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BRIEF NOTE

THE THIRD FAMILY OF HB HOSHIDA IN JAPAN-A HEMOGLOBINOPATHIC PEDIGREE DISCOVERED BY TRACING A MINOR ABNORMAL HEMOGLOBIN COMPONENT INCIDENTALLY FOUND IN A CORD BLOOD SAMPLE

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In 1979 a minor hemoglobin component electrophoretically reminiscent of Hb G was encountered in our laboratory during a hemoglobin survey of cord blood samples by isoelectric focusing technique[1,2] of the hemolysates. Only one out of 98 samples was positive for this hemoglobin. The blood was from a male baby. The content of the abnormal hemoglobin component in the hemolysate was 4.9 per cent, and the hemoglobin composition was Hb A 7 : Hb F 88 : abnormal Hb 4.9. This abnormal hemoglobin component was not thought to be of γ or α chain variant, because its content was too low. A β chain variant seemed to be most likely. Therefore, family study was soon undertaken. Six members were examined, and hemoglobin electrophoretically the same as that of the baby was detected in three subjects, namely in his mother and his two aunts (Fig. 1). All of the three were apparently healthy

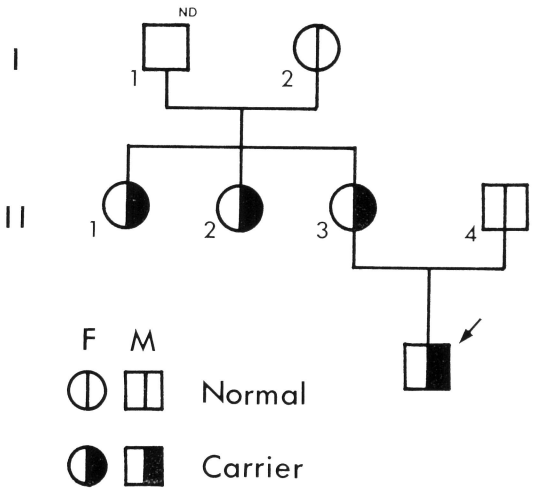

Fig. 1. Family tree of the patient with abnormal hemoglobin.
↙ : Propositus. ND : Not determined.

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and normal by routine hematological and clino-chemical examinations.

Structural, functional and biosynthetic studies on this abnormal hemoglobin were carried out mainly with the blood samples collected from the mother of the baby.

Hemolysate was prepared by the conventional method[3]. Isoelectric focusing of the hemolysate on ampholine-polyacrylamide gel plate (pH range 6-9) gave discretely separated bands of Hb A, Hb F, abnormal hemoglobin and Hb A_2 in the order mentioned from the anode to the cathode (Fig. 2). Hemoglobin

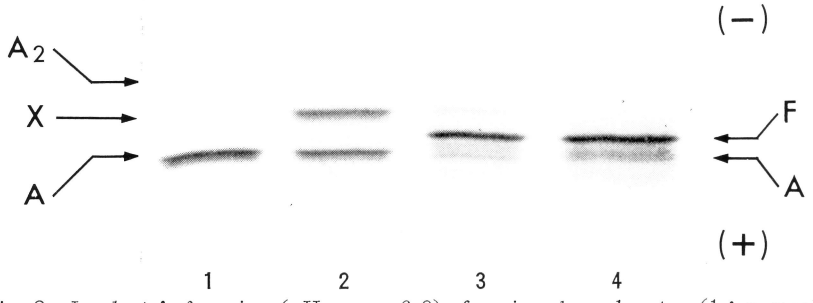


Fig. 2. Isoelectric focusing (pH range 6-9) of various hemolysates (1 : normal adult control, 2 : mother of the propositus, 3 : propositus and 4 : normal cord blood). A : Hb A. X : Abnormal hemoglobin. F : Hb F. A_2 : Hb A_2.

