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Brief Note

Hemoglobin J-Norfolk or α57 (E6) Gly→Asp Found in the Kobe District

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In April 1985, during a survey of hemoglobinopathy in the Kobe district we detected a patient who was heterozygous for a fast-moving abnormal hemoglobin.

Structural analysis demonstrated that the Hb variant was identical to Hb J-Norfolk (α57[E6]Gly→Asp) which was first observed in an English family in 1958[1] and then was discovered in some Japanese.[2,3]

The propositus was an apparently healthy 40-yr-old male. His hematological examinations provided us with following data : Hb 15.6 g/dl, RBC 5.05 $\times 10^{12}$/l, Reticulocyte 0.9%, PCV 0.45 1/l, MCV 90 fl, MCH 30.9 pg and MCHC 34.4 g/dl. The abnormal Hb (Hb X) of the hemolysate was isoelectrically focused at a more acidic pH than Hb A on polyacrylamide slab gel[4] and its content was 28.6%. Hb F was 0.8% by the alkali denaturation test.[5] An isopropanol precipitation test of the hemolysate proved negative.[6]

Hb X was separated from Hb A by DEAE-cellulose column chromatography[7] and globin was prepared from Hb X with 1% HCl-acetone in ice-water. An α chain anomaly was confirmed by urea dissociation cellulose acetate electrophoresis of the globin.[8] An abnormal α chain (α^{X}) was isolated from a normal β chain by CM-cellulose column chromatography.[9] After digestion of the α^{X} chain with TPCK-trypsin for 3 hr at 37°C and at pH 8.4, the resulting soluble peptides were separated by reverse phase high performance liquid chromatography (HPLC).[10] The chromatogram showed that both αT-7 and αT-7·8 were missing in their place, however, two new peaks (α^{X}T-7, α^{X}T-7·8) appeared slightly later than their normal places. These new peptides were hydrolysed with 6N-HCl at 110°C for 20 hr. Their amino acid compositions are listed in Table 1. The α^{X}T-7 peptide had the same composition as the normal αT-7 peptide (2Gly, His Lys) except that in place of one of two glycine residues, there was an Asx residue. The α^{X}T-7·8 peptide consisted of a combination of the α^{X}T-7 peptide and Lysine. The abnormal peptide had substitution of Asx for Gly at position 57 or 59 of the α chain. This Asx

TABLE 1. Amino acid composition of the abnormal peptide. The numbers in the parentheses are the values of residues in the same fragment of the normal α chain.

amino acid	α^{X}T-7	α^{X}T-7·8
Asp	0.91 (0)	0.91 (0)
Gly	1.18 (2)	1.11 (2)
Lys	0.93 (1)	1.99 (2)
His	0.98 (1)	0.98 (1)

日高和夫，井内岩夫

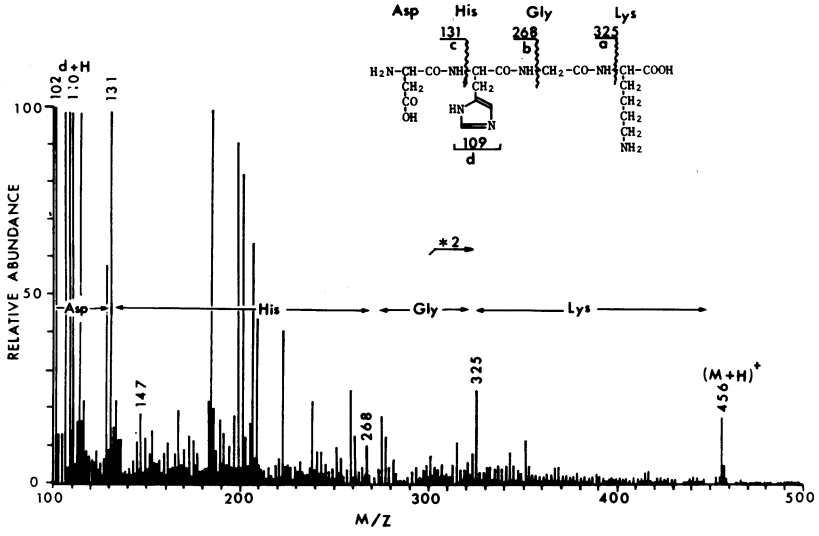

Fig. 1. Secondary ion mass spectrum for the α^{X}T-7 peptide. The protonated molecular ion $(M+H)^+$ at m/z 456 is clearly observed for the α^{X}T-7 peptide (Asp-His-Gly-Lys) and fragments at m/z 325, 268 and 131 have been produced by bond cleavages at a, b and c, respectively. The ions at m/z 147 and 110 are from lysine and histidine parts.

residue was expected to be an Asp residue because the abnormal Hb showed a more acidic isoelectric pH than Hb A. Determination of the amino acid substitution of the α^{X}T-7 peptide was performed by molecular secondary ion mass spectrometry (SIMS).[11] The resulting mass spectrum is shown in Figure 1. The protonated molecular ion $(M+H)^+$ at m/z 456 indicates that the α^{X}T-7 peptide consists of four residues, Asp, Gly, His, and Lys. Bond cleavages at a, b and c produced m/z 325, 268 and 131 ions, respectively. The ions at m/z 147 and 110 are lysine and histidine parts. These results indicate that this abnormal peptide has the sequence of Asp-His-Gly-Lys from the N-terminus. Therefore, it is suggested that the Gly residue was replaced by the Asp residue at the 57th position of the α chain and that the abnormal Hb is Hb J-Norfolk.

The normal α57(E6) glycine residue is the external residue of the α chain and is next to the distal heme linked histidine residue α58(E7).[12] Nevertheless, the substitution of Asp for Gly at this position is not expected to change the function or stability of the Hb molecule. The carrier for this abnormal Hb is apparently healthy and has shown no significant abnormalities clinically or in hematological findings.

Kazuo HIDAKA and Iwao IUCHI

Department of Biochemistry,
Kawasaki Medical School,
Kurashiki 701-01, Japan

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