**β₂-Glycoprotein I: A Plasma Inhibitor of the Contact Activation of the Intrinsic Blood Coagulation Pathway**

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The general hypothesis for the biological function of β₂-glycoprotein I is that it neutralizes all negatively charged macromolecules that might enter the bloodstream and diminishes unwanted activation of the blood coagulation. In the present study we report that β₂-glycoprotein I inhibits the activation of the contact phase system of the intrinsic pathway of blood coagulation. Activation was accomplished by an ellagic acid-phospholipid suspension (Cephotest) and measured by the appearance of amidolytic activity using the chromogenic substrate H-D-Pro-Phe-Arg-p-nitroanilide (S-2302). This inhibitory effect of β₂-glycoprotein I was observed both when Cephotest was preincubated with β₂-glycoprotein I and when the amount of β₂-glycoprotein I in plasma was increased by addition of β₂-glycoprotein I to either normal or β₂-glycoprotein I-deficient plasma. The inhibitory effect of β₂-glycoprotein I on the contact phase activation could be one of the physiological functions of this protein.

**MATERIALS AND METHODS**

Purification of β₂-glycoprotein I. β₂-Glycoprotein I was purified from human plasma by a combination of several of previously published methods. Plasma was chromatographed on heparin-Sepharose, and the fractions containing β₂-glycoprotein I were pooled and treated with perchloric acid (PCA) for ten hours at 4°C. After neutralization and dialysis, the protein was further purified by anion exchange chromatography on DEAE-cellulose. The fractions containing β₂-glycoprotein I were pooled, concentrated, and analyzed for purity by crossed immunoelectrophoresis using antibody to total human serum and by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (Fig I A and 1B). The purified protein was stored at −20°C in 50 mmol/L Tris, HCl, pH 8.0, at a protein concentration of 5 to 20 mg/ml.

Immunological methods. Antibody to β₂-glycoprotein I was produced in rabbits immunized with antigen mixed with equal volumes of Freund’s incomplete adjuvant. The antigen–adjuvant mixture was injected intercutaneously every second week with approximately 100 μL of the mixture containing 40 μg of β₂-glycoprotein I. Ten days after the fourth injection the rabbits were bled for 40 mL of blood. Booster injections were given every fourth week, followed by new bleedings. The γ-globulin fraction was isolated from the blood according to Harboe and Ingild.

If not mentioned otherwise, immunoelectrophoresis in agarose gels was performed according to standard procedures. The gel consisted of 1% (wt/vol) agarose in electrophoresis buffer that consisted of either 0.1 mol/L glycine, 0.038 mol/L Tris, pH 8.7, and 0.5% (vol/vol) Triton X-100 (Bie and Berntsen, Copenhagen, Denmark) or 0.02 mol/L Barbitol buffer, pH 8.6. Concentration of β₂-glycoprotein I in plasma was determined by electroimmunoassay.
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was applied in the well. After electrophoresis in the first dimension brilliant blue R250 (250 μL) or by amplifying the immunoprecipitates by methods previously described.2 Asialo-β2-glycoprotein I (30 μg) and asialo-β2-glycoprotein II were obtained by neuraminidase treatment of β2-glycoprotein I as described previously.2

Electrophoresis in SDS-polyacrylamide gel electrophoresis (B) of purified β2-glycoprotein I. In the crossed immunoelectrophoresis, 10 μL of β2-glycoprotein I (17.8 mg/mL) was applied in the well. After electrophoresis in the first dimension (60 minutes, at 10 V/cm) the plate was turned 90 degrees, and electrophoresis was carried out in the second dimension gel (20 hours, at 2 to 3 V/cm) containing 50 μL/mL of agarose of anti-whole-human serum (DAKO A 206). SDS-polyacrylamide gel electrophoresis was performed in a 10% gel (see Materials and Methods). Lane 1 consists of a mixture of molecular weights standards; lane 2 consists of β2-glycoprotein I (10 μg); and lane 3 consists of asialo-β2-glycoprotein I (30 μg). Asialo-β2-glycoprotein I was obtained by neuraminidase treatment of β2-glycoprotein I as described previously.7

under special conditions.13 After termination of the immunoreactions, the precipitates were detected either by staining with Coomassie brilliant blue R250 or by amplifying the immunoprecipitates by peroxidase-labeled antibodies.28

Polyacrylamide gel electrophoresis. Electrophoresis in SDS 10% (wt/vol) polyacrylamide gel was performed29 using a slab apparatus equipped with notches.28 Before electrophoresis, samples were denatured by boiling for five minutes in the presence of 1% (wt/vol) SDS. After electrophoresis, the proteins were fixed and stained with Coomassie brilliant blue R250 dissolved in 50% (vol/vol) methanol, 7% (vol/vol) acetic acid.

