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Interaction of high-molecular-weight kininogen with endothelial cell binding proteins suPAR, gC1qR and cytokeratin 1 determined by Surface Plasmon Resonance (BiaCore)

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Summary

The physiologic activation of the plasma kallikrein-kinin system requires the assembly of its constituents on a cell membrane. High-molecular-weight kininogen (HK) and cleaved HK (HKa) both interact with at least three endothelial cell binding proteins: urokinase plasminogen activator receptor (uPAR), globular C1q receptor (gC1qR), and cytokeratin 1 (CK1). The affinity of HK and HKa for endothelial cells are $K_D=7-52$ nM. The contribution of each protein is unknown. We examined the direct binding of HK and HKa to the soluble extracellular form of uPAR (suPAR), gC1qR and CK1 using surface plasmon resonance. Each binding protein linked to a CM-5 chip and the association, dissociation and K_D (equilibrium constant) were measured. The interaction of HK and HKa with each binding protein was zinc-dependent. The affinity for HK and HKa was $gC1qR > CK1 > suPAR$, indicating that gC1qR is dominant for binding. The affinity for HKa compared to HK was the same for gC1qR, 2.6-fold tighter for CK1 but 53-fold tighter for suPAR. Complex between binding proteins was only observed between gC1qR and CK1 indicating that a binary CK1-gC1qR complex can form independently of kininogen. Although suPAR has the weakest affinity of the three binding proteins, it is the only one that distinguished between HK and HKa. This finding indicates that uPAR may be a key membrane binding protein for differential binding and signalling between the cleaved and uncleaved forms of kininogen. The role of CK1 and gC1qR may be to initially bind HK to the membrane surface before productive cleavage to HKa.

Keywords

Kininogen; surface plasmon resonance; suPAR; gC1qR; cytokeratin 1

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Conflict of interest

None declared.

Introduction

High-molecular-weight kininogen (HK) is the substrate and cofactor of the plasma kallikrein-kinin system (KKS) (1). HK is present on plasma at a concentration of 660 nM (1) binds to platelet GPI-V-IX complex, and inhibits binding of thrombin to platelets (2) serving as an antithrombotic protein *in vivo* (3). Domain 2 of HK exhibits antiplatelet activity by inhibiting platelet calpain, a cysteine protease which is a signalling molecule in platelets (4). HK inhibits adhesion of neutrophils to blood compatible surfaces under flow conditions (5) and enhances cellular fibinolysis (6). The proenzyme, prekallikrein, after activation forms an active enzyme plasma kallikrein which can cleave HK to two chain HKa and bradykinin (7), a potent bioactive peptide which stimulates endothelial cell prostacyclin synthesis and nitric oxide formation (8) resulting in vasodilation. The formation of HKa occurs on platelet or endothelial cell surfaces where kallikrein is activated even in the presence of plasma protease inhibitors (9, 10).

The transformation of HK to HKa is accompanied by dramatic conformational changes detected by circular dichroism (11) and electron microscopy (12). These physical alterations result in new functional activities. HKa stimulates monocytes to synthesise and secrete cytokines and chemokines (13) and thus is anti-inflammatory in experimental inflammatory arthritis (14,15). The action of HKa on monocytes is mediated by integrin receptors such as $\alpha v \beta 3$ and $\alpha 5 \beta 1$ as well as the adaptor protein uPAR (16). HKa is also antiangiogenic by virtue of inhibiting proliferation and migration of stimulated endothelial cells (17) as well as interfering with tube formation *in vitro* (18) and angiogenesis *in vivo* (19).

On the surface of human umbilical vein endothelial cells (HU-VECs) both HK and HKa associate with binding proteins of uPAR (20), gC1qR (21) and CK1(22). Each of these three proteins are required for binding of HKa to endothelial cells because antibodies to any one protein block binding(18, 23)and a peptide from the light chain of HK, isolated by an affinity column inhibits both binding and contact activation (24). The sub-domains by which HK and HKa binds to each binding protein are known. For example, a peptide derived from domain 5 of HK, G486-K502 (25) inhibits cell adhesion to vitronectin. In this paper, we confine ourselves to cell surface binding proteins which are intrinsic to endothelial cells. To better understand the function of each binding protein, we now determine the relative affinity of each soluble purified binding protein for both HK and HKa using surface plasmon resonance. This method also allows evaluation of multiple interactions including the possible occurrence of binary binding protein complexes. The differential binding of HK and HKa to each binding protein was assessed in a quantitative approach in the presence of zinc ion.

