Mapping of the Discontinuous Kininogen Binding Site of Prekallikrein

A DISTAL BINDING SEGMENT IS LOCATED IN THE HEAVY CHAIN DOMAIN A4*

(Received for publication, December 29, 1995, and in revised form, February 29, 1996)

Heiko Herwaldt‡, Thomas Renné, J oost C. M. Meijer‡, Dominic W. Chung, J immy D. Page†‡, Robert W. Colman**, and Werner Müller-Esterl††§§

From the Institut für Physiologische Chemie und Pathobiochemie, Johannes Gutenberg University at Mainz, Duesbergweg 6, D-55099 Mainz, Germany, the §Department of Pathology, University Hospital Utrecht, P. O. Box 85500, 3508 GA Utrecht, The Netherlands, the ¶Department of Biochemistry, University of Washington, Seattle, Washington 98195, and the §§Sol Sherry Thrombosis Research Center, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

Prekallikrein, the precursor to the serine proteinase kallikrein, circulates in plasma in an equimolar complex with H-kininogen. The binding to H-kininogen heavy chain consisting of four "apple" domains, A1-A4, which attaches to H-kininogen with high specificity and affinity (Kd = 83 nM). At least two distinct portions of the kallikrein heavy chain form this H-kininogen binding site: a proximal segment located in the NH2-terminal fragment of the heavy chain encompassing A1, and distal segment(s) located in COOH-terminal fragment spanning domains A2-A4. The proximal binding segment has been located to amino acid positions 56–86 of A1. To precisely map the distal binding segment, we have identified monoclonal antibodies directed to the COOH-terminal fragment which interfere with the H-kininogen-prekallikrein complex formation. Monoclonal antibody 13G11 binds to recombinant apple domain A4 but not to domain A3 of the prekallikrein heavy chain. Deletion mutagenesis of domain A4 narrowed down the target epitope of 13G11 to the center portion of domain A4, positions 284–331. Direct binding studies of H-kininogen to various domain A4 constructs revealed that the distal H-kininogen binding segment is located on a segment of 48 residues, which overlaps the 13G11 epitope. Hence the tight interaction of H-kininogen and prekallikrein is mediated by at least two separate sequence segments located in domains A1 and A4, respectively, of the prekallikrein heavy chain. The isolated distal binding segment significantly prolongs the partial thromboplastin time of reconstituted Williams plasma thus stressing the critical role of the prekallikrein-H-kininogen complex formation in the initiation of the endogenous blood coagulation cascade.

Plasma prekallikrein, the 86-88-kDa precursor to the serine protease, α-kallikrein, participates in the inflammatory response (1), in prourokinase-dependent fibrinolysis (2), and in blood coagulation on artificial surfaces (3). Factor XIIa converts prekallikrein to the active protease, α-kallikrein, on cellular and artificial surfaces via a single cleavage at position 371. This cleavage generates a heavy chain of 50 kDa and a light chain of 35 kDa (4). The light chain harbors the catalytic domain, whereas the heavy chain binds to the non-enzymatic cofactor, H-kininogen. Sequence analysis has revealed that the heavy chain consists of four repetitive units, "apple" domains A1-A4, characterized by a unique disulfide loop pattern (5). Each apple domain comprises 90–91 amino acid residues; the only other known protein containing apple domains is the heavy chain of factor XI (6). Autocatalytic cleavage of α-kallikrein at Lys140-Ala141 generates β-kallikrein, with two heavy chain portions, Gly1-Lys140 ("fragment N") and Ala141–Arg371 ("fragment C"), connected by two disulfide bridges (5, 7, 8).

Prekallikrein circulates in plasma as a heterodimeric complex with the non-enzymatic cofactor, H-kininogen (Kd = 8.3 × 10⁻⁵ M⁻¹) (9). The prekallikrein binding site on H-kininogen has been mapped to a "continuous" sequence segment of 25 amino acid residues located at the extreme carboxy-terminal part of the H-kininogen light chain (10–12). The corresponding H-kininogen binding site on prekallikrein is located in the heavy chain region (8, 13). At least two separate sequence segments, namely fragment N and fragment C of β-kallikrein, contribute to this binding site: an amino-terminal ("proximal") segment has been mapped to domain A1, positions Phe66–Gly80 of fragment N (14, 15), whereas the precise location of the carboxy-terminal ("distal") binding segment of fragment C remains unclear. Binding studies with synthetic peptides have indicated that at least one portion of the A4 domain might be involved in the binding (15).

