Hemoglobin Aichi [α50(CE8) His → Arg]: a new slightly unstable hemoglobin variant discovered in Japan

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A new abnormal hemoglobin, Hb Aichi [α50(CE8) His → Arg], was discovered in a young Japanese man. This variant was isoelectrofocussed between Hb A and Hb A_2 and amounted to about 21% of the total hemoglobin in the hemolysate. This hemoglobin showed normal oxygen affinity but slight instability.

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1. INTRODUCTION

In April 1983, an abnormal hemoglobin that isoelectrofocussed between Hb A and Hb A_2 was detected in a 19-year-old Japanese man in the course of a mass screening hemoglobinopathy survey [1]. Structural analysis of this hemoglobin showed that the His residue at the 50th position of the α chain was replaced by Arg. This amino acid substitution has not yet been recorded [2]; we call this variant Hb Aichi after the name of the Prefecture where the carrier lived. A family study showed that the father and the older sister of the propositus had the same variant. We shall describe the results of our experiments with this new abnormal hemoglobin.

2. MATERIALS AND METHODS

Hematological and chemical laboratory examinations were carried out by conventional procedures. Isoelectric focusing for the detection and purification of the abnormal hemoglobin was done on thin-layer ampholine–polyacrylamide gel (pH range 6–9) as in [1]. The hemoglobin composition of the hemolysate was measured spectrophotometrically at 415 nm on individual eluates of isoelectrofocussed hemoglobins. The detection of the abnormal polypeptide chain was made by cellulose acetate electrophoresis in urea–Tris–EDTA–borate buffer (pH 8.3) [3]. Oxygen equilibrium curves of the purified Hbs were automatically determined in 0.05 M Bis–tris buffer (pH 7.4 and 7.9) with or without 2,3-DPG (2 mM) or IHP (2 mM) at 25°C [4]. Instability test of the abnormal hemoglobin was done as in [5] and compared with normal hemoglobin. The globins obtained by treatment with cold HCl–acetone were chromatographed on a CM–cellulose (CM-52, Whatman) column to isolate the abnormal chain [6]. The soluble fraction of the TPCK-tryptic digest of the α chain was fingerprinted on cellulose thin-layer (Chromagram Sheet, Eastman) [7], and the amino acid composition was determined in an automatic amino acid analyzer.

3. RESULTS

Hematological and chemical laboratory examinations of the carrier of this abnormal hemoglobin were within the normal range. The abnormal hemoglobin component constituted 21.2–21.8% of the total hemoglobins of the hemolysate. Oxygen binding properties of the purified hemoglobin were normal; [P_{50} 4.1 mm Hg (Hb A = 4.0) at pH 7.4, P_{50} 2.6 (Hb A = 2.4) at pH 7.9]. The Bohr effect was −0.40 (Hb A = −0.44) at pH 7.4–7.9 and the organic phosphate effects at pH 7.4 were $P_{50}^{DPG}/P_{50}^{free} = 2.37$ (Hb A = 2.65) and $P_{50}^{IHP}/P_{50}^{free} = 9.00$ (Hb A = 10.23).

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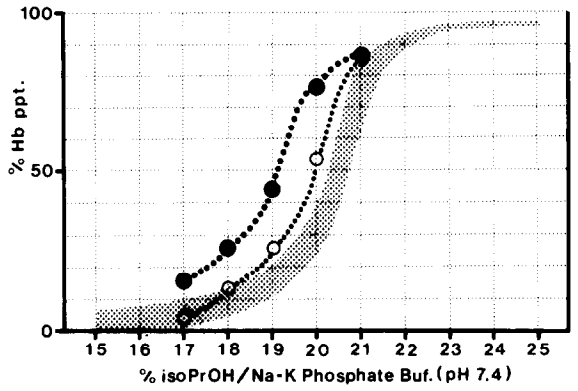

Fig.1. Instability test of the purified hemoglobins: (●---●) Hb Aichi, (○---○) Hb A.

The isopropanol precipitation test for 1 h incubation at 37°C was slightly positive (fig.1).

Despite the fact that isoelectric focusing of the whole hemolysate from the propositus showed a discrete abnormal hemoglobin band (fig.2), detection of the abnormal polypeptide chain by urea dissociation electrophoresis (pH 8.3) yielded only a slight difference between the α chain and the slow-moving abnormal α (α^{Aichi}) chain.

