beta2-glycoprotein-I (apolipoprotein H) and beta2-glycoprotein-I-phospholipid complex harbor a recognition site for the endocytic receptor megalin.

S K Moestrup, …, E I Christensen, T E Willnow

*J Clin Invest.* 1998;102(5):902-909. [https://doi.org/10.1172/JCI3772](https://doi.org/10.1172/JCI3772).

Screening of serum by using a surface plasmon resonance analysis assay identified beta2-glycoprotein-I/apolipoprotein H as a plasma component binding to the renal epithelial endocytic receptor megalin. A calcium-dependent megalin-mediated beta2-glycoprotein-I endocytosis was subsequently demonstrated by ligand blotting of rabbit renal cortex and uptake analysis in megalin-expressing cells. Immunohistochemical and immunoelectron microscopic examination of kidneys and the presence of high concentrations of beta2-glycoprotein-I in urine of mice with disrupted megalin gene established that megalin is the renal clearance receptor for beta2-glycoprotein-I. A significant increase in functional affinity for purified megalin was observed when beta2-glycoprotein-I was bound to the acidic phospholipids, phosphatidylserine and cardiolipin. The binding of beta2-glycoprotein-I and beta2-glycoprotein-I-phospholipid complexes to megalin was completely blocked by receptor-associated protein. In conclusion, we have demonstrated a novel receptor recognition feature of beta2-glycoprotein-I. In addition to explaining the high urinary excretion of beta2-glycoprotein-I in patients with renal tubule failure, the data provide molecular evidence for the suggested function of beta2-glycoprotein-I as a linking molecule mediating cellular recognition of phosphatidylserine-exposing particles.

Find the latest version:

[http://jci.me/3772-pdf](http://jci.me/3772-pdf)
**Abstract**

Screening of serum by using a surface plasmon resonance analysis assay identified \( \beta_2 \)-glycoprotein-I/apolipoprotein H as a plasma component binding to the renal epithelial endocytic receptor megalin. A calcium-dependent megalin-mediated \( \beta_2 \)-glycoprotein-I endocytosis was subsequently demonstrated by ligand blotting of rabbit renal cortex and uptake analysis in megalin-expressing cells. Immunohistochemical and immunoelectron microscopic examination of kidneys and the presence of high concentrations of \( \beta_2 \)-glycoprotein-I in urine of mice with disrupted megalin gene established that megalin is the renal clearance receptor for \( \beta_2 \)-glycoprotein-I. A significant increase in functional affinity for purified megalin was observed when \( \beta_2 \)-glycoprotein-I was bound to the acidic phospholipids, phosphatidylserine and cardiolipin. The binding of \( \beta_2 \)-glycoprotein-I and \( \beta_2 \)-glycoprotein-I–phospholipid complexes to megalin was completely blocked by receptor-associated protein.

In conclusion, we have demonstrated a novel receptor recognition feature of \( \beta_2 \)-glycoprotein-I. In addition to explaining the high urinary excretion of \( \beta_2 \)-glycoprotein-I in patients with renal tubule failure, the data provide molecular evidence for the suggested function of \( \beta_2 \)-glycoprotein-I as a linking molecule mediating cellular recognition of phosphatidylserine-exposing particles. (J. Clin. Invest. 1998. 102:902–909.) Key words: \( \beta_2 \)-glycoprotein-I • apolipoprotein H • megalin • phosphatidylserine • Fanconi syndrome

**Introduction**

\( \beta_2 \)-glycoprotein-I (\( \beta_2 \)-gpI)\(^1 \) is a predominant plasma protein (0.2 mg/ml) of 50 kD consisting of five short consensus repeat/Sushi modules. 30% of \( \beta_2 \)-gpI in plasma is associated with lipoprotein particles, probably due to its binding to acidic phospholipids, e.g., phosphatidylserine (PS) (1, 2). Therefore, \( \beta_2 \)-gpI is also designated as apolipoprotein H in the literature (3, 4).

