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Molecular cloning and mammalian expression of human β_2-glycoprotein I cDNA*

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Human β_2-glycoprotein ($\beta_2$gpI) cDNA was isolated from a liver cDNA library and sequenced. The cDNA encoded a 19-residue hydrophobic signal peptide followed by the mature $\beta_2$gpI of 326 amino acid residues. In liver and in the hepatoma cell line HepG2 there are two mRNA species of about 1.4 and 4.3 kb, respectively, hybridizing specifically with the $\beta_2$gpI cDNA. Upon isoelectric focusing, recombinant $\beta_2$gpI obtained from expression of $\beta_2$gpI cDNA in baby hamster kidney cells showed the same pattern of bands as $\beta_2$gpI isolated from plasma, and at least 5 polypeptides were visible.

β_2-Glycoprotein I; Apolipoprotein H; Short consensus repeat; Expression; Cloning; *Homo sapiens*

1. INTRODUCTION

β_2-glycoprotein ($\beta_2$gpI) or apolipoprotein H is a single chain perchloric acid-soluble plasma protein of about 55 000 M_r [1,2]. $\beta_2$gpI interacts strongly with anionic surfaces such as DNA, heparin, or vesicles of negatively charged phospholipids or activated platelets, and is thereby capable of inhibiting the prothrombinase activity of activated platelets [3] as well as the activation of the intrinsic pathway of blood coagulation [4]. The latter function is shared with the homologous b-subunit of blood coagulation factor XIII [5]. A large part (~35%) of $\beta_2$gpI in plasma is found in association with lipoprotein fractions (LDL, VLDL, HDL) and chylomicrons [6]. Recently, $\beta_2$gpI in complex with negatively charged phospholipids was found to be the major antigen recognized by anticardiolipin autoantibodies [7]. The complete amino acid sequence of $\beta_2$gpI, the assignments of positions of *N*-glycosylation as well as partial localization of disulfide bridges have been published [2].

$\beta_2$gpI is composed of 5 short consensus repeats (SCRs), a common structural motif found in a number of complement and non-complement proteins [8,9]. Due to microheterogeneity in sialic acid content and also to differences in asialo-$\beta_2$gpI, several bands are observed on isoelectric focusing [10,11].

In this work we present the cDNA and derived amino acid sequences of human $\beta_2$gpI. Human $\beta_2$gpI cDNA was expressed in the baby syrian hamster kidney fibroblast cell line (BHK) and the expression product was characterized.

2. EXPERIMENTAL

2.1. *cDNA cloning and sequencing*

cDNA libraries were prepared as previously described from human liver and HepG2 (grown in the presence of poly-lysine) RNA [12]. Two mixed-sequence deoxyribonucleotide probes corresponding to amino acid residues 4–9 and 19–24 (underlined in Fig. 1) were synthesized. cDNA library screening, colony purification, cDNA sequencing and compilation of data were performed as described earlier [13]. The cDNA and derived amino acid sequences were compared to those in the GenEMBL (release 26) and the MIPSX (release 28) databases, respectively [14]. Northern blot analyses were performed as in [15].

2.2. *Construction of a $\beta_2$gpI expression vector*

*Bam*HI sites were introduced at the 3′ and 5′ ends of the cDNA. By site-specific mutagenesis [16] a *Bam*HI site was introduced at position 1139 in the 3′ non-translated region of the $\beta_2$gpI cDNA employing the deoxyribonucleotide:5′(TTTAATTCAATTAGGATCCATGGATG-AACAAGAAAC)3′. An additional *Bam*HI site was introduced by ligation of the adaptor (5′(GGATCCACCATGATATCTCCAGTG-CTCATATTGTTC)3′ to the *Xho*I site located 27 bp 3′ to the translation initiation site. The modified $\beta_2$gpI cDNA was introduced at a *Bam*HI site of the mammalian expression vector Zem219b [17] to generate plasmid p1099.

* The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X53595.

