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The Survey of Abnormal Hemoglobins in the Kobe District : I. Hb Ube-2 (α68 Asn → Asp) and Hb Syracuse (β143 His → Pro) with High Oxygen Affinity

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ABSTRACT. In a hemoglobin survey conducted in Kobe for the past three years, ten instances of abnormal hemoglobin were discovered. This paper describes two instances of Hb Ube-2 (α68 Asn → Asp) and one instance of Hb Syracuse (β143 His → Pro) with high oxygen affinity detected in this survey.

Key words : Hb Ube-2 — Hb Syracuse — Hemoglobino variant

Kobe, Hyogo Prefecture, is one of the representative port cities of Japan. It has been a prosperous city from ancient times and has a population of about one and a half million. A survey of hemoglobinopathy in this district has been conducted by us since May, 1981 to examine the population genetics. Ten instances of abnormal hemoglobin have been detected in eight families and in this paper we describe three of them, two identified as Hb Ube-2 and one as Hb Syracuse.

METHODS

Whole blood samples (2.0 ml) were drawn from patients for routine hematologic examination in the Kobe Municipal Central Hospital and then were refrigerated for about a week until they reached our laboratory, 80 miles away. Hemolysate (10 g/dl) containing KCN were prepared in the usual way[1] and screened by isoelectric focusing on polyacrylamide slab gels with ampholine at a pH of 5-9[2]. When an abnormal Hb was detected, physical examination and family studies of the patient and hematologic and biochemical examinations of the proband were performed.

Abnormal Hb fractions were purified either chromatographically[3] or electrophoretically.[4] Functional studies, including the oxygen equilibrium curve,[5] Hb stability,[6] auto-oxidation[7] and absorption spectra of hemoglobin were performed when indicated. Structural analysis of the abnormal hemoglobin was undertaken to determine the location and nature of the amino acid substitution. Sequences were established both by amino acid analysis of the various protease digested peptides of the abnormal chain and by Edman's stepwise sequential degradation[8] of the abnormal peptides.

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RESULTS

1. Hb Ube-2 (α68 Asn→Asp)

An electrophoretically abnormal Hb was found in a 19-year-old female with right-migraine but with no hematologic abnormalities (Hb 14.2 g/dl, PCV 0.44 1/1, RBC 4.75×10^{12}/1, MCV 93 fl, MCHC 32.3 g/dl, reticulocyte 1.2%, serum bilirubin 0.6 mg/dl).

Her apparently healthy father and elder sister had the same abnormal hemoglobin.

The abnormal Hb (Hb X) of the hemolysate was isoelectrically focused at a more acid pH than Hb A. Hb X constituted 22.4 percent of total hemoglobin, and Hb F was 0.38 percent.

The propositus of the second family was a 47-year-old female who was admitted for surgical therapy of cerebrovascular disease. Her hematological and blood chemistry examinations at the time of admission were not remarkable. She had no children and her parents were dead. The isoelectric focusing of her hemolysate produced the same result as that in the first case. Hb X constituted 21.5 percent and Hb F was 0.98 percent of the total hemoglobin.

Structural and functional analyses of the hemoglobin of each propositus were carried out by the methods followed in our previous analysis of Hb Ube-2,[9] since the same results were obtained. The abnormal hemoglobins were identified as Hb Ube-2 (α68 Asn→Asp) which is distributed sporadically among the Japanese.

No kinship between the two families could be established.

2. Hb Syracuse (β143 His→Pro)

The propositus was a 22-year-old female who visited the hospital because of rhinitis in Sept., 1981. Her hematologic examination revealed polycythemia without cyanosis (RBC 6.29×10^{12}/l, Hb 15.8 g/dl, PCV 0.52 1/1, MCV 83 fl, MCH 24.2 pg, MCHC 29.6 g/dl, reticulocyte 1.0%, serum bilirubin 0.6 mg/dl, platelet $43.2\times10^{4}/\mu$l). She returned to the hospital with pyelitis in Dec., 1981, and her polycythemia was found to be persistent.