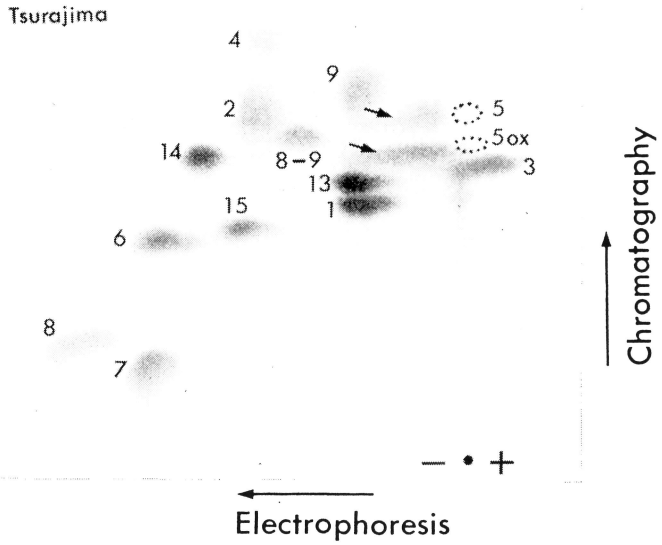
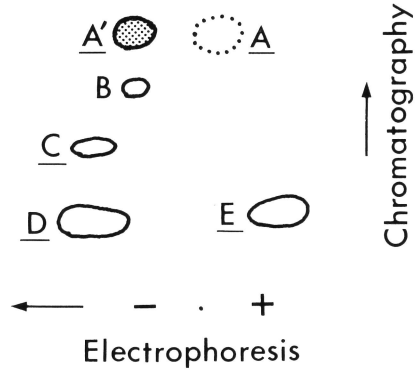


Fig. 3. Fingerprint of tryptic digest of abnormal β chain. Arrows indicate the abnormal peptide spots and dotted-line circles usual positions of peptides (βTp-5 and βTp-5ox) from normal β chain.

composition of the hemolysate was determined by spectrophotometry (at 415 nm) of the eluates of the individual hemoglobin bands into 0.1M phosphate buffer solution (pH 7.4) containing 0.01% KCN. Hb A_2 content was measured by cellulose acetate membrane electrophoresis elution method[4] and Hb F content by Betke's method[5]. Instabilily of hemoglobin was examined by isopropanol precipitation test[6] of the hemolysate, and it was negative.

Test for the chain anomaly by urea-dissociation cellulose acetate membrane electrophoresis[7] showed a β chain abnormality.

The abnormal β chain was isolated by urea CM-cellulose column chromatography of globin prepared from the whole hemolysate using gradient Na-phosphate developers (pH 6.8 ; Na^+ ion gradient : 8 mM→40 mM) containing 8 M urea and 50 mM mercaptoethanol[8]. Abnormal β chain emerged between



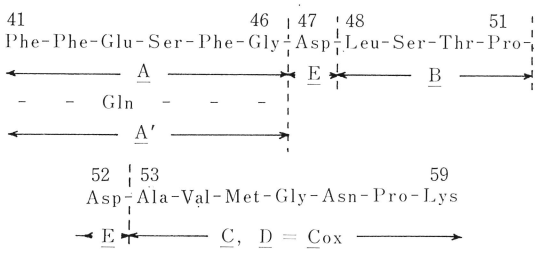


Fig. 4. Fingerprint of acetic acid hydrolysate of abnormal βTp-5 peptide and Edman degradation analysis.

the normal β^A chain and the normal α^A chain.

Fingerprint of the tryptic digest of the isolated abnormal β chain was compared with that of β^A chain[9]. The spots of βTp-5 and βTp-5ox were absent from the usual place, and instead two extra spots were seen dislocated to the cathode side (Fig. 3).

Amino acid analysis of these abnormal peptides disclosed their identity with the normal βTp-5 and βTp-5ox.

The fingerprint of acetic acid (5%) hydrolysate of the abnormal βTp-5 peptides (Fig. 4) suggested β^{43} Glu→Gln and, finally, this was established by Edman degradation studies[10] of the abnormal βTp-5. Phenylalanine were released in the first and the second cycles, and glutamine and traces of glutamic acid were liberated in the third cycle.

