Human cytokeratin 1 (CK1) in human umbilical vein endothelial cells (HUVEC) is expressed on their membranes and is able to bind high molecular weight kininogen (HK) (Hasan, A. A. K., Zisman, T., and Schmaier, A. H. (1998) *Proc. Natl. Acad. Sci. U. S. A.*, 95, 3615–3620). Recent investigations indicate that when the proteins of the plasma kallikrein/kinin system (high molecular weight kininogen (HK), prekallikrein (PK), and factor XII (XII)) assemble on their binding sites, putative receptor(s) on endothelial cells, PK activation is required for kininogen binding to endothelial cells (7), and very few copies are found on the endothelial cell membrane (8). Furthermore, investigations have shown that endothelial cell urokinase plasminogen activator receptor (uPAR) can also serve as a kininogen-binding site on endothelial cells (9). However, this protein is not found on the platelet surface and alone cannot account for the total number of kininogen-binding sites that number 10 million versus 0.25 million for uPAR (10).

Additional studies from our own laboratory have identified that cytokeratin 1 (CK1) is a kininogen-binding protein, and CK1 is found on the surface of endothelial cells (11). The present studies map the HK binding region on CK1 and show that peptides of the kininogen-binding site on CK1 block PK activation on HUVEC. These data indicate that CK1 is a component of the kininogen multiprotein receptor which participates in PK activation.

**EXPERIMENTAL PROCEDURES**

**Materials**—A biotinylation kit and ImmunoPure streptavidin hors eradish peroxidase dihydrochloride (3,3′,5,5′-tetramethylbenzidine dihydrochloride) were supplied by Pierce. Prestained and low molecular weight standards, nitrocellulose, and polyacrylamide were purchased from Bio-Rad. Purified human cytokeratin was obtained from DAKO Corp., Carpintera, CA. Human umbilical vein endothelial cells (HUVEC), endothelial cell growth medium, and trypsin-neutralizing buffer were purchased from Clonetics, San Diego, CA. Trypsin-EDTA was obtained from Life Technologies, Inc. GST gene fusion system was purchased from Amersham Pharmacia Biotech.

**Proteins, Peptides, and Antibodies**—Single chain high molecular weight kininogen (HK) with a specific activity of 13 units/mg in 4 mM sodium acetate-HCl and 0.15 M NaCl, pH 5.3, was purchased from Enzyme Research Laboratories, Inc., South Bend, IN. HK was biotinylated according to the procedure of Pierce (see Ref. 12). Briefly, 5 mg of HK was diazylated against 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4. Sulfo-NHS-LC-biotin was added to HK to give 12-fold molar excess of sulfo-NHS-LC-biotin to HK. After incubation for 2 h in ice, the sample was then loaded onto 10 ml of Econo-Pac10 DG column (Bio-Rad). Biotinylated HK (biotin-HK) was monitored by absorbance at 280 nm using an extinction coefficient of 7.0 for HK and a protein assay (Bio-Rad). Biotin-HK had a specific activity of 17 units/mg. Low molecular weight kininogen (LK) was prepared as previously reported (7).
Mapping Binding Domains of Cytokeratin 1

Fig. 1. Diagram of exon structure of human cytokeratin 1 and overlapping recombinant proteins and peptides used to characterize the high molecular weight kininogen binding domain. The middle structure shows a schematic diagram of the first 7 exons of human cytokeratin 1 according to Johnson et al. (13). The numbers within each exon diagram represent the number of amino acids (aa) in each of the exons. The three dark lines above the exon diagram represent the span of each of three recombinant CK1s (rCK131, rCK128, and rCK114) that were used to locate the HK binding region. The three letters and numbers 20 and 20 between the heavy black lines below the exon diagram (GY20, NQS20, SRE20, VRF20, VD720, SRR20, and NMQ20) represent 7 sequential 20 amino acid peptides that partially span the protein coded by exons 1–3. The three peptides (GPV15, PGG15, and EVT14) below peptides GY20 and NQS20 represent peptides used for the fine mapping of the HK-binding site on cytokeratin 1. The full sequence of each of these peptides along with their location on cytokeratin 1 is given under “Experimental Procedures.”
human CK1 were confirmed by DNA sequence analysis.