The present study was undertaken to define the distal H-kininogen binding site(s) of the prekallikrein heavy chain. Employing an antibody-directed strategy, we have identified a critical segment of 48 residues of domain A4 that contributes to the binding of H-kininogen. Our results suggest that at least two separate regions of the kallikrein heavy chain, domains A1 and A4, contribute to the H-kininogen binding site, thereby mediating the interaction of the prehormone

*This work was supported in parts by Grant Mu 598/4-1 from the Deutsche Forschungsgemeinschaft, Grant 163323 from the Fonds der Chemischen Industrie (to W. M. E.), National Institutes of Health Grant HL16929 (to D. W. C.), and a fellowship from the Royal Netherlands Academy of Arts and Sciences (to J. C. M. M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Present address: Institute of Cell and Molecular Biology, Section of Molecular Pathogenesis, Lund University, P. O. Box 94, S-222 22 Lund, Sweden.
‡ Present address: Cardiovascular Diseases Research Dept., G. D. Searle, 4901 Searle Pkwy., Skokie, IL 60031.
†† Present address: Cardiovascular Diseases Research Dept., G. D. Searle, 4901 Searle Pkwy., Skokie, IL 60031.
§§ To whom correspondence should be addressed: Institute of Physiological Chemistry and Pathobiochemistry, Johannes Gutenberg University at Mainz, Duesbergweg 6, D-55099 Mainz, Germany. Tel.: 49-6131-395890; Fax: 49-6131-395792; E-mail: mueller@mzdmza.zdv.uni-mainz.de.
and its processing enzyme in the fluid phase and on the cell membrane.

**EXPERIMENTAL PROCEDURES**

Sources of Proteins and Peptides—Prekallikrein was isolated from human plasma following established protocols (16). Factor XII was from Enzyme Research Laboratories, South Bend, IN. H-kininogen was purified from human plasma (17) with minor modifications described elsewhere (18). The generation and characterization of monoclonal antibodies PKH1, PKH4, PKH6 (16), PKH3 (14), and 130G1 (8, 19) has been detailed previously. Proteins were desalted and dialyzed as described (16) except that the final buffer change was made to 150 mM NaCl, 10 mM NaH2PO4, 10 mM Na2HPO4, pH 7.4, by centrifuging three times at 10,000 × g for 10 min. The low molecular weight electrophoresis calibration kit was from Pharmacia Biotech Inc. The resolved proteins were visualized by the silver staining technique (21) or transferred to nitrocellulose at 100 mA for 30 min by the semi-dry technique (22). The membranes were blocked with 50 ml KH2PO4, 2 mM NaCl, pH 7.4, containing 5% (v/v) dry milk powder, for 1.05% (w/v) Tween 20 (buffer A). Immunostaining of the purified proteins was done according to Towbin et al. (23) with modifications specified elsewhere (11). Typically the first antibody was diluted 1:1000 in buffer A. Bound antibody was detected by a horseradish peroxidase-coupled secondary antibody against rabbit or mouse immunoglobulin, followed by the chemiluminescence detection according to the manufacturer's instructions (Amersham Corp.).

Limited Proteolysis of Prekallikrein—To produce α- and β-kallikrein, 100 μg of human prekallikrein and 4 μg of human factor XIIa (molar ratio 1:50) were dissolved in 250 μl of 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, and incubated for 3 days at 37°C. Aliquots of the reaction mixture were removed after 2 h and 72 h. Analysis of the reaction mixture showed that the majority of the kallikrein was converted to kallikrein fragments in 100 mM sodium acetate, 100 mM NaCl, pH 5.5, was used for amino-terminal sequence analysis, the proteins were electrophoresed on polyvinylidene difluoride sheets and microsequenced by Edman degradation using a 470A pulsed-laser phase sequencer (Applied Biosystems).

Indirect and Competitive ELISAs—The indirect ELISA was performed essentially as described previously (14). A 10–20 μg/ml stock solution of fragments in 100 mM sodium acetate, 100 mM NaCl, pH 5.5, was used for coating. The probe was 5 μg/ml H-kininogen in the same buffer, and monoclonal antibody HKH14 (4.5 μg/ml) against the H-kininogen heavy chain (25) served as the reporter antibody. To test for the interference of unlabeled antibodies with the H-kininogen-prekallikrein complex formation, the same ELISA was employed except that biotinylated H-kininogen (0.5 μg/ml) served as the reporter. For the mutual displacement of monoclonal antibodies from the prekallikrein heavy chain, the ELISA in the competitive mode was applied (16) using biotinylated antibody 13G11 (1 μg/ml) as the probe.

