Chapter 1

INTRODUCTION

Hemoglobin is a conjugated protein. Its function is to transport oxygen from the lungs to the tissues.

By fundamental x-ray crystallographic studies of Perutz and his colleagues (Perutz et al. 1960), a hemoglobin molecule had been shown to consist of four polypeptide chains, each of which enfolds a single heme. It has been described that there are at least five different types of human globin chains designated as α , β, δ, γ, and ε chains. The complete amino acid sequences of the α , β, γ and δ chains have been published (Branitzer et al. 1961; Konigberg et al. 1961; Schroeder et al. 1963; Schroeder & Jones. 1965). Most hemoglobin molecules consist of two pairs of identical polypeptide chains except for the hemoglobin Gower I. The structural formulae of the human hemoglobin can be written as the following.

Hemoglobin A (Hb A)	:	$\alpha_2\beta_2$
Hemoglobin A_2 (Hb A_2)	:	$\alpha_2\delta_2$
Hemoglobin F (Hb F)	:	$\alpha_2\gamma_2$
Hemoglobin Gower I	:	ε_4
Hemoglobin Gower II	:	$\alpha_2\varepsilon_2$

Normal adults hemoglobins.

These are a heterogenous mixture of hemoglobin fractions. The major fraction, Hb A ($\alpha_2\beta_2$) is present to the extent of 97 % of

total hemoglobin. Other three minor fractions are Hb A_2, Hb A_3 and Hb F. The Hb A_2 ($\alpha_2 \delta_2$) is found ranging 2-3 % of total hemoglobin. The Hb A_3 is present in varying amounts but usually less than 5 % in adult (Kunkel & Wallenius, 1955). The Hb A_3 is believed to be a derivative of Hb A, since it increases on in vitro storage of Hb A. Another minor hemoglobin is Hb F ($\alpha_2 \gamma_2$), which normally comprises less than 1 % of total hemoglobin in normal adult.

Hemoglobin in fetus

Hb F is the main hemoglobin component throughout intrauterine life of fetus and comprises 60-70 % of total hemoglobin at birth. Hb F will rapidly fall during the first few months after birth and it persists less than 1 % after the age of one year.

Embryonic hemoglobins

Two hemoglobin components described by Butler and her associates as Hb Gower I and Hb Gower II were found in human embryo (Butler et al. 1960). Their molecular structures differ from other hemoglobins, and their structural formulae are believed to be ε_4 and $\alpha_2 \varepsilon_2$ respectively (Huehns et al. 1964). The Gower I is present at a gestational age of about 40 days, while the Gower II, the second embryonic hemoglobin, appears after α chain begin to be synthesized. The amounts of embryonic hemoglobins rapidly fall as fetal development proceeds and the pigments completely disappear after the fetus reaches a crown-rump length of 100 mm.

Genes Control Hemoglobin Synthesis

There are good evidence of separated pairs of genes controlling the synthesis of α , β, γ, and δ chains respectively. (Smith & Torbert, 1958; Atwater et al. 1960; Pugh et al. 1964). It was also evident that the β and δ chain loci are closely linked. (Weatherall, 1965). However the information about the location of locus controlling γ chain synthesis of Hb F is not yet available.

These loci direct the globin chain synthesis and the subunits finally conform a tetrameric hemoglobin molecules as shown in Figure 1.

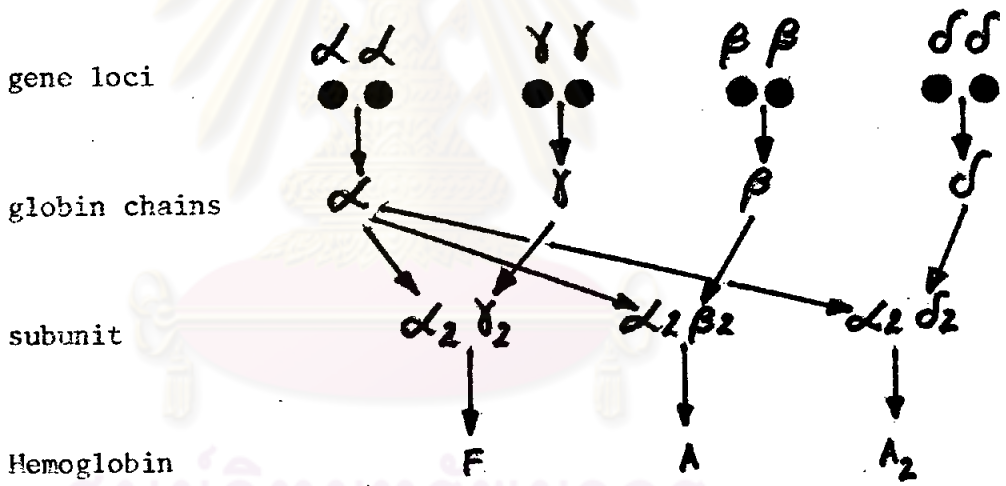

Figure 1: The genetic control of hemoglobin synthesis in normal adult.