Expression of Recombinant Cytokeratin 1 (rCK1)—The purified cDNA were cloned into the EcoRI-SalI restriction sites of vector pGEX-5X-1 (Amersham Pharmacia Biotech) and transformed into INVaF9 competent cells (Invitrogen, Carlsbad, CA). All positive colonies were screened and analyzed by restriction digest using EcoRI and SalI. Additional digestion by BglII was performed to confirm the proper orientation of the insert. Recombinant CK1 proteins were produced in Escherichia coli from pGEX-5X-1 plasmid by the GST gene fusion system (Amersham Pharmacia Biotech). Briefly, CK1 fusion proteins expression were induced by adding 0.6 mM isopropyl-D-thiogalactoside to the E. coli transformed with the pGEX-5X-1 recombinants. Induced cultures were incubated for an additional 4 h at 25–30 °C to express GST-CK1 fusion proteins. The bacteria were harvested and lysed by sonicating for 4 cycles (30 s each) at 4 °C. Solubilization of the fusion proteins was performed by 1% Triton X-100, and it was centrifuged at 10,000 × g for 10 min to remove the insoluble material and applied directly to glutathione-Sepharose 4B. The GST-CK1 fusion proteins were washed and eluted from the column by the manufacturer’s elution buffer (Amersham Pharmacia Biotech). The isolated GST-CK1 fusion proteins were digested with 1% (w/w) bovine factor Xa for 16 h at room temperature. The cleaved GST fusion protein was then re-applied to the glutathione-Sepharose 4B affinity column, and the recombinant CK1 proteins were recovered in the fractions not binding to the column, whereas the GST mostly remained bound. The recombinant proteins were confirmed to be CK1 by immunoblotting with antibodies C1801.

**Fig. 2.** Characterization of recombinant CK1. Top panel, SDS-PAGE. Two to 3 μg of three glutathione transferase (GST) recombinant cytokeratin 1 (rCK1) fusion proteins (GST-rCK114, GST-rCK128, and GST-rCK131) were subjected to 12% SDS-PAGE after reduction with 2% β-mercaptoethanol and boiling. Isolated rCK1s (rCK114, rCK128, and rCK131) separated from the GST fusion protein also were subjected to 12% SDS-PAGE and electrophoresed onto nitrocellulose paper. An immunoblot was performed with monoclonal antibody C1801 (middle panel, C 1801 Ab) and anti-CAE18 (bottom panel, Anti-CAE18) as described under the “Experimental Procedures.” The top figure is a photograph of a SDS-PAGE stained with Coomassie Blue R250, and the middle and bottom figures are photographs of autoradiograms of chemiluminescence. The numbers to the right of the gels represent molecular mass markers in kilodaltons.
and anti-CAE18. The isolated recombinant CK1s (rCK1) were named according to the size of the protein as determined by reduced SDS-PAGE.

**Endothelial Cell Culture—HUVEC** were obtained and cultured according to the recommendations of Clonetics Corp. Cells between the 1st and 5th passage were subcultured onto fibronectin-treated, 96-well plates 24 h prior to the start of the experiment as previously reported (19). Cell viability was determined using trypan blue exclusion. Cell number were determined by counting on a hemocytometer.