Thus, the abnormal hemoglobin found in the cord blood of the baby and the peripheral blood of the family members was identified with Hb Hoshida (β^{43} Glu→Gln)[11].

The oxygen affinity[12] of the abnormal hemoglobin purified by isoelectric focusing was within the normal range (log P_{50}, 0.841 (Hb A = 0.903), at pH 7.4, 25°C). Bohr's effect was $\triangle \log P_{50}/\triangle pH$, −0.54 (Hb A = −0.43) at pH 7.0—7.4. Hill's n values was n = 3.0 (HbA = 3.1) at pH 7.4. The 2,3-DPG

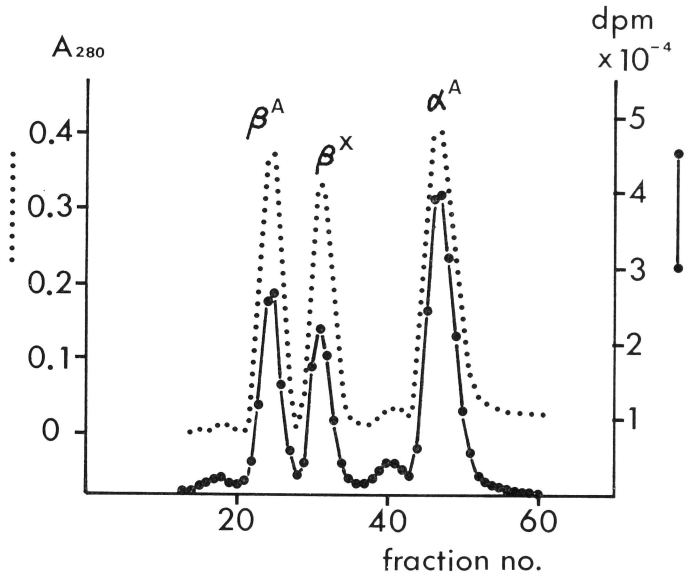


Fig. 5. Chain separation of globin prepared from biosynthesized hemoglobin hemolysate with Hb Hoshida on CM-cellulose column chromatography. Globin was used 10mg. Flow rate of elution buffer solution was 0.2ml/min. Fraction size was 1.2 ml.

effects was $\triangle$ log P_{50}, 0.309 (Hb A = 0.302) at pH 7.4 by addition of 300 μM 2,3-DPG per 15 μM Hb solution.

Hemoglobin biosynthesis experiment by incubation of reticulocytes collected from fresh venous blood in Lingrel-Borsook's culture medium[13] containing ^{3}H-Leu for 2 hrs followed by Clegg-Naughton-Weatherall's urea CM-cellulose chromatography[8] revealed that the β^X ($\beta^{Hoshida}$) was equal to β^A chain in production rate (specific radioactivity ratio $\beta^{Hoshida}/\beta^A$ was 0.91) and the production of the β chains ($\beta^A+\beta^{Hoshida}$)was in good equilibrium with that of the α^A chain (β/α ratio 0.95 - 1.04) (Fig. 5 and Table 1).

TABLE 1

Incorporation of ^{3}H-leucine into the normal β chain (β^A), the normal α chain (α^A) and the abnormal β chain (β^X) composing the blood with abnormal hemoglobin.

Chains	Activities	
	Specific (dpm/A_{280})	Total (dpm)
α^A	105,316	179,285
β^A	109,563	88,191
β^X	100,075	82,358
β^A/α^A	1.04	0.49*
β^X/α^A	0.95	0.46*
β^X/β^A	0.91	0.93

*The values of $(\beta^X+\beta^A)/\alpha^A$ in total activities was 0.95

This is the third instance of Hb Hoshida families. The distribution of this abnormal hemoglobin is restricted to kurashiki district (Hoshida, Chaya[14] and Tsurajima) of Okayama Prefecture. Our meticulous inquiry into the ancestors of these families failed to establish kinship between them. They are independent from each other so far as four generations are dated back.

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