From this model for genetic control of hemoglobin synthesis, the hemoglobin constitutions of a homozygote or heterozygote for a hemoglobin mutant or a double heterozygote for different mutants can

be predicted. For example a heterozygous Hb E has hemoglobin type of Hb A + Hb E besides the normal Hb A_2 and Hb F (Figure 2).

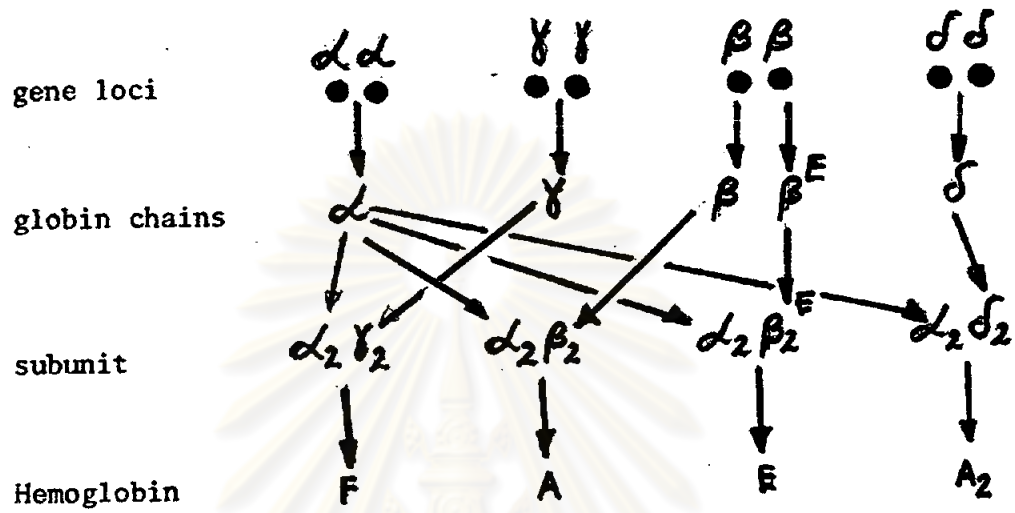

Figure 2: Explanation of the finding of hemoglobin components in heterozygote for hemoglobin E variant.

Since the Hb F, normally presenting in less than 1 % in adult, will not be detectable in electrophoresis and the electrophoretic mobility of Hb A_2 migrates at the same rate as Hb E, only the two major components, Hb A and Hb E in a heterozygous Hb E are observed on starch gel electrophoresis.

Hemoglobinopathies

In 1949, the begining of the abnormal hemoglobin era, Pauling and his colleagues (Pauling et al. 1949) discovered that the hemoglobin in sickle cell anemia patients possess a mobility slower

than that of normal adult hemoglobin during electrophoresis. Since the electrophoretic mobility of protein depends on its net charge which, in turn, depends on its amino acid composition. Pauling and his coworkers called sickle cell disease a "Molecular disease". Since then there has been a rapid growth of knowledge in fields of structural studies and of the genetic controls of hemoglobin synthesis leading to the understanding with the molecular defects of the disease.

The hemoglobinopathies, the disorder of hemoglobin structure and synthesis are classified into two groups, namely abnormal hemoglobins and thalassemias.

The abnormal hemoglobins are mostly resulted from a single amino acid alteration in a globin chain. This is generally due to a mutation of one base of a triplet codon. It is described, for example that glutamic acid at residue 26 of β-chain of Hb E is substituted by lysine, and the abnormal hemoglobin is written as $\alpha_2\beta_2^{26Glu\rightarrow Lys}$. Over one hundred abnormal variants have now been established.

Thalassemias are defined as hereditary defects with reduce rate of globin synthesis and present as a hypochromic microcytic anemia without iron deficiency. Other hematologic parameters e.g. decrease in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) as well as decrease osmotic fragility of red cells are very helpful to designate the stigmata of the thalassemias.

Two major types of thalassemias namely α-thalassemias and β-thalassemias are generally accepted.