Expression of Apple-Tissue Plasminogen Activator (tPA) Fusion Proteins in Baby Hamster Kidney Cells—The A3 (Gly179–Glu271) and A4 (Pro272–Ser262) domains of prekallikrein with flanking 5′ and 3′ XhoI sites were amplified by the polymerase chain reaction (PCR) with Taq polymerase. The PCR products were cloned into the TA cloning vector pCR1 (Invitrogen), and the sequence was verified by dideoxy sequencing. The DNA segments corresponding to the A3 and A4 domains were excised by digestion BgIII and XhoI and cloned into the HindIII and the restriction enzyme-modified TPA expression vector Z-plasmid (ZpLM(75478BA) (26, 27). The constructs encode fusion proteins, which contain the signal sequence and propeptide of tPA, followed by the prekallikrein apple domain A3 or A4, kringle 1 and kringle 2 domains of tPA, and the active site-mutated tPA light chain. The expression plasmids were transfected into BHK cells as described previously (28). The fusion proteins were expressed in serum-free medium (Oph MEM, Life Technologies, Inc.), and purified with a monoclonal antibody to tPA as described (27).

Expression of Recombinant Apple Domains in E. coli—The pMAL™-c2 (New England Biolabs) expression vector was used for the expression of fusion proteins consisting of the maltose-binding protein (MBP) and apple domain A4 constructs in Escherichia coli (29). PCR with Taq polymerase was applied to generate DNA fragments encoding portions of prekallikrein domain A4 using upstream and downstream specific primers, respectively (Roth, Karlsruhe, Germany), 5′-CTCTTGTGGATATAGCCTCTGC-3′ and 5′-GGAAGCTTATTTTGTGTTGACGACGACGACGACGAGATCGTTATT-3′ (construct A4); 5′-GGTCTCTTTGACCCTTTTGGGACGACGACGACGAGATCGTTATT-3′ (construct A4.1); 5′-GCAAGCTTATTTTGTGTTGACGACGACGAGATCGTTATT-3′ (construct A4.2); 5′-GCAAGCTTATTTTGTGTTGACGACGACGAGATCGTTATT-3′ (construct A4.3); 5′-GCAAGCTTATTTTGTGTTGACGACGACGAGATCGTTATT-3′ (construct A4.4); 5′-GCAAGCTTATTTTGTGTTGACGACGACGAGATCGTTATT-3′ (construct A4.5). Plasmid pPK was used as the template for the polymerase chain reactions; 40 cycles of denaturation at 95°C for 1 min, annealing at 45°C for 2 min, and extension at 72°C for 3 min were done in a thermal cycler (Perkin Elmer). To insert the A4 DNA fragments into the pMAL™-c2 plasmid, the vector and the constructs were cleaved by the restriction enzymes XbaI and HindIII for constructs A4, A4.2, and A4.4; XbaI and PstI for A4.1; BamHI and HindIII for A4.3; and PstI and BamHI for A4.5. The E. coli strain XLI-Blue was employed for recombinant expression of the domain A4 variants.

Purification of Domain A4 Variants—The bacteria transformed with the prekallikrein fusion constructs were stimulated with 0.3 mM isopropyl-p-thio-β-thiogalactopyranoside for 2 h, followed by centrifugation at 4,000 × g for 10 min at room temperature. The resulting pellet was resuspended in 10 mM Tris, pH 7.4, 200 mM NaCl, 1 mM EDTA, 10 μg/ml benzamidine hydrochloride, 10 μg/ml phenylmethylsulfonyl fluoride, and sonicated in short pulses for 3 min. The suspension was centrifuged at 15,000 × g for 20 min at 4°C. The supernatant was removed and transferred to an amyllose affinity matrix (New England Biolabs). Unbound proteins were washed off with 10 mM Tris, pH 7.4, 200 mM NaCl, 1 mM EDTA, 0.1% benzamidine hydrochloride, 10 μg/ml phenylmethylsulfonyl fluoride. Proteins bound to the column via MBP were eluted with the same buffer including 10 mM maltose. The eluted proteins were separated from maltose by gel filtration over Superdex 200 (Pharmacia) using 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, as the effluent. The purification procedure was monitored by SDS-PAGE.

Partial Thromboplastin Time—To study the effect of kallikrein heavy chain fragments on the kaolin-activated partial thromboplastin time, we used the original assay (30) with modifications described previously (14). Typically 50 μl of kininogen-deficient human plasma (Williams trait) reconstituted with 0.5 μg of purified H-kininogen in the presence or absence of 200 μg of recombinantly expressed kallikrein A4 domain variants were used, and the assay was performed on an Amelung coagulometer.

