Chapter 1

INTRODUCTION

There are at least 5 different human globin chains designated as α-, β-, γ-, δ- and ε- chains. The α-chain contains 141 amino acid residues (Hill and Konigsberg, 1962), while each of the β-, γ-, and δ- chains contains 146 amino acid residues (Rhinesmith et al., 1958; Schroeder et al., 1963). Although the sequence of ε- chain has not been described, it is believed, based on the peptide mapping study by Huehens et al., (1964) that the ε- chain has a different amino acid sequence from the other haemoglobin chains. Normal human haemoglobins are at least comprised of haemoglobin A (Hb A), haemoglobin F (Hb F), and haemoglobin A_2 (Hb A_2). Hb A is the major component and is present at about 97% of total haemoglobin in adult. Its molecule consists of two α-chains joined to two β-chains and the formula is thus written $\alpha_2 \beta_2$. Hb F or foetal haemoglobin, with the formula of $\alpha_2 \gamma_2$, is the prominating pigment in foetal life and is present to approximately 60-70% at birth. In postneonatal period, Hb F is rapidly decreased in synthesis and is present in less than 1% at the end of one year of age (Garby et al., 1962). Hb A_2 ($\alpha_2 \delta_2$) (Kunkel and Wallenius, 1955) which is synthesized at perinatal period reaches the level around 1.5-3.5% in a normal person (Kunkel et al., 1957).

Haemoglobin A_3, another minor component resulting from an ageing process of Hb A (Huisman et al., 1966), may be found in a normal

haemolysate. Hb A_3 is a product of a modification at the N-terminal of β-chain of Hb A with glutathione (Holmquist and Schroeder, 1964).

Haemoglobinopathies.

Haemoglobinopathies, the disorders of haemoglobin synthesis, can be classified into two categories, namely abnormal haemoglobins and thalassaemia.

1. Abnormal haemoglobin

Over one hundred abnormal haemoglobins have now been documented. Most of the variants result from an amino acid substitution in a globin chain of the haemoglobin molecule. The basic mechanism of the amino acid alteration is due to a mutation of one base of the triplet for coding of amino acid (Beale and Lehmann, 1965). For example, for Hb E it is evident that the glutamic acid at residue 26 of β-chain is replaced by lysine (Hunt and Ingram, 1961), and in Hb Q aspartic acid residue at position 74 of α-chain is substituted by histidine (Lorkin et al., 1970).

Besides the single amino acid substitution, other mechanisms e.g. amino acid deletion as in Hb Gun Hill (Bradley and Wohl, 1967), Hb Frieburg (Jones and Brimhall, 1966) and unequal crossing over of β - and δ -genes as in Hb Lepore (Baglioni, 1962) and Hb Miyada the anti-Lepore haemoglobin (Ohta et al., 1971) have also been established.

Recently, a slow haemoglobin variant has been found to associate with haemoglobin H disease in many countries, which was

designated as Hb Thai in Thailand (Wasi, 1970), as Hb Athens in Greece (Sofraniadou et al., 1968), as Hb Constant Spring (HbCS) in Jamaica (Clegg et al., 1971). These slow variants have now been structurally studied and they are identical in biochemical abnormalities (Fessas et al., 1972). It is of interest that the Hb Thai (Constant Spring) has 31 amino acid residues extending form the C-terminal of the normal α -globin chain. Thus the α -chain of Hb Thai is 172 amino acid residues long while the normal α -globin chain contains 141 amino acid residues.

Hb Thai can be detected as the slower migrating haemoglobin compared to Hb A_2 on starch-gel electrophoresis at alkali pH. It is composed of two slow haemoglobin bands of Hb CS_1 or Y and Hb CS_2 or X. The proportion of the two components varies among samples of fresh red cell lysate. The more anodal fraction of Hb CS_1 (Y) always exceed that of Hb CS_2 (X), and occasionally Hb CS_1 was the only fraction observed. Since the length of mRNA which directs a corresponding globin chain synthesis has beenknown to be longer than that required to code for 141 amino acids normally found in the α^A -chain. (Gaskill and Kobat, 1971; Blobel 1971) and the amino acid residue 142 of the α -globin chain of Hb Thai, corresponding to the terminating codon in mRNA of normal α^A -globin chain (Figure 1) is glutamine, it is strongly suggested that uracil of the terminating codon (UAA, UAG) is mutated to cytosine resulting to code for glutamine (CAA, CAG). Therefore the process of translation must proceed on until next terminating codon is reached, which is believed to be located in α -globin mRNA at the

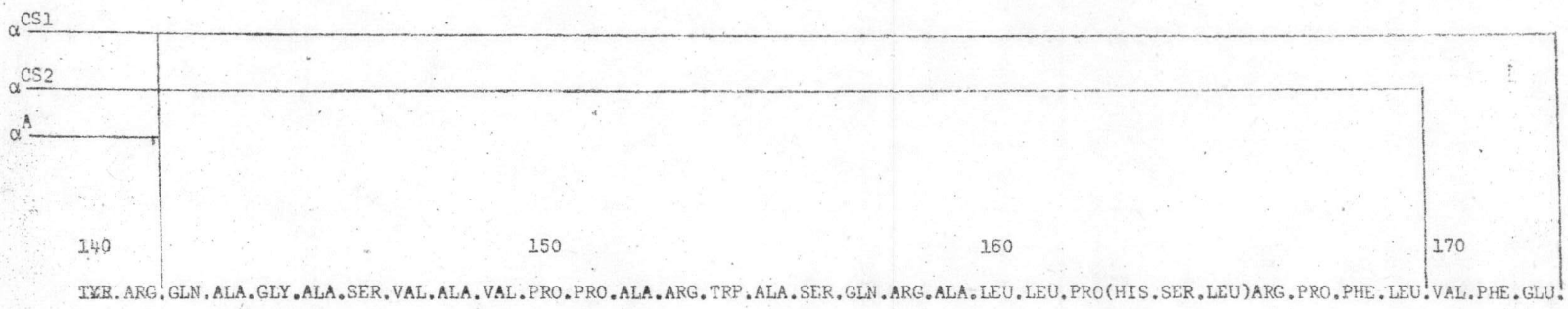

Figure 1 Amino acid sequences of the α^{CS1}- and α^{CS2}-chains.