α-thalassemias

α-thalassemias are characterized by decreased production in α chain. The diagnosis of the α-thalassemias in neonates has no problems since all are associate with Hb Bart's (γ_4) in the cord blood study. The α-thalassemia results in the α chain deficit, thus the γ chain which joined to α chains forming Hb F, would polymerize to tetrameric molecule- γ_4, Hb Bart's. The diagnosis of the α-thalassemia in adult is, rather difficult, based on hypochromic microcytic red cell without increased Hb A_2. The presence of Hb Bart's (γ_4) and Hb H (β_4) are also suggestion of α-thalassemia.

Base on the previous studies (Wasi et al. 1964; Na-Nakorn & Wasi, 1970; Pootrakul et al. 1970), α-thalassemias are devided into two types designated as α-thalassemia$_1$ (classical α-thalassemia) and α-thalassemia$_2$ (mild α-thalassemia). Two clinical important disorders of the α-thalassemia syndromes are Hb Bart's hydrops fetalis and hemoglobin H disease, which genetically represent the homozygosity for α-thal$_1$ and double heterozygote for α-thal$_1$ and α-thal$_2$ respectively. (Wasi et al. 1969).

β-thalassemias

β-thalassemias are defective in rate of β chain synthesis. Normally, the genotype designations of β-thalassemia in adult has no

problems since they are characterized by hypochromic microcytic red cells with elevation in Hb A_2 and Hb F. The β-thalassemia can be classified into two types namely A_2-thalassemia and F-(γδ) thalassemia.

In A_2-thalassemia which is a characteristic of a heterozygous state, there is an elevation of Hb A_2 with a slight increase of Hb F.

From a study of the combination of A_2-thalassemia with Hb E in Thailand (Wasi et al. 1969), the A_2-thalassemia can be divided into two subtypes called classical β-thalassemia (β-$thal_1$) and mild β-thalassemia (β-$thal_2$) on the basis of interaction of Hb E. The former shows severe suppression of β chain synthesis resulting in the absence of detectable Hb A, while the latter shows only partial depression of β chain synthesis leading to the appearance of some Hb A on starch gel electrophoresis.

F-(γδ) thalassemia is similar to the A_2-thalassemia except that there is decreased Hb A_2 and elevated Hb F. Only the presence of Hb F without Hb A and Hb A_2 has been described in homozygous state (Braucati & Baglioni, 1966).

β-thalassemia syndromes in Thailand

β-thalassemia and Hb E ($\alpha_2\beta_2^{26Glu \rightarrow Lys}$) are highly prevalent in Thailand. The incidence of the two abnormal genes is about 5 and 13 % respectively in the population. The different combinations of the abnormal genes cause at least two disorders of clinical impor-

tance. They are homozygous β-thalassemia and β-thalassemia/Hb E disease. The former has been known as thalassemia major or Cooley's anemia, of which hemoglobin types are Hb F + Hb A or entirely Hb F. This disorder generally manifests severe hemolytic anemia and almost of the patients expire during child-hood. The β-thalassemia/Hb E disease results in a clinical disorder characterized by chronic hemolytic anemia, growth retardation, hepatosplenomegaly and severe thalassemic red cells changes. Hemoglobin electrophoresis of the patients hemolysate shows only two major components, Hb E and Hb F. The explanation of the absence of Hb A is due to the interaction of β^E gene and β-thalassemic gene leading to completely depress the β chain synthesis (Figure 3).

Wasi and his colleagues (Wasi et al. 1969) described patients with clinical features resembling to the mentioned group except that the clinical symptoms are apparently, less severe. Hemoglobin types are Hb E, Hb F and Hb A, without a history of previous blood transfusion. The presence of Hb A suggested that the β-thalassemic gene to partially suppress β^A synthesis. (Figure 4,p.9). Family studies indicate that the disease is caused by a double heterozygous for another β-thalassemia and Hb E.

Based on the interaction of Hb E gene, β-thalassemias can be, at least, classified into two types, namely classical β-thalassemia trait (β-thal$_1$), complete suppression of β^A chain synthesis and mild β-thalassemia (β-thal$_2$), partial suppression of β^A chain synthesis.

