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Brief Note

Hb J Norfolk [α57 (E 6) Gly→Asp], Discovered in the People Living in Hyogo Prefecture

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We screened 12,391 inhabitants in Hyogo Prefecture for abnormal hemoglobins by isoelectric focusing[1] of hemolysates during the period from May, 1980 to March, 1981, and encountered four abnormal hemoglobins : Hb G Waimanalo [α64 (E 13) Asp→Asn][2], Hb Ankara [β10 (A 7) Ala→Asp][3], Hb Ube-2 [α68 (E 17) Asn→Asp][4] and another abnormal hemoglobin which is to be described here. This was a fast-moving hemoglobin. Structural analysis revealed that the hemoglobin was identical to Hb J Norfolk [α57 (E 6) Gly→Asp], which was discovered by Ager et al.[5] in an English family in 1958 and the amino acid substitution of which was established by Baglioni[6] in 1962. This hemoglobin was discovered also in Japanese families by Imamura[7] in 1966 and Yanase et al.[8] in 1968. They named it Hb Kagoshima, and Hb Nishiki-I, -II and -III, respectively. In our study, testing of family members demonstrated that two of the propositus' daughters have the same abnormal hemoglobin.

The propositus was a 52-year-old apparently healthy woman. Hematological and chemical laboratory examinations showed no abnormalities (RBC $4.37\times10^6/\mu l$, WBC $4.6\times10^3/\mu l$, Hb 13.2 g/dl, Ht 38.9%, MCV 88 μm^3, MCH 30.1 pg, MCHC 33.9%, total bilirubin 0.5 mg/dl, reticulocyte count 0.4%, serum iron 122 μg/dl, TIBC 317 μg/dl). The erythrocyte 2,3-DPG content was within the normal range (2.02 μmol/ml whole blood, 15.3 μmol/gr Hb, and 5.2 μmol/ml packed cells).

The hemolysate was prepared by the standard method[9]. Isoelectric focusing of the propositus' hemolysate on an ampholine-polyacrylamide gel plate (pH

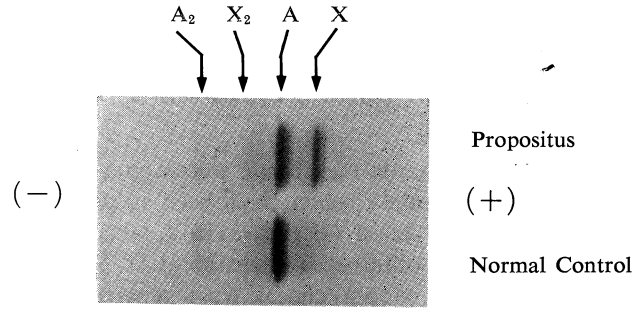

Fig. 1. Isoelectric focusing of hemolysates. A_2=Hb A_2. X_2=Abnormal Hb A_2 (=Hb X_2). A=Hb A. X=Abnormal Hb (=Hb X).

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range 6–9) gave discrete bands of Hb A_2, Hb X_2 (Hb J Norfolk$_2$), Hb A and Hb X (Hb J Norfolk) in order from the cathode to the anode (Fig. 1). The hemoglobin composition of the hemolysate, as examined by isoelectric focusing, elution of individual hemoglobin bands from the gel plate into Na-K phosphate buffer (pH 7.4, containing 0.01% KCN) and measurement of absorbances at 415 nm, was Hb A_2-Hb X_2 : 2.3%, Hb A : 69.3%, and Hb X : 28.4%. The abnormal hemoglobin contents of her daughters' hemolysates were nearly equal to hers (28.4 to 29.0%). The Hb F content by the alkali denaturation method[10] of the propositus' hemolysate was 0.35%. Carrell's isopropanol precipitation test[11] of the hemolysates carried out at 37°C for 5 min was negative.

Hb X was purified by elution of hemoglobin bands from the isoelectro-focused gel plates into distilled water, followed by concentration by vacuum aspiration using collodion bags[12].

Absorption spectra of the purified oxy-Hb X in alkaline buffer solution (Tris-EDTA-Borate buffer solution, pH 8.6) and of met-Hb X in acidic buffer solution (sodium-potassium phosphate buffer solution, pH 6.5) were in good agreement with those of isomolar oxy-Hb A and met-Hb A solutions respectively over the ultraviolet and visible ranges.

Analysis of oxygen equilibrium curves of the purified Hb X (60 μM, basis on heme) dissolved in 0.1M potassium phosphate buffer solution (pH 6.9–7.9), as examined by the method of Imai et al.[13], is shown in Table 1. The P_{50} values of Hb X at various pHs were slightly lower than those of Hb A, but the differences were not significant. The co-operativity (Hill's n constant) and Bohr effect were normal.

TABLE 1. Oxygen binding properties of Hb X and Hb A, and methemoglobin content after the measurement (at 25°C).

pH	Hb X				Hb A			
	P_{50} (mmHg)	Hill's n	Bohr eff.	metHb %	P_{50} (mmHg)	Hill's n	Bohr eff.	metHb %
6.9	10.53	2.35	−0.45	26	11.15	2.30	−0.45	22
7.4	6.29	2.55	−0.60	14	6.67	2.72	−0.63	8
7.9	3.14	2.38		23	3.23	2.50		17