Materials and methods

Materials

Purified proteins were made or obtained from participants in this study. Cytokeratin 1 was as purified and characterised in Joseph et al.(23). Soluble expressed urokinase plasminogen activator receptor (suPAR) was produced as described previously (26). Single-chain urokinase-type plasminogen activator (scuPA or PUK) was purchased from American Diagnostica Inc. (Stamford, CT, USA). gC1qR (also known as p32, p33, C1QBP, TAP) was purified as described(27). Single-chain high-molecular-weight kininogen (HK) and two-chain high-molecular-weight kininogen (HKa) was purchased from Enzyme Research Laboratories (South Bend, IN, USA). Low-molecular-weight kininogen (LK) was purchased from Calbiochem (Darmstadt, Germany). Ovalbumin was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA).

Methods

Purified proteins were examined for purity by Sodium Dodecyl Sulfate (SDS) polyacrylamide gel electrophoresis (PAGE). The samples 3–5 µg (reduced and non-reduced), were applied to a 4–20% NuPage gel using MOPS buffer (Invitrogen, Carlsbad, CA, USA). Invitrogen-SeeBlue Plus2 was used as standard and Simply blue Safe stain used to observe the position of the proteins.

Real-time biomolecular interaction analysis was performed using a BiaCore 2000 instrument (GE Healthcare, Piscataway, NJ, USA). Purified proteins were covalently linked at pH 5.0 in 10 mM sodium acetate buffer to a CM5 Chip using EDC/NHS amine coupling of the primary amine of the protein to a carboxyl group of a chip linked carboxymethylated dextran using HBS-P running buffer (10 mM HEPES, 0.15 M NaCl, 0.005% polysorbate 20, pH 7.4) at 25°C. Ethanolamine was used to block unwanted carboxy groups after linkage. The base values of suPAR (950 RU), gC1qR (600 RU), cytokeratin 1 (750 RU), HK (924), HKa (1400 RU) and LK (1190 RU) were used as the specific ligands where 1 RU represents the mass of 1 pg of linked protein. Each RU represents 1 pg of mass giving the number of molecules linked to the chip. The mass of gC1qR, suPAR, cytokeratin 1, HK, HKa and LK; when used for calculations were 33 kDa (as a non-covalent trimer), 40 kDa (20), 54 kDa, 120 kDa, 110 kDa and 64 kDa respectively. An ethanolamine blocked Fc1 was used as blank for background subtraction of any non-specific response to the derivatised dextran chip. In kinetic determinations, concentrations of different proteins were produced by programmed dilution from a stock solution using the BiaCore software starting from low concentrations to higher concentrations. Kinject was used for injection. Flow rate was 30 µl/minute (min) for suPAR immobilised competition studies (► Fig. 2) and for HKa/PUK competition studies on suPAR. Other studies were at 60 µl/min. Temperature for all studies were set at 25°C. The running buffer used was BiaCore HBS-P (10 mM HEPES, 0.15 M NaCl, 0.005% polysorbate 20, pH = 7.4) containing 5 µg/ml (111 nM) chicken egg albumin (Sigma Chemical Co.) as well as either 10 µM ZnCl₂ or 3 mM EDTA (absence of Zinc runs). After each analyte injection, the chip surfaces were regenerated by two injections of 100 mM EDTA + 2 M NaCl. In the PUK/HKa competitive study, regeneration was performed by short injection series of 0.1 M acetic acid, 2 M NaCl, and 3 mM EDTA. For affinity constant determinations, merging of triplicate injections of each concentration was performed to obtain a single sensorgram line at each concentration. In determining the affinity constants, to stay well below R_{max} values, only the lower three or four concentrations were used to fit curves for kinetic determinations shown in ► Table 1. The remaining lines are for illustrative purposes. A Lang-muir binding model with local fit (stoichiometry of 1:1) was used to analyse *kon* (association rate constant), *koff* (dissociation rate constant) and K_D (equilibrium dissociation constant). Chi² for the fits were usually <1 or some studies or <4.

Results

The purity and functionality of each of the proteins were tested by gel electrophoresis patterns and by competitive tests. ► Figure 1 shows SDS gel electrophoretic patterns for HK, HKa, suPAR and gC1qR at the expected locations under non-reduced and reduced conditions. Each binding protein was tested for interaction with HKa using 1:1 ratio mixing studies of HKa with each binding protein. Lowering of the ΔRU of HKa sensorgram binding to immobilised suPAR in the presence of the soluble binding protein indicates there is competition between HKa binding to the immobilised suPAR and the tested soluble purified binding protein (► Fig. 2). Immobilised suPAR was also tested for functional activity. The suPAR is a specific receptor for single-chain urokinase plasminogen activator (scuPA or prourokinase), and was found to bind to suPAR at a high affinity as previously described (K_D = 0.1–1 nM, data not shown) (26) indicating its functionality. The results indicate that

after complex formation with HKa in solution, suPAR, gC1qR and CK1 competed with HKa binding to the immobilised suPAR.