In isoelectric focusing of her hemolysate, the abnormal Hb (Hb X) was observed as a clearly isolated band on the acid side close to Hb A (Fig. 1). Hb X constituted 41.4 percent of the total Hb. Hb F constituted 1.5 percent. Efforts to purify the Hb X for further analysis were unsuccessful in several electrophoretic methods, including electrophoresis in cellulose acetate membrane (Tris buffer, pH 6.2, 8.0), agar gel (citrate, pH 6.2 and 7.0), agarose gel (Bis-tris, pH 6.2) and starch gel (Tris buffer, pH 8.0). Hb stability by Carrell's method was normal. Erythrocyte 2,3-DPG was 16.0 μM/g-Hb, which is within the normal limits. By CM-sephadex column chromatography (Pharmacia G-50 ; developing, 0.05 M phosphate buffer, pH 6.0, with a linear sodium gradient from 0.06 to 0.15 M) the Hb X was eluted faster than Hb A.

The absorption spectrum of the purified Hb X in the visible and UV regions was identical to that of isomolar Hb A in the oxy and met Hb forms. The auto-oxidation velocity of Hb X from oxy Hb to met Hb in 0.05 M phosphate buffer at pH 6.6 at 37°C was slightly but significantly slower than that of control Hb A.

The oxygen equilibrium curves (OEC) of the purified Hb X demonstrated

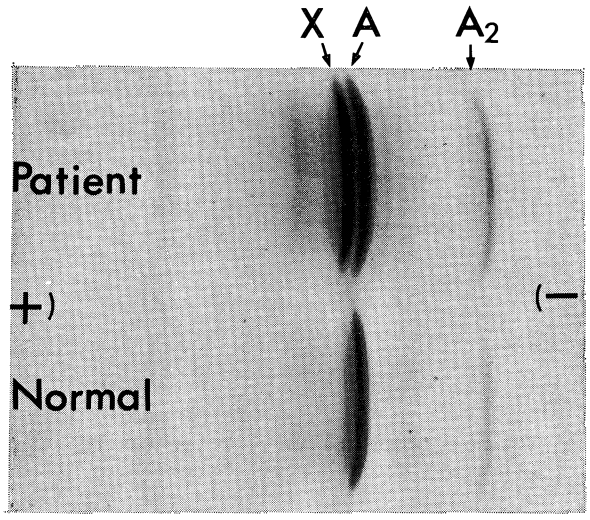

Fig. 1. Isoelectric focusing on polyacrylamide slab gel of the hemolysate. X, A and A_2 indicate abnormal Hb, Hb A and Hb A_2 respectively.

high oxygen affinity as shown in Table 1.

Remarkably less homotropic and heterotropic effects on the allosteric properties of the Hb molecule were seen in the purified Hb X than in Hb A. The effects of the hemolysate were moderate in magnitude. It was noteworthy that the plot of log pO_2 vs log oxygen saturation of the hemolysate was bent at its half point, probably because it was a mixture of Hb X and Hb A (Fig. 2).

Isoelectric focusing of PCMB-treated Hb X revealed a β chain (β^X) anomaly. The β^X chain was obtained in preparative amount by use of CM-cellulose column chromatography, and the β^X chain was digested with TPCK-trypsin (pH 8.0, 37°C for 3 hrs). The map of the digest of the β^X chain lacked βT-14 and had a new spot (peptide S) behind and above the expected βT-14. The other spots on the map were in the same location as those of the control β^A chain digest (Fig. 3).

The amino acid analysis of the acid hydrolysate of peptide S revealed the residues Lys 0.96 (1), His 0.0 (1), Asp 1.08 (1 as Asn), Pro 0.88 (0), Gly 1.20 (1), Ala 3.86 (4), Val 2.43 (3, including one at the N terminus) and Leu 1.05

TABLE 1 Examination of oxygen equilibrium curve of the hemolysate and the purified Hb X at 25°C

specimen	pH	log P_{50} (mmHg)	Hill's constant n	Bohr effect ($\triangle$log $P_{50}/\triangle$pH)	2,3-DPG effect ($\triangle$log P_{50}/[μM DPG]*)
purified Hb X	7.0	−0.01 (1.02)	1.23 (2.54)		
	7.4	−0.14 (0.85)	1.24 (2.58)	−0.33 (−0.44)	0.09 (0.14)

Values in parentheses indicate those of control Hb A measured in the same run.
* : $\triangle$log P_{50}/[μM DPG] measured at DPG concentration of zero and ten times as high as the Hb concentration.

