Studies on specific interaction of beta-2-glycoprotein I with HBsAg

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Abstract

AIM: To observe the binding activity of beta-2-glycoprotein I
(β2GPI) to hepatitis B surface antigen (HBsAg) and the
possible roles of β2GPI in hepatitis B virus (HBV) infection.

METHODS: The rationale of ELISA methods and ELISA-
based research method and ligand-blotting technique were
used to detect the specific interaction of β2GPI with HBsAg.

RESULTS: With the increase of rHBsAg, the binding of β2GPI
to rHBsAg elevated, and these changes had statistic
significance. When we added non- biotinlyated β2GPI, the
OD value significantly decreased though they still were
positively relevant to rHBsAg, suggesting non- biotinlyated
β2GPI competed with biotinylated β2GPI to saturate the
binding sites on rHBsAg. Meanwhile BSA was used as
negative control to substitute for rHBsAg coating the plates.
The results indicated no interaction between β2GPI and BSA,
suggesting the affinity of β2GPI to HBsAg was specific. The
ligand blotting indicated that β2GPI might bind to HBsAg no
matter whether it was under reduced condition or not.

CONCLUSION: The binding of β2GPI to HBsAg suggests
that β2GPI may be a carrier of HBV and that β2GPI may play
important roles in HBV infection.

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INTRODUCTION

β2GPI (beta-2-glycoprotein I) is a plasm glycoprotein
circulating as a free protein and is associated to lipoproteins.
This protein is also referred to as apolipoprotein H(Apo H).
Human β2GPI is a single-chain molecule consisting of 326
amino acid residues and 5 carbohydrate chain and has a
molecule mass of approximately 55 kDa[1-3]. The amino acid
sequences of β2GPI in human, bovine, mouse, and rat appear
to be highly conserved[4,5]. The protein contains 5 internal repeat
unit of 60 amino acid residues, each with 2 internal disulfide
bonds, known as Sushi domain[7].

Although quite a lot is known about the structure of β2GPI,
its biological function remains unclear. It is known that β2GPI
can bind to negatively charged substances such as DNA and
heparin and negatively charged phospholipids etc in vivo[8,9],
but the meaning of such interaction is still unclear. It is known
that β2GPI may serve as a major factor in clearing the plasma
liposome[10] as well as an anticoagulant in blood[11,12]. β2GPI may
also modulate the function of kidney and placenta. The
abnormality of plasma β2GPI level has been shown to be
associated with many diseases such as arterial and venous
thrombosis, recurrent abortion and alcoholic liver disease[12,13].
In addition, it has been found that, in diabetes or atherosclerosis
patients, the concentration of plasma β2GPI increases and
distribution of the protein among different types of lipoprotein
is perturbed[14,15]. Recent research on β2GPI has given a further
impetus to the discovery that lipid- associated β2GPI can bind
to some pathogenic antigens or proteins such as HBV Dane
particles, protein p18, p26, gp160 of HIV and so on[16,17], and
perhaps hepatitis virus antigen[17,18]. These findings highlighted
a potentially critical role of β2GPI in the mechanism of hepatitis
B, AIDS, and systemic lupus erythematosus, etc.

The lipid-binding and transportation functions are
considered as a basic mechanism related to its physiological
and pathogenic functions. It has been demonstrated by several
labs that β2GPI prefers to bind negatively charged
phospholipids[9,19-21]. It was reported that β2GPI could be
removed from the membranes with a weakly acidic buffer and
it was partly associated with chylomicrons and high-density
lipoproteins, both of which were targeted to hepatocytes during
the normal course of lipid metabolism[22]. In the present paper,
the characteristics of β2GPI interacting with HBsAg were
further examined by several measurements. As a result, the
standpoints that β2GPI might participate in HBV infection were
held out accordingly. Our results may also help to explain how
β2GPI facilitates HBV transportation, location and the
important roles of β2GPI in HBV infection.

MATERIALS AND METHODS

Reagents

TEMED, APS, PMSF, and aprotinin purchased from Sigma
Chemical Co., acrylamide and biacrylamide purchased from
Serva, human β2GPI provided by Prof. Zhang GR from Jilin
University, rHBsAg offered by Zhang HY from Jilin Institute
of Family Plan, Hybond obtained from Amersham, SDS, NP-40,
Coomasie brilliant blue obtained from Fluka (Finland). The
other chemicals used were of analytical grade made in China.

