Site-directed Mutagenesis of Recombinant Human β_2-Glycoprotein I

Effect of Phospholipid Binding and Anticardiolipin Antibody Activity

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β_2-Glycoprotein I (β_2GPI), a phospholipid-binding plasma protein, has been shown to be the target antigen for antiphospholipid (aPL) antibodies purified from patients with autoimmune disease.[1] Antiphospholipid antibodies that occur in association with infections are not associated with an increased risk of thromboembolic complications[2] and are directed against anionic phospholipids and not β_2GPI.[1]

These antibodies preferentially bind β_2GPI that has been immobilized on anionic phospholipid surfaces, whereas binding in the fluid phase is weak and nondetectable. A number of hypotheses have been put forward to explain this reactivity. It has been proposed that the binding of β_2GPI to PL induces a conformational change in β_2GPI, thus exposing a cryptic epitope for aPL antibodies to bind.[1] Alternatively, it has been suggested that binding of β_2GPI to phospholipid increases the local concentration of β_2GPI, thus promoting an increase in affinity of the aPL antibodies for β_2GPI.[3] β_2GPI is composed of five highly conserved domains of 60 repeating amino acids called complement control protein (CCP) repeats. In β_2GPI, the fifth domain is the most variable and contains a region that has been predicted to be critical for phospholipid binding. To study the interaction between native β_2GPI and anionic PL such as cardiolipin in more detail, we first calculated a 3D model of the fifth domain of β_2GPI (β_2GPI-5), relying on its similarity to the 15th module from human factor H, whose 3D structure has been determined by NMR. The electrostatic calculations confirm that the loop Lys282–Lys287 is likely to be part of the PL-binding site. To test this prediction, the cDNA for human β_2GPI was inserted into a baculovirus vector for expression in insect cells. Site-directed mutagenesis was then performed to assess the role of individual amino acids in the Lys282–Lys287 loop on the phospholipid binding and cofactor activity of β_2GPI. Expressed wild-type β_2GPI had activity equivalent to native β_2GPI. Four mutants were generated: mutants 1, Lys286 to Glu286; 2, Lys286,

287 to Glu286, 287; 3, Lys284, 287 to Glu284, 287; and 4, Lys284, 286, 287 to Glu284, 286, 287. Affinity-purified antibodies from patients with aPL syndrome exhibited binding in a CL-ELISA system only in the presence of recombinant wild-type β_2GPI in a dose-dependent manner similar to that obtained with native human β_2GPI (FIGURE 1). In contrast, no cofactor activity was obtained with mutants 2, 3, and 4 when these were tested up to a concentration of 64 μg/mL (FIGURE 1). However, mutant 1 exhibited a dose-dependent increase in binding that was only

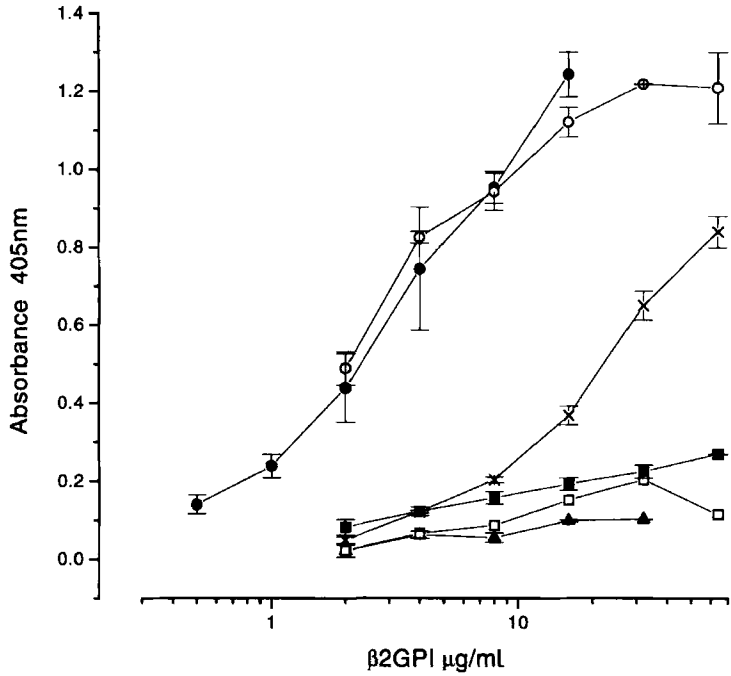

FIGURE 1. Dose response of binding activity of affinity-purified aPL antibody at 2 μg/mL from an autoimmune patient to CL in a modified CL-ELISA in the presence of different preparations of β_2GPI: native β_2GPI (●), recombinant wild-type β_2GPI (○), mutant 1 (×), mutant 2 (■), mutant 3 (▲), and mutant 4 (□). Results are expressed as the mean ± SE of duplicates.

demonstrated at high concentrations of β_2GPI and only reached approximately 50% of that obtained with wild-type β_2GPI (FIGURE 1).

In summary, comparative molecular modeling has predicted that a highly positive charged amino acid sequence, Lys282-Asn-Lys-Glu-Lys-Lys287, located in the fifth domain of β_2GPI is the major PL-binding site. We have tested this hypothesis and have shown that amino acid residues Lys284, 286, and 287 are critical for β_2GPI binding to PL and for cofactor activity for aPL antibodies.

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