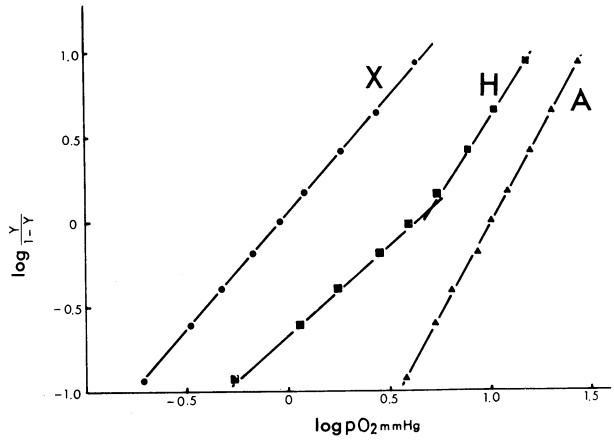

Fig. 2. Hill plots for the reaction of oxygen with hemoglobin at pH 7.4 and 25°C. X : Hb X, H : hemolysate, A : Hb A.

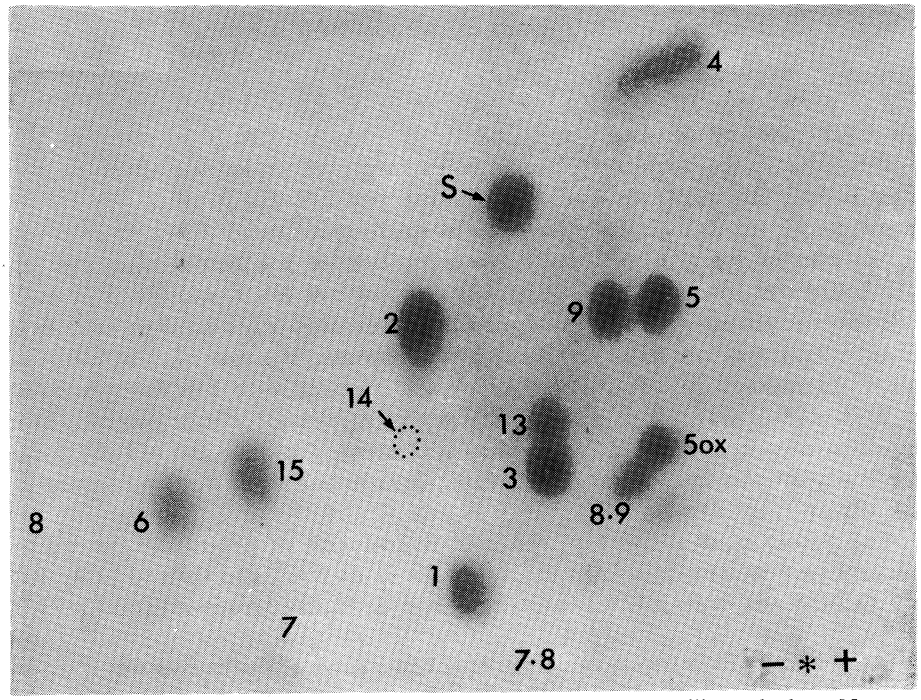

Fig. 3. Fingerprint of the tryptic digest of the β^X chain on silica gel plate. Note for absence of βT-14 and presence of a new spot S.

(1). (The values in parentheses are the residue numbers present in normal βT-14.) The peptide was the same as peptide βT-14 except for the substitution of a proline residue for a histidine residue at 143 of the β chain.

The amino acid composition of other tryptic peptides and chymotryptic peptides of the "core" were normal. The structural anomaly was thus found to be β143 His→Pro, identifying it as Hb Syracuse.