Abbreviations: $\beta_2$gpI, β_2-glycoprotein I; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; BHK, baby hamster kidney cells; DMEM, Dulbecco's Modified Eagle Medium; FCS, fetal calf serum; SCR, short consensus repeat

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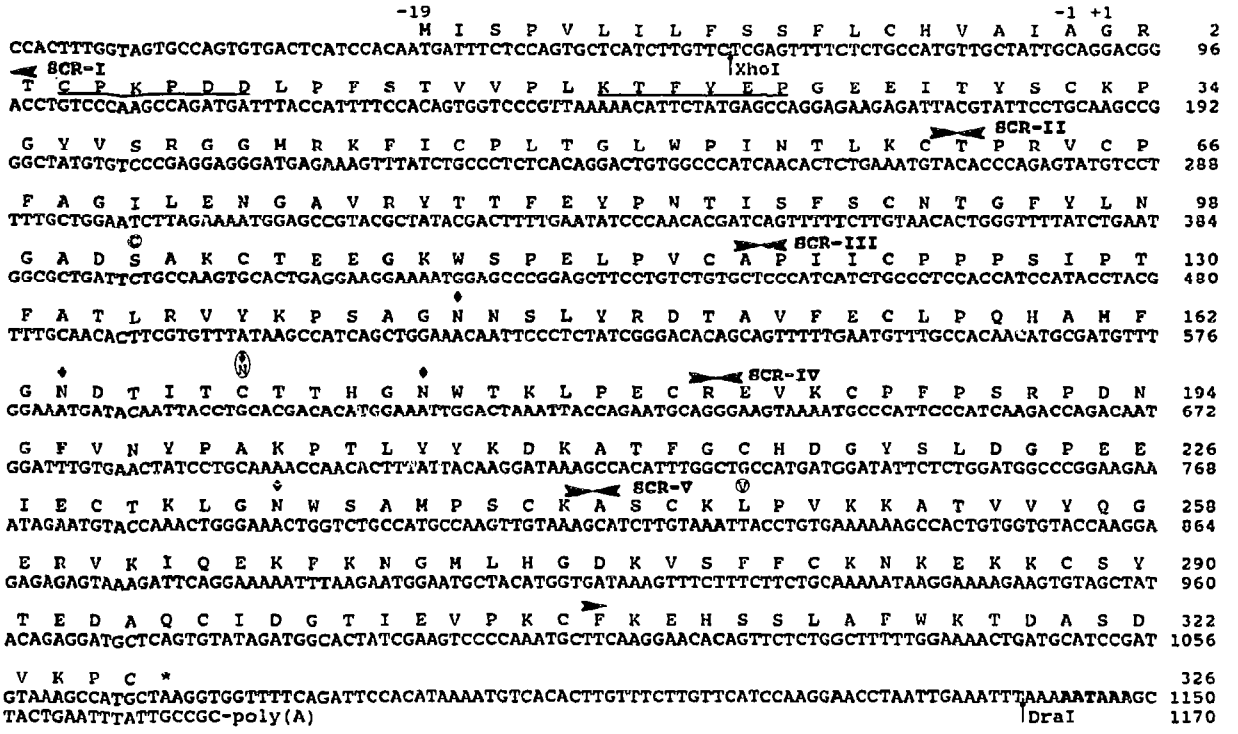

Fig. 1. The cDNA and derived amino acid sequence of human $\beta_2$gpI. The SCRs are indicated above the amino acid sequence. The segments used as probes are underlined and the poly-A signal is in bold. Three amino acid differences [2] are encircled. ♦, *N*-bound carbohydrate.

2.3. *Expression of recombinant $\beta_2$gpI in mammalian cells*

Cells were grown in DMEM supplemented with 10% FCS. The expression vector p1099 was transfected into subconfluent BHK cells by the calcium phosphate method, trypsinized after 48 h and diluted into medium containing 400 nM methotrexate. After 10–12 days individual colonies are cloned out and expanded separately.

A rabbit anti-human $\beta_2$gpI antibody (titre: 1 mg/ml) was used for immunoblotting [18]. Aliquots were drawn from the isolated media and spotted onto a nitrocellulose membrane which was subsequently incubated with buffers containing (i) 2% (w/v) bovine serum albumin (1 h); (ii) rabbit anti-human $\beta_2$gpI antibody, diluted 1:500 (overnight); (iii) peroxidase conjugated swine antirabbit-IgG, diluted 1:1000 (1 h); and (iv) 1,4-phenylenediamine.

Quantification of recombinant $\beta_2$gpI (r$\beta_2$gpI) was performed by electroimmunoassay in agarose gels containing 0.2% (w/v) deoxycholate [19] and amplification of the immunoprecipitates by reaction with a secondary peroxidase-labeled antibody [20]. Prior to quantification, the media were concentrated 40-fold on a Minicon 15 spinal cord fluid concentrator. It was assumed that proportional amounts of calf albumin (from the culture media) and r$\beta_2$gpI adhered unspecifically to the Minicon membrane. A serum standard containing 180 mg/l of $\beta_2$gpI was used as reference [19].

