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Brief Note

Three Electrophoretically Fast-moving Hemoglobin Variants in the Niigata District: Hb J-Amiens [β17(A14) Lys→Asn], Hb Hope [β136(H14) Gly→Asp] and Hb J-Cape Town [α92(FG4) Arg→Gln]

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In this paper we describe three electrophoretically fast-moving hemoglobins (Hb J-Amiens, Hb Hope, and Hb J-Cape Town) found in eight cases showing an abnormal high performance liquid (HPL) chromatographic pattern. These cases were detected during a screening for diabetes mellitus in all people who were admitted to the ward for routine health examinations. The screening was done using the Hb A_{Ic} level of the hemolysate, which was automatically estimated by cation-exchange HPLC (HLC-723GHb, Tosoh Ltd. Co.).

Hb J-Amiens [β17(A14) Lys→Asn].[1] The proband was an apparently healthy 57-year-old Japanese male. The hematological findings of his peripheral blood were normal: RBC $510\times10^4/\mu l$, Hb 14.3 g/dl, Ht 45.0%, MCV 88 fl, MCH 28.0 pg, Total bilirubin 0.4 mg/dl, Hb F 0.4%, and Hb A_2 3.3%. Isoelectrofocusing of his hemolysate revealed discrete bands of an abnormal Hb, Hb A and Hb A_2, in that order from the anode to the cathode (Fig. 1A). The abnormal Hb was amounted to 40.1% of the total Hb. The isopropanol precipitation test[2] was negative.

Cellulose acetate electrophoresis of globins[3] prepared from the hemolysate showed a β chain anomaly, the behavior of which was very similar to those of Hb Takamatsu (β120 Lys→Gln)[4] and Hb Riyadh (β120 Lys→Asn).[5] An abnormal β chain (β^X) was eluted before the normal β chain by CM-cellulose (CM-52, Whatman BioSystems Co.) column chromatography.[6] The β^X chain was aminoethylated[7] and digested with TPCK-trypsin at room temperature at pH 8.3-9.0 for 20 hrs. The resulting peptides were separated on the Cosmosil $5C_{18}$-P column (4.6 mm I.D.×250 mm, Nacalai tesque Co.) of a HPL-chromatograph (Shimadzu LC-4A, Shimadzu Corp.).[8] The chromatogram obtained (Fig. 2) showed an absence of βT-3 peptide and a slight migration of βT-2 peptide to the side of βT-9 peptide. The amino acid composition of the aberrant peptide is listed in Table 1. This corresponded to a combination of the βT-2 and βT-3 peptides except that a Lys residue at the β17 position was replaced by an Asp or Asn residue. Although the substitution of Lys→Asn was expected from the electrophoretic behavior of the β^X chain, to determine which of these was substituted, amino acid sequence analysis of the peptide was done according to the method of Chang *et al.*[9] The results

原野昭雄，原野恵子，櫛田由美，上田　智

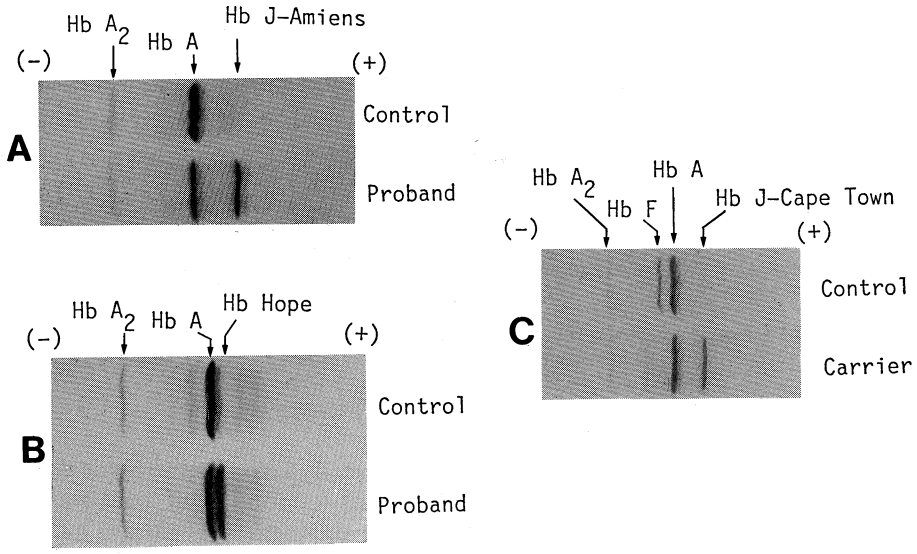

Fig. 1. Isoelectrofocusing of the hemolysates (pH range: 6-9).
A: Hb J-Amiens, B: Hb Hope, C: Hb J-Cape Town

showed that the 9th (β17) amino acid of this peptide was an Asn residue and, additionally, that the 7th (β15) was a Trp residue. This, therefore, identified the variant as Hb J-Amiens [β17(A14) Lys→Asn].[1]

The normal functional properties of Hb J-Amiens do not cause any hematological abnormalities although it was first discovered in a Spanish female with polycythemia.[1] The present carrier of Hb J-Amiens, the first Japanese case, has no hematological or clinical abnormalities.