Contact activation and determination of amidolytic activity. The contact system was activated by Cephotest, a stable suspension of brain cephalins (1 mL Cephotest stock solution contains cephalins from 1 mg dried brain), containing 15 mmol/L ellagic acid. In a total volume of 800 μL, a tenfold dilution of a commercial stock solution of Cephotest was preincubated in 50 mmol/L Tris, HCl, pH 7.8, containing 12 mmol/L NaCl. The amidolytic activity was determined using ϵ = 10 × mmol−1 × cm−1 for pNA,32

Plasma. Normal blood was collected from healthy volunteers and delivered as citrate–phosphate–dextrose blood from the State University Hospital. After centrifugation, EDTA were added to the plasma, giving a final concentration of 10 mmol/L. Electroimmunoassay showed that the plasma β2-glycoprotein I concentration was 200 μg/mL.

β2-Glycoprotein I-deficient plasma was obtained from a female donor (courtesy of Dr H. Eiberg, Institute of Medical Genetics, Panum Institute, University of Copenhagen). Electroimmunoassay showed that the β2-glycoprotein I-deficient plasma contained 9 μg/mL of β2-glycoprotein I. Forty milliliters of blood were drawn into 2.5 mL of 1% (wt/vol) potassium–EDTA–dihydrate. Some of the β2-glycoprotein I-deficient plasma was normalized by addition of purified β2-glycoprotein I to a final concentration of 300 μg/mL. β2-Glycoprotein I concentrations in purified preparations were determined by measuring the extinction of the protein at 280 nm using E1% = 9.4.33

Materials. Cephotest was obtained from Nyegaard & Co (Oslo), and S-2302 was purchased from Kabi Diagnostica (Stockholm). Heparin-Sepharose-CL-6B was from Pharmacia (Uppsala, Sweden). All other chemicals were of purest grade available from regular commercial sources.

RESULTS

The effect of β2-glycoprotein I on contact activation. The amidolytic activity in normal plasma, activated for 120 seconds with different amounts of Cephotest, which had been preincubated in the absence and in the presence of β2-glycoprotein I is shown in Fig 2. The figure shows that when Cephotest was preincubated with β2-glycoprotein I, the capacity of Cephotest to activate the contact system was strongly diminished. Figure 3 shows a dose–response curve, which indicates that the inhibitory effect of β2-glycoprotein I...
is present at amounts as low as 5 \mu g, which is about twice as much \beta_2-glycoprotein I as is present in the plasma sample in the assay (10 \mu L, 200 \mu g/mL \beta_2-glycoprotein I).

The effect of \beta_2-glycoprotein I on the rate of contact activation. The progress curve for the contact activation showed a decrease in the activation rate from 0.26 microkatal (\mu Kat) \times 1^{-1} \times sec^{-1} to 0.093 and 0.044 (\mu Kat) \times 1^{-1} \times sec^{-1} when 400 \mu L of Cephotest was preincubated with 5 \mu g and 50 \mu g of \beta_2-glycoprotein I, respectively (Fig 4). Increasing the \beta_2-glycoprotein I concentration in normal plasma from 200 \mu g/mL to 700 \mu g/mL gave the result shown in Fig 5. A 36% decrease in the rate of formation of amidolytic activity (from 0.25 \mu Kat \times 1^{-1} \times sec^{-1} to 0.16 \mu Kat \times 1^{-1} \times sec^{-1}) was seen. This decrease varied with the \beta_2-glycoprotein I concentration in plasma as shown in Fig 6. The relative decrease in the rate of formation of amidolytic activity in \beta_2-glycoprotein I-enriched plasma was, however, unaffected by changes in the Cephotest concentrations (Fig 7).

A possible inhibition of the amidolytic activity of factor XIIa and kallikrein by \beta_2-glycoprotein I was investigated by activating \beta_2-glycoprotein I-deficient plasma and measuring the amidolytic activity by addition of S-2302 containing buffer or \beta_2-glycoprotein I (125 \mu g/mL). In duplicate experiments the same amidolytic activity was measured in the presence as well as in the absence of \beta_2-glycoprotein I.