**RESULTS**

Our strategy to localize the distal H-kininogen binding segment of the prekallikrein heavy chain involved: (i) the selection of monoclonal antibodies that bind to fragment C and interfere with the H-kininogen-prekallikrein complex formation, (ii) the precise mapping of the target epitope(s) of the selected antibodies, (iii) the recombinant expression of prekallikrein heavy chain fragments encompassing the relevant epitope, (iv) the analysis of the binding capacity of the recombinant constructs for H-kininogen, and (v) the identification of the critical binding segment and its application to a biological test.

Monodonal Antibodies Interfering with H-Kininogen-Prekallikrein Complex Formation—In previous studies we have described a panel of 20 monoclonal antibodies to prekallikrein (14, 16, 19); of these, 11 antibodies were found to be directed to the kallikrein heavy chain. Ten antibodies have been mapped to four distinct epitope classes exposed on the kallikrein heavy chain, arbitrarily named A–D. To test whether these antibodies interfere with H-kininogen-prekallikrein complex formation, we established a competitive ELISA where prekallikrein was immobilized on the titer plate and biotinylated H-kininogen served as the probe. Increasing concentrations of monoclonal antibodies PKH1 (epitope class A), PKH4 (B),
PKH6 (C), PKH19 (D), and 13G11 (unclassified) were applied and tested for their capacity to prevent H-kininogen binding to prekallikrein (Fig. 1A). Antibody PKH6 almost completely blocked H-kininogen binding with an apparent IC50 of 3.9 nM. Antibodies PKH1 and 13G11 prevented binding of biotinylated H-kininogen with IC50 values of 42 and 59 nM, respectively, whereas anti-peptide antibody PKH19 was less effective with an IC50 of 760 nM, and PKH4 did not interfere at all, IC50 > 1 µM.

Epitope Classification of Antibody 13G11—The observation that antibody 13G11 efficiently inhibited H-kininogen binding to prekallikrein prompted us to identify its epitope class. The competitive ELISA was modified such that titer plate-bound prekallikrein was probed by the biotinylated antibody 13G11 in the presence of increasing concentrations of antibodies PKH1 (epitope class A), PKH4 (B), PKH6 (C), and PKH19 (D) (Fig. 1B). As a control unlabeled antibody 13G11 was applied and found to displace its biotinylated homologue almost completely. Similarly antibody PKH1 inhibited binding of biotinylated 13G11 in a concentration-dependent manner, whereas antibodies PKH4, PKH6, and PKH19 were without effect. Hence antibodies PKH1 and 13G11 are members of the same epitope class, A, suggesting that they might recognize overlapping, if not identical target sequences in the prekallikrein heavy chain. Other members of class A epitope-specific antibodies are PKH2, PKH8, and PKH9 (16), indicating that their target sequence(s) might represent a major immunogenic epitope of the prekallikrein heavy chain.

Identification of Antibodies Directed to Fragment C—To identify antibodies that interfere with complex formation and bind to fragment C containing the distal H-kininogen binding site, we employed immunoprinting. A mixture of α-kallikrein and β-kallikrein was separated by SDS-PAGE under reducing conditions, electroblotted on nitrocellulose, and probed by the various antibodies (Fig. 2). For control a polyclonal antibody was used (lane 1), which detected multiple kallikrein fragments. All of the monoclonal antibodies bound to the kallikrein heavy chain, although with varying intensities. Antibodies PKH1 (lane 2), PKH4 (lane 3), and 13G11 (lane 6) recognized fragment C, and PKH19 (lane 5) bound to fragment N, whereas PKH6 (lane 4) decorated the heavy chain but failed to detect a heavy chain breakdown product. Hence epitope classes A and B are located on fragment C, whereas epitope class D is positioned on fragment N; the relative position of epitope class C remains unknown.

