted amino acid residue at the 2,3-diphosphoglycerate binding site

Teruo Harano, Keiko Harano, Susumu Shibata*, Satoshi Ueda+, Hiroo Mori+ and Naomichi Arimasa*

*Departments of Biochemistry, *Internal Medicine* and +*Clinical Pathology, Kawasaki Medical School, Kurashiki 701-01, Japan*

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A new 'silent' abnormal hemoglobin, Hb Okayama [β2 (NA 2) His → Gln], happened to be discovered in a diabetic Japanese female living in Okayama Prefecture, Japan, in the course of glyco-Hb measurement of the blood samples of diabetic patients. This variant did not differ from Hb A by conventional electrophoretic tests. Only the isoelectric focusing on PAG plate for the determination of glyco-Hb and the cation exchanger chromatography were successful in the separation of this abnormal variant from Hb A and glyco-Hb. Functional study of the whole blood demonstrated a slight increase of oxygen affinity.

*Hb Okayama [β2 (NA 2) His → Gln]* *Silent Hb variant*

## 1. INTRODUCTION

Although most of the abnormal hemoglobins discovered to date are different in electrophoretic mobility from Hb A, a number of variants are almost indistinguishable from Hb A electrophoretically. These are called 'silent' variants, and they are detected only by chance when hematological abnormality of the carrier's blood is investigated by specialized techniques; e.g., oxygen-binding property, isopropanol stability test, heat lability test. Otherwise, if these variants are normal in function and in physicochemical tests, they will escape detection.

Here, a silent hemoglobin variant, Hb Okayama, detected in a diabetic woman through the determination of glycosylated hemoglobin (glyco-Hb) will be described.

## 2. MATERIALS AND METHODS

Hematological studies were done by conventional routine procedures. Isoelectric focusing (IEF) was performed on Ampholine-PAG plate for the determination of glyco-Hb (LKB-Produkter AB). Hemoglobin composition of hemolysate was examined by elution of the isoelectrofocused individual Hb bands and spectrophotometry of the eluates. Glyco-Hb component was also analyzed by microcolumn chromatographic assay (NC-Roppet, Nihon Chemipher) and microcolumn affinity chromatography using glyco·gel B (Pierce Chemicals) [1] as well as by IEF. The Hb F content was determined by alkali denaturation [2]. The erythrocyte 2,3-DPG content was measured by use of 2,3-DPG measurement kit (Sigma). Instability test was done as in [3].

The abnormal hemoglobin fraction was isolated from Hb A and purified by large column (25 mm × 30 cm) chromatography, on BioRex 70 (Bio-Rad Chemical Labs.) as in [4] with slight modification. After removal of heme from purified hemoglobins with HCl–acetone, globins were chromatographed on urea–CM-cellulose (CM-52, Whatman) as in [5]. A β-chain anomaly was demonstrated for the abnormal hemoglobin



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by this chromatography. The abnormal $\beta$-chain ($\beta^X$) was aminoethylated [6], digested with TPCK–trypsin (Worthington) and fingerprinted on cellulose thin-layer sheet (Chromagram Sheet, Eastman-Kodak) [7]. The abnormal peptide was eluted, hydrolyzed in constant-boiling HCl and analyzed for amino acid composition in an automatic amino acid analyzer (Yanaco L-7).

The oxygen binding properties of a whole blood sample (not a hemolysate) studied in phosphate buffer solution of pH 7.3 at 37°C by a Hemox-Analyzer (TCS, USA) [8].

## 3. RESULTS

The existence of this abnormal hemoglobin was noticed during the process of glyco-Hb examination of a 64-year-old woman living in Okayama Prefecture who had insulin-dependent diabetes. She had been receiving insulin injection repeatedly for these 10 years. Hematological studies of her peripheral blood were within the normal range (RBC 3.97 × $10^6/\mu$l, Hb 13.9 g/dl, Ht 38.5%, MCV 97 fl, MCH 35.0 pg, Retics 0.9% and Bil(T) 0.6 mg/dl). The erythrocyte 2,3-DPG content was normal: 14.2 $\mu$mol/g Hb (normal: 11.0–16.2). By IEF the abnormal hemoglobin component focused anodally from the Hb A band, and overlapped the Hb $A_{Ic}$ fraction to a certain extent. The band composed of the abnormal hemoglobin and Hb $A_{Ic}$ together occupied 47.4% of the total hemoglobin of the hemolysate. Other hemoglobin components were: Hb $A_2$ (2.6%), Hb A (42.1%), and abnormal glyco-Hb (Hb $X_{Ic}$) (7.9%), as shown in fig.1. The first portion which was eluted early from the column of microcolumn chromatographic assay (belonging to Hb $A_I$) was 43.1% (normal: 5–7). The glyco-Hb level measured simultaneously by affinity chromatography on glyco·gel B was 13.8% (normal: 4.5–6.5). This corresponded to ~2-times the level of the abnormal glyco-Hb (Hb $X_{Ic}$) fraction estimated by IEF. Instability test of the whole hemolysate was almost negative.