The function of \( \beta_2 \)-gpI is not fully elucidated, but via binding of PS it is suggested to regulate platelet-dependent thrombosis as well as the clearance of liposomes, PS-expressing cells, and foreign particles (5–7). A substantial interest has also been attributed to pathological properties of \( \beta_2 \)-gpI. One aspect is the presence of antiphospholipid antibodies in plasma of patients with antiphospholipid syndrome and other autoimmune diseases (8, 9). In fact, recent reports indicate that most of the antiphospholipid antibodies do not bind to phospholipids, but instead bind to \( \beta_2 \)-gpI when it is in complex with the acidic phospholipids PS and cardiolipin or immobilized on negatively charged surfaces (10, 11). Another pathological aspect of \( \beta_2 \)-gpI relates to the high urinary excretion of the protein in patients with Fanconi-type renal tubular failure (12). Accordingly, \( \beta_2 \)-gpI is a superior marker of this disease (12).

This study was initiated in order to identify novel renal-filtered plasma ligands for megalin (13, 14), an endocytic receptor expressed in various absorptive epithelia including kidney, yolk sac, lung, and the ependyma of the central nervous system (15–17). In kidney, a very high megalin expression is seen in renal proximal tubules (18), where megalin has been demonstrated to mediate uptake of glomerular-filtered polybasic drugs (19) and vitamin B\(_12\)-transcobalamin (20). Several high molecular weight proteins including apolipoprotein B (21), apolipoprotein E (22), and clusterin (23) are also reported to bind to megalin in vitro, but due to the glomerular filtration barrier their recognition by megalin may occur predominantly in extrarenal tissues and in glomerular podocytes (24).

A surface plasmon resonance (SPR) analysis screening procedure of fractionated serum led to the identification of \( \beta_2 \)-gpI as a novel megalin ligand. The physiological relevance of this observation was validated by a number of studies including uptake analysis in megalin-expressing cells, determination of the renal fate of \( \beta_2 \)-gpI in mice with disrupted megalin gene, and evaluation of the effect of acidic phospholipids on the binding of \( \beta_2 \)-gpI to megalin.

**Methods**

**Receptors, ligands, and antibodies.** Megalin was purified by receptor-associated protein (RAP) affinity chromatography of renal cortex membranes (25). Low density lipoprotein receptor–related protein was purified by \( \gamma_1 \)-macroglobulin affinity chromatography of solubilized human placenta membranes (26). Human RAP was a recombinant protein produced in *Escherichia coli*. \( \beta_2 \)-gpI was purified from human plasma as described (27). Iodination was performed by the chloramine T method (28). The specific activity was \( \sim 1 \) MBq/\( \mu \)g. The phospholipids were from Sigma Chemical Co. (St. Louis, MO) and homogenized by sonication before use essentially as described previously (27). Two rabbit polyclonal antibodies against human \( \beta_2 \)-gpI were used, one purchased from PerImmune Inc. (Rockville, MD) and another characterized previously (29). The latter antibody

---

1. Abbreviations used in this paper: \( \beta_2 \)-gpI, \( \beta_2 \)-glycoprotein-I/apolipoprotein H; PS, phosphatidylserine; RAP, receptor-associated protein; SPR, surface plasmon resonance.

was affinity-purified on a β-gpl column. A monoclonal antibody 5B-B11 raised against rabbit megalin (30) and a sheep polyclonal IgG raised against rat megalin (20) have been described previously.

**Gel filtration and ion exchange chromatography.** 2 ml of rabbit serum was loaded onto a 175-ml Superdex-200 (Pharmacia Biotech AB, Uppsala, Sweden) column using 10 mM Hepes, 150 mM NaCl, and 1.5 mM CaCl₂, 1 mM EGTA, pH 7.4, as running buffer. Cation exchange chromatography was carried out on a 10-ml Mono S (Pharmacia Biotech AB) FPLC column and bound proteins were eluted with a linear gradient of 0–1 M NaCl in 20 mM Tris, pH 7.

**Gel electrophoresis, protein sequence analysis, and ligand blotting.** Protein was electroblotted from a 4–16% polyacrylamide SDS gel onto a polyvinylidene difluoride membrane (Problot; Applied Biosystems, Inc., Foster City, CA). The electroblotted protein was cut out and subjected to Edmann degradation using an Applied Biosystems 477 A sequencer equipped with a 120 A online chromatograph. Ligand blotting was essentially carried out as described (25).