Recombinant $\beta_2$gpI grown in serum-free media was partially purified and concentrated by affinity chromatography on a heparin-Sepharose 4B column.

Isoelectric focusing of r$\beta_2$gpI desorbed from heparin–Sepharose 4B and concentrated on Minicon CS 15 was performed as previously described [10]. Pharmalytes pH 5–8 and 3–10 were used. Following incubation with the rabbit anti-human $\beta_2$gpI antibody, the focusing pattern was developed using a secondary peroxidase-conjugated antibody.

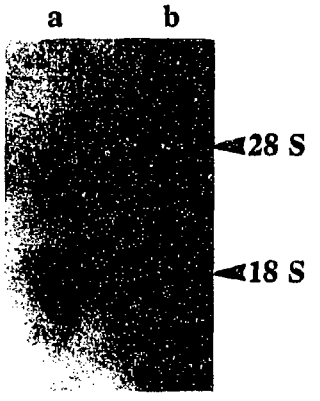

Fig. 2. Northern blot analysis of $\beta_2$gpI RNA. (a) human liver RNA; (b) HepG2 cell (poly-lysine added to the medium) RNA.

3. RESULTS AND DISCUSSION

3.1. *cDNA sequence of human $\beta_2$gpI*

A total of 160 000 recombinant clones from human liver and HepG2 cDNA libraries were initially screened with 2 synthetic probes and later with a *XhoI–DraI* cDNA fragment (position 61–1141; Fig. 1). Several positive clones were isolated and an estimated full-length $\beta_2$gpI (liver) cDNA insert was randomly sequenced at least twice on each strand. The nucleotide sequence of 1170 bp exclusive of the poly-A tail contains 33 bp of 5′-untranslated sequence preceding a translation initiation codon. The open reading frame encodes a 19 amino acid residue hydrophobic signal peptide and the 326

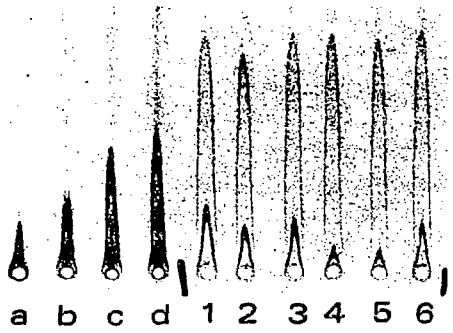

Fig. 3. Electroimmunoassay of r$\beta_2$gpI. Three μl samples of conditioned media from transfected cells (wells 1–6). Tall and weak precipitates represent bovine $\beta_2$gpI (control not shown), while low and dense precipitates represent r$\beta_2$gpI. Wells a–d contain human serum $\beta_2$gpI standards (90, 180, 270, and 360 ng/well, respectively).

residues of mature $\beta_2$gpI determined earlier [2]. Following the stop codon (TAA) are 99 bp of 3′-untranslated sequence including the canonical poly-A signal sequence AATAAA positioned 26 bp upstream from the poly-A tail (Fig. 1).

Recently rat $\beta_2$gpI cDNA was sequenced [21] and the degree of identity with human $\beta_2$gpI cDNA between positions 24 and 97, and 240 and 1068 (the coding region; human numbering) was 83%. This high degree of identity indicates an evolutionarily rather well conserved sequence. The rat nucleotide sequence, however, does not contain the region between positions 97 and 240 when compared to the human $\beta_2$gpI cDNA sequence. This region does not represent a separate exon when compared to the human $\beta_2$gpI gene structure (H. Okkels and T. Kristensen, unpublished results). It cannot be ruled out that there may be a major difference between rat and human $\beta_2$gpI similar to the differences seen between mouse and human C4b-binding proteins, a protein homologous to $\beta_2$gpI [15].

3.2. *Northern blot analysis*

Northern blots of total RNA from human liver, HepG2 and placenta revealed two RNA species hybridizing with the *Xho*I–*Dra*I cDNA fragment in liver and HepG2 cells (Fig. 2) while no $\beta_2$gpI mRNA could be detected in placenta (results not shown). The 1.4 kb band represents the $\beta_2$gpI mRNA. Attempts to obtain cDNA clones representing the 4.3 kb RNA species were unsuccessful, even after close inspection of 125 positive clones by restriction analyses. It was therefore concluded that the 4.3 kb RNA species may represent a non-polyadenylated precursor of $\beta_2$gpI mRNA and therefore is not represented in the oligo-(dT)-primed cDNA libraries. The intensity of the hybridization signals in the Northern blots of liver and HepG2 RNA were compared to that of human α_2-macroglobulin (results not shown) indicates a high level of $\beta_2$gpI mRNA.

