Chapter 5

CONCLUSION AND RECOMMENDATION

Hb Thai is an α-chain haemoglobin variant with a unique primary structure. It is present in very small amounts, less than 1 % in a heterozygote. Interaction of Hb Thai with either $\alpha\text{-thal}_1$ or $\alpha\text{-thal}_2$ gene results in clinical disorders of α-thalassaemia syndromes. It is of interest to study the haemoglobin synthesis of Hb Thai in heterozygote and in double heterozygote of Hb Thai and α-thalassaemia in order to get a better understanding the nature of Hb Thai.

The studies can be sumarrized as follows.

1 Haematological Studies and Globin Chain Synthesis.

Twelve cases of Hb H disease, 5 with $\alpha\text{-thal}_1/\alpha\text{-thal}_2$ and the remainders with $\alpha\text{-thal}_1$/Hb Thai were studied. The clinical and haematological data of the two groups were similar but the means of radioactivity α/β ratio were 0.57 ± 0.15 and 0.70 ± 0.14 respectively, whereas the α/β ratio of 4 normal controls was 1.08 ± 0.02. The marked decrease in α-chain synthesis led to the polymerized β-chain as β_4 (Hb H).

The haematological mean values of the 14 heterozygous Hb Thai revealed within normal limits and the radioactivity α/β ratio was 1.34 ± 0.09.

Three cases of homozygous Hb Thai were also studied to compare with one case of α-thal$_2$/Hb Thai. The haematological data of both revealed haemoglobin around 8-10 %, and definite hypochromic microcytic red cells. The haemoglobin types of both showed Hb Thai + Hb A + trace Hb Bart's. The α/β ratio of the homozygous Hb Thai was 1.84 ± 0.11, whereas the α-thal$_2$/Hb Thai was 1.09. This indicated that the measurement of globin chain synthesis was useful to designate the genotypes.

2 Delta-Chain in Hb H Disease with Hb Thai.

Either α-thal$_2$ or Hb Thai would give rise to Hb H disease upon the interaction with α-thal$_1$. The α-thal$_1$/α-thal$_2$ disease has Hb types of A_2 + A + H while the α-thal$_1$/Hb Thai has Hb types of Hb Thai + A + H. Whole haemolysate or the isolated slow haemoglobin by DEAE-Sephadex chromatography from a patient of α-thal$_1$/Hb Thai disease revealed no observable Hb A_2 on starch-gel electrophoresis at alkali pH. The studies of CM-cellulose chromatography of globin from either whole haemolysate or haemolysate after Sephadex G-100 gel-filtration showed the presence of a peak corresponding to δ-chain. This globin chain was structurally characterized by peptide mapping study and the result was consistent with the peptide map of δ- chain. Since the δ-chain was detectable in the haemolysate after Sephadex G-100 chromatography which was believed to exclude the free chain from tetramer haemoglobin molecules, the δ-chain in the patient might be present in polymerized form δ_4, or in other

unusual haemoglobin molecules. The fraction which contained entirely Hb H (β_4), on Sephadex G-100 chromatography, showed a band migrating at Hb A in starch-gel electrophoresis. This haemoglobin band presumably corresponded to δ_4, as previously described by Dance et al., (1963). However the CM-cellulose chromatography of globin from the isolated slow haemoglobin which was separated from the whole haemolysate of the patient by DEAE-Sephadex chromatography also revealed a peak whose peptide map identical to δ-chain, besides the globin chains corresponding to β, α and two slow α-chains of Hb Thai. Thus the δ-chain in the isolated slow haemoglobins probably presented in form of $\alpha_2^{Thai}\delta_2$ or unusual molecule of $\alpha_2^{Thai}\beta\delta$. This hypothesis needs further investigations especially an attempt to fractionate the slow haemoglobin components in a patient with α-thal$_1$/ Hb Thai disease.