**SPR analysis.** For the SPR analyses, the BIAcore sensor chips (type CM5: Biosensor, Uppsala, Sweden) were activated with a 1:1 mixture of 0.2 M N-ethyl-N’-(3-dimethlaminopropyl) carbodiimide and 0.05 M N-hydroxysuccinimide in water according to the manufacturer. Rabbit renal megalin was immobilized as described (20). The SPR signal from immobilized rabbit megalin generated 15–21 × 10⁶ BIAcore response units (RU) equivalent to 25–35 fmol/mm². The flow cells were regenerated with 20 μl 1.5 M glycine-HCl, pH 3.0. The flow buffer was 10 mM Hepes, 150 mM NaCl, and 1.5 mM CaCl₂, 1 mM EGTA, pH 7.4. The binding data were analyzed using the BIA evaluation program. The number of ligands bound per immobilized receptor was estimated by dividing the ratio RUliquid/masssignal with RUreceptor/massreceptor.

**Uptake of 125I-β-gpl in rat yolk sac cells.** Megalin-expressing Brown Norway rat yolk sac epithelial cells transformed with mouse sarcoma virus (31) were grown to confluence (∼300,000 cells/well) in 24-well plates (Nunc A/S, Roskilde, Denmark) in MEM (Gibco, Paisley, UK) containing 10% FCS. Incubation with 125I-β-gpl was carried out in MEM supplemented with 0.1% BSA. Degradation of the proteins was measured by precipitation of the incubation medium in 12.5% trichloracetic acid. Cell-associated radioactivity was measured after three 5-min washes of the cells with ice-cold 20 mM EDTA in PBS in order to dissociate calcium-dependent surface-associated ligand. Radioactivity was subsequently counted in the cells released by trypsination. Chloroquine and leupeptin (both from Sigma Chemical Co.) were used as inhibitors of lysosomal enzyme activity.

**Analysis of megalin gene knockout mice.** Mice genetically deficient for megalin were generated by gene targeting as described previously (32). Although most megalin−/− mice die perinatally, ∼2% of the animals survive to adulthood (Nykjaer, A., H. Vorum, D. Dragan, D. Walther, C. Jacobsen, J. Herz, F. Melsen, E.I. Christensen, and T.E. Willnow, manuscript submitted for publication). For urine collection, these adult megalin knockout mice and control littermates were placed in metabolic cages for 1 h and given 10% sucrose in drinking water. Urine samples were collected on ice and were qualitatively indistinguishable from samples obtained without sucrose load. Creatinine and protein concentrations as well as total urine volume per hour were similar in knockout mice and control animals, indicating normal glomerular filtration rates in receptor-deficient kidneys.

**Preparation of renal tissue.** Normal human renal tissue was obtained from resected renal carcinoma kidneys and fixed in 8% paraformaldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. Kidneys from megalin-deficient and control mice were fixed by perfusion through the heart with 4% paraformaldehyde in the same buffer. Rat kidneys were fixed by retrograde perfusion through the abdominal aorta with 1% paraformaldehyde. The tissues were trimmed into small blocks, further fixed by immersion for 1 h in 1% paraformaldehyde, infiltrated with 2.3 M sucrose containing 2% paraformaldehyde for 30 min and frozen in liquid nitrogen.

**Immunocytochemistry.** For light microscopy, 0.8-μm cryosections were obtained at −80°C with an FCS Reichert Ultracut S cryoultra-
Figure 2. Ligand blotting with $^{125}$I-$\beta$-gpI. Triton X-100-solubilized rabbit renal cortex membranes ($\sim 500 \mu$g/lane) were electroblotted from 4-16% SDS-polyacrylamide gels (nonreducing conditions) and incubated with $^{125}$I-$\beta$-gpI in the absence (lane 1) or presence of 10 mM EDTA (lane 2) or 1 \( \mu \)M RAP (lane 3). The ligand blots were incubated with 35,000 Bq $^{125}$I-ligand/ml at 4°C for 20 h and exposed on Amersham Hyperfilm at −70°C for 72 h. Lane 4 is an antimegalin immunoblot of the same blot as in lane 1 after it had been subjected to ligand blotting and autoradiography. The primary antibody is monoclonal antibody 5B-B11 recognizing rabbit megalin (30). Alkaline phosphatase–conjugated anti-mouse IgG was used as secondary antibody.