► Figure 3 shows the sensorgrams for the BiaCore method determinations of the kinetic binding of HK and HKa to each binding protein gC1qR (► Fig. 3A), suPAR (► Fig. 3B) and CK1 (► Fig. 3C) in the presence of 10 μ M ZnCl₂ or in the presence of 2 mM EDTA (to assure the absence of bivalent cations). Each concentration used were diluted and injected in triplicate and the three curves were averaged to one curve per concentration (as shown) and analysed using the Biaevaluation program as described in *Methods*. In the presence of EDTA, there is no apparent binding of HK nor HKa in the range of 25–400 nM examined. In the presence of 10 μ M ZnCl₂, there is significant binding of both HK and HKa with all the linked binding proteins with HKa having a greater Δ RU binding peak than HK using the same concentrations. These results confirm the requirement for Zn²⁺ for both HK and HKa binding to endothelial cells.

The sensorgram is displayed for the binding of HK and HKa to immobilised gC1qR (► Fig. 3A) and the data in the presence of 10 μ M zinc is displayed in ► Table 1. The *kon* is twice as fast for HKa as for HK and as for all three binding proteins is absolutely dependent on the presence of Zn²⁺. The *koff* is very slow almost flat which accounts for the very tight binding K_d = 0.7–0.8 nM for both HK and HKa (► Table 1). There is little selectivity with gC1qR for either form of kininogen. The sensorgram for CK1 is dominated by the slow *kon*, but due to the low *koff* the affinity is 6–15 nM (► Fig. 3C, ► Table 1). There is little preference for HKa over HK.

The sensorgram for immobilised suPAR is strikingly different (► Table 1 and Fig. 3B). The lower *kon* and higher *koff* of HK over HKa accounts for the lower K_d (53-fold higher affinity) of HKa binding to suPAR.

We probed whether binding to the cell binding proteins involved the heavy chain of HK. LK contains only the heavy chain (domains 1–3) and does not contain the light chain components domain 5 and 6. We compared the sensorgram binding of LK and HKa to immobilised CK1, suPAR and gC1qR in the presence of 10 μ M ZnCl₂. No binding of LK was observed in the presence or absence of zinc ions to any of these immobilised binding proteins (data not shown). Therefore it is likely that the light chain domain of HK or HKa is required for the binding of HKa binding to these membrane proteins.

Up to this point we have only considered the interaction of kininogens with each of the three binding proteins separately. In the next set of experiments, the binding proteins were studied as soluble proteins and as immobilised proteins. We first examined the higher affinity proteins gC1qR and CK1. First it is shown that HKa (arrow indicates injection) binds to both immobilised binding proteins (► Fig. 4, left side of graph, base CK1 and gC1qR). With base gC1qR, when additional soluble gC1qR or CK1 (arrow indicated) was injected to the bound gC1qR-HKa complex (► Fig. 4, top line, middle and right graph), no additional trimolecular gC1qR-HKa-gC1qR (A) or gC1qR-HKa-CK1 complex formation are observed. This finding indicates that once HKa is bound to gC1qR, the binding sites can no longer bind to additional CK1 and gC1qR. In contrast, when base CK1 with HKa bound is injected with gC1qR (► Fig. 4, middle line, middle graph), additional binding is observed forming a CK1-HKa-gC1qR complex (► Fig. 4B). Additional injection of CK1 does not add nor displace the complex (► Fig. 4, middle line, right graph). This raises the question as to whether CK1 and gC1qR can associate with each other independent of HKa. Using base CK1 with addition of gC1qR (► Fig. 4, bottom lines, mid graph) additional binding of gC1qR to immobilised CK1 was observed (► Fig. 4C) and the RU displacement was similar to that observed with base CK1-HKa-gC1qR complex (► Fig. 4B). When CK1 was added to

base gC1qR (► Fig. 4, bottom lines, right graph), no additional binding occurred. This indicates that CK1-gC1qR complex can form, but the immobilised structure of gC1qR may interfere with the association site for CK1. Additionally from these experiments, dual complexes of gC1qR-gC1qR (► Fig. 4, bottom lines, middle graph) or CK1-CK1 (► Fig. 4, bottom lines, right graph) are not observed.