DISCUSSION

The two Hb Ube-2 families were not consanguineous, and there was no kinship with other Hb Ube-2 families reported in the past among the Japanese. This evidence supports our previous conclusion that Hb Ube-2 are distributed sporadically among the Japanese and that the structural anomaly is harmless to the carrier, so that there is no selective pressure.[10]

Hb Syracuse was first detected by Jensen *et al.*[11] Our patient was also female, but the polycythemia was milder than in the first instance, perhaps because of her weakened general condition due to rhinitis and pyelitis.

The use of isoelectric focusing on gel in the hemoglobin survey was fortunate since Hb Syracuse has never been separated electrophoretically. It therefore appears advisable to use isoelectric focusing on gel in hemoglobin surveys, at least in patients with polycythemia. It should be noted, however, that ampholine is eluted together with hemoglobin from the gel and interferes with further studies of the function and structure since ampholine is hard to remove from hemoglobin solution.

The mechanism of the high affinity for oxygen, lowered Hill's constant and decreased Bohr effect of Hb Syracuse has been elucidated in detail on the basis of the conformational structure of the hemoglobin molecule by Jensen,[11] and Bunn and Forget.[12] Our results were in agreement with theirs, and further explanation is unnecessary.

REFERENCES

1) Huisman, T.H.J. and Jonxis, J.H.P. : The Hemoglobinopathies : Technique of Identification. New York, Marcel Dekker Inc. 1977
2) Harano, T., Iuchi, I. and Shibata, S. : A simple isoclectric focusing procedure for screening human hemoglobin components on polyacrylamide gel. Kawasaki Med. J. **4** : 53-56, 1978
3) Dozy, A.M. and Huisman, T.H.J. : Studies on the heterogeneity of hemoglobin. XIV. Chromatography of normal and abnormal human hemoglobin types on CM-Sephadex. J. Chromtogr. **40** : 62-70, 1969
4) Ueda, S., Shibata, S., Miyaji, T. and Ohba, Y. : Routine Hb A_2 estimation by cellulose acetate membrane electrophoresis. Kawasaki Med. J. **1** : 113-120, 1975
5) Imai, K., Morimoto, H., Kotani, M., Watari, H., Hirata, W. and Kuroda, M. : Studies on the function of abnormal hemoglobins. 1. An improved method for automatic measurement of the oxygen equilibrium curve of hemoglobin. Biochim. Biophys. Acta **200** : 189-196, 1970
6) Carrell, R.W. and Kay, R. : A simple method for detection of unstable hemoglobins. Br. J. Haematol. **23** : 615-619, 1972
7) Antonini, E. and Brunori, M. : Hemoglobin and myoglobin in their reactions with ligands. Amsterdam and London, North-Holland Pub. Co. 1971
8) Koningsberg, W. : *In* Methods in Enzymology, vol. 25, ed. by Hirs, C.H.W. and Timasheff, S.N. New York and London, Academic Press. 1972, p. 326
9) Iuchi, I., Shimasaki, S., Hidaka, K., Ueda, S. and Shibata, S. : Hb Ube-2 (α68[E17] Asn→Asp). The second instance in Japan. Hemoglobin **5** : 599-603, 1981
10) Iuchi, I., Hidaka, K., Shimasaki, S., Shibata, S., Ueda, S., Mizushima, M. and Aoba, H. : Abnormal hemoglobins in the Takamatsu district with emphasis on epidemiological characteristics. Hemoglobin **6** : 493-502, 1982
11) Jensen, M., Oski, F.A., Nathan, D.G. and Bunn, H.F. : Hemoglobin Syracuse ($\alpha_2\beta_2$143 (H21) His→Pro) a new high-affinity variant detected by special electrophoretic methods. Observations on the auto-oxidation of normal and variant hemoglobins. J. Clin. Invest. **55** : 469-477, 1975
12) Bunn, H.F., Forget, B.G. and Ranney, H.M. : Human Hemoglobins. Philadelphia-London-Toronto, W.B. Saunders Co. 1977