**Direct Biotin-HK Binding to Recombinant Cytokeratin 1—** Recombinant CK128, rCK131, and deleted-rCK131 at 1 μg/well were incubated in microtiter plate cuvette wells in 0.1 mM Na2CO3, pH 9.6, overnight at 37 °C. After blocking the wells with 0.2% BSA for 1 h at 37 °C, the wells were washed and then incubated with biotin-HK (7 nM) in 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4, containing 1% BSA and 0.05% Tween 20 in the absence or presence of a 50-fold molar excess of HK. The relative binding of biotin-HK binding to the cytokeratins was determined using ImmunoPure streptavidin peroxidase conjugate (Pierce) and peroxidase-specific fast reacting substrate, 3,3',5,5'-tetramethylbenzidine dihydrochloride (Pierce), as described previously (4). Bound biotin-HK was quantified by measuring the absorbance of the reaction mixture at 450 nm using a microplate auto reader EL 311 (Bio-Tek Instrument, Winooski, VT). Total biotin-HK binding to each recombinant protein in the presence or absence of 50 μM Zn2+ was determined and compared with the level of binding seen in the presence of zinc ion and a 50-fold molar excess of HK.

**Inhibition of Biotin-HK Binding to rCK128—** Recombinant CK128 was coupled to microtiter plates in 0.1 mM Na2CO3, pH 9.6, overnight at 37 °C. After blocking the wells with 0.2% BSA for 1 h at 37 °C, the wells were washed and then incubated with biotin-HK (7 nM) in 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4, containing 1% BSA and 0.05% Tween 20 and 50 μM Zn2+ in the absence or presence of a 50-fold molar excess of HK or various concentrations of peptides (HVL, HKH, LDC, and FNQ) or low molecular weight kininogen (LK) for 1 h at 37 °C. After blocking the wells with 0.2% BSA for 1 h at 37 °C, the wells were washed and then incubated with biotin-HK (7 nM) in the same buffer and hydrolysis proceeded for 1 h at 37 °C. Additional experiments were performed to determine if increasing concentrations of peptide PGG15 (0.003–500 μM) inhibited biotin-HK binding (7 nM) and PK activation in a concentration-dependent fashion.

**RESULTS**

**Characterization of Recombinant Cytokeratins—** In order to map the domains on CK1 which HK binds, recombinant CK1s were expressed in *E. coli* by a GST gene fusion system (Figs. 1 and 2). Since both the amino- and carboxyl-terminal globular end domains of CK1 are highly enriched with glycine, and glycine is toxic to cells, we designed recombinant proteins that avoided these regions (13, 16). Three recombinant proteins were produced. Recombinant CK114 was protein-coding by exons 3–5; rCK118 was protein partially coded by exon 1 and all of exons 2–5; and rCK131 was protein partially coded by exons 1 and 7 and all of exons 2–6 (Fig. 1). A fourth recombinant protein (deleted-6 CK118) was identical in size and immunoreactivity to rCK118 but was missing the first 6 amino acids of its amino terminus (data not shown). The name of each recombinant protein is based upon the presence or absence of the isolated fragment on 12% SDS-PAGE (data not shown). The GST-CK1 fusion proteins migrated on 12% SDS-PAGE with an apparent molecular mass of 40- (rCK118), 55- (rCK118), and 58-kDa (rCK131) protein bands when stained with Coomassie Blue (Fig. 2, top panel). Since GST migrated with the apparent molecular mass of 26–31 kDa, small amounts of GST alone comigrated with isolated rCK118 and rCK131. Therefore we examined the isolated rCK1s by immunoblot for molecular

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**Mapping Binding Domains of Cytokeratin 1**

**Table I**

The variable influence of overlapping amino acid sequences of recombinant proteins and peptides coded by exon 1 of CK1 on biotin-HK binding to endothelial cells

| Sequence | Name | IC50 μM |
|----------|------|---------|
| G137G... | Wild type | 0.4–0.5 |
| GPTSGP... | rCK131 | >30 |
| GPTSGP... | rCK131 | >30 |
| GPTSGP... | rCK131 | >30 |
| GPTSGP... | rCK131 | >30 |

| Name | IC50 μM |
|------|---------|
| GYG20 | 35 |
| GPV15 | 18 |
| PGG15 | 9–10 |
| EVT14 | >300 |