Results

Identification of $\beta$-gpI as a megalin ligand. The screening strategy used here for identifying filtered ligands in plasma was based on the facts that small and cationic molecules preferentially are filtered in the glomerulus and that cationic features of megalin ligands are important for recognizing megalin (19) on the apical surface of the proximal tubules (18). Initially, SPR analysis measured megalin binding activity in serum fractionated by gel filtration. Subsequently, the proteins in the most binding-active fractions were further separated by cation exchange chromatography. Fig. 1 shows rabbit serum fractionated by gel filtration on a Superdex-200 column and the SPR signal of each fraction applied to a flow cell with immobilized purified rabbit megalin (Fig. 1 A). A peak in binding activity was seen in fractions 38–42 and a smaller peak in fraction 50–54. SDS-PAGE of the fractions showed a wide spectrum of proteins of 20–150 kD in the high peak and 67 kD and less in the smaller peak (not shown). Cation exchange (Fig. 1 B) chromatography of fractions of the two peaks showed that one predominant 50 kD protein from the high peak fraction was strongly bound to the cation exchange matrix. The fraction containing this protein exhibited megalin-binding activity, and amino-terminal sequencing of the electroblotted 50-kD rabbit protein yielded the sequence Tyr-Arg-Thr-X-Pro-Lys-Asp-

Figure 3. Uptake and degradation of $^{125}$I-$\beta$-gpI in the rat yolk sac carcinoma cell line, BN/MSV. (A) Time courses for uptake (filled circles) and degradation (open circles). Confluent cell layers (300,000 cells/well) in 24-well plates were incubated with $^{125}$I-$\beta$-gpI (400 Bq = 8 fmol). Degradation was measured as the increase in trichloroacetic-soluble radioactivity in the medium. Uptake was measured as the cell-associated radioactivity plus the degraded fraction. The uptake/degradation rate of the $^{125}$I-$\beta$-gpI was 15% (~1 fmol) per hour. (B) The inhibitory effect on uptake and degradation by leupeptin (100 \( \mu \)M), chloroquine (100 \( \mu \)M), RAP (1 \( \mu \)M), anti-rat megalin IgG (100 \( \mu \)g/ml), and rat nonimmune IgG (100 \( \mu \)g/ml). All values are means of triplicates.

Figure 4. Immunohistochemical and immunoelectron microscopic detection of $\beta$-gpI in human, rat, and mouse renal cortex. (Four top panels) Light microscope immunohistochemical labeling for $\beta$-gpI using horseradish peroxidase, in proximal tubules from a human, a rat, a wild-type mouse (+/+), and a megalin-deficient mouse (−/−). Labeling is seen as a brown granular staining. The proximal tubules of the megalin-deficient mouse (−/−) did not show staining for $\beta$-gpI. (Bottom panel) Electron microscopic immunocytochemical labeling for $\beta$-gpI in a human renal proximal tubule using 10 nm colloidal gold coupled to goat anti–rabbit IgG. Gold particles are strongly accumulated in lysosomes.
Demonstration of a Novel Receptor Recognition Site in β₂-Glycoprotein-I
Asp-Leu. The SwissProt protein database identified the protein as the rabbit homologue of human β₂gpI which has Gly-Arg-Thr-Cys-Pro-Lys-Asp-Asp-Leu as the amino-terminal sequence. Binding of β₂gpI to megalin was subsequently confirmed by SPR analysis of purified human β₂gpI (Fig. 1 C). This analysis estimated a $K_d$ of $1.7 \times 10^{-7}$ M at 20°C.