Urea-dissociation cellulose acetate membrane electrophoresis[14] of the purified Hb X disclosed an α chain anomaly which had been suspected because of the presence of an abnormal Hb A_2 band (Hb X_2=Hb J Norfolk$_2$) on the electrophoresis gel plate. Globin was obtained by treatment of Hb X with 1% HCl-acetone[15] in ice-water, and the globin chains were separated chromatographically on CM-cellulose (CM-52, Whatman Co.) to isolate the abnormal α chain (α^X)[16]. The α^X chain was digested with TPCK-trypsin (Worthington Co.) and the soluble fraction of the digest was fingerprinted on cellulose thin layers (Chromagram Sheet, Eastman Kodak Co.)[17] (Fig. 2). The fingerprint revealed

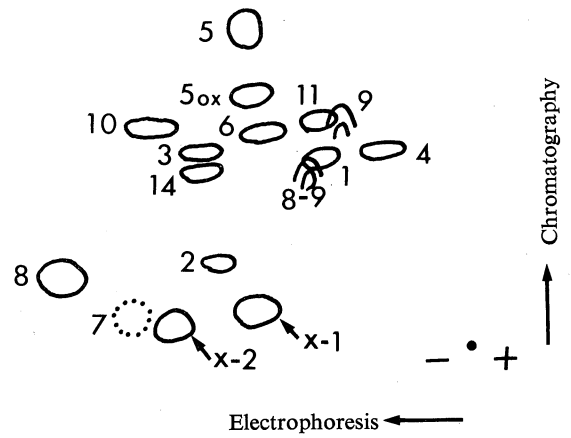

Fig. 2. Fingerprint of the soluble fraction of the tryptic digest of α^X chain. Dotted circle indicates the proper site of the normal αTp-7. Two new abnormal peptide spots (X-1 and X-2) were indicated by arrows.

absence of the normal αTp-7 peptide spot at its proper site and appearance of two new abnormal peptide spots located electrophoretically on the anode side of the normal αTp-7 peptide spot (indicated by arrows and designated X-1 and X-2 in the figure). The abnormal peptide spots were individually cut out from the map, eluted into 10% acetic acid and lyophilized. A portion of lyophilized residue was hydrolyzed with constant-boiling HCl at 105°C for 20 hrs. The amino acid composition of the acid hydrolysates (X-1 and X-2) is presented in the Table 2. The peptide X-1 corresponded in composition to the

TABLE 2. Amino acid composition of abnormal peptide spots, X-1 and X-2, and the expected number of amino acid residues composed the normal αTp-7 and αTp-7-8 peptides.

amino acids	X-1	normal αTp-7	X-2	normal αTp-7-8
Lys	1.09	1	2.00	2
His	1.08	1	1.05	1
Asp	0.83	0	0.86	0
Gly	1.00	2	1.08	2

Amino acid sequence of normal αTp-7 and αTp-8 :

57 58 59 60 61
—Gly–His–Gly–Lys—Lys—
← αTp-7 → αTp-8 ←

peptide derived from the normal αTp-7 peptide ($\overset{57}{\text{Gly}}$–$\overset{58}{\text{His}}$–$\overset{59}{\text{Gly}}$–$\overset{60}{\text{Lys}}$) by displacement of one of the two glycine residues by aspartic acid or asparagine, and the peptide X-2 was the peptide X-1 combined with peptide αTp-8 (consisting of a lysine residue only). Thus the abnormal peptide had one Gly residue

less and one Asx (Asp or Asn) residue in excess in comparison with the normal αTp-7. This suggested a Gly→Asx substitution. The Asx residue substituted for Gly ought to be Asp, because the abnormal hemoglobin is electrophoretically fast-moving. There are two Gly residues, α57 and α59, in the normal αTp-7. The Edman degradation procedure successfully localized the substitution position[18]. The first cycle of the normal degradation of abnormal peptide, when compared with that of the normal αTp-7 peptide, showed that aspartic acid was present in place of glycine at the 57th position of the abnormal peptide (Fig. 3). Thus, the abnormal hemoglobin was identified as Hb J Norfolk [α57 (E 6) Gly→Asp][6].

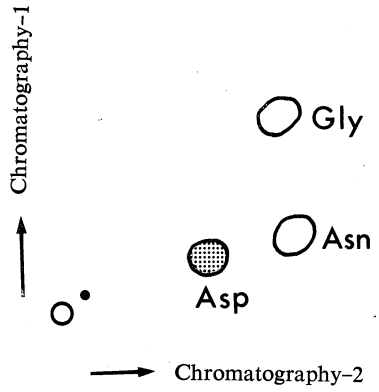

Fig. 3. Two dimensional chromatography of PTH-amino acid produced in the first cycle of Edman degradation of abnormal peptide (X-1) on polyamide durable layer (Cheng Chin Trading Co. Ltd.) is indicated by the darkened circle. Asp, Asn and Gly are at the pertinent positions of their standard PTH-derivatives. Solvent system used for developing the chromatography-1 is a solution of 25 mg butyl-PBD in 10 ml of the mixture of toluene, n-pentane and acetic acid (6 : 3 : 1.6, by vol.), and 25% acetic acid is used as developer of chromatography-2. O : indicates the sample origin.

According to the three dimensional hemoglobin molecular model[19], the normal α57 glycine residue occupies the sixth position in the helical E segment of the α chain, and is located on the surface of the hemoglobin molecule next to the distal heme linked histidine residue at α58 (E 8). Nevertheless, the substitution of Gly→Asp occurring at this position in this hemoglobin molecule does not seem to affect its stability or its functional properties and does not cause clinical symptoms. In addition, this abnormal hemoglobin is not susceptible to easy methemoglobinization through binding of the carboxyl group of the substituting aspartic acid residue to the heme iron. Another abnormal hemoglobin possessing amino acid substitution at the same position (α57) is Hb L Persian Gulf [α57 (E 6) Gly→Arg][20], which is not associated with appreciable clinical consequences.

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