THE ABNORMALITY OF THE PRIMARY STRUCTURE OF HEMOGLOBIN SHIMONOSEKI*

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In 1960 Yamaoka et al. described one of the first well-documented abnormal hemoglobins to be found in Japan. This hemoglobin, designated Hb Shimonoseki for the city in which the family resides, constitutes approximately 20% of the hemoglobin of the heterozygotes and migrates with Hb S on paper electrophoresis at pH 8.6. The present communication concerns its further characterization.

On starch gel electrophoresis (Smithies, 1959), using borate buffer at pH 8.6, Hb Shimonoseki migrated very slightly cathodally to Hb S, even when the hemolysates were adjusted so as to contain equal amounts of the abnormal components. On Amberlite CG-50 column chromatography (Allen et., 1958) the Hb Shimonoseki peak was eluted with 110 ml. of developer compared with 65 ml. for normal Hb A and 130 ml. for Hb S. Hybridization of Hb Shimonoseki with other hemoglobins known to have abnormal α- or β-polypeptide chains was performed on starch gel using the technique of Singer and Itano (1959) demonstrating that the amino acid substitution resides in the α-chain.

Normal adult hemoglobin (Hb A) and Hb Shimonoseki were separated and purified by starch block electrophoresis. Heat denatured hemoglobi solutions were digested with TCA trypsin and fingerprinted (Ingram,

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1958; Chernoff and Liu, 1961) by performing descending chromatography first, using the n-butanol-acetic acid-pyridine-water solvent (90:18:60:72) of Swenson et al., (1962), followed by high voltage electrophoresis at pH 6.4 in a water-cooled plastic tank containing Varsol. Fingerprints were stained with ninhydrin and for specific amino acids. The composition of individual peptides was qualitatively determined by two-dimensional paper chromatography (Chernoff and Liu, 1961). Amino acids were estimated quantitatively by elution from carefully controlled chromatograms and the optical density compared with standardization curves (Fisher and Doerfer, 1953).

The fingerprints of Hb A and Hb Shimonoseki were remarkably similar. Six peptides of Hb Shimonoseki were positive by the Sakaguchi stain for arginine, compared with only five for Hb A (Figure 1). The amino acid composition of the peptide in question, equivalent to Ingram's number 10 and Chernoff and Lui's number 17 in Hb A, is shown for hemoglobins A and Shimonoseki in Table 1. This peptide in Hb A has been identified (Braunitzer et al., 1961; Konigsberg et al., 1961) as the sixth tryptic peptide from the N-terminus of the α-chain ($\alpha TpVI^{A}$). Its amino acid sequence is shown in Fig. 2. In $\alpha TpVI^{Shimonoseki}$ lysine, valine, and glutamic acid are missing; arginine is added.

Table 1. Amino acid composition of peptides expressed as molor ratios. Proline is present but not quantitated.

	Lys	Asp	Glu	His	Gly	Ser	Thre	Ala	Val	Leu	Phe	Tyr	Arg
$\alpha TpVI^{A}$	1.1	1.1	0.9	1.8	1.0	1.6	0.9	1.1	1.2	1.0	1.8	0.8	0.0
$\alpha TpVI^{Shimo}$	<.1	0.9	<.1	1.6	1.0	1.6	0.8	1.0	<.1	0.9	1.6	0.8	0.8

