Effect of Synthetic Peptide Fragments of β_2-Glycoprotein-I on the Binding of Antiphospholipid Antibodies to Cardiolipin

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The synthetic fragment of β_2-glycoprotein-I peptide P5 (Phe280-Ser289) maximally inhibits the binding of anticardiolipin antibodies to cardiolipin. This β_2-glycoprotein-I sequence probably reacts with negatively charged phospholipids and antibodies.

Key Words: *β_2-glycoprotein-I; synthetic peptide fragments; anticardiolipin antibodies*

Autoantibodies in the blood of patients with the antiphospholipid syndrome (APS) recognize antigenic determinants formed as a result of reaction between negatively charged phospholipids with β_2-glycoprotein-I (β_2-GP-I) and some other plasma proteins. β_2-GP-I (apoprotein H) is a glycoprotein consisting of 326 amino acid residues with molecular weight of 60 kD. In normal blood plasma this natural anticoagulant is associated with lipoproteins. The structure of β_2-GP-I molecule has five homologous domains, four of which include 50 amino acid residues each. The phospholipid-binding site of β_2-GP-I is the lysine-rich fifth domain of the molecule [4,5], which consists of 82 amino acid residues. Its main antigenic determinants are probably the linear sequences of β_2-GP-I located in the C-terminal [10,11] or other sites of the molecule [9], which is confirmed by the presence of antibodies reacting with β_2-GP-I in the absence of cardiolipin in the plasma of patients with APS [3,7,8].

We attempted to identify the antigenic determinants of β_2-GP-I using synthetic peptide fragments of the molecule and studied their effect on the binding of autoantibodies isolated from the plasma of APS patients to cardiolipin.

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MATERIALS AND METHODS

The following synthetic antigenic determinants β_2-GP-I selected by computer analysis with consideration for the hydrophilic characteristics of amino acid residues, mobility of amino acid α-carbon atom, and incidence of amino acid combinations in the known determinants [2] were used:

1-10 Gly-Arg-Thr-Cys-Pro-Lys-Pro-Asp-Asp-Leu — P1
1-13 Gly-Arg-Thr-Cys-Pro-Lys-Pro-Asp-Asp-Leu-Pro-Phe-Ser — P2
96-112 Tyr-Leu-Asn-Gly-Ala-Asp-Cys-Ala-Lys-Cys-Thr-Glu-Glu-Gly-Lys-Trp-Ser — P3
120-138 Pro-Ile-Ile-Cys-Pro-Pro-Pro-Ser-Ile-Pro-Thr-Phe-Ala-Thr-Leu-Arg-Val-Tyr-Lys — P4
280-289 Phe-Cys-Lys-Asn-Lys-Glu-Lys-Lys-Cys-Ser — P5
313-326 Leu-Ala-Phe-Trp-Lys-Thr-Asp-Ala-Ser-Asp-Val-Lys-Pro-Cys — P6

and the new synthetic peptide fragments of β_2-GP-I:

268-279 Lys-Asn-Gly-Met-Leu-His-Gly-Asp-Lys-Val-Ser-Phe — P7
249-267 Val-Lys-Lys-Ala-Thr-Val-Val-Tyr-Gln-Gly-Glu-Arg-Val-Lys-Ile-Gln-Glu-Lys-Phe — P8
200-220 Pro-Ala-Lys-Pro-Thr-Leu-Tyr-Tyr-Lys-Asp-Lys-Ala-Thr-Phe-Gly-Cys-His-Asp-Gly-Tyr-Ser — P9

0007-4888/99/0001-0044 $22.00

β_2-GP-I was isolated by affinity chromatography from normal donor serum by a modified method [3]. Pooled sera was fractionated first on a Hi Trap-Protein A Sepharose column (Pharmacia) equilibrated with phosphate buffered saline (PBS), pH 7.4. The passing solution was collected and applied onto a Hi Trap-Protein A Sepharose column (Pharmacia). Fractions containing β_2-GP-I were eluted by 0.35 M NaCl solution in PBS. IgG of a patient with APS were isolated by affinity chromatography on a Hi Trap-Protein A Sepharose column (Pharmacia). IgG-containing fractions were eluted by 0.2 M glycin buffer, pH 2.8.

The effects of peptides on the reaction between anticardiolipin autoantibodies and cardiolipin were evaluated by modified enzyme immunoassay [1] and competitive peptide inhibition of IgG, isolated from the plasma of APS patients, by cardiolipin in the presence of β_2-GP-I. Cardiolipin was left overnight in 96-well plates (Flow Lab.) in ethanol (50 μg/ml) at 4°C until complete evaporation of ethanol. Nonspecific binding was blocked by incubation with 1% milk solution and 0.3% gelatin in PBS for 1 h at 18-20°C.

