"By three methods we may learn wisdom:
First, by reflection, which is noblest;
Second, by imitation, which is easiest;
and third by experience, which is the bitterest."

Confucius

CHAPTER

1

GENERAL INTRODUCTION

Çetin Ağar

INTRODUCTION

History of β2-glycoprotein I

β2-Glycoprotein I (β2GPI) was described in literature for the first time in 1961[1] and seven years later the first β2GPI deficient, seemingly healthy individual was identified[2]. β2GPI's alternative name, apolipoprotein H, suggests a function in lipid metabolism but this was only based on a single publication that dates from 1979 in which it was shown that β2GPI was distributed over different human lipoproteins[3]. Since 1983 the names β2GPI and apolipoprotein H were used side by side for the same protein[4], and the official designation for the β2GPI gene has become APOH. From 1990 on, the interest in this protein has increased significantly when β2GPI was identified as the most important antigen in the antiphospholipid syndrome (APS), which is amongst others characterized by the presence of antibodies directed to β2GPI[5,6].

Proposed functions of β2-glycoprotein I

Individuals and mice deficient in β2GPI appear to be healthy, indicating that the presence of β2GPI is not essential for life. β2GPI, however, is a highly abundant protein present in blood, and it is unlikely that it should not have a function. Because of the affinity of β2GPI for anionic phospholipids, it was thought that β2GPI could play a role in maintaining the haemostatic balance by inhibition of the contact phase activation of coagulation[7-9]. It was suggested that binding of β2GPI to either FXI or FXII results in inhibition of the intrinsic pathway of coagulation in *in vitro* systems[7-9]. Furthermore, it has been suggested that β2GPI is involved in platelet prothrombinase activity and ADP-mediated platelet aggregation[10,11]. β2GPI binds liposomes and microparticles via an interaction with phosphatidylserine and is also involved in the clearance of these negatively charged cellular fragments in mice[12-14]. β2GPI has also been identified in atherosclerotic plaques[15] and a number of studies

have suggested that the presence of antibodies against β_2GPI resulted in accelerated atherosclerosis[16,17]. The first publication suggesting a role of β_2GPI in angiogenesis showed that clipped or nicked β_2GPI was able to inhibit bladder cancer development in mice[18]. β_2GPI levels increase with age and are reduced in pregnant women and in patients with stroke and myocardial infarction[19].

β_2-glycoprotein I and the antiphospholipid syndrome

APS is an auto-immune disease defined by the presence of antiphospholipid antibodies in blood of patients in combination with thrombotic complications in arteries or veins as well as pregnancy-related complications[20]. In APS patients, the most common venous event is deep vein thrombosis and the most common arterial event is stroke. In pregnant women with APS early and late miscarriage can occur. Next to miscarriages also placental infarctions, early deliveries and stillbirth are reported. Antiphospholipid antibodies are found in 1% of the general population, however, the incidence increases with age and coexistent chronic disease[21]. The syndrome occurs more in women than in men, and is most common in young to middle-aged adults but can also occur in children and the elderly. Among patients with systemic lupus erythematodes, or lupus, the prevalence of antiphospholipid antibodies ranges from 12% to 30% for anticardiolipin antibodies, and 20% to 35% for lupus anticoagulant antibodies[21]. It is now generally accepted that the relevant auto-antibodies are not directed against phospholipids but towards proteins bound to these phospholipids[5,6]. β_2GPI has a relative low affinity towards these negatively charged phospholipids but its affinity increased more than 100 times in the presence of auto-antibodies. β_2GPI is now accepted as the most prominent antigen for the auto-antibodies in APS[22]. Recently, three independent groups have shown the importance of antibodies against β_2GPI. Mice that were challenged by injection of these antibodies had an

increased thrombus formation[23-26] and showed increased foetal resorption and a significant reduction in foetal and placental weight[27,28]. Despite the significant role of β_2GPI in the pathophysiology of APS, all these in vivo and in vitro experiments did not reveal a convincing physiological function for β_2GPI.