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*The numbering of the amino acid sequences shown in this table is based upon the work of Johnson et al. (12). The I50 represents the concentration of the recombinant protein or peptide needed to inhibit 50% of biotin-HK binding to HUVEC. The amino terminus of the named recombinants or synthetic peptides are shown.*
mass. Using monoclonal antibody C1801 on immunoblot, detectable major bands of the rCK1s were seen at 14, 28, and 31 kDa, respectively, corresponding to the predicted size of the recombinant CK1 proteins (Fig. 2, middle panel). Using a monoclonal antibody that is not directed to CK1 (C2931), no protein bands were seen (data not shown). Furthermore, using an affinity purified polyclonal antibody reared to a peptide from exon 5 of CK1 (anti-CAE18), the isolated rCK114, rCK128, and rCK131 were also detected by immunoblot at the predicted size (Fig. 2, bottom panel). The higher bands seen on the immunoblots with rCK128 and rCK131 with both antibodies probably represented residual intact fusion protein detected by immunoblot. These results indicated that the recombinant proteins were recognized by antibodies that identify cytokeratin 1.

**Biotin-HK Binds to Recombinant CK1**—Initial investigations determined if the rCK1s could bind HK in a Zn\(^{2+}\)-requiring mechanism as native CK1 (11) (Fig. 3). Recombinant CK1 (rCK128, rCK128, and Deleted1–6rCK131) were coupled to microtiter plates followed by treatment with biotin-HK (Fig. 3). Incubation of biotin-HK with the microtiter plates coated with the rCK131 and rCK128, but not Deleted1–6rCK131, resulted in increased specific biotin-HK binding over time only in the presence of 50 \(\mu M\) Zn\(^{2+}\) (Fig. 3). These data indicated that the HK specifically bound directly to rCK128 and rCK131 but not Deleted1–6rCK131, and the binding was Zn\(^{2+}\)-dependent. These data suggested that the six amino acids at the amino terminus of rCK131 and rCK128, but not Deleted1–6rCK131, participated in HK binding to CK1 (Table I).

**Domains of HK That Participate in Biotin-HK Binding to rCK128**—Since biotin-HK bound to certain rCK1, studies were first performed to determine which cell binding domain(s) on kinogen bound to CK1 (5, 12). Investigations determined which synthetic peptides from each cell binding region on domains 3 and 5 of kinogen blocked biotin-HK binding to rCK128 (12, 20) (Fig. 4). By using peptides from domain 5 of HK (HVL, HKH) and domain 3 of both kinogens (LDC), there was progressive inhibition of biotin-HK binding to rCK128 over a range of concentrations from 1 to 300 \(\mu M\) (Fig. 4). At 300 \(\mu M\), HVL, HKH, and LDC blocked biotin-HK binding to rCK128 by 90 \(\pm\) 3.8, 70 \(\pm\) 0.5, and 74 \(\pm\) 1.7%, respectively. Under the same conditions of this experiment, 0.5 \(\mu M\) LK blocked the biotin-HK binding to rCK128 by 77 \(\pm\) 1.4%. Alternatively, a peptide from human coagulation factor X (FNQ) had no significant inhibitory effect. These data indicated that HK interacted with rCK128 by regions on both its heavy chain domain 3 and light chain domain 5.