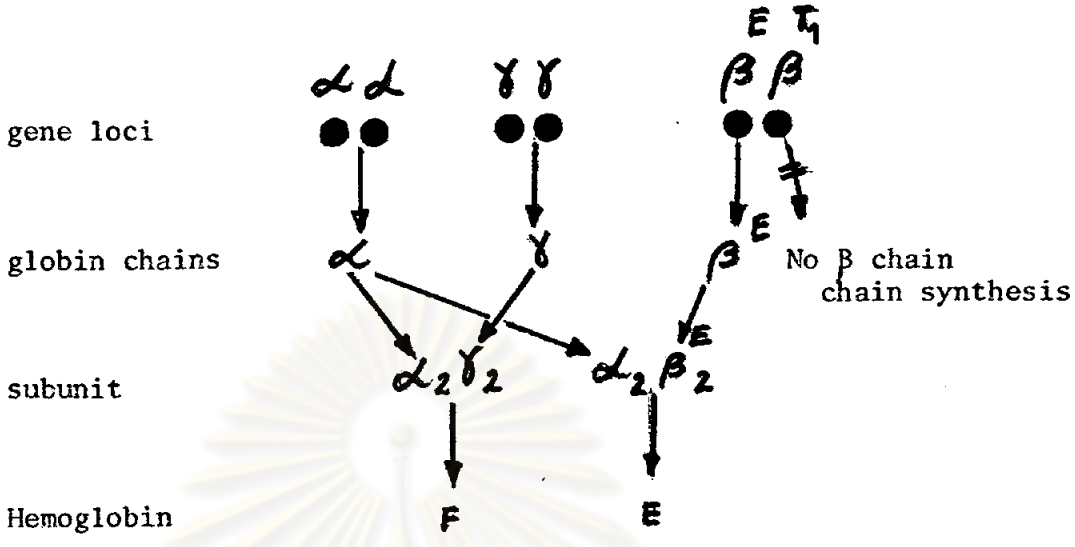

Figure 3: Explanation of abnormal hemoglobin type found in double heterozygous β-thal$_1$ and Hb E

T_1 = Classical β-thalassemia (β-thal$_1$).

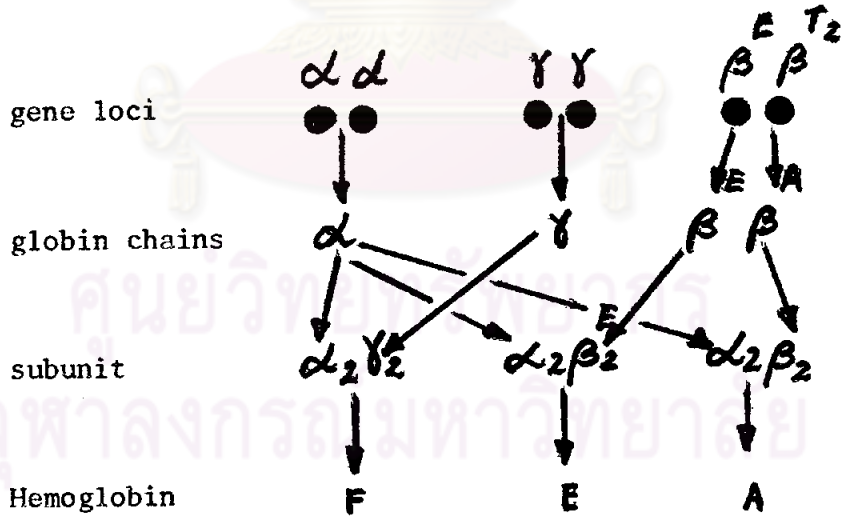

Figure 4: Explanation of hemoglobin type found in double heterozygous β-thal$_2$ and Hb E

T_2 = Mild β-thalassemia (β-thal$_2$)

Diagnosis of β-thalassemia

Evidences for designation of β-thalassemia are the following

1. Hematologic evidence. The hypochromic microcytic red cells with an increased Hb A_2 or Hb F is generally a characteristic of β-thalassemia.

2. Genetic evidence. Either parents or offspring with a normal hemoglobin type from a patient with β-thalassemia/Hb E disease is known to be an obligatory case of β-thalassemia.

3. Hemoglobin chain synthesis. The radioactivity of α/β chain ratio in a nonthalassemic reticulocytes appears to be approximately one. β globin chain synthesis in β-thalassemia is evidently decreased. It is now believed that the globin chain incorporative study is the most sensitive method to detect the stigmata of a thalassemia.

Objective of this study

The precise diagnosis of the classical β-thalassemia (β-$thal_1$) trait and mild β-thalassemia (β-$thal_2$) trait is difficult because the hematologic findings including the amounts of Hb A_2 and Hb F are similar to each other. This study attempts to characterize the heterozygotes in obligatory cases of β-$thal_1$ and β-$thal_2$ trait and also in heterozygous Hb E, β-$thal_1$/Hb E disease and β-$thal_2$/Hb E disease as well by measuring the globin chain synthesis in reticulocytes.