In the next experiments, we examined the possible formation of protein complexes with the lower HKa affinity binding protein suPAR (► Fig. 5). Binding of HKa to immobilised suPAR occurred in the presence of zinc indicating suPAR-HKa complexes formed (► Fig. 5, top line). Injections of soluble gC1qR, CK1 or suPAR resulted in no additional observed binding (► Fig. 5), indicating the absence of suPAR-gC1qR, suPAR-CK1 or suPAR-suPAR complex formation.

In the next experiment to test the presence of tri-molecular complexes once HKa binds to suPAR, HKa was first injected over immobilised suPAR (50 seconds, in the presence of zinc) which resulted in formation of suPAR-HKa complexes (► Fig. 6). Subsequent addition of gC1qR, CK1 or suPAR did not result in any increase of the sensorgram signal (► Fig. 6). In fact, the sensorgram indicates that addition of gC1qR or CK1 may enhance the removal of HKa from the immobilised suPAR as exhibited by an enhanced off rate. This result suggests that HKa binds suPAR independent of gC1qR or CK1.

Discussion

This investigation focuses on the binding of HKa and HK to each of the endothelial cell kininogen binding proteins (20) uPAR, gC1qR and CK1. Our challenge was to define how each endothelial protein relates to the functions of HK and HKa. We have recently reviewed the mechanisms by which cleaved kininogen inhibits endothelial differentiation and signalling (28) emphasizing the critical role of uPAR.

The urokinase plasminogen activator receptor (uPAR) modulates cell adhesion/migration and fibrinolysis. uPAR is a 55 kDa, glycosylphosphatidylinositol (GPI)-linked protein consisting of three domains (29). uPAR binds urokinase-type plasminogen activator (uPA) through specific interactions with uPAR domain 1. This interaction promotes the focused expression of cell surface plasminogen activator activity. We also demonstrated the formation of a zinc-dependent complex between suPAR and HKa (20) on the surface of endothelial cells. HKa inhibits endothelial cell proliferation and induces apoptosis (17). The functional results of these changes is the inhibition of angiogenesis *in vitro* (19). The mechanism by which HKa down-regulates endothelial cell tube formation is by disrupting the uPA-uPAR complex and inhibiting its signalling and utilisation (30). A similar mechanism has been described to explain how uPA binding to uPAR mediates intracellular signalling as reviewed by Schmaier and McCrae (31). However, the other receptors of HK and HKa must also be considered.

Human gC1qR/p33 is a ubiquitously expressed, multi-compartmental and multifunctional protein expressed on a wide range of tissues and cell types including endothelial cells. The gC1qR can serve as a receptor for diverse proinflammatory ligands including proteins of the plasma kallikrein-kinin system, most notably high-molecular-weight kininogen (HK and HKa; $K_D=9$ nM) (21). The gC1qR is a trimer of 33 kDa and HKa binds to this binding protein via its light chain(domain 5) in a zinc-dependent manner (21,23). Nevertheless, there is no evidence for the participation of gC1qR in cell signalling after the binding of HK or factor XII to human vascular smooth muscle (32).

Human cytokeratin 1 (CK1) is a 54-kDa protein isolated from endothelial cells by a biotin-high-molecular-weight-mass kininogen affinity column (22). HK bound specifically to CK1

only in the presence of zinc, as is the case of HK or HKa binding to HUVEC, and CK1 blocks HKa binding to HUVEC (22). Binding of HK to CK1 involves all the cell binding regions of HK on domains 3,4, and 5 which are concerned with binding of HK or HKa to endothelial cell (22, 33–35). Additional studies have identified that HK interacts with purified cytokeratin 1 by the same regions of the protein (i.e. its heavy and light chain) that interact with endothelial cells (36). However, at present, there is no evidence that upon binding to CK1, HK or HKa induces a signalling event.

Another proposed binding protein for the binding of HKa to endothelial cells is tropomyosin (37) to which HKa binds with high affinity through interactions involving HKa or D5. Inhibition of this interaction blocks the induction of endothelial apoptosis and inhibition of angiogenesis by HKa. These authors demonstrated that the effects of HKa on proliferating endothelial cells required direct binding to tropomyosin. When the binding of HK or HKa was examined using confluent, static endothelial monolayers, tropomyosin was minimally exposed on the cell surface. Hence, other binding proteins play a more prominent role in the binding of single- or two-chain HKa (33, 34, 38–41).