**Recombinant CK1 That Inhibit Biotin-HK Binding to HUVEC**—Investigations next turned to map the HK binding region on CK1. Since HK bound to CK1 (11) and rCK128 and rCK131, we postulated that rCK1s may inhibit biotin-HK binding to HUVEC if they contained a cell binding domain for kinogens. Recombinant CK128 inhibited biotin-HK binding to HUVEC with an IC\(_{50}\) of 0.4 \(\mu M\) (Fig. 5A). Alternatively, rCK114 did not inhibit binding (Fig. 5A). Recombinant CK131 also inhibited biotin-HK binding to HUVEC with an IC\(_{50}\) of 0.5 \(\mu M\) (Fig. 5B). At 1 \(\mu M\), rCK128 and rCK131 inhibited biotin-HK binding 65% and 75%, respectively (Fig. 5, A and B). Alternatively, a six-amino acid, amino-terminal deletion mutant of rCK131 (Deleted1–6rCK131) did not inhibit binding of biotin-HK to HUVEC to the same extent (Fig. 5B and Table I). Rather, 1 \(\mu M\) Deleted1–6rCK131 blocked HK binding by 25%, a level of inhibition similar to that seen with GST alone. These data suggested that protein coded by exon 1 of CK1 including the amino-terminal portion of rCK131 contained a region that participated in HK binding to CK1.

**Inhibition of Biotin-HK Binding to HUVEC by Peptides of CK1**—Investigations next were performed to map the biotin-HK binding region on CK1 by using 7 sequential 20-amino acid peptides that spanned portions of the protein partially coded by exons 1 and 3 and all of exon 2 (amino acids 137–276, Fig. 1 and Table I) (13). The peptide strategy was developed to confirm the recombinant protein strategy. On close inspection, the rCK1s began 17 amino acids carboxyl-terminal to the end of the glycine-rich region of the amino terminus of the protein coded by exon 1 of CK1 (Table I). Only peptide GYG20, which is 17 amino acids amino-terminal to the amino terminus of rCK128 and rCK131, inhibited biotin-HK binding to HUVEC with an IC\(_{50}\) of 35 \(\mu M\) (Figs. 6A, Table I). None of the remaining 6 sequential peptides, containing amino acids 157–276, significantly inhibited biotin-HK binding to HUVEC (Fig. 6, A and B). These data also suggested that a HK-binding site on CK1 was localized to protein coded by exon 1.

**Fine Mapping of the HK-binding Site on CK1**—In order to map further the sequence on CK1 that bound HK, we synthe-
sized three overlapping 15 amino acid peptides localized to the inhibitory region of the protein produced by exon 1 (Fig. 1 and Table I) and determined which peptide(s) produced the greatest inhibition of biotin-HK binding (Fig. 7). Peptides GPV15 and PGG15 inhibited biotin-HK binding to HUVEC with IC50 at 18 and 9 μM, respectively (Fig. 7A and Table I). These data indicated that a biotin-HK binding domain on HUVEC is localized to a 20-amino acid sequence on the protein coded by exon 1 of CK1. Peptides GPV15 and PGG15 also blocked biotin-HK binding to cytokeratin with IC50 of 20 and 3 μM, respectively, as shown in Fig. 7B. The next overlapping peptide, EVT14, did not achieve an IC50 at 300 μM on HUVEC or cytokeratin, respectively (Fig. 7). The specificity of these interactions was shown by the fact that a scrambled peptide of PGG15 did not inhibit biotin-HK binding to HUVEC or purified cytokeratin (Fig. 7, A and B). These data indicated that a biotin-HK binding domain for CK1 is localized to a 20-amino acid sequence between Gly143 and Gln162 on the protein coded by exon 1 of CK1.

Fig. 5. Inhibition of biotin-HK binding to HUVEC by recombinant cytokeratin 1. Monolayers of HUVEC were incubated with biotin-HK (7 nM) in the absence or presence of increasing concentrations of isolated recombinant cytokeratins or GST protein. A shows the concentration-dependent inhibition of biotin-HK binding by rCK114 (○) and rCK128 (□). B shows the concentration-dependent inhibition of biotin-HK binding by GST (□), rCK131 (○), or Deleted-rCK131 (○). The figure represents the mean ± S.E. of the percent biotin-HK bound when compared with uninhibited binding of each data point in triplicate from 3 to 5 separate experiments.