Biochemistry of β_2-glycoprotein I

β_2GPI is a 43 kDa protein, consists of 326 amino acid residues[29] (Figure 1). β_2GPI is synthesized in the liver and it circulates in blood at variable levels (1-10 μM)[30]. β_2GPI is an anionic phospholipid binding glycoprotein composed of five homologous complement control protein repeats (CCP-I to CCP-V)[31,32]. These CCPs are generally found in proteins from the complement system and they could mediate binding of complement factors to viruses and bacteria[33,34]. The first four domains contain about 60 amino acids each, whereas the fifth domain has a 6 residues insertion and an additional 19 amino acid C-terminal extension. The extra amino acids are responsible for the formation of a large positive charged patch within the fifth domain of β_2GPI[35] that forms the binding site for anionic phospholipids (Figure 1). Human β_2GPI contains one O-linked sugar on Threonine 130 and four N-glycosylation sites, at Arginines 143, 164, 174 and 234, localized in the third and fourth domain. The glycans account for 20% of the total molecular mass[36]. The crystal structure of β_2GPI has been solved in 1999 by two groups[32,37] and revealed a structure that looked like a J-shaped fishhook. The phospholipid binding site is located at the bottom side of CCP-V and consists of two major parts, a large positive patch of 14 charged amino acid residues and a flexible hydrophobic loop. This flexible loop contains a Tryptophan-Lysine sequence, giving the loop the potential to insert into the membranes[38]. Of the many single-nucleotide polymorphisms in the promoter region of the β_2GPI gene, only two have been identified that correlate with a significant reduction of plasma levels of β_2GPI[39,40].

An interesting polymorphism is Cysteine to Glycine at position 306, a polymorphism that disrupts the phospholipid binding site within β_2GPI, and which is also correlated with plasma levels of β_2GPI[41].

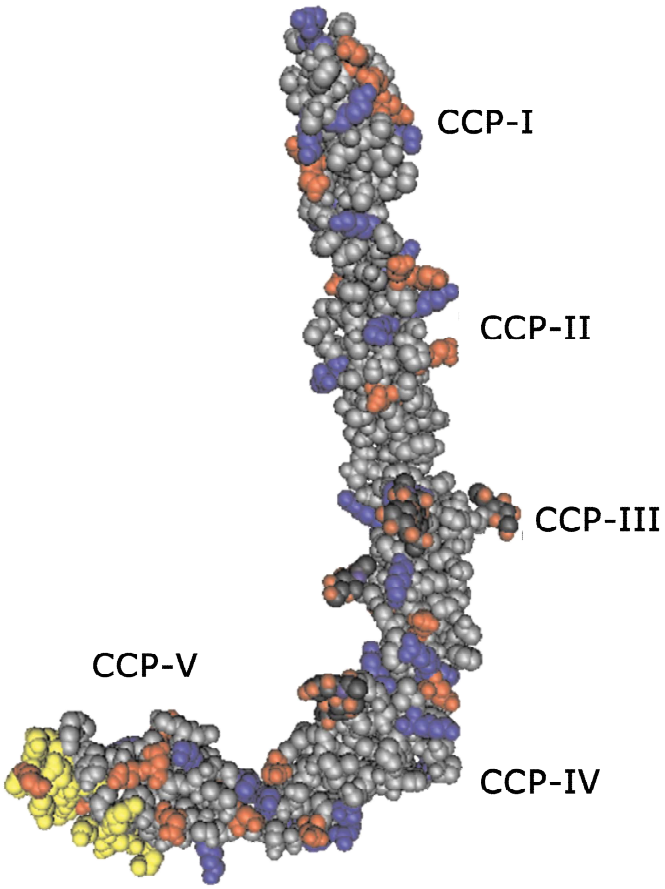

Figure 1. Crystal structure of β_2GPI with the five domains (CCP-I to CCP-V). In blue the negatively charged amino acids and in red the positively charged amino acids. In yellow the large positive charged patch within the fifth domain of β_2GP that forms the binding site for anionic phospholipids. Picture was made using Cn3D version 4.1, produced by the National Center for Biotechnology Information (http:// www.ncbi.nlm.nih.gov).