Another candidate is the epidermal growth factor binding protein (EGFR). EGFR is known to transduce uPAR signalling (42). Recently factor XII has been shown to initiate angiogenesis by stimulating ERK 1/2 and Akt through uPAR, integrins and the EGFR (43). This signalling pathway results in HUVEC stimulation and growth *in vitro* and *in vivo*. Now HKa has been shown to inhibit migration and invasion of prostrate cancer cells through the EGFR pathway (44).

In this study, in the presence of zinc, we found no differences between HK and HKa binding to similar amounts of immobilised gC1qR and CK1. The affinity of HK and HKa for these binding proteins was greater in the order of gC1qR > CK1 > suPAR indicating a dominant role in initial binding for gC1qR as has been suggested by a previous studies (24,45). However, only suPAR showed a 50-fold higher affinity for HKa than for HK. Therefore, it is relatively selective for HKa. Other studies have shown that factor XII can signal by binding to uPAR (43) This suggests that factor XII may compete with HKa binding after cleavage of HK.

Previous studies using dot blot, Western blot and gel filtration techniques, CK1-gC1qR and CK1-uPAR complexes were found (24). In this study, we found only one receptor-receptor complex of CK1-gC1qR formation confirming previous findings for this complex. This study indicated that the uPAR-CK1 association does not occur when using the SPR technique.

We suggest that gC1qR, CK1 (or possibly CK1-gC1qR complex) may function to bind initially a fluid HK to the membrane surface, due to their higher affinity contributed by their slow off-rate. These encounters can serve as a cofactor surface for activation of the kallikrein-kinin system resulting in the release of bradykinin and the formation of HKa. The presence of HKa allows its selective binding to uPAR which can interfere with uPAR-integrin association affecting cell signalling pathways and cell adhesion. As a result, HKa will down regulate the signalling cascade involving uPAR and can result in an inhibition of angiogenesis (18, 19).

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What is known about this topic?

- The activation of the plasma kallikrein-kinin system on a cell membrane requires the binding of factor XII, plasma prekallikrein, and high-molecular-weight kininogen (HK).
- It is known that both HK and cleaved HK (HKa) bind to three different binding proteins, uPAR, gC1qR and cytokeratin 1(CK1) on endothelial cells.
- HK by releasing bradykinin initiates angiogenesis, while HKa terminates the angiogenic process by inhibiting endothelial cell proliferation, migration, and tube formation.

What does this paper add?

- Using surface plasmon resonance, the affinity for HK and HKa is gC1qR > CK1 > suPAR due to a very slow koff (dissociation rate constant).
- Complex formation between the binding proteins was only observed between gC1qR and immobilised CK1 and was independent of HK.
- Of the three membrane binding proteins, only suPAR has significantly tighter binding to HKa than HK which may explain their differences in regulating angiogenesis.

Figure 1. SDS PAGE of purified suPAR, gC1qR, HK and HKa

SDS PAGE was performed on purified HK, HKa, suPAR and gC1qR under reduced (left) and non-reduced (right) conditions on using a 4–20% Bis/Tris gel using MOPS buffer. To the left of the gel are the positions of molecular standard markers.

Figure 2. Binding protein integrity sensorgram

In the presence of 10 μM ZnCl_2 , HKa (100 nM) alone or preincubated with gC1qR (100 nM), cytokeratin-1 (100 nM) or suPAR (100 nM) was injected over a suPAR immobilised CM5 chip. All reagents were found to be functionally active by HKa competing with the soluble binding protein to prevent binding to the chip immobilised suPAR. HKa alone (solid line), HKa + gC1qR (dotted line), HKa + cytokeratin 1 (short dashed line), HKa + suPAR (long dashed line). Each line represents a mean of two similar injections. Analyte injection was terminated at 50 seconds.

Figure 3. Sensorgrams of HK and HKa binding to immobilised binding proteins in the presence and absence of 10 μ M ZnCl₂