3 A Fast Component in Hb H Disease.

A fast component in starch-gel electrophoresis of a patient with Hb H disease has been described as a polymerized of β-chain-β_4 (Hb H) (Jones et al., 1959). Based on the studies of haemoglobin components on Sephadex G-100 chromatography in this study, the fast component was evidently eluted after the peak corresponding to Hb A. Since the principle of separation on Sephadex G-100 is depended on size of the proteins, the fast component of Hb H (fraction C) in figure 13, p 43, was most likely to be a monomer of β-chain. Since the tetrameric molecule of Hb H has been known to be unstable, it

readily dissociate into dimer and into monomer during the cause of Sephadex G-100 chromatography.

4 Studies on Haemoglobin Synthesis of Hb Thai.

The results of this study can be concluded as follows.

4.1 The slow haemoglobin components from a haemolysate of Hb H disease with Hb Thai separated by DEAE-Sephadex contained two major components; X and Y of Hb Thai. It was evident that the two abnormal pigments were unstable and gradually converted to another-Z component. Furthermore, the Z component was believed to be further degraded to a band with a mobility at Hb A. This implied that the Hb Thai molecule was unstable and degraded to a band-presumably Hb A. The CM-cellulose chromatography of the haemoglobin band is recommened in order to characterize the haemoglobin band as being Hb A.

4.2 The radioactive slow haemoglobin-Hb Thai was isolated from the whole haemolysate of Hb H disease with Hb Thai by DEAE-Sephadex chromatography. Globin prepared from the labelled slow haemoglobin mixed with non-radioactive Hb A as carrier was fractionated on the CM-cellulose chromatography. Radioactivity was incorporated mainly into peaks corresponding to β- and normal α-chain and very small peaks corresponding to α^{Y} and α^{X} of the abnormal α-chains. The evidence from starch-gel electrophoresis indicated that the isolated haemoglobin contained entirely Hb Thai which presumably consists of α^{Thai}(α^{Y} and α^{X}) and β-chain. Thus major radioactivity peaks were expected to correspond to β- and α^{X} and α^{Y} in the CM-

cellulose chromatography. Since the major radioactive peak was observed at the normal α-chain, instead of α^{Y} and α^{X}, this strongly indicated that the α^{Y} and α^{X} were probably unstable and degraded to normal α-chain, even during the DEAE-Sephadex chromatography.

In order to demonstrate that the degraded globin chain was the normal α-chain, a preparative isolation of the slow haemoglobin from a haemolysate of Hb H disease with Hb Thai by DEAE-Sephadex was carried out. The CM-cellulose chromatography of the globin showed five peaks, which by peptide mapping studies, corresponded to β-, δ -, normal α, α^{Y}- and α^{X}-chain. This confirmed the presence of normal α-chain which presumably was the product of the degradation of α^{Y} and α^{X}-chain.

4.3 The synthetic rate of the slow haemoglobin-Hb Thai and Hb A was studied in reticulocytes and bone marrow of a patient with $\alpha\text{-thal}_1$/Hb Thai disease. The specific activity of the Hb Thai at one hour incubation exceeded that of Hb A being approximately 1.6 and 8 times in reticulocytes and bone marrow respectively. The specific activity of the Hb Thai after four hours of incubation in reticulocytes was less than that of Hb A. This suggested that the turnover rate of Hb Thai was rapid, probably due to the breakdown of the unstable abnormal haemoglobin molecule. This would explain the levelling of specific activity of Hb Thai at four hour incubation in reticulocytes study.

The high specific activity of Hb Thai in the bone marrow was presumably due to the rapid synthetic rate of the α^{Thai} chain and

this abnormal chain was evidently degraded to the normal α-chain. The newly synthesized α-chain including the degradation of α^{Thai} would readily join to the non-radioactive β-chain in the pool leading to the increase in radioactivity α/β ratio in Hb Thai trait and homozygous Hb Thai. This was consistent with the means of α/β ratio of Hb Thai trait and homozygous Hb Thai of 1.34 and 1.64 respectively.