The antigen-antibody reaction was carried out in 50 μl of reaction mixture containing 15 μg IgG, 3.3 μg β-GP-I, tested peptide fragment β_2-GP-I, 0-100 μg in buffer solution (0.3% gelatin in PBS). The plates were incubated for 3 h at ambient temperature. The immune complex was detected by adding goat monospecific IgG to human IgG labeled with horse radish peroxidase (Sigma) and 1-h incubation under the same conditions. After each incubation the plate was washed three times in PBS. The peroxidase reaction was carried out in a solution containing orthophenylene diamine and hydrogen peroxide, stopped by adding 50% hydrochloric acid, and light absorbance was recorded at 492 nm.

RESULTS

P5 peptide in concentrations 20-500 μg/ml inhibited the binding of IgG isolated from the plasma of patients with APS to cardiolipin in the presence of β_2-GP-I [2]. We extended the range of P5 peptide concentrations to 2 mg/ml. The inhibitory effect of all concentrations of P5 peptide on the binding of IgG from an APS patient (IgG2) to cardiolipin in enzyme immunoassay is shown on Fig. 1. The binding decreased as the concentration of the peptide in the reaction mixture increased, the minimum values being observed at concentrations >1 mg/ml. The concentration 1.7 mg/ml was selected as the optimum for investigating the inhibitory effect of P5 peptide and other synthetic peptide fragments of β_2-GP-I on the binding of anticardiolipin autoantibodies to cardiolipin.

The effects of the synthetic peptide fragments of β_2-GP-I on the binding of APS patient's IgG (IgG1) to cardiolipin in enzyme immunoassay are illustrated by Fig. 2. P5 peptide had the highest inhibitory effect (95.7%), peptides P2 and P4 had no inhibitory effect, and peptides P3, P6, P7, P8, and P9 suppressed IgG-cardiolipin binding by 21, 22, 29, 42, and 25%, respectively. The peptide inhibition of β_2-GP-I-dependent reaction between autoantibodies and phospholipid is probably due to competition of the peptide fragment and whole β_2-GP-I molecule for IgG binding sites. On the other hand, the peptide fragment containing Cys281-Cys288 sequence is the main phospholipid-binding site of β_2-GP-I molecule [4]. Peptide P5 (Phe280-Ser289), which includes this sequence, may bind both to cardiolipin and autoantibodies. This may account for the maximum inhibitory effect of P5 peptide on the binding of IgG fractions of APS patients (IgG1 and IgG2) to cardiolipin in the presence of β_2-GP-I.

Suppression of the anticardiolipin autoantibody-cardiolipin binding by peptides P3, P6, P7, P8, and P9, which were selected by computer analysis as probable antigenic determinants of β_2-GP-I, does not rule out their possible participation in the reaction with autoantibodies. This is consistent with other reports on

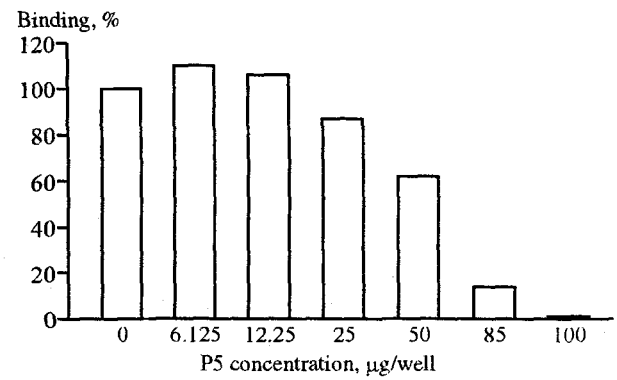

Fig. 1. Relationship between the binding of anticardiolipin autoantibodies (IgG2) to cardiolipin and the concentration of P5 peptide in enzyme immunoassay. Here and in Fig. 2: binding without peptide is taken as 100%.

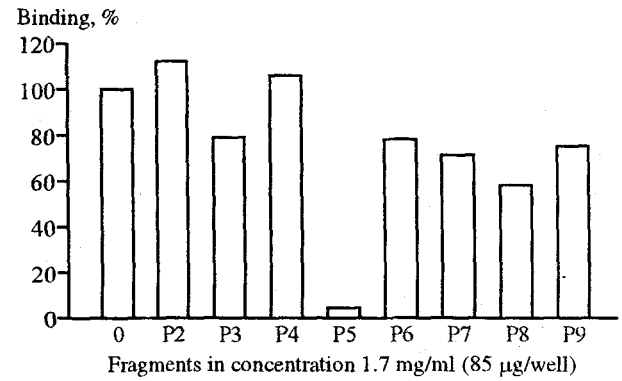

Fig. 2. Effect of β_2-glycoprotein peptides on the binding of anticardiolipin autoantibodies (IgG1) to cardiolipin in enzyme immunoassay.

the presence of antigenic determinants in the fourth and fifth domains of β_2-GP-I [4,9,10].

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