A) gC1qR in the presence and absence of 10 μ M ZnCl₂. Top graphs: HKa (bottom sensorgrams to top 0, 25, 50, 100, 150, 200, 250, 300, 350 nM) were injected over a gC1qR immobilised (base 600 RU) CM5 chip by an automated method file. Top left: in the presence of 10 μ M ZnCl₂. Top right graph: sensorgram in the presence of 3 mM EDTA. Bottom graphs: HK (bottom sensorgrams to top 0, 50, 100, 200, 300, 400 nM) were injected. Bottom left graph: sensorgram in the presence of 10 μ M ZnCl₂. Bottom right graph: sensorgram in the presence of 3 mM EDTA. Each sensorgram line is an average of three injection runs in the method. Analyte injection was terminated at 120 seconds. B) suPAR in the presence and absence of 10 μ M ZnCl₂. Top graphs: HKa (bottom sensorgram to top 0, 25, 50, 100, 150, 200, 250, 300, 350 nM) were injected over a suPAR immobilised CM5 chip (base 950 RU) by an automated method file. Top left graph: in the presence of 10 μ M ZnCl₂. Top right graph: in the presence of 3 mM EDTA. Bottom graphs: HK (bottom sensorgram to top 0, 50, 100, 200, 300, 400 nM) were injected. Bottom left graph: sensorgram in the presence of 10 μ M ZnCl₂. Bottom right graph: sensorgram in the presence of 3 mM EDTA. Each sensorgram line is an average of three injection runs. Analyte injection was terminated at 120 seconds. C) Cytokeratin 1 in the presence and absence of 10 μ M ZnCl₂. Top graph: HKa (bottom sensorgram to top 0, 25, 50, 100, 150, 200, 250, 300, 350 nM) were injected over a cytokeratin 1 immobilised (base 750 RU) CM5 chip by an automated method file. Top left graph: sensorgram in the presence of 10 μ M ZnCl₂. Top right graph: sensorgram in the presence of 3 mM EDTA. Bottom graph: HK (bottom sensorgram to top 0, 50, 100, 200, 300, 400 nM) were injected. Bottom left graph: sensorgram in the presence of 10 μ M ZnCl₂. Bottom right graph: sensorgram in the presence of 3 mM EDTA. Each sensorgram line is an average of three injection runs in the method. Analyte injection was terminated at 120 seconds.

Figure 4. CK1-gC1qR interactions

Sensorgrams show comparative binding of purified binding proteins and HKa in the presence of 10 μM ZnCl_2 to immobilised cytokeratin 1 and gC1qR. HKa, cytokeratin 1, and gC1qR (all 100 nM) were injected in the sequence indicated in the figure. Injection times were adjusted, but the ΔRU were not adjusted, for comparative illustrative purposes. Arrows with vertical dotted line indicates the position where each analyte was injected. The chip-linked binding protein base is indicated on the right each line. Each analyte injection was for 50 seconds. A: peak of suPAR-HKa-gC1qR complex; B: peak of CK1-HKa-gC1qR complex; C: peak of CK1-gC1qR complex.

Figure 5. CK1, gC1qR, suPAR interactions

Sensorgram indicating lack of binding of CK1, gC1qR and suPAR to immobilised suPAR in the presence of $10\mu\text{M}$ ZnCl_2 (no binding was found in the absence of Zinc). 100 nM of HKa, gC1qR, CK1 and suPAR were each injected separately at zero time. Each plot represents the mean of duplicate runs. Each analyte was injected for 60 seconds.

Figure 6. HKa binding protein complexes on suPAR

Sensorgram indicating lack of binding of CK1, gC1qR and suPAR to suPAR bound HKa. HKa (100 nM) in the presence of 10 μ M ZnCl₂ was first injected over an immobilised suPAR chip. Subsequent addition of 100 nM CK1, gC1qR, or suPAR did not increase the binding signal. Each analyte injection was for 50 seconds.

Table 1
Binding constants of HK and HKa to immobilised kininogen binding proteins in the presence of 10 μ

M ZnCl₂. Mean ± SD of n=3–4 of combined sensorgrams of each concentration using BiaCore Langmuir binding model (see *Materials and methods*).

	HK			HKa		
	K _D	kon	koff	K _D	kon	koff
Immobilised Protein	nM	M ⁻¹ s ⁻¹ × 10 ⁴	s ⁻¹ × 10 ⁴	nM	M ⁻¹ s ⁻¹ × 10 ⁴	s ⁻¹ × 10 ⁻⁴
gC1qR (p32)	0.8 ± 0.7	12.3 ± 5.0	0.8 ± 0.5	0.7 ± 0.5	24.4 ± 10.9	1.4 ± 0.5
CK1	15.1 ± 1.0	3.6 ± 0.3	5.4 ± 0.7	6.3 ± 1.9	5.8 ± 0.3	3.6 ± 0.9
suPAR	2,313.5 ± 1,465.3	1.0 ± 0.7	164.8 ± 22.2	43.6 ± 16.6	21.3 ± 11.9	78.5 ± 10.6