Epitope Mapping Using Cyanogen Bromide Cleavage Products of Prekallikrein—Our results indicate that epitope class A antibodies 13G11 and PKH1 might be useful probes for the localization of the distal H-kininogen binding segment because they (i) interfere efficiently with the H-kininogen-prekallikrein complex formation and (ii) bind to fragment C of the kallikrein heavy chain. To characterize their target sequence(s) more precisely, prekallikrein was chemically cleaved by cyanogen bromide. The resultant fragments were reduced and separated by SDS-PAGE; four identical blots on nitrocellulose were prepared, and the transferred proteins were probed by the anti-prekallikrein antibodies described above (Fig. 3). Control antibodies PKH6 (lane 3) and PKH19 (lane 4) were applied, which either failed to recognize a cleavage product (PKH6) or produced a complex staining pattern (PKH19). Antibody 13G11 (lane 2) recognized two CNBr cleavage products of 19 and 23 kDa, respectively. Antibody PKH1 (lane 1) bound to the same fragments, although with different intensity. These results suggest that its target sequences of PKH1 and 13G11 might be overlapping, although not necessarily identical. Edman degradation of the 19-kDa fragment, which was readily detected by both antibodies, revealed a single amino-terminal sequence of Asn-Ile-Phe-Gln-His-Leu-Ala. This heptapeptide maps to positions 185–191 located in the center portion of domain A3. Considering the size of the 19-kDa fragment and that of a single apple domain (~10 kDa corresponding to 90–91 residues), we conclude that the relevant epitopes for 13G11 and PKH1 must be located in domains A3 and/or A4 of the prekallikrein heavy chain.
A4—To identify the target domain(s) of antibodies 13G11 and PKH1, domains A3 (Gly179–Glu271) and A4 (Pro272–Ser362) were expressed in baby hamster cells in the form of fusion proteins with tPA (27). The purity of the isolated fusion proteins was tested by SDS-PAGE under reducing conditions (Fig. 4, lanes 1 and 2). Immunoprint analysis demonstrated that antibodies 13G11 (lanes 3 and 4) and PKH1 (lanes 5 and 6) bound to tPA-A4 but not to tPA-A3. The same antibodies failed to bind to wild-type tPA (data not shown). Together these experiments demonstrate that antibodies 13G11 and PKH1 bind to overlapping although distinct target sequences of prekallikrein’s domain A4.

Epitope Mapping Using Truncated Domain A4 Constructs—For precise epitope mapping, we chose an expression system in E. coli, where domain A4 and its truncated versions were fused to the maltose binding protein, MBP. Initial experiments indicated that MBP-A4 (Thr257–Arg371) but not MBP-A3 (Gly179–Thr257) or MBP alone was recognized by 13G11 and PKH1 (data not shown) indicating that post-translational modifications such as glycosylation and/or disulfide bridging are not critical to antibody binding. Apple domain constructs were engineered such that domain A4 was either truncated at the carboxyl terminus (MBP-A4.1, Thr257–Arg31) or Escaped from antibody detection (data not shown). Results with antibody PKH1 were indistinguishable from those obtained with 13G11 (data not shown). Therefore we conclude that 13G11 and PKH1 are directed to the center portion of domain A4 which covers residues 284 to 331. Our attempts failed to further narrow down the target sequences of 13G11 and PKH1 by a synthetic peptide library covering the relevant sequence segments (data not shown).

Mapping of the Distal H-kininogen Binding Site by Domain A4 Constructs—The recombinant expression of overlapping sequences encompassing the 13G11 epitope allowed us to test these segments for their H-kininogen binding capacity. To this end, a direct binding assay was designed where fixed molar concentrations of the MBP fusion proteins of domain A4 and its truncated versions, A4.1 to A4.5, were used to coat titer plates, followed by H-kininogen, a monoclonal antibody to the heavy chain of H-kininogen, HKH14, and a peroxidase-coupled secondary antibody. The change in absorbance at 405 nm was followed; all values were corrected for the nonspecific binding of H-kininogen to wild-type MBP (≤20% of the maximum binding). For control, the intact heavy chain of prekallikrein including domains A1–A4 was recombinantly expressed as a MBP fusion protein and compared to the native β-kallikrein. Under these conditions H-kininogen bound readily to the recombinant heavy chain of prekallikrein (Fig. 6, column 2), although with less affinity than to β-kallikrein (column 1). Recombinant domain A4 (column 3) bound significant amounts of H-kininogen as did the truncated versions A4.1, A4.2, and A4.3 (columns 4, 5, and 6) but not A4.4 (column 7). Therefore, we conclude that the distal binding segment of the prekallikrein heavy chain is located in the center portion of domain A4, Gly284–Arg331, where it overlaps the epitope of monoclonal antibodies 13G11. We were unable to map more accurately the
Functional Interference of Domain A4 Fragments with Partial Thromboplastin Time—Given that domain A4 and its fragments bind to H-kininogen without conveying an enzymatic function to the resulting complex, one might expect that domain A4 constructs interfere with the physiological interaction of H-kininogen with prekallikrein in surface-dependent blood coagulation. To test this hypothesis, we employed the kaolin-activated partial thromboplastin time (aPTT) assay using a kininogen-deficient plasma (Williams trait) (Fig. 7). This plasma was reconstituted with a limited amount of purified H-kininogen, and the competition of recombinant fusion proteins MBP-A4 and its fragments with plasma kallikrein for the limited amount of H-kininogen was measured by a prolongation of the aPTT time. Recombinant MBP alone had no effect on the aPTT of reconstituted Williams plasma (column 9). MBP-A4 efficiently competed with plasma prekallikrein and caused a significant prolongation of the aPTT (column 3), as did MBP-A4.1, and less efficiently MBP-A4.2, MBP-A4.3, and MBP-A4.4 (columns 4-8). These findings support our notion that A4.5 is the minimum fragment which causes a significant prolongation of the aPTT (column 9), added to Williams plasma, and the clotting times were measured as above.