Hb Hope [*β136(H14) Gly→Asp*].[10] The proband, a healthy 55-year-old Japanese female whose hematological findings were within the normal range: RBC $408\times10^4/\mu$l, Hb 12.2 g/dl, Ht 37.0%, MCV 90.7 fl, MCH 29.9 pg, Total bilirubin 0.5 mg/dl, Hb F 0.4% and Hb A_2 3.3%. Isoelectrofocusing of her hemolysate revealed an abnormal Hb band close to the anodic side of the Hb A band, which amount to about 43% of the total Hb (Fig. 1B). The isopropanol precipitation test of her hemolysate gave a slightly positive result.

An abnormal β chain was separated from other chains by CM-cellulose column chromatography and, after aminoethylation, it was digested with trypsin. The resulting peptides were applied to the reverse phase HPLC described above. On her chromatogram (Fig. 3) the βT-14 peptide seemed to be eluted later than its usual position. The amino acid composition of the peptides was as follows: Lys 1.09(1), His 0.97(1), Asx *2.11(1)*, Gly *0.05(1)*, Ala 4.13(4), Val 2.63(3), and Leu 1.08(1). The numbers in parentheses refer to the expected values for the normal βT-14. The results showed that the peptide was the same as the βT-14 peptide except that a Gly residue at the β136 position of the β chain was replaced by an Asx residue, which was expected to be the Asp residue because of the electrophoretic behavior of the abnormal β chain. Accordingly, this Hb was identified as Hb Hope [β136(H14) Gly→Asp].[10]

To date, excluding the present case, there have been three cases reported Hb Hope,[11–13] two of which were found in patients with either diabetes[12] or

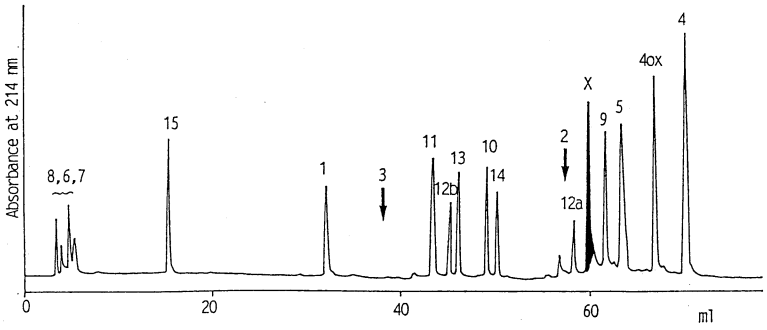

Fig. 2. Separation of the tryptic digest of the AE$\beta^{J\text{-}Amiens}$ chain by HPLC with a linear gradient of acetonitrile (from 0 to 50%) in 9 mM trimethylamine-acetic acid buffer (pH 6.0) at a flow rate of 1.0 ml/min for 100 min. The position of the missing peptide is indicated by an arrow. X (darkened) indicates a new peptide.

TABLE 1. Amino acid composition of the aberrant peptide and the theoretical values of the normal βT-2 and βT-3 peptides

Amino Acids	Found (mol. ratio)	Theoretical Values	
		βT-2	βT-3
Asp	2.87		2
Thr	0.92	1	
Ser	1.00	1	
Glu	2.19		2
Gly	4.02	1	3
Ala	3.28	2	1
Val	4.15	1	3
Leu	2.39	1	1
Lys	0.10	1	
Arg	1.02		1
Trp*		(1)	

*degradated in acid hydrolysis.

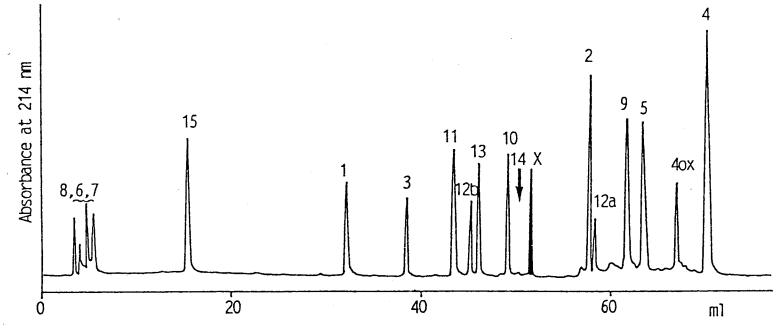

Fig. 3. Separation of the tryptic digest of the AEβ^{Hope} chain by HPLC. The position of the missing peptide is indicated by an arrow. X (darkened) indicates a new peptide.

basal cell carcinoma of the genial region.[13] However, the present carrier of this Hb, like the first case,[11] showed no clinical symptoms. The relationship among these carriers has not as yet been established.