Fig. 6. Inhibition of biotin-HK binding to HUVEC by CK1 peptides. Biotin-HK (7 nM) was incubated with endothelial cells in the absence or presence of 50-fold molar excess HK or various concentrations of 20 amino acid, sequential peptides of human CK1 (A, GYG20 (□), SRE20 (○), and VRF20 (□); B, NQS20 (□), VDT20 (○), SRR20 (○), and NMQ20 (○); see “Experimental Procedures”). After incubation, the level of bound biotin-HK was determined as described under “Experimental Procedures.” The data are mean ± S.E. of triplicate determinations from three different experiments.
prepared. Increasing concentrations of biotin-GPV20 bound to HK linked to microtiter plates, and this binding was blocked by a 50-fold molar excess peptide GPV15 or HK (Fig. 8).

**Influence of CK1 Peptides on Prekallikrein Activation on HUVEC**—Previous investigations have shown that the binding of HK to endothelial cells is essential for PK activation by a cell-associated cysteine protease (1). Investigations next were performed to determine if peptides of the HK binding domain on CK1 blocked PK activation on endothelial cells with peptides of its binding domain on CK1 blocked PK activation on endothelial cells.

Further studies were performed to determine if there was a correlation between inhibition of biotin-HK binding to HUVEC and inhibition of prekallikrein activation by peptide PGG15 (Fig. 9B). As the concentration of peptide PGG15 increased from 0.003 to 100 μM, there was a progressive decrease in prekallikrein activation and HK binding (Fig. 9B). The IC₅₀ of peptide PGG15 on prekallikrein activation and HK binding was 1 and 10 μM, respectively. These data indicated that at 3 μM peptide PGG15, there was over 80% inhibition of PK activation when there was about 20% inhibition of HK binding.

**DISCUSSION**

This study has four major findings. First, synthetic peptides of domains 3 and 5 of HK inhibited the biotin-HK binding to rCK128. These data are similar to those found previously that HK interacts with purified cytokeratin by the same regions of the protein (i.e. its heavy and light chain) that interact with endothelial cells (11, 12, 20). Second, rCK1₂₈ and rCK1₃₁ are inhibitors of biotin-HK binding to HUVEC with IC₅₀ of 0.4 to 0.5 μM. Previously, we reported that commercially prepared cytokeratin partially blocked biotin-HK binding to HUVEC (11). The present data confirm those findings and suggest that the HK binding competitor may be localized to a portion of cytokeratin 1 coded by exon 1. Further investigations revealed that a biotin-HK binding domain on cytokeratin 1 is localized to 20 amino acids (Gly¹⁴³ to Gln¹⁶²) beyond the glycine-rich region.
in the globular region of human CK1 of the protein coded by exon 1. Last, peptides of the HK binding region on cytokeratin 1 interfere with prekallikrein activation on endothelial cells (1, 3). These data confirm that HK binding to HUVEC is an essential step for endothelial cell prekallikrein activation (1, 3).

Both the amino- and carboxyl-terminal globular end domains of CK1 are known to be enriched with glycine. CK1 is known to be insoluble because of its glycine-rich regions (21). Preparation of the full-length recombinant CK1 and peptides extending through the glycine-rich region for this investigation was not feasible. We succeeded in preparing recombinant protein fragments of CK1 carboxyl-terminal to its glycine-rich region. It was helpful to find that both rCK131 and rCK128 directly bind to HK and inhibit biotin-HK binding to HUVEC. These data