Binding Properties of Heavy Chain Variants—Our previous work (14, 15) and this study suggest that a “discontinuous” binding site for H-kininogen is formed by at least two distinct portions located on domains A1 and A4 of the kallikrein heavy chain. To address if a proteolytic cleavage between domains A1 and A4 would influence the relative binding affinity for H-kininogen, we tested various forms of kallikrein including prekallikrein (column 1), MBP fused to the kallikrein heavy chain (column 2), MBP-A4 (column 3), MBP-A1 (column 4), MBP-A4.2 (column 5), MBP-A4.3 (column 6), MBP-A4.4 (column 7), or MBP-A4.5 (column 8). H-kininogen purified from human plasma (42 nM corresponding to 5 µg/ml) was added, followed by the monoclonal antibody HKH14 to the kininogen heavy chain (27 nM = 4 µg/ml). Bound antibody was detected by a peroxidase-labeled secondary antibody. The nomenclature of the domain A4 constructs is that of Fig. 5.

An ELISA was set up where titer plates were coated with 200 nM of β-kallikrein (column 1), MBP fused to the kallikrein heavy chain (column 2), MBP-A4 (column 3), MBP-A1 (column 4), MBP-A4.2 (column 5), MBP-A4.3 (column 6), MBP-A4.4 (column 7), or MBP-A4.5 (column 8). H-kininogen purified from human plasma (42 nM corresponding to 5 µg/ml) was added, followed by the monoclonal antibody HKH14 to the kininogen heavy chain (27 nM = 4 µg/ml). Bound antibody was detected by a peroxidase-labeled secondary antibody. The nomenclature of the domain A4 constructs is that of Fig. 5.
complex protein is prekallikrein, which most likely arose by fusion of a particular motif, the “apple” domain, to a catalytic domain, and by quadruplication of the exon set specifying the apple domain. In this way a multidomain protein was generated which subserves functions such as kinin release, initiation of the endogenous blood coagulation cascade, stimulation of the profibrinolytic pathway (1), and stimulation of neutrophils (31).

Sequence analysis has revealed that prekallikrein is 58% identical on the protein sequence level with another plasma protein, factor XI (32). Prekallikrein and factor XI share their overall structure, except that the latter is a homodimer with the two disulfide-bridged heavy chains connected in the A4 domains (5, 27, 28). Factor XI has developed multiple interaction sites, which have been analyzed in great detail by Walsh and co-workers: (i) domain A1 binds H-kininogen and thrombin (33, 34); (ii) domain A2 exposes a substrate binding site for factor IX (35); (iii) domain A3 serves as the cell binding site (36); and (iv) domain A4 provides a factor XII recognition site (37). Some of these interactions are not found in prekallikrein, e.g. the recognition of factor IX; others are yet ill-defined, e.g. the interaction with factor XII (8); and still others are modified, e.g. the binding of H-kininogen.

The detailed analysis of the interaction of prekallikrein with H-kininogen reveals that at least two domains of the heavy chain, namely A1 (14, 15) and domain A4 (this work), are involved in H-kininogen binding. Our present findings suggest a “discontinuous” binding site formed by two segments, which are almost 200 residues apart in the primary sequence. We cannot entirely exclude the possibility that the “bridging” domains, A2 and A3, also contribute directly or indirectly to H-kininogen binding although the failure of recombinantly expressed A3 to bind H-kininogen (data not shown) does not support such a view. Thus prekallikrein and factor XI, although similar in structure, seem to have evolved distinct H-kininogen interaction sites(s). This notion is corroborated (i) by the observation that their corresponding binding sites on the H-kininogen light chain are overlapping although not identical (10); and (ii) by the finding that a synthetic peptide spanning positions 317 to 350 of domain A4 of factor XI is without effect on the complex formation between H-kininogen and factor XI (38). It is tempting to speculate that, in the case of factor XI, two A1 domains contributed by the two heavy chains in the dimeric molecule might suffice to secure binding to H-kininogen, whereas in the monomeric prekallikrein, two apple domains in the same chain must interact to tether this cofactor with sufficient affinity.