ELISA-based determination of β2GPI-rHBsAg interaction

rHBsAg was diluted into 0.125 µg/ml, 0.5 µg/ml and 2 µg/ml
in 0.05 NaHCO3 (pH 9.4) and added to 96-well plates
respectively at 4°C overnight (8 wells/group). Non-specific
sites were blocked with PBS containing 1% BSA for 1.5 h at
37°C. Plates were washed three times between the different
incubation steps. Biotinylated β2GPI was added to every well
followed by adding HRP-Avidin and substrate consequentially.
Simultaneously, 40 ng of non-labeled β2GPI was added to
Student’s RESULTS ±SD and comparisons of which were performed using the Luzex-F Image-analysis System. The density was analyzed with Luzex-F Image-analysis System. Biotinylated rHBsAg was used at a dilution of 1/200 and HRP-avidin (Huamei Company), a dilution of 1/200. The blotting buffer consisted of phosphate-buffered saline (10 mM phosphate, pH 7.5, 138 mM NaCl, 2.7 mM KCl) containing 5 % dried skimmed milk powder and 1 % Tween 20 detergent (Sigma). Biotinylated rHBsAg was used at a dilution of 1/200 and HRP-avidin (Huamei Company), a dilution of 1/200. The density was analyzed with Luzex-F Image-analysis System.

Statistical analysis

Results from quantitative parameters were presented as mean ±SD and comparisons of which were performed using the Student’s t test.

RESULTS

ELISA-based determination of β2GPI- rHBsAg interaction

rHBsAg was diluted into 0.125 µg/ml, 0.5 µg/ml and 2 µg/ml and used as capture antibody to coat the plates, then biotinylated β2GPI was added to interact with rHBsAg, simultaneously we used non-biotinylated β2GPI to observe the competitive binding interaction. The following figure indicated that with the increase of HBSAg the binding of β2GPI into rHBsAg elevated and these changes had statistic significance (vs 0.125 µg/ml group, P<0.05). When we added non-biotinylated β2GPI, the OD value significantly decreased though they still were positively relevant to rHBsAg, suggesting that non-biotinylated β2GPI competed with biotinylated β2GPI to saturate the binding sites on rHBsAg (Figure 1). Meanwhile BSA was used as negative control to substitute for rHBsAg coating the plates, the results indicated no interaction between β2GPI and BSA, (Figure 2) suggesting the affinity of β2GPI to rHBsAg was specific.

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interaction.

β-GPI is a glycoprotein with four N-linked carbohydrate chains[21,22] present at concentrations of approximately 200 μg/ml in serum[23]. The 326-residue mature protein is composed of a 61 amino acid motif repeated four times, followed by one longer, modified repeat. Each of the first four repeats contains four cysteines in conserved positions, at least one of which is critical for its rHBsAg-binding activity. This pattern of disulfide bonds is similar to the short consensus repeat units found in a family of approximately 20 proteins which includes many of the complement control proteins. It is interesting that the measles virus receptor, CD46[24], also belongs to this short consensus repeat family.

The work described in the present report was to reveal the possible roles of β-GPI in HBV infection. As we have known that β-GPI may target to hepatocytes in the form of chylomicrons and high-density lipoproteins. The result of the present paper suggested that β-GPI might specifically bind to HBsAg no matter whether it was under reduced condition or not. We might well reckon that β-GPI might contribute to transportation of HBV to hepatocytes as a kind of carrier. Since β-GPI is associated with lipoproteins, particularly chylomicrons and HDL, it is possible that HBV binds to β-GPI on the surface of these lipoprotein particles. In the bloodstream, chylomicrons are partially degraded by lipoprotein lipase, resulting in chylomicron remnants, which are taken up by hepatocytes. HDLs are also taken up by hepatocytes in the process of “reverse cholesterol transport”. They may have been associated with the process of HBV infection. It is tempted to speculate that infectious HBV might bind to chylomicron or HDL particles by interacting with β-GPI and be taken into hepatocytes as a “hitchhiker” along with these lipoproteins. Our results showed that β-GPI I might bind to rHBsAg no matter whether it was under reduced condition or not manifested difference from that reported by Mehdi et al[17]. In Mehdi experimental system, they used human serum to observe the relationship between β-GPI and HBsAg, while we used purified β-GPI. There might exist differences between the two kinds of β-GPI sources in their first, second or third structure, which might lead to the differences of sensitivity to reduced-agents. Moreover, the genetic heterogeneity of β-GPI could also cause mutations of sequences of some domains, which eventually affect the binding of β-GPI to rHBsAg. Related issues should be further investigated.

To sum up, if HBV infection to hepatocytes involves β-GPI on chylomicrons and/or HDL, it is a novel mechanism for virus attachment and entry.

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