**Megalin mediates endocytosis of β₂gpI.** Ligand blotting (Fig. 2) of rabbit renal cortex membranes with $^{125}$I-labeled β₂gpI identified megalin as the only β₂gpI-binding protein in renal cortex membrane. The binding was completely abolished by the addition of EDTA (lane 2) or RAP (lane 3). Ligand blotting to purified megalin revealed a similar staining (not shown), whereas no significant binding to the homologous human LDL receptor–related protein was evident by ligand blotting (Fig. 3) or SPR analysis (data not shown).

Endocytosis of $^{125}$I-β₂gpI was studied (Fig. 3 A) in a rat yolk sac epithelial cell line which exhibits a high expression of megalin (31). $^{125}$I-labeled β₂gpI was effectively taken up and radioactive degradation products appeared in the medium after a lag time of ~20 min. The uptake was effectively inhibited by RAP and a polyclonal antibody raised against megalin (Fig. 3 B).

Chloroquine, a weak membrane-permeable base raising pH in intracellular compartments, and leupeptin, a membrane-diffusible protease inhibitor, were used to show that the degraded ligand in the medium was accounted for by receptor-mediated endocytosis and lysosomal degradation. Both reagents inhibit lysosomal enzyme activity (33, 34) and, in addition, chloroquine is an inhibitor of membrane trafficking (34). In accordance with these effects, leupeptin and chloroquine caused a strong decrease in degradation of $^{125}$I-β₂gpI leading to accumulation of nondegraded radiolabel within the cells (Fig. 3 B). Furthermore, cells incubated with chloroquine, but not those incubated with leupeptin, exhibited a partial inhibition in ligand uptake (Fig. 3 B) in agreement with the additional cellular effect of chloroquine.

**β₂gpI is excreted in the urine of megalin-deficient mice.** To determine whether megalin is the renal proximal tubule component responsible for the uptake of β₂gpI in the ultrafiltrate, we investigated the renal content of β₂gpI in the kidney cortex of a human, a rat, and mice, including one with a disrupted megalin gene. Light microscopic immunohistochemistry of normal kidneys of the three species revealed a granular staining of β₂gpI in renal proximal tubules (Fig. 4). The staining was only observed in segment 1 of the proximal tubule, indicating that the protein under physiological conditions is removed very efficiently in the early part of the proximal tubule after glomerular filtration. No other nephron segments showed any immunoreactivity. Electron microscopic examination of immunogold-labeled kidney sections demonstrated that the granular staining was accounted for by staining of lysosomes. Fig. 4, (bottom) shows the accumulation of gold particles in lysosomes of a human proximal tubule cell. In contrast to the staining in normal mice (Fig. 4, Mouse +/+), there was virtually no staining in kidneys of mice with disrupted megalin gene (Fig. 4, Mouse −/−).

In accordance with the kidney data, no β₂gpI was detected in the urine of normal mice, whereas the megalin-deficient mice exhibited a high excretion as evaluated by immunoblotting (Fig. 5 A). The urine from two myelomatosis patients suffering from a Bence-Jones protein-induced Fanconi syndrome (renal tubule failure) contained in accordance with previous data (12, 35) also a significant content of β₂gpI (Fig. 5 B).

**Binding of PS to β₂gpI increases the functional affinity for megalin.** The acidic phospholipids PS and cardiolipin bind strongly to the basic region of the fifth SCP repeat of β₂gpI (36) and an SPR analysis was therefore performed to study the effect of phospholipids on the binding of β₂gpI to megalin. A strong increase in the SPR signal was seen (Fig. 6 A), when PS and cardiolipin were added to β₂gpI, whereas no effect of the neutral phosphatic acid and phosphatidylcholine was observed (Fig. 6 B). A decreased dissociation rate of the β₂gpI–PS complexes ($k_{off} = 1.33 \times 10^{-3}$ s$^{-1}$) compared with native β₂gpI ($k_{off} = 4.75 \times 10^{-3}$ s$^{-1}$) demonstrated a higher functional affinity of liposome-bound β₂gpI. An exact $K_d$ for the interaction of the phospholipid-bound β₂gpI to megalin was not measurable by SPR due to the fact that the binding of several β₂gpI molecules per liposome may cause multisite interactions and different size of the particles.