In this work we have used recombinant prekallikrein domains expressed in eukaryotic and in prokaryotic systems. Similar binding characteristics were observed for the constructs made in kidney cells or in E. coli, implying that the expressed sequence segments are able to spontaneously acquire a “correct” folding. This finding is unexpected because reduction and denaturation of prekallikrein result in a complete loss of its H-kininogen binding properties (16). The cysteine-rich core of apple domain A4 seems to be of critical importance for the formation of an “active” binding site. These notions are consistent with the finding that a synthetic peptide, which spans the segment Leu262–Gly295 of prekallikrein domains A3 and A4 and overlaps the G1v284–Arg331 segment by 12 residues, shows binding affinity for H-kininogen (15). It should be noted that in the latter study the H-kininogen was present on the surface of a microtitre plate, where its conformation may have been altered.

The precise functional role of the circulating complex of prekallikrein and H-kininogen is still obscure. Recent studies have demonstrated that these two proteins are present on the surface of cardiovascular cells such as neutrophils (39, 40) and endothelial cells (41). Prekallikrein and H-kininogen, possibly in concert with other proteins such as U-kininogen and factor X, are likely to subserve the local kinin release on endothelial cells and circulating blood cells (18, 42, 43). The potent biological effects exerted by kinins and their extremely short half-life in plasma (8–15 s) demand for efficient control mechanisms to initiate, sustain, and terminate the cell-associated kinin generation. A powerful mechanism controlling kallikrein activity is provided by the endogenous inhibitors such as C1-inhibitor and α2-macroglobulin (44). Our finding that the proteolytic cleavage of the kallikrein heavy chain drastically reduces its ability to bind H-kininogen might provide yet another regulatory mechanism. If H-kininogen is the major anchoring protein for (pre)kallikrein on cell surfaces, the loss of the intrinsic binding affinity for kininogen would result in a rapid release of the processed kallikrein from the cell surface, thereby terminating local kinin release. This notion is supported by the finding that the procoagulant activity of kallikrein is critically dependent on the integrity of its heavy chain (45). Likewise the blockage of the H-kininogen binding site in prekallikrein by synthetic peptides or monoclonal antibodies drastically reduced its procoagulant activity (14, 15). We envisage that the assembly and disassembly of the prekallikrein-H-kininogen complex on the cellular H-kininogen-binding protein is a critical factor in the regulation of circumscribed kinin release.

Acknowledgments—We are thankful to Dr. J. Godovac-Zimmermann, Institute of Molecular Biotechnology, Jena, Germany, for sequence analysis; to Dr. G. Hafner, University of Mainz, for experimental help with the aPTT assay; and to Dr. J. Hock, Behring Inc., Marburg, for stimulating discussions.

REFERENCES

1. Kaplan, A. P., and Silverberg, M. (1987) Blood 70, 1–15
2. Ichinose, A., Fujikawa, K., and Suyama, T. (1986) J. Biol. Chem. 261, 3486–3489
3. Cochrane, C. G., Revak, S. D., and Wuepper, K. D. (1973) J. Exp. Med. 138, 1546–1583
4. Mandle, R. J., and Kaplan, A. P. (1977) J. Biol. Chem. 252, 6097–6104
5. McMullen, B. A., Fujikawa, K., and Davie, E. W. (1991) Biochemistry 30, 2050–2056
6. Chung, D. W., Fujikawa, K., McMullen, D. A., and Davie, E. W. (1986) Biochemistry 25, 2410–2417
7. Berger, D., Schleuning, W. D., and Schapira, M. (1986) J. Biol. Chem. 261, 324–327
8. Page, J. D., and Colman, R. W. (1991) J. Biol. Chem. 266, 8143–8148
9. Mandle, R. J., Colman, R. W., and Kaplan, A. P. (1977) Proc. Natl. Acad. Sci. U.S.A. 73, 4179–4183
10. Tait, J. F., and Fujikawa, K. (1987) J. Biol. Chem. 262, 11651–11656
11. Vogt, R., Kaufmann, J., Chung, D. W., Keilermann, J., and Müller-Esterl, W. (1990) J. Biol. Chem. 265, 12494–12502
12. You, S. L., Page, J. D., Scarsdale, J. N., Colman, R. W., and Harris, R. B. (1993) Blood 81, 1301–1306
13. Van Der Graaf, F., Tans, G., Bouma, B. N., and Griffen, J. H. (1982) J. Biol. Chem. 257, 14300–14305
14. Harrell, H., Jahn, H., Detert, W., Allia, S. A., Hock, J., Bonna, B. N., and Müller-Esterl, W. (1993) J. Biol. Chem. 268, 14527–14535
15. Page, J. D., You, J. L., Harris, R. B., and Colman, R. W. (1994) Arch. Biochem. Biophys. 314, 159–164
16. Hock, J., Vogt, R., Linke, R. P., and Müller-Esterl, W. (1990) J. Biol. Chem. 265, 12005–12011
17. Salvesen, G., Parkes, C., Abrahamsson, M., Grubb, A., and Barrett, A. J. (1986) J. Biol. Chem. 261, 429–434
18. Hasan, A. A., Cines, D. B., Zhang, J., and Schmaier, A. H. (1994) J. Biol. Chem. 269, 31822–31830
19. Vélez, D., Silver, L. D., Hahn, S., and Colman, R. W. (1987) Blood 70, 1503–1502
20. Laennec, U. K. (1970) Nature 227, 680–685
21. Heukeloven, J., and Deminh, R. (1985) Eutrophoraphia 6, 103–112
22. Kyllonen, S., and Jock, J. (1988) Biochem. Biophys. Methods 20, 203–209
23. Tobin, H., Stahelin, T., and Gorden, J. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 4350–4354
24. Good, E. (1967) Methods Enzymol. 13, 238–255
25. Kaufmann, J., Haasemann, M., Modrow, S., and Müller-Esterl, W. (1993) J. Biol. Chem. 268, 9079–9091
26. Johannessen, J., Drines, V., Ringd, K., Petersen, L. C., Rao, D., Lioubin, P., O’Hara, P., and Mulvihill, E. (1990) Thromb. Haemostasis 63, 54–59
27. Meijers, J. C. M., Mulvihill, E. R., Davie, E. W., and Chung, D. W. (1992) Biochemistry 31, 4680–4684
28. Meijers, J. C. M., Davie, E. W., and Chung, D. W. (1992) Blood 79, 1435–1440
29. Maina, C. V., Rigs, P. D., Grandea, A. G., III, Slatko, B. E., Moran, L. S.,
Discontinuous Kininogen Binding Site of Prekallikrein