3.3. *The human $\beta_2$gpI amino acid sequence*

There is good agreement between the cDNA-derived amino acid sequence shown in Fig. 1 and the amino acid sequence published by Lozier et al. [2]. However, 3 differences are seen (encircled in Fig. 1): (1) Val^{247} [2] to Leu; (2) Cys^{102} [2] to Ser; and (3) Asn-(carbohydrate)^{169} [2] to Cys. The differences (1) and (2) may be the results of single base mutations while (3) involves changes in at least 2 base pairs. The five SCRs of $\beta_2$gpI are indicated in Fig. 1. The differences, Cys^{102} [2] and Asn-(carbohydrate)^{169} [2], do not conform to the SRC consensus [10], and they were also found to be Ser and Cys, respectively, in the cDNA-derived amino acid sequences of rat $\beta_2$gpI [21].

The fifth and most C-terminal of $\beta_2$gpI shares a very low degree of identity with the other SCRs of $\beta_2$gpI and with other known SCR sequences, and even though this sequence contains 6 instead of 4 half-cystine residues, it does not belong to the novel classes of SCRs found among the selectins or in Pregnancy Associated Plasma Protein-A ([9] and T. Kristensen, O. Sand and Sottrup-Jensen, unpublished results). In the SCRs Cys^1 is bound to Cys^3 and Cys^2 to Cys^4 [2,22]. However, this pattern may be violated in the fifth SCR of human $\beta_2$gpI where

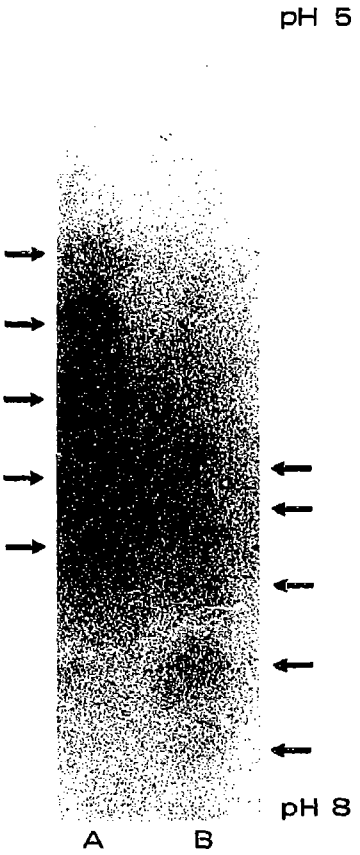

Fig. 4. Isoelectric focusing at pH 5–8 of human serum $\beta_2$gpI (lane A) and of r$\beta_2$gpI (lane B). Arrows mark the positions of bands. pH values are shown at the right.

Lozier et al. [2] have found Cys^2 to be disulfide bound to Cys^3 although C^2 would be expected to be bound to Cys^5, with residues 307–326 being arranged as a disulfide bridged extension.

3.4. *Characterization of recombinant human $\beta_2$gpI*

Dotblots of aliquots of media collected from transfected BHK cells grown in the presence of 10% FCS suggested that low amounts of r$\beta_2$gpI were being synthesized and secreted into the media. Fig. 3 shows that two immunoprecipitates were formed in electroimmunoassays of all samples originating from media drawn from cultured transfected cells. The weakest precipitate forming the tallest rockets, however, was found to be due to pre-existing $\beta_2$gpI originating from the FCS added to the cell culture medium. The low rockets were due to r$\beta_2$gpI secreted from the transfected cells. Quantification of r$\beta_2$gpI showed that it was present at 0.2–2 μg/ml. For further characterization of r$\beta_2$gpI selected clones were grown in serum-free media, and r$\beta_2$gpI was partially purified. Forty μg of r$\beta_2$gpI constituting less than 1% of the total protein desorbed from the heparin–Sepharose 4B column was obtained. Isoelectric focusing of the preparation of r$\beta_2$gpI revealed that r$\beta_2$gpI showed the same type of microheterogeneity as native human $\beta_2$gpI (Fig. 4). The isoelectric points of the different types of r$\beta_2$gpI were, however, higher than the isoelectric points for the native human $\beta_2$gpI. The reason for this could be that r$\beta_2$gpI is less sialylated than native human $\beta_2$gpI.

The perspectives of the results presented here are that expression of recombinant human $\beta_2$gpI cDNA gives a tool for studying the anticoagulant function of $\beta_2$gpI in greater detail using cDNA expression mutants.

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