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**FIG. 9. Influence of CK1 peptides on PK activation on HUVEC.** A, confluent monolayers of endothelial cells were incubated with 20 nM HK in the absence (HK±PK) or presence of 100 or 500 μM GYG20, GPV15, or PGG15. After incubation, the cells were washed, and PK (20 nM) was added. The cells then were washed again and 0.4 mM H-d-Pro-Phe-Arg-p-nitroanilide was added and hydrolysis of the substrate was measured for 1 h. B, confluent monolayers of endothelial cells were incubated with 20 nM HK or biotin-HK in the absence or presence of increasing concentrations of PGG15 for 1 h at 37 °C. After incubation, the cells were washed, and those wells that were incubated with unlabeled HK received PK (20 nM) for an additional hour of incubation. These cells then were washed again, and 0.4 mM H-d-Pro-Phe-Arg-p-nitroanilide was added in the same buffer, and hydrolysis of the substrate (PK activation) was measured for an hour. After the first incubation, those wells that received biotin-HK (○, % biotin-HK bound) were examined for the level of bound biotin-HK as described under “Experimental Procedures.” In both panels, the data represent the mean ± S.E. of three triplicate experiments.
suggest that the six amino acids ( . . . VTINQS . . . ) seen in rCK1\textsubscript{31} but not Deleted1\textsubscript{a}rCK1\textsubscript{31} participate in HK binding to isolated cytokeratin and cytokeratin on HUVEC (11). The observation that inhibition of biotin-HK binding by rCK1\textsubscript{31} and rCK1\textsubscript{316} is not stronger is probably due to the fact that only a small portion of the domain on CK1 that HK binds is actually contained in these recombinant proteins. Combining synthetic peptide studies with the recombinant protein data serves to define better the region on the protein coded by exon 1 of CK1 that interacts with HK. By using native sequences, scrambled peptides, and biotinylated peptides of CK1, we are able to localize a 20-amino acid region from Gly\textsuperscript{143} to Gln\textsuperscript{162} which binds HK. Since we were unable to prepare either peptides or recombinant proteins more amino-terminal to this region, we cannot exclude an additional role of the first 136 amino acids of CK1 in HK binding. Our data map one site on CK1 where HK cannot exclude an additional role of the first 136 amino acids of recombinant proteins more amino-terminal to this region.

The possible role of CK1 in the plasma kallikrein/kinin system was recognized by our observation that biotin-HK binds to commercially prepared cytokeratin in a concentration-dependent fashion in the presence of Zn\textsuperscript{2+} (11). In addition to showing that various forms of recombinant CK1 interfere with HK binding to HUVEC, the present report shows that PK activation is inhibited by peptides of CK1 that block HK from binding to endothelial cells. Inhibition of prekallikrein activation by CK1 peptides of the cell binding region of HK occurred at a 10-fold lower concentration than inhibition of HK binding itself. It is not known at this time whether the effect of the CK1 peptide to block PK activation is merely the inhibition of HK binding to HUVEC, an essential requirement for PK activation on these cells, or an actual interference with PK activation that is signaled through CK1 on the membrane of HUVEC (1, 3).

The finding, however, that a 3 μM concentration of peptide PGG15 blocks 80% of prekallikrein activation while it only inhibits 20% of HK binding suggests that HUVEC CK1 may participate in the events that lead to PK activation (1). These data also suggest that modulation of expression of CK1 could result in regulation of prekallikrein activation and thus any proteolytic reactions dependent upon it, i.e. bradykinin liberation and factor XII and single chain urokinase activation (1, 3). This interpretation indicates a new regulatory mechanism of the plasma kallikrein/kinin system.

The present report solidifies the recognition of CK1 as a kininogen-binding protein, putative receptor, on the membranes of HUVEC. Recent preliminary studies from another laboratory (22) also confirm that HK binds to CK1. CK1 along with gc1qR and urokinase plasminogen activator receptor may constitute a multiprotein receptor complex for kininogens on cells in the intravascular compartment (23). Regulation of kininogen binding on cells in the intravascular compartment may have a number of consequences on vascular biology. The presence of HK on endothelial cells is a necessary component for PK assembly and activation (1–3). PK activation on endothelial cells results in bradykinin liberation, a potent mediator of vascular cell stimulation, and cellular fibrinolysis independent of tissue plasminogen activation and fibrin (1–3). Further investigations are needed to determine how the binding of the HK-PK complex to CK1 initiates prekallikrein activation.

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