In order to investigate whether the inhibitory effect of \beta_2-glycoprotein I could be observed also on the rate of formation of amidolytic activity in \beta_2-glycoprotein I-deficient plasma, rate experiments were performed using this plasma as well as \beta_2-glycoprotein I-deficient plasma normalized by addition of \beta_2-glycoprotein I to a final concentration of 300 \mu g/mL (Fig 8). As expected, the rate of contact activation was diminished in deficient plasma to which purified \beta_2-glycoprotein I was added. In Table 1, the rate of formation of amidolytic activity of the \beta_2-glycoprotein I-

![Fig 3. Effect of increasing amount of \beta_2-glycoprotein I on the amidolytic activity measured after 120 seconds' activation of plasma with 400 \mu L of Cephotest preincubated with the given amount of \beta_2-glycoprotein I.](image)

![Fig 4. Amidolytic activity of plasma activated for increasing periods of time with Cephotest preincubated in the absence and in the presence of \beta_2-glycoprotein I. The amount of Cephotest was 400 \mu L and the amount of \beta_2-glycoprotein I with which Cephotest was preincubated was 50 \mu g (O——O), 5 \mu g (Δ——Δ), and 0 \mu g (●——●). The amount of \beta_2-glycoprotein I added concomitantly with the plasma was 3 \mu g.](image)

![Fig 5. The rate of appearance of amidolytic activity in normal plasma and in plasma enriched with \beta_2-glycoprotein I. Normal plasma was enriched with 500 \mu g \beta_2-glycoprotein I/per milliliter before dilution of the plasma with buffer. Normal plasma (●——●). \beta_2-glycoprotein I-enriched plasma (O——O). The amount of Cephotest in the assay was 400 \mu L.](image)

![Fig 6. Rate of formation of amidolytic activity in plasma measured at various \beta_2-glycoprotein I concentrations. Plasma was diluted and mixed with various amounts of \beta_2-glycoprotein I before determination of the amidolytic activity. The final amount of plasma in the assay was 5 \mu L containing 0.95 \mu g \beta_2-glycoprotein I. The amount of Cephotest in the assay was 600 \mu L. The curve was drawn after correction for plasma \beta_2-glycoprotein I content by adding the plasma \beta_2-glycoprotein I content and the additional \beta_2-glycoprotein I.](image)
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The rate of contact activation determined at varying amounts of Cephotest. The rate was determined in normal plasma (O—O) and in plasma enriched with 1.2 mg of β₂-glycoprotein I per milliliter of plasma (O—O).

deficient plasma was compared statistically with that of normalized and normal plasma having a β₂-glycoprotein I concentration of 200 μg/mL. The difference in rate between β₂-glycoprotein I-deficient and normalized or normal plasma was statistically significant (P < .01), whereas the difference in rate between normalized and normal plasma was insignificant.

All of the progress curves shown have been corrected for the reaction blank. This value was higher in the assays containing Cephotest preincubated with β₂-glycoprotein I than in those preincubated without β₂-glycoprotein I. Therefore, β₂-glycoprotein I was tested for kallikrein-like activity but was found neither to possess such activity nor prekallikrein-like activity as tested by Cephotest activation of β₂-glycoprotein I in the presence of buffer instead of plasma.

DISCUSSION

β₂-Glycoprotein I, as a protein that neutralizes all negatively charged macromolecules that might enter the bloodstream and prevents unwanted activation of the blood coagulation, was investigated. Because β₂-glycoprotein I reacts with the same kind of surfaces as the blood coagulation factors, Hageman factor (factor XII), Fletcher factor (prekallikrein), and Fitzgerald factor (HMW kininogen), it was tempting to investigate the effect of β₂-glycoprotein I on the activation of the contact system of blood coagulation.

The data reported here clearly indicate that β₂-glycoprotein I diminishes the rate of formation of amidolytic activity of factor XIIa and kallikrein in EDTA-plasma activated by Cephotest. This inhibitory effect of β₂-glycoprotein I is not restricted to the Cephotest-activated contact system, since it was also observed when dextran sulfate and sulfatide were used as the activator (results not presented). The decrease in the formation of amidolytic activity is seen both when β₂-glycoprotein I is added to β₂-glycoprotein I-deficient plasma or to normal plasma before contact phase activation and when Cephotest has been preincubated with β₂-glycoprotein I. However, the inhibiting effect of β₂-glycoprotein I on the rate of formation of amidolytic activity is stronger when the protein has been preincubated with the activator than when plasma has been enriched with β₂-glycoprotein I before activation, indicating a competition between β₂-glycoprotein I and HMW kininogen/Hageman factor/prekallikrein for binding to the activator, Cephotest.