OUTLINE OF THIS THESIS

This thesis started with a general introduction on β_2GPI of what was known until the year 2007 in which I started my PhD project. My work focused on the search for a physiological function of β_2GPI, an abundant plasma protein and the major antigen for the antiphospholipid syndrome, but whose physiological function is still an enigma. The second chapter describes the distribution of β_2GPI (apolipoprotein H) over the different lipoprotein fraction to confirm or falsify observations made in the literature. Since patients with antiphospholipid antibodies do not have circulating antibody antigen complexes in the presence of large amounts of β_2GPI in the circulation, we hypothesized that the conformation of β_2GPI in plasma may be different than when used in tests for the antiphospholipid syndrome. Therefore in chapter three, we focused on different conformations that β_2GPI can adopt in response to changes in its environment. Due to the fact that β_2GPI can adopt different conformations and the description in literature that domain V of β_2GPI shows antibacterial activity, we hypothesized that β_2GPI has the capacity to bind to LPS. This novel interaction between LPS en β_2GPI is described in chapter four. In chapter five we show that the β_2GPI protein isconserved across the animal kingdom. More and more evidence supports the association between infectious agents and APS, and it has been suggested that many autoimmune diseases are caused or triggered by infections. Despite this, the exact nature of their contribution is not deciphered. In chapter six we try to give an answer on the etiology of the antiphospholipid syndrome. In chapter seven I summarize and discuss all chapters described above and finish this thesis with a Dutch summary.

REFERENCES

1. Schultze HE, et al. Naturwissenschaften. 1961; 48: 719.
2. Haupt H, et al. Humangenetik. 1968; 5: 291-293.
3. Polz E and Kostner GM. Febs Letters. 1979; 102: 183-186.
4. Lee NS, et al. J Biol Chem. 1983; 258: 4765-4770.
5. McNeil HP, et al. Proc Natl Acad Sci USA. 1990; 87: 4120-4124.
6. Galli M, et al. Lancet. 1990; 335: 1544-1547.
7. Schousboe I. Blood. 1985; 66: 1086-1091.
8. Brighton TA, et al. Br J Haematol. 1996; 93: 185-194.
9. Shi T, et al. J Biol Chem. 2005; 280: 907-912.
10. Nimpf J, et al. Thromb Haemost. 1985; 54: 397-401.
11. Nimpf J, et al. Biochim Biophys Acta. 1986; 884: 142-149.
12. Nomura S, et al. Br J Haematol. 1993; 85: 639-640.
13. Chonn A, et al. J Biol Chem. 1995; 270: 25845-25849.
14. Balasubramanian K, et al. J Biol Chem. 1997; 272: 31113-31117.
15. George J, et al. Circulation. 1999; 99: 2227-2230.
16. Vaarala O. Lupus. 1996; 5: 442-447.
17. Staub HL, et al. Autoimmun Rev. 2006; 6: 104-106.
18. Beecken WD, et al. Ann Surg Oncol. 2006; 13: 1241-1251.
19. Lin F, et al. Lupus. 2006; 15, 87-93.
20. Miyakis S, et al. J Thromb Haemost. 2006; 4: 295-306.
21. Gezer S. Dis Mon. 2003; 49: 696-741.
22. Willems GM, et al. Biochemistry. 1996; 35: 13833-13842.
23. Fischetti F, et al. Blood. 2005; 106: 2340-2346.
24. Romay-Penabad Z, et al. Blood. 2009; 114: 3074-3083.
25. Ramesh S, et al. J Clin Invest. 2011; 121 :120-131.
26. Romay-Penabad Z, et al. Blood. 2011;117: 1408-1414
27. García CO, et al. Am J Reprod Immunol. 1997; 37: 118-124.
28. Ikematsu W, et al. Arthritis Rheum. 1998; 4: 1026-1039.
29. Lozier J, et al. Proc Natl Acad Sci USA. 1991; 81: 3640-3644.
30. Rioche M, et al. Biomedicine. 1974; 21: 420-423.
31. Bouma B, et al. EMBO J. 1999; 18: 5166-5174
32. Schwarzenbacher R, et al. EMBO J. 1999; 18: 6228-6239.
33. Brier AM, et al. Science. 1970; 170: 1104-1106.
34. Pangburn MK, et al. Biochem Soc Trans. 2002; 30: 1006-1010.
35. Hunt JE, et al. Proc Natl Acad Sci U S A. 1993; 90: 2141-2145.

36. Kondo A, et al. J Proteomics. 2009; 73: 123-133.
37. Bouma B, et al. EMBO J. 1999; 18: 5166-5174.
38. de Planque MR, et al. J Biol Chem. 1999; 274: 20839-20846.
39. Kamboh MI, et al. Lupus. 1999; 8: 742-750.
40. Mehdi H, et al. Hum Genet. 1999; 105: 63-71.
41. Suresh S, et al. FEBS J. 2010; 277: 951-963.