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Brief Note

A Slow Moving Hb F-Kotobuki or $^{A}\gamma^{I}6$ (A3) Glu→Gly Detected in Fukuyama District

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During a survey of hemoglobinopathy in the Fukuyama district, a slow-moving γ chain variant was discovered from a cord blood specimen in July 1987. Structural analysis showed the Hb variant to be identical to Hb F-Kotobuki or $^{A}\gamma^{I}6$ Glu→Gly, which was first found in a Japanese in 1982 by Yoshinaka *et al.*[1] and subsequently reported by Wada *et al.*[2] Therefore, the present Hb variant was the third case in Japan. This paper aims to characterize this variant.

Isoelectricfocusing (IEF) of the hemolysate of the blood specimen on polyacrylamide slab gel (pH 5-9) revealed discrete bands of Hb A. Hb Fo and Hb Fx from the anode to the cathode.[3] The percentage of Hb Fx was 9.2 of the total hemoglobin, which corresponds to 14.6% of the total Hb F (Hb Fo +Hb Fx). The composition of γ chains of the hemolysate by reverse phase high performance liquid chromatography (HPLC) on μBondapak C18 (3.9× 300 mm)[4] was shown to have a $^{G}\gamma$ over $^{A}\gamma$ ratio of 82 to 18 (normal ratio : 70 to 30), which might suggest that the abnormal $\gamma(\gamma^{X})$ chain and the $^{G}\gamma$ chain were superimposed and appeared as one peak, that of the $^{G}\gamma$ chain, on the chromatogram. If so, Hb Fx is a variant of $^{A}\gamma$ type.

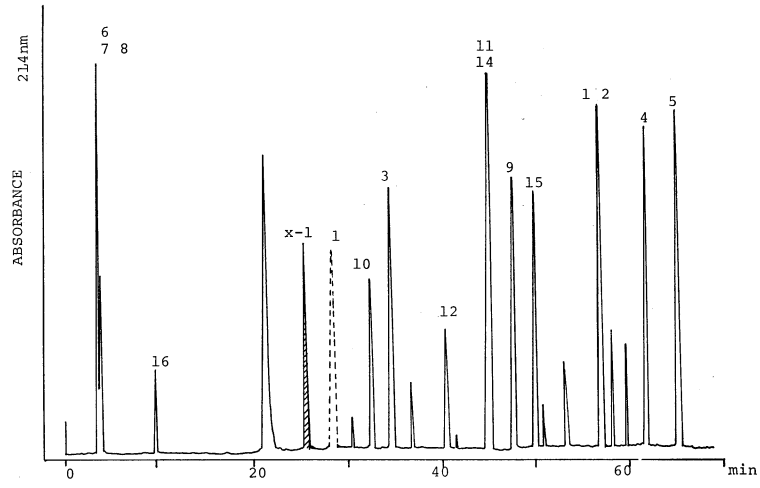

Fig. 1. Separation of the tryptic peptides of γ^{X} chain by HPLC. The shaded area indicates the abnormal peptide.

A large quantity of Hb Fx was obtained by IEF and the α and the γ^{X} chains were separated by 8M urea buffered CM-cellulose column chromatography,[5] after which the γ^{X} chain was digested with TPCK-trypsin (Worthington,

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Inc.) for 3 hr at 37°C and at pH 8.5. The HPLC elution map of the soluble fraction of the digest revealed the appearance of an aberrant γ^{X}T-1 peptide eluted slightly faster than normal γT-1 and the absence of normal γT-1 from its usual position (Fig. 1).[6] Amino acid analyses of the acid hydrolysate of the γ^{X}T-1 peptide showed it to consist of Lys 1.03(1), His 0.95(1), Asp 0.91(1), Thr 0.96(1), Glu 1.07(2), Gly 2.00(I) and Phe 1.01(1) (the numbers in the parentheses are those of amino acid residues for the normal γT-1 peptide). This peptide had the same amino acid residues as normal γT-1 peptide except that a glycine residue was substituted for one of two glutamic acids at the 5th or 6th position of the γ chain. Therefore, the γ^{X}T-1 peptide was hydrolysed with staphylococcus aureus V8 protease, producing two peptide fragments, A and B, as expected.[7] The results of amino acid analyses showed that peptide A referred to a residue 1-5 and the peptide B to a residue 6-8, indicating clearly a γ6 Glu→Gly replacement, as shown below.

1	2	3	4	5	6	7	8
Gly	His	Phe	Thr	Glu	Gly	Asp	Lys
———			A	———	———	B	———

The amino acid analyses of the peptide γT-9 and γT-15 of the aberrant γ chain demonstrated that the residues at γ75 and γ136 were an isoleucyl residue and an alanyl residue, respectively, indicating $^{A}\gamma^{I}$ chain. Accordingly, the abnormal Hb was identical as Hb F-Kotobuki or $^{A}\gamma^{I}$ 6 Glu→Gly.

The cord blood of this study was obtained from a healthy male neonate weighing 3020 g of 40 week gestation. No hematologic abnormalities were found in the infant or his parents.

Two other γ chain variants with a substitution at the same position, namely, Hb F-Texas II (γ6 Glu→Lys) and Hb F-Pordone ($^{A}\gamma^{I}$6 Glu→Gln) have been reported.[8,9] In addition, Hb S (β6 Glu→Val), Hb G-Makasser (β6 Glu→Ala) and Hb Machida (β6 Glu→Gln), which possess an amino acid substitution at the same site in the non α chain, have been reported.[10] The hydrophobic branched side chain of Val in Hb S is known to play an important role in polymerization of the hemoglobin molecule, but the side chain of other amino acids at position 6 of non α chains do not give any pathologic nature to the Hb molecule. The present Hb variant might belong to the latter group and be harmless to the carrier.

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