**Concentration dependence of the binding of β₂gpI–PS to megalin.** The maximum PS-augmented binding was seen when β₂gpI (10 μg/ml) was added to ~10 μg/ml PS. The binding of β₂gpI to megalin (Fig. 7 A) was reduced at higher concentrations of PS, indicating that diluting the density of β₂gpI

![Figure 5. Urinary excretion of β₂gpI in megalin-deficient mice and patients with Fanconi syndrome. (A) Analysis of urine from megalin −/− and control mice. 15 μl of urine from mice either wild-type (+/+) (lane 1, 4, and 5), heterozygous (+/−) (lane 3), or homozygous for a disruption of the megalin gene (−/−) (lanes 2, 6, and 7) was subjected to 4–15% nonreducing SDS-PAGE and subsequent immunoblot analysis using rabbit anti–human β₂gpI polyclonal antibody and the enhanced chemiluminescence system (Amersham, Arlington Heights, IL). 1 μg purified human β₂gpI (lane 8) was used as control. (B) Analysis of human urine samples. 0.5 μl of midstream urine samples from two individuals with myeloma-associated Fanconi syndrome (lanes 2, 3, 5, and 6) or 10 μl of urine sample from a healthy control individual (lanes 1 and 4). Samples in lanes 1–3 were stained with silver nitrate and lanes 4–6 were subjected to immunoblotting with the anti-β₂gpI antibody.
molecules in the liposomes decreases the functional receptor affinity of \( \beta_{\text{gpI}} \)-PS complexes. In accordance with this observation, the inverse experiment (Fig. 7 B), where the PS concentration was constant and the \( \beta_{\text{gpI}} \) was varied, showed that adding \( > 10 \mu g/ml \beta_{\text{gpI}} \) to 10 \( \mu g/ml \) PS or cardiolipin caused an increase in binding.

\( \beta_{\text{gpI}} \)-PS binds to the high-affinity RAP binding site(s) in megalin. RAP binds to multiple sites in the ligand-binding regions of megalin. Previous SPR analysis (20) of the RAP binding to megalin showed that 50\% of the RAP molecules dissociated rapidly after the receptors were saturated with RAP (20), whereas the remaining 50\% were almost irreversibly bound.

Fig. 8 (top curve) shows the binding of RAP and dissociation of \(~50\%\) of the RAP molecules. When \( \beta_{\text{gpI}} \)-PS subsequently was exposed to the megalin-RAP sensor chip, no significant binding was seen in contrast to the binding seen to the megalin-sensor chip without RAP (Fig. 8, bottom curve). This demonstrates that the high-affinity bound RAP completely blocks the megalin binding site(s) for \( \beta_{\text{gpI}} \)-PS.

**Discussion**

These data including investigation of mice with disrupted megalin gene demonstrates megalin as an efficient and physiological relevant receptor of \( \beta_{\text{gpI}} \). Furthermore, we show that binding of acidic phospholipids to \( \beta_{\text{gpI}} \) increases the functional affinity in analogy with the stabilizing effect of lipids on the receptor binding of apolipoproteins B and E.

\( \beta_{\text{gpI}} \) and kidney disease. Previous analyses of the urine from patients suffering from renal tubule failure have shown that \( \beta_{\text{gpI}} \), compared with other known urinary proteins, is a superior diagnostic marker for renal proximal tubule failure seen after drug- or toxin-induced intoxication of renal tubules and in a number of chronic diseases including adult Fanconi syndrome, nephrocalcinosis associated with autoimmune diseases, Lowe’s syndrome, and Dent’s disease (12, 35). 16 renal proximal tubule failure patients in the study of Norden et al. (12) excreted between 2 and 40 \( \mu g \) of \( \beta_{\text{gpI}} \) per millimole of creatinine, whereas healthy controls had nondetectable amounts. Previous data demonstrating \( \beta_{\text{gpI}} \) in the glomerular filtrate of Bowman’s capsule (37) and the present data on megalin, including absent renal uptake of \( \beta_{\text{gpI}} \) in megalin-deficient mice, indicate that an insufficient activity of renal megalin during renal proximal tubule failure causes the urinary excretion of the ligand. The apparent high filtration rate of \( \beta_{\text{gpI}} \) is remarkable in view of its size. The high content of basic amino acids in \( \beta_{\text{gpI}} \) may in part account for this.