Oxygen-binding properties of the whole blood showed that the $P_{50}$-value was 22.5 mm Hg [25–27]. This indicates that the abnormal hemoglobin has a raised oxygen affinity.

The fingerprint of the tryptic digest of the aminoethylated $\beta^X$ chain (AE-$\beta^X$) revealed absence of the $\beta$T-1 peptide spot at the proper site and

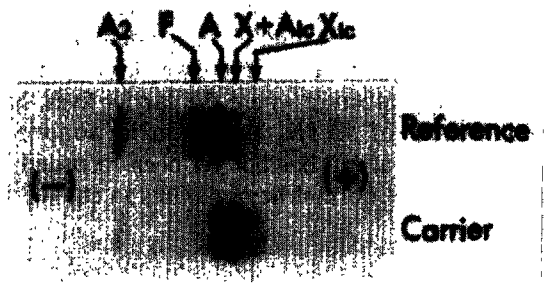


Fig.1. IEF of hemolysates: $A_2$, Hb $A_2$; F, Hb F; A, Hb A; X, abnormal Hb; $A_{Ic}$, glyco-Hb A; $X_{Ic}$, glyco-Hb X (Hb $X_{Ic}$).

presence of a new abnormal peptide spot which was shifted electrophoretically to the anode side (fig.2). The amino acid composition of the acid hydrolysate of the new peptide indicates that the histidine residue at $\beta$2 is substituted by either glutamic acid or glutamine. The abnormal hemoglobin is electrophoretically hardly separable from Hb A which indicates that they have nearly the same electric charge. An amino acid substitution His → Gln which causes a slight increase in negative charge will be more likely for this abnormal hemoglobin than that of His → Glu which would result in a distinct rise in negative charge. Also, the RNA codon for His at $\beta$2 is CAC [9]; this can be converted into CAA or CAG which specifies Gln by mutational change of one base (C → A or G), whereas conversion of His into Glu

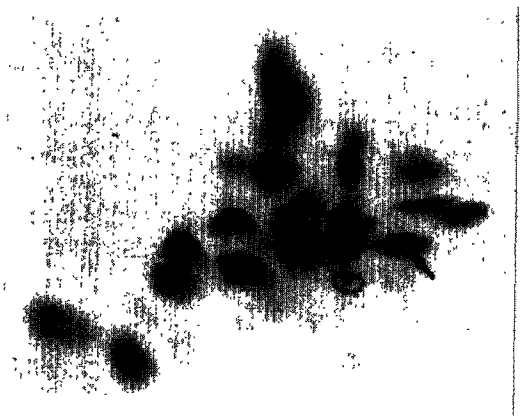

Fig.2. Fingerprint of the tryptic digest of the AE-$\beta^X$ chain. The dotted circle shows the missing spot of $\beta^A$T-1 peptide. The arrow indicates the spot of the new peptide $\beta$T-1.

(GAA or GAG) is not possible by the change of one base. It is, accordingly, concluded that this hemoglobin, which we call Hb Okayama after the name of the Prefecture where the carrier lives, has a replacement of $\beta$2 His by Gln in its abnormal chain. Such a variant has not yet been recorded in the list of the abnormal hemoglobins in the world [10].

## 4. DISCUSSION

The His residues possessing positive electric charge at the 2nd position of the $\beta$ chains of the normal hemoglobin tetramer belong to the 4 pairs of cationic groups (Val-1, His-2, Lys-82 and His-143 residues of the $\beta$ chains) which are directly involved in accommodation of organic phosphate (such as 2,3-DPG) in the central cavity of the hemoglobin molecule [11]. A 2,3-DPG molecule binds to the cluster of the cationic groups lining the wall of the central cavity, thus stabilizing the deoxy-Hb conformation which has a lowered oxygen affinity. In Hb Okayama deoxygenation of its molecule is expected to be incomplete due to loss of one of the 2,3-DPG binding sites on account of the amino acid substitution of His → Gln at the $\beta$2 position. This was corroborated by the raised oxygen affinity of the whole blood of the carrier of Hb Okayama ($P_{50}$-value: 22.5 mm Hg). However, the oxygen affinity was not so much increased as to cause polycythemia in the carrier.

There is another hemoglobin variant possessing an amino acid substitution at the $\beta$2 site. It is Hb Deer Lodge ($\beta$2 His → Arg) [12]. Like Hb Okayama this variant showed an increased oxygen affinity, but no clinical signs [12,13].

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