Previous investigation of the effect of positively charged molecules such as protamine, cytochrome C, or hexadimethrine bromide on the contact activation has shown that pretreatment of the activator with these compounds blocks the adsorption of Hageman factor.34 β₂-Glycoprotein I behaves as a positively charged molecule35 and, in contrast to the previously mentioned molecules, is a natural constituent of the blood. Furthermore, at least two histidine residues in β₂-glycoprotein I are involved in the binding of β₂-glycoprotein I to negatively charged surfaces, such as suspensions of phosphatidic acid/phosphatidyl choline vesicles and mitochondria.36 Apparently, the binding of HMW kininogen to negatively charges surfaces also involves a histidine-rich cluster.

In 1958, it was reported,35 and later confirmed,36,37 that Hageman factor-deficient plasma contained agents that inhibited the clot-promoting effects of glass, and later a crude inhibitor fraction was isolated from normal plasma.38 This fraction exhibited inhibitory activity in three fractions containing IgG—fibrinogen, ceruloplasmin, and β1C/β1A globulin—but incubating the fractions with each of the specific antisera did not abolish the inhibitory activity. It is possible that β₂-glycoprotein I and the inhibitor described by Ratnoff and coworkers38 are identical proteins, but the inhibitor described by Ratnoff and coworkers38 was incompletely characterized. β₂-Glycoprotein I shows electropho-

![Graph of Rate of Contact Activation](image)

**Fig. 7.** The rate of contact activation determined at varying amounts of Cephotest. The rate was determined in normal plasma (O—O) and in plasma enriched with 1.2 mg of β₂-glycoprotein I per milliliter of plasma (O—O).

![Graph of Amidolytic Activity](image)

**Fig. 8.** Contact activation of β₂-glycoprotein I-deficient plasma determined as a function of the activation period. β₂-Glycoprotein I-deficient EDTA-plasma (O—O), normalized EDTA-plasma (O—□), and Cephotest preincubated with 0.1 mg β₂-glycoprotein I (Δ—Δ). Cephotest was present in an amount of 400 μL.
retic mobility,\textsuperscript{2,3} identical to the inhibitor described by Ratnoff. \(\beta_2\)-Glycoprotein I can, due to its sialo microheterogeneity, be separated into several subfractions\textsuperscript{3} analogous to the electrophoretic separation of the previously mentioned inhibitor into three fractions.\textsuperscript{38}

Recently, Canfield and Kisiel\textsuperscript{39} partially characterized a plasma protein that neutralized the amidolytic and anticoagulant activity of radioiodinated activated protein C (APC). The neutralization was apparently accomplished by formation of a complex between APC and the plasma protein. This plasma protein has the same mol wt, the same low electrophoretic mobility, and the same N-terminal amino acid sequence as \(\beta_2\)-glycoprotein I. However, purified APC-binding protein failed to form a complex with unlabeled preparations of APC, and the presence of heparin was found to be essential for complex formation between radiolabeled APC and APC-binding protein.

In plasma, \(\beta_2\)-glycoprotein I occurs in concentrations varying from 0 to 300 \(\mu\)g/mL.\textsuperscript{40} The actual concentration is determined by two codominant autosomal alleles, \(B^gN\) and \(B^gH\) with a \(B^gH\) frequency of 0.94 in Caucasians.\textsuperscript{41} The results so far obtained on possible functions for the protein raise the question of whether people with low or deficient \(\beta_2\)-glycoprotein I levels in plasma are more easily exposed to blood coagulation defects, such as disseminated intravascular coagulation (DIC).

So far, all of four blood samples from patients with clinical DIC appeared to have extremely low \(\beta_2\)-glycoprotein I (<50 \(\mu\)g/mL). One of these, an abruptio placentae who survived the coagulation defect was later found to have a normal \(\beta_2\)-glycoprotein I concentration of 175 \(\mu\)g/mL (unpublished observations, March, 1981). An extension of this investigation must be performed in order to show a correlation between DIC and low concentrations of \(\beta_2\)-glycoprotein I.

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**REFERENCES**

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