Cloning and Sequence Analysis of cDNAs for Human High Molecular Weight and Low Molecular Weight Prekininogens

PRIMARY STRUCTURES OF TWO HUMAN PREKININOGENS*

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The abbreviations used are: HMW, high molecular weight; LMW, low molecular weight; H chain, heavy chain; L chain, light chain; bp, base pair(s).

Kinin has a number of pharmacological actions including muscle contraction, hypotension, pain generation, and increase of vascular permeability (1-4). This peptide is liberated from two distinct precursor proteins, designated HMW1 and LMW kininogens, which differ in molecular weight and in susceptibility to different kallikreins (5, 6). Both HMW and LMW kininogens are single-chain glycoproteins which carry a bradykinin moiety in the interior of the polypeptide chains bridged by a disulfide linkage and thus consist of three domains: an amino-terminal H chain, the bradykinin moiety, and a carboxyl-terminal L chain (7-9). Recent findings on human genetic deficiency of plasma kininogen show another role of HMW kininogen in the initial step