next triplet code from the C-terminal of Hb Thai.

Besides the difference of its unique primary structure from other variants, Hb Thai is present in only small amounts, less than 2% in a heterozygote, and easily denatured leading to technical problems to detect the abnormal pigment (Wasi et al., 1972). It is also evident that the abnormal gene of Hb Thai can interact with a classical α-thalassaemia (α -thal$_1$) gene causing a type of haemoglobin H desease.

2. Thalassaemia.

Thalassaemias are hereditary defects resulting in a decreased synthesis of a globin chain and the presence of hypochromic microcytic anaemia without iron deficiency. It has been accepted that thalassaemias can be classified into two major groups, namely α -thalassaemia and β -thalassaemia. The biochemical diagnosis of the β -thalassaemia is based on the elevation of Hb A_2 and/or increased Hb F (Kunkel et al., 1957). This is due to the decrease of β -globin chain leading to the compensation by δ - and γ -chain. The α -thalassaemia is characterized, in addition to hypochromic microcytic red cells, by the normal or decreased Hb A_2 and Hb F, and by the presence of Hb H (β_4) and Hb Bart's (γ_4) (Jones et al., 1959; Hunt and Lehmann, 1959). The deficient α -globin chain due to α -thalassaemia gene expression causes the excess of β - and γ -globin chains and the free β - and γ -chains are tetramerized to form β_4 (Hb H) and γ_4 (Hb Bart's) respectively.

It is evident that the α -thalassaemias are at least divided into two types, designated as classical α -thalassaemia or α -thalassaemia$_1$

(α -thal$_1$) and mild α -thalassaemia or α -thalassaemia$_2$ (α -thal$_2$) (Wasi et al., 1964; Na-Nakorn et al., 1969; Na-Nakorn and Wasi, 1970). The α -thal$_1$ gene expression has been described to a complete suppression of the α -globin chain synthesis while the α -thal$_2$ gene expresses partial suppression of the α -chain synthesis. The genotype assignment for the α -thalassaemia genes in adult is very difficult since the haematological findings of both show minimal changes or are apparently normal. It is rather easy to designate the α -thal$_1$ and the α -thal$_2$ gene in a newborn on the basis of the presence of 5-6% and 1-2% Hb Bart's respectively (Pootrakul et al., 1970, Na-Nakorn and Wasi, 1970).

Based on cord blood studies (Pootrakul et al., 1970), an incidence of α -thal$_1$ is about 10%, as high as the α -thal$_2$. Thus the total incidence of α -thalassaemias in Thailand is very high, estimated to be 20%. Interaction of the two abnormal genes results in at least two important clinical syndromes, i.e, Hb Bart's hydrop feetalis and Hb H disease. Hb Bart's hydrop foetalis is clinical disorders characterized by a hydropic baby with intrauterine or perinatal death. Haematological data indicates thalassaemic red cell changes with severe haemolysis. Haemoglobin electrophoresis shows almost 100% of Hb Bart's. The genetics of the disease is evidently a homozygous α -thal$_1$ gene (Pootrakul et al., 1967). The interaction of the genes lead to the complete suppression the α -chain synthesis (Weatherall et al., 1970), therefore, the excess γ -chain is polymerized to be tetramer γ_4 (Hb Bart's).

Haemoglobin H disease is a clinical disorder characterized by

a moderate chronic haemolytic anaemia, splenomegaly, positive for inclusion test in almost all red cells and Hb types of A and H. From the cord blood studies by Pootrakul et al., (1967;1970) apparently healthy neonates presenting definite hypochromic microcytic red cells associated with 20-30% Hb Bart's would subsequently develope into typical cases of Hb H disease.

It is evident that Hb H disease can be classified into two types on the basis of haemoglobin typing, one with Hb A and H, another with Hb A + H + Constant Spring (or Hb Thai), which genetically correspond to the double heterozygous state for α -thal$_1$ and α -thal$_2$; and for α -thal$_1$ and Hb Thai respectively (Wasi 1970, Clegg et al., 1971). Since either α -thal$_2$ or Hb Thai interacting with α -thal$_1$ would result in a similar clinical disorder of Hb H disease, it suggested that the Hb Thai expression is equivalent to the α -thal$_2$ gene in terms of the suppression effect on the normal α -chain synthesis.

Objectives of the study

Hb Thai has the following special characteristics:

1. a unique structure
2. very small amounts, less than 3% is detectable in haemolysate.
3. unstable haemoglobin molecule.
4. gene expressivity is equivalent to α -thal$_2$ gene.
5. high incidence (about 4%) of the Thai population.

It is of interest to study the haemoglobin biosynthesis of the mutant in order to better understand the nature of the mutant.