Hb J-Cape Town [*α92(FG4) Arg→Gln*].[14] Six individuals possessing Hb J-Cape Town were detected. Their hematological findings were all within normal range as shown in Table 2. The abnormal Hb was isoelectrofocused to Hb A at the anodic side and it amounted to 21.3–24.1% of the total Hb (Fig. 1C). An instability test of the hemolysate was negative.

TABLE 2. Hematological findings of the carriers of Hb J-Cape Town

Subjects	Sex	RBC ($\times 10^4/\mu l$)	Hb g/dl	Ht %	MCV fl	Hb F %	Hb A_2 %	Hb X %
Y.I.	M	491	16.1	46.0	93.7	0.4	3.0	21.4
E.S.	M	509	15.9	47.7	93.7	0.8	3.1	21.3
N.T.	F	423	12.0	37.1	87.7	0.7	2.6	22.5
K.S.	M	455	14.9	42.4	93.1	0.2	2.9	22.7
K.H.	F	426	14.1	42.0	98.6	0.3	2.8	24.1
M.M.	F	528	15.0	47.2	89.4	0.1	2.5	21.8

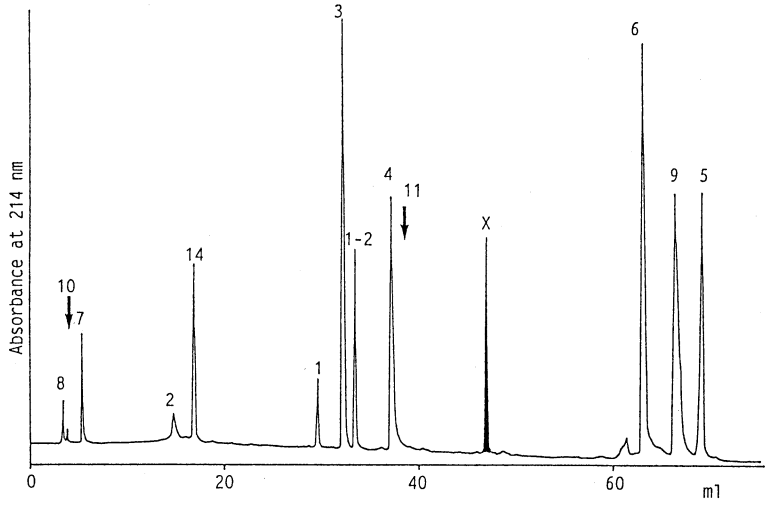

Fig. 4. Separation of the tryptic digest of the $\alpha^{J\ Cape\ Town}$ chain by HPLC. The position of the missing peptide is indicated by an arrow. X (darkened) indicates a new peptide.

An abnormal α chain (α^X) was obtained in the usual manner and digested with trypsin. The HPL-chromatographic pattern of the soluble fraction of the tryptic digest of the α^X chain is illustrated in Fig. 4, showing the absence of αT-10 and αT-11 peptides and the presence of a new peptide eluted later than the usual position of the normal αT-11 peptide. An amino acid analysis of the new peptide demonstrated that the abnormal peptide corresponded to a combination of the αT-10 and αT-11 peptides except that an Arg residue at the α92 position of the α chain was replaced by a Glx residue. This substitution was readily identified as a Gln residue by the amino acid sequence

analysis. Therefore, this was identified as Hb J-Cape Town [α92(FG4) Arg→Gln].[11]

A family study to establish the relationship among these carriers has not as yet been performed. However, a family study of one carrier (Subject: Y.I.) was performed and one of his sons was found to be a carrier of the same abnormal Hb.

The amino acid residue at α92 in the Hb molecule is in the region $\alpha_1\beta_2$ contact which plays an important role in Hb function.[15] This is the reason for the increased affinity for oxygen of Hb J-Cape Town. However, unlike previously reported cases,[14,16–18] hematological and clinical abnormalities, such as polycythemia, were not encountered in the present carriers of this abnormal Hb variant.

Teruo HARANO, Keiko HARANO, Yumi KUSHIDA* and Satoshi UEDA*

Department of Biochemistry and
**Department of Clinical Pathology,*
Kawasaki Medical School,
Kurashiki 701-01, Japan

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