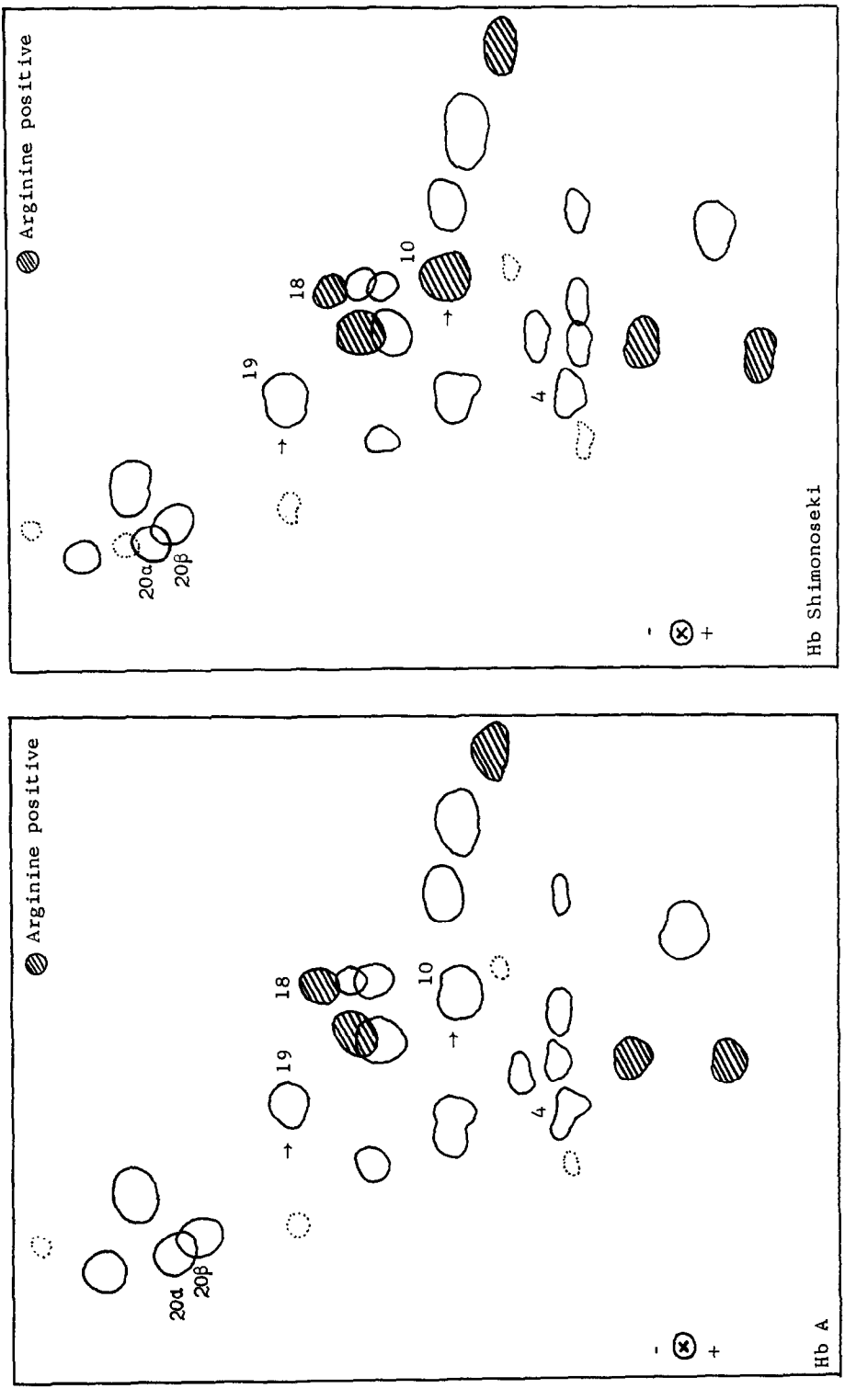

Fig. 1. Tracing of fingerprints of the tryptic digests of hemoglobin A and Hb Shimonoseki showing the location of the arginine positive peptides.

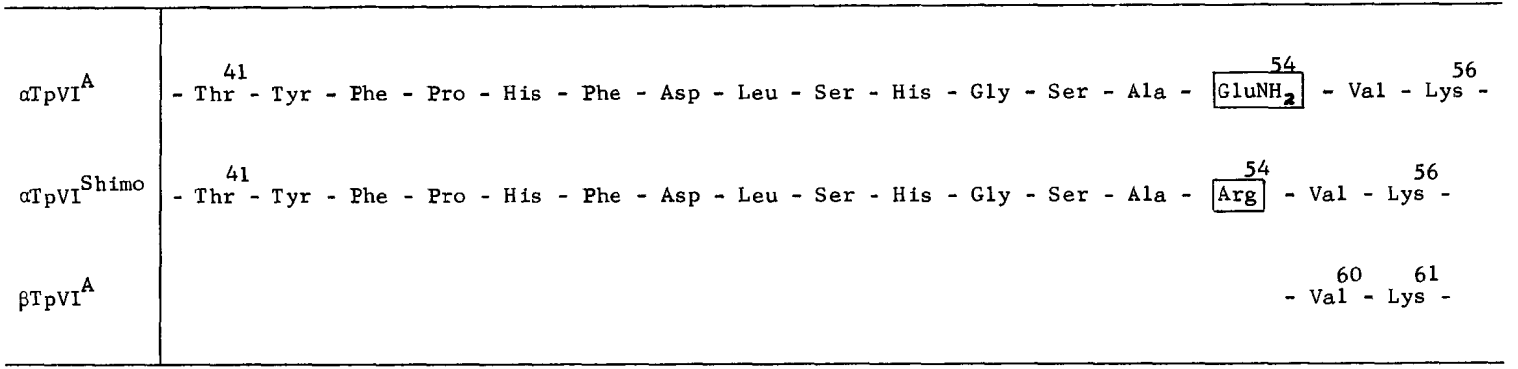

Fig. 2. Sequences of peptides $\alpha TpVI^{A}$, $\alpha TpVI^{Shimonoseki}$, and $\beta TpVI^{A}$.

These findings suggest that the glutamine, normally present as the 54th residue (converted to glutamic acid in the process of acid hydrolysis) of Hb A, is substituted by arginine in Hb Shimonoseki (Fig. 2). This should create an additional peptide, valyl lysine, which is also a tryptic peptide of the β-chains ($\beta TpVI^{A}$). Upon gross comparison of Hb Shimonoseki and Hb A fingerprints, this peptide (Ingram's number 19; Chernoff and Liu's number 6) was clearly relatively more dense in the former hemoglobin. This has been further documented by comparing the $O.D._{504}$ of eluates of ninhydrin-copper nitrate complexes of this peptide with that of other control peptides on the same fingerprint. In Table 2 the ratio of optical density of this peptide to that of the control peptides βTpI, αTpVII + βTpVII, and αTpX is approximately twice as great in Hb Shimonoseki as in Hb A.

Table 2. $O.D._{504}$ of eluates of $\alpha TpVI^{A}$ and $\alpha TpVI^{Shimonoseki}$ relative to control peptides. The ratio is $O.D._{504}$ of $\alpha TpVI/O.D._{504}$ of the respective control peptides.

Peptide number		Control peptides					
	αTpVI	βTpI		αTpVII+ βTpVII		αTpX	
Ingram	19	4		20α + 20β		18	
Chernoff & Liu	6	14		2 + 3		7	
	O.D.	O.D.	Ratio	O.D.	Ratio	O.D.	Ratio
Hb A	.095	.085	1.1	.322	.3	.070	1.4
Hb Shimonoseki	.346	.168	2.0	.556	.6	.122	2.8

Thus, Hb Shimonoseki can be designated as $\alpha_2^{54\ Arg}\beta_2^{A}$. The fact that its net ionic charge is +2 relative to Hb A verifies the findings of Konigsberg et al. and Braunitzer et al. that residue 54 is glutamine rather than glutamic acid, since the substitution of arginine

for the latter would result in a net charge difference of +4 for Hb Shimonoseki. To our knowledge, this is the first amino acid substitution to be shown in αTpVI.

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