

Brief Note

Hb Riyadh [β 120 (GH3) Lys \rightarrow Asn] a Second Case in Japan

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Hemoglobin Riyadh, β 120 (GH3) Lys \rightarrow 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 $3.94 \times 10^{12}/l$, PCV 0.38 l/l, 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 A₂ 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 A₂ 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

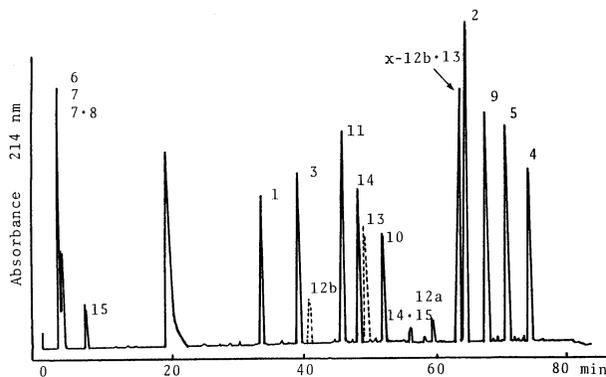


Fig. 1. Separation of the tryptic peptides of the AE- β^x chain by HPLC. The chromatogram was developed by use of a linear gradient device making a mixture of from 0 to 40% of acetonitril with 0.01 M acetic acid-triethylamine, pH 6.0.

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

in ice-cooled water. An abnormal β chain (β^x) was eluted in front of normal β and α chains by column chromatography on CM-cellulose.⁹⁾ The amino-ethylated β^x chain was digested with TPCK-trypsin and the digest was separated by high performance liquid chromatography (HPLC) on Cosmosil 5 C₁₈P ($4.6 \times$

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250 mm) (Fig. 1).¹⁰⁾ The chromatogram revealed the absence of both normal β T-12b and β T-13 and the appearance of an extra peak just ahead of β T-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 β T-12b and β T-13. This analytical result showed that a Lys residue of C-terminus of β T-12b was substituted by an Asx residue. Therefore, the binding site between β T-12b and β T-13 could not be cleaved by trypsin and the new peptide combining β T-12b with β T-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 [β 120 (GH3) Lys \rightarrow Gln],¹¹⁾ Hb Jianghua [β 120 (GH3) Lys \rightarrow Ile]¹²⁾ and Hb Hijiyama [β 120 (GH3) Lys \rightarrow 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^0 -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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REFERENCES

- 1) EI-Hazrai, M.A.F. and Lehman, H. : Hemoglobin Riyadh $\alpha_2\beta_2$ (120[GH3] Lys \rightarrow Asn) A new variant found in association with α -thalassemia and iron deficiency. *Hemoglobin* 1 : 59-74, 1976-77
- 2) Budge, L.J., Bradly, T.B. and Graham, J.L. : Hemoglobin Riyadh in a Mexican American family of Spanish ancestry. *Hemoglobin* 1 : 283-295, 1977
- 3) Miyaji, T., Ohba, Y., Matsuoka, M., Kudoh, H., Asano, M., Yamamoto, K. and Satoh, T. : Hemoglobin Karatsu : β 120(GH3) Lysine \rightarrow Asparagine, An example of Hb Riyadh in Japan. *Hemoglobin* 1 : 461-466, 1977

- 4) Pinkerton, P.H., Wilson, J.B., Lam, D., William, D. and Huisman, T.H.J. : Hemoglobin Riyadh- β^0 -Thalassemia in an Indian family. *Hemoglobin* 3 : 451-458, 1979
- 5) Harano, T., Iuchi, I. and Shibata, S. : A simple isoelectric focusing procedure for screening human hemoglobin components on polyacrylamide gel. *Kawasaki Med. J.* 4 : 53-56, 1978
- 6) Betke, K., Marti, H.R. and Schlicht, I. : Estimation of small percentage of foetal haemoglobin. *Nature* 184 : 1877-1878, 1957
- 7) Ueda, S., Shibata, S., Miyaji, T. and Ohba, Y. : Routine Hb A₂ estimation by cellulose acetate membrane electrophoresis. *Kawasaki Med. J.* 1 : 113-120, 1975
- 8) Carrell, R.W. and Kay, R. : A simple method for detection of unstable haemoglobins. *Br. J. Haematol.* 23 : 615-619, 1972
- 9) Clegg, J.B., Naughton, M.A. and Weatherall, D.J. : Abnormal human haemoglobins, separation and characterization of the α and β chains by chromatography and determination of the new variants, Hb Chesapeake and Hb J (Bangkok). *J. Mol. Biol.* 19 : 91-108, 1966
- 10) Hidaka, K., Iuchi, I., Shimasaki, S., Mizuta, W., Takatsuka, M., Mori, T., Toda, A. and Matsuo, M. : The survey of abnormal hemoglobin in the Kobe district : Hb G Coughatta, Hb Ankara, Hb Handa and Hb J Habana. *Hemoglobin* 10 : 65-72, 1986
- 11) Iuchi, I., Hidaka, K., Harano, T., Ueda, S., Shibata, S., Shimasaki, S., Mizushima, J., Kubo, N., Miyake, T. and Uchida, T. : Hemoglobin Takamatsu [β 120 (GH3) Lys \rightarrow Gln] : A new abnormal hemoglobin detected in three unrelated families in the Takamatsu area of Shikoku. *Hemoglobin* 4 : 165-176, 1980
- 12) Lu, Y.-Q., Fan, J.-L., Liu, J.-F., Hu, H.-L., Peng, X.-H., Huang, C.-H., Huang, P.-Y., Chen, S.-S., Jia, P.-C., Yang, K.-G., Liang, C.-C., Ren, X.-D. and Zuo, C.-R. : Hemoglobin Jianghua [β 120 (GH3) Lys \rightarrow Ile] : a fast-moving variant found in China. *Hemoglobin* 7 : 321-326, 1983
- 13) Miyaji, T., Ohba, Y., Yamamoto, K., Shibata, S., Iuchi, I. and Hamilton, H.B. : Hemoglobin Hijiya : a fast-moving hemoglobin in a Japanese family. *Science* 159 : 204-206, 1968