Chapter 1. INTRODUCTION

1.1 Biological functions of Haptoglobin (Hp)

Human haptoglobin (Hp), is known as an acute phase protein, produced mainly by the liver and secreted into the circulation (1, 2). In response to inflection, inflammation, tissue injury and malignant proliferation, its plasma level is increased (3, 4). Its production is regulated by IL-6 (5, 6) and is part of the acute phase reaction, with rapid turnover rates (< 3 days) (7) and marked upregulation in the setting of inflammation (8). Hp stimulates angiogenesis (9), inhibits prostaglandin synthesis (10-12), and promotes cholesterol crystallization (13). The Hp binding to CD22-positive B cells (14) and antigen presenting skin cells (15) was described; anti-inflammatory (16) or immunosuppressive properties (17, 18) of this protein were proposed. Furthermore, Hp was suggested to inhibit the reverse transport of cholesterol in the ovarian follicular fluid (19). Thus, to quantify the Hp concentrations have been used in the diagnosis for some infection diseases (2, 20-23). Hp can capture the hemoglobin (Hb) by forming a high affinity Hp-Hb complex (21, 24, 25), and acts as a scavenger that transporting this complex to the liver for protein degradation and iron recycling. This Hp-Hb complex is cleared from

circulation by the reticuloendothelial system to alleviate the oxdative stress of intravascular hemolysis (26). It was suggested that Hp plays an important antioxidant role in vivo by preventing iron-stimulated formation of hydroxyl radicals in the Fenton reaction (27, 28).

1.2 The structural arrangement of Hp

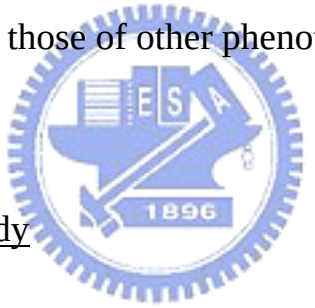
Hp is known to be synthesized as a single precursor (αβ), that is processed posttranslationally into α and β subunits by proteolytic cleavage (29). These subunits are held together by interchain disulfide bridges in mature Hp (21). In humans, Hp has a complex gel electrophoretic pattern due to the heterogeneity of the α-subunit which are *Hp*^{1} and *Hp*^{2} alleles on chromosome 16q22.1 (30, 31). There are three phenotypes (1-1, 2-1, and 2-2) in the population results from the length of α-chains. The Hp α1-chain exists in two additional allelic forms, 1F and 1S (2, 32). All the phenotypes share the same β (Mr 40000, each 245 residues, containing 30% carbohydrate) chain. Hp 1-1 represents the simplest combination with dimeric α1-β chains [or $(\alpha1\text{-}\beta)_2$]. Hp 2-2 is heterogeneous in size, composed of trimeric α2-β chains [or $(\alpha2\text{-}\beta)_3$] and other cyclic polymers. Hp 2-1 is also heterogeneous, but composed of a dimeric [$(\alpha1\text{-}\beta)_2$], trimeric α-β chains [or $(\alpha\text{-}\beta)_3$], and other linear polymers (Fig. 1); where α represents a

mixture of α1 and 2 chains. α1 is a polypeptide containing 83 amino-acids (NCBI Accession # CAA25267). Uniquely, α2 is identical to α1, but with an insertion of a 3/4 repeat of α1 (residues 12-70 or 59 amino-acids). Therefore, α2 sequence consists of 142 amino-acid (NCBI Accession # AAA52685). Due to an extra insertion of Cys-74 in α2, Hp 2-1 and 2-2 form complicated polymers (Fig. 1). Marked geographical differences exist with respect to the distribution of Hp phenotypes, with the lowest *Hp*^{1} allele frequency in South-East Asia and the greatest *Hp*^{1} frequency in South America (30). The allelic subtypes Hp1F and Hp1S also appear to show geographical variability (33-37).

1.3 Clinical conception with Hp phenotypes

Several biological functions among haptoglobin phenotypes are different which include the binding affinity of Hp and Hb (38), the antioxidant activity towards Hb-stimulated lipid peroxidation (39-42), and the inhibitory effect on prostaglandin synthesis (11). Interestingly, the activity in all cases was shown that Hp 1-1 is higher compared with 2-1 and 2-2 types. Molecular heterogeneity of Hp may play a role in certain clinical settings. For example, patients with polymeric form of Hp 2-1 or 2-2 are associated with an increased

risk of atherosclerotic coronary disease (43), kidney failure (44), and diabetics (45, 46). In addition, the different penetration of Hp 2-1 and 2-2 into human ovarian follicles was found correlated with the oocyte fertilization (47). This was supported by the observation that the levels of an antioxidant vitamin C are lower in Hp 2-2 individuals than in individuals with other phenotypes (42), and the finding that serum iron, ferritin and transferring are higher in Hp 2-2 individuals (48). Additionally, altered serum lipid profile, atherosclerosis, hypertension and cardiovascular disorders are more common in Hp 2-2 phenotype individuals than those of other phenotypes (43, 49-55).



1.4 The purpose of this study

Recently, we and others have shown that Hp is an extremely potent antioxidant, which directly prevents LDL from Cu^{2+}-induced oxidation (39, 41). Its potency is markedly superior to probucol: one of the most potent antioxidants used in antioxidant therapy (39, 56). Transfection of Hp cDNA into CHO cells protects them against oxidative stress (39). Epidemiological studies show that patients with polymeric form of Hp 2-1 or 2-2 are associated with the complications of myocardial infarction (46), kidney failure (57), and diabetes (58). Presumably, this is due to the markedly complicated

arrangement of Hp 2-1 and 2-2 in which the biologically functional groups are not fully expressed on the surface.

The distinct antigenic surface structure in Hp phenotype, however, is rarely reported. This is probably because of the difficult purification procedures for each Hp type (59, 60). We hypothesized that the unique surface epitopes of each type might be probed by specific monoclonal antibodies (mAb). Thus, the purpose of this study was to explore the structural difference among the Hp types using unique lines of mAb and to elucidate the subunit arrangement. Studies by antigenic mapping suggest that α chains are rearranged in the polymers of Hp 2-1 and 2-2. Combining the results from the unique conformation-dependent mAb prepared, the present study shows that the antigenic structure of Hp 2-1 and 2-2 is markedly different from that of 1-1. Hp 1-1 is essentially the only type in all non-human species. Therefore from an evolutionary point of view, the evolved α2 chain with an extra repeated copy of residues 12-70 (containing Cys-74) could moderate some of the biological functions of Hp. The relationship between immunochemical structure of Hp and its biological implications are discussed in detail.