30. Proctor, R. R., and Rapaport, S. L. (1961) Am. J. Clin. Pathol. 36, 212–219
31. Schapira, M., Scott, C. F., and Colman, R. W. (1982) J. Clin. Invest. 69, 462–468
32. Fujikawa, K., Chung, D. W., Hendrickson, L. E., and Davie, E. W. (1986) Biochemistry 25, 2417–2424
33. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1992) J. Biol. Chem. 267, 4247–4252
34. Baglia, F. A., and Walsh, P. N. (1996) J. Biol. Chem. 271, 3652–3658
35. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1991) J. Biol. Chem. 266, 24190–24197
36. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1995) J. Biol. Chem. 270, 6734–6740
37. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1993) J. Biol. Chem. 268, 3838–3844
38. Baglia, F. A., Seaman, F. S., and Walsh, P. N. (1995) Blood 85, 2078–2083
39. Henderson, L. M., Figueroa, C. D., Müller-Esterl, W., and Bhoola, K. D. (1994) Blood 84, 474–482
40. Gustafson, E. J., Schmaier, A. H., Wachtfolg, Y. T., Kaufman, N., Kucich, U., and Colman, R. W. (1989) J. Clin. Invest. 84, 28–35
41. Schmaier, A. H., Kuo, A., Lundberg, D., Murray, S., and Cines, D. B. (1988) J. Biol. Chem. 263, 16327–16333
42. Herwald, H., Hasan, A. A. K., Godovac-Zimmermann, J., Schmaier, A. H., and Müller-Esterl, W. (1995) J. Biol. Chem. 270, 14634–14642
43. Hasan, A. A. K., Cines, D. B., Herwald, H., Schmaier, A. H., and Müller-Esterl, W. (1995) J. Biol. Chem. 270, 12956–12961
44. Schapira, M., Despland, E., Scott, C. F., Boxer, L. A., and Colman, R. W. (1982) J. Clin. Invest. 69, 1199–1202
45. Colman, R. W., Wachtfolg, Y. T., Kucich, U., Weinbaum, G., Hahn, S., Pixley, R. A., Scott, C. F., de Agostini, A., Burger, D., and Schapira, M. (1985) Blood 65, 311–318
Mapping of the Discontinuous Kininogen Binding Site of Prekallikrein: A DISTAL BINDING SEGMENT IS LOCATED IN THE HEAVY CHAIN DOMAIN A4
Heiko Herwald, Thomas Renné, Joost C. M. Meijers, Dominic W. Chung, Jimmy D. Page, Robert W. Colman and Werner Müller-Esterl

J. Biol. Chem. 1996, 271:13061-13067.
doi: 10.1074/jbc.271.22.13061

Access the most updated version of this article at http://www.jbc.org/content/271/22/13061

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 28 of which can be accessed free at http://www.jbc.org/content/271/22/13061.full.html#ref-list-1