Thesis Title STRUCTURAL STUDIES ON HEMOGLOBIN ß-CHAIN VARIANTS

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ABSTRACT

This thesis presents the characterization of the primary structure of four different abnormal hemoglobins from three individuals in Thailand.

Propositus R.K. : A healthy twenty-two year old male with normal hematological parameters was found to have an abnormal hemoglobin migration electrophoretically towards the anode slower than Hb A in electrophoresis under non-denaturing conditions. The non α-chain variant had a cathodal electrophoretic mobility faster than the $ß^A$ chain on cellulose acetate electrophoresis under denaturing conditions. DEAE-cellulose column chromatography was used to prepare the abnormal hemoglobin, which was estimated to be 35% of total hemoglobin. Purification of the abnormal globin chain carried out on CM-cellulose column chromatography. The tryptic peptide map of the abnormal

globin chain, in comparison with that of β^A chain, revealed a more cathodal displacement of peptide β^{PK} Tp13, which had an amino acid composition identical to that of normal β^A Tp13. Since the abnormal peptide β^{PK} Tp13, had additional positive charge, it was concluded that abnormal hemoglobin resulted from a Glu-Gln replacement at position 121 of the β chain. The abnormal hemoglobin was identified as Hb D Punjab ($\alpha_2\beta_2^{121\ Glu-Gln}$).

Proposita C.S. : The hemolysate of an asymptomatic female with a normal hematological profile was shown to be composed of two abnormal bands, Hb E and a fast-moving hemoglobin (Hb C.S.), on cellulose acetate electrophoresis under non-denaturing conditions. Globin separation by cellulose acetate electrophoresis under denaturing conditions indicated that the β^{CS} chain moved towards the cathode more slowly than the β^A chain. Hb E (32%) and Hb C.S (68%) were well separated by DEAE-cellulose column chromatography, after which the purified β^E and β^{CS} globin chains were prepared by CM-cellulose column chromatography and digested with trypsin. In comparison to the β^A chain, the β^E fingerprint showed a lack of peptide β^A Tp3 and the appearance of two extra peptides β^E Tp3a and β^E Tp3b, suggesting the substitution of glutamic acid by lysine at position β26. The β^{CS} fingerprint indicated an abnormality in peptide Tp5, which had more negative charge and moved

towards the anode faster than the corresponding peptide of the β^A chain. The results from both amino acid analysis and CNBr cleavage disclosed that the fast-moving hemoglobin had a β chain variant with a replacement of glycine by aspartic acid at position 56, characterized as Hb J (Bangkok). It was concluded that the proposita was doubly heterozygous, for Hb J Bangkok ($\alpha_2\beta_2^{56\ Gly-Asp}$) and Hb E ($\alpha_2\beta_2^{26\ Glu-Lys}$), the second such report in Thailand.

Propositus D.R. : A thirty-six year old male with anemia and a moderate thalassemia-like hematological profile was observed to possess an abnormal hemoglobin inherited from his father. The abnormal hemoglobin was detectable by cellulose acetate electrophoresis pH 9.1 as a very faint band moving towards the anode more slowly than Hb A, presumably due to a non α-type variant. Globin typing by cellulose acetate electrophoresis in acid-urea-tris-EDTA-borate buffer, pH6.5 indicated that the abnormal globin chain had a cathodal electrophoretic mobility slightly slower than or equal to the $\beta^{Leper-Boston}$ chain. The abnormal hemoglobin was isolated in good purity by DEAE-cellulose column chromatography and estimated as 10% of total hemoglobin, while the remainder was Hb F in a very large amount. The purified abnormal globin chain, prepared by CM-cellulose column chromatography, was digested with trypsin and fingerprinted in comparison to the β^A chain. Peptide mapping studies of

the abnormal globin chain showed the presence of peptides δTp2, δTp3, δTp5 and ßTp13, and loss of peptides ßTp2, ßTp3 and ßTp5, implying that the abnormal globin chain was a δß fused globin chain or Hb Lepore. Peptide mapping of the aminoethylated chain followed by amino acid analysis showed that the $ß^{pr}$ chain contained peptides δTp10 and ßTp12b. This indicates that the Hb Lepore was of the Washington-Boston type. The existence of a fusion δß globin gene was confirmed by the finding of a deletion of 7 kb XbaI fragment on analysis of the propositus DNA, which also revealed that the propositus should have δß-thalassemia inherited from his mother.