Furthermore, when the urea CM–cellulose chromatographic separation (pH 6.8) of whole globin chains was attempted, to detect the abnormal polypeptide chain, there was difficulty in obtaining a clear-cut separation from the normal α chain. It is therefore believed that the charge difference between the normal and abnormal chains will be small. α^{Aichi} chain for structural analysis was obtained by CM–cellulose chromatography of the globin from the purified abnormal hemoglobin. The fingerprint of the soluble fraction of the tryptic digest of α^{Aichi} chain revealed the absence of the αT-6 (α41–56) peptide at the proper site and the

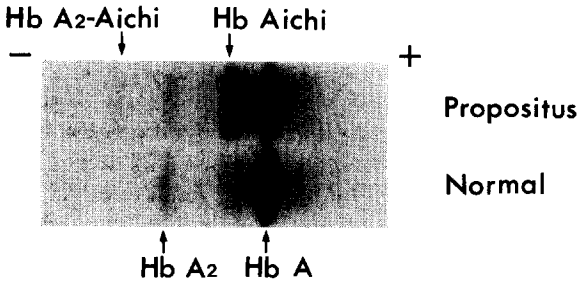

Fig.2. Isoelectric focusing of the hemolysates.

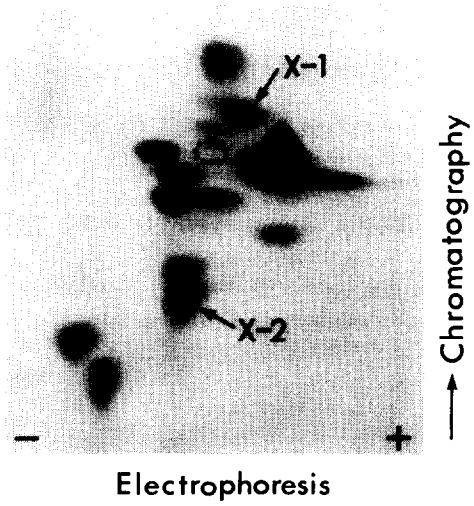

Fig.3. Fingerprint and the amino acid analyses of the soluble fraction of the tryptic digest of the α^{Aichi} chain. The dotted circle shows the missing spot of αT-6 peptide. The arrows (X-1 and X-2) indicate the newly appeared peptides. X-1 (actually analyzed): Thr (1.02), Tyr (0.85), Phe (2.05), Pro (1.10), His (0.87), Asp (0.99), Leu (1.09), Ser (1.06), Arg (0.96). X-2 (actually analyzed): Gly (1.02), Ser (1.22), Ala (0.97), Gln (1.15), Val (0.92), Lys (0.72). αT-6 (α41–56) (theoretical): α41–50: Thr-Tyr-Phe-Pro-His-Phe-Asp-Leu-Ser-His. α51–56: Gly-Ser-Ala-Gln-Val-Lys.

presence of two new abnormal spots on the map (fig.3, X-1 and X-2). The amino acid composition of these peptides indicated that the X-1 peptide corresponded to residues α41–50, where the His residue at position α50 was replaced by Arg, and X-2 was identical with residues α51–56 (fig.3). It was therefore thought that the abnormal αT-6 (α41–56) was cleaved at α50 by trypsin due to the α50 His → Arg substitution.

4. DISCUSSION

According to the three-dimensional hemoglobin model, the residue at position α50(CE8) occupies an external nonhelical segment of the α chain, without being involved in the heme contact sites [8,9], but the CE nonhelical segment might play a significant role in maintaining the structure of the heme pocket composed of C and E helices. Furthermore, the His residue at α50 of the normal α chain is situated close to the α30 Glu residue in the same chain, which is involved in the $\alpha_1\beta_1$ inter-

chain contact affecting the stability of the molecule. In reality a slight molecular instability was noticed in Hb Aichi. The replacement of the His residue by an Arg residue possessing a bulky branched side chain will not result in formation of salt bridge between α30 Glu and the newly introduced Arg at α50, but may disturb the quaternary structure around the heme contacts, and will consequently cause some instability and functional abnormality. The other abnormal hemoglobin with a mutation at the same position as Hb Aichi is Hb Sardegna [α50(CE8) His → Asp] [10]. Its instability and functional abnormality have not yet been reported. Hb Aichi is not associated with any clinical disturbances, probably because its instability is very slight.

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