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Brief Note

Hb Riyadh [β120 (GH3) Lys→Asn] a Second Case in Japan

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Key words : Hb Riyadh — HPLC

Hemoglobin Riyadh, β120 (GH3) Lys→Asn, a fast moving human hemoglobin (Hb) variant, was first detected in a Saudi Arabian woman by EI-Hazmi *et al.*[1] and was subsequently discovered in three ethnic groups, Spanish, Japanese and Indian.[2-4] Recently we detected the same variant in a Japanese female. In this paper we report its characterization.

The propositus was a 78-yr-old female with renal calculi. Her hematological data was as follows : Hb 12.6 g/dl, RBC 3.94×10^{12}/1, PCV 0.38 1/1, MCV 95 fl, MCH 32.0 pg and MCHC 33.9 g/dl. Isoelectric focusing of the hemolysate of the propositus showed discrete bands of an abnormal Hb (Hb X), Hb A and Hb A_2 in that order from the anode to the cathode.[5] The percentage of Hb X was 48.0 of the total hemoglobin and those of Hb F and Hb A_2 were 0.62 and 1.20, respectively.[6,7] An isopropanol denaturation test of the hemolysate of the propositus gave a negative result.[8]

Globin was prepared from the hemolysate by treatment with 1%HCl-acetone

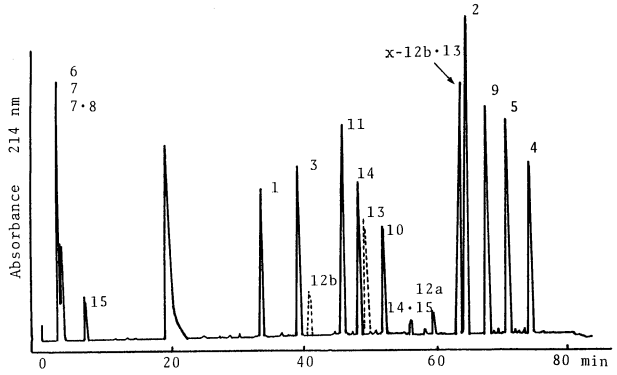

Fig. 1. Separation of the tryptic peptides of the AE-β^X chain by HPLC. The chromatogram was developed by use of a linear gradient device making a mixture of from 0 to 40% of acetonitril with 0.01 M acetic acid-triethylamine, pH 6.0.

Note that βT-12b and βT-13 were missing and a new peak was eluted just before βT-2.

in ice-cooled water. An abnormal β chain ($\beta^\times$) was eluted in front of normal β and α chains by column chromatography on CM-cellulose.[9] The aminoethylated $\beta^\times$ chain was digested with TPCK-trypsin and the digest was separated by high performance liquid chromatography (HPLC) on Cosmosil 5 C_{18}P (4.6×

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250 mm) (Fig. 1).[10] The chromatogram revealed the absence of both normal βT-12b and βT-13 and the appearance of an extra peak just ahead of βT-2. The amino acid composition of the acid hydrolysate of the new peptide was Lys 0.93(2), His 1.87(2), Asp 1.08(0), Thr 0.90(1), Glu 2.88(3), Pro 1.91(2), Gly 1.12(1), Ala 2.96(3), Val 2.00(2), Leu 1.11(1), Tyr 0.97(1) and Phe 1.84(2). The numbers in parentheses refer to the number of amino acid residues expected for the normal βT-12b and βT-13. This analytical result showed that a Lys residue of C-terminus of βT-12b was substituted by an Asx residue. Therefore, the binding site between βT-12b and βT-13 could not be cleavaged by trypsin and the new peptide combining βT-12b with βT-13 was present. The Asx residue substituted for the Lys residue was assumed to be an Asn residue, because the abnormal Hb was electrophoretically fast moving corresponding to one more negative charge than Hb A.

The direct Edman degradation procedure using a Solid Phase Sequencer (LKB 4020) demonstrated that the substitution of the Asn residue for the Lys residue at position 120 of the β chain was evident. Accordingly, this Hb variant was identified as Hb Riyadh. This variant was the second instance in Japan and the carrier bore no blood relationship to the first Japanese case.

Since position 120 (GH3) of the β chain is on the surface of the Hb molecule, it seems that it might not result any severe functional alteration of the Hb molecule.

In addition, Hb Takamatsu [β120 (GH3) Lys→Gln],[11] Hb Jianghua [β120 (GH3) Lys→Ile][12] and Hb Hijiyama [β120 (GH3) Lys→Glu],[13] which possess a different amino acid substitution at the same position, have shown neither hematological nor clinical abnormalities.

Two of the four cases of Hb Riyadh reported previously were associated with thalassemia (a Saudi Arabian with α-thalassemia, an Indian with β°-thalassemia), but this appears to be the instances of the place where thalassemia is endemic. The other two cases were asymptomatic as ours.

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