Using SPR for ligand screening. Although the present SPR screening strategy identified a novel ligand, the screening as-
specific binding was subtracted as described in the legend to Fig. 6.

The binding of 28 to low density lipoprotein receptor–related protein.
demonstrates that binding to phospholipids causes a conformational
noreactivity and infrared spectroscopy studies (38), demon-
sate uptake of PS-containing particles.

SPR sensogram of the binding of βgpl–PS to megalin–RAP. The top curve shows the binding of RAP (40 μg/ml). After dissociation of the loosely bound RAP (~50% of total bound), βgpl (10 μg/ml) was exposed to the sensor chip. Only a weak increase in response is seen compared with the response by exposure of βgpl to the same megalin chip without prior binding of RAP (bottom curve). Non-specific binding was subtracted as described in the legend to Fig. 6.

say was not successful in respect to identifying other megalin ligands. This can partly be explained by the fact that ligands with low plasma concentrations will not generate a measurable SPR signal above background. Another problem is that protein fractions with very high amounts of abundant plasma proteins such as albumin, which does not generate any SPR signal itself, may mask the SPR signal generated by high affinity proteins in much lower concentrations. Other methods for fractionating serum, including removal of albumin before the initial SPR screening, are currently being evaluated in order to improve the efficiency of the screening method.

Binding of phospholipid increases the functional affinity of βgpl. SPR analyses revealed that the phospholipids augment the binding of βgpl to megalin. In view of previous immunoreactivity and infrared spectroscopy studies (38), demonstrating that binding to phospholipids causes a conformational change of βgpl, it is tempting to speculate that this neoconformation favors receptor binding. In this case the receptor recognition site must be only partially cryptic, since megalin binds and mediates endocytosis of the free form of βgpl as well. Alternatively, the binding of multiple βgpl molecules associated to each phospholipid vesicle may explain the increase in functional affinity. The decreased binding observed, when very high concentrations of PS were added (Fig. 7), does in fact indicate this. A similar bonus effect of multivalency is suggested for the binding of apolipoprotein E–containing lipoprotein particles (39) and α2–macroglobulin–proteinase complexes (28) to low density lipoprotein receptor–related protein.

βgpl and clearance of PS-expressing cells and particles. It is well established that surface expression of PS occurs in apoptotic and senescent cells due to decreased activity of the ATP-dependent aminophospholipid translocase (40) and several mechanisms for PS-facilitated cellular recognition apparently exist. A high density lipoprotein receptor related to scavenger receptor I (41) and a microsialin/CD68 (42) have both been reported to bind PS directly. However, recent data also suggest an important role for βgpl. βgpl binds to rapidly cleared PS liposomes (7) and to apoptotic thromocytes (43), and can increase the uptake of erythrocyte ghosts and apoptotic thromocytes in macrophages (6). The receptor in macrophages must be different from megalin but in some of the megalin–expressing epithelia, e.g., from the central nervous system (ependyma), lung, yolk sac, intestine, and placenta as well as in the ectoderm and neuroderm of the fetus, megalin may facilitate uptake of PS-containing particles.

In conclusion, this study has established the existence of a novel physiological important receptor recognition site in βgpl. In addition to providing the molecular background for the urinary excretion of βgpl in patients suffering from renal tubule failure, these data add further evidence for the function of βgpl as a linking molecule facilitating cellular uptake of PS-exposing particles.

Acknowledgments

The technical assistance of Kirsten Lassen, Anne Marie Bundsgaard, and Hanne Sidelman is gratefully acknowledged. Urine from Fanconi patients was kindly provided by Dr. P. Aucloutier, Hôpital de Paris, France.

This work was financially supported by the Novo Nordisk Foundation, the Danish Medical Research Council, the Danish Biomembrane Center, Aarhus University Research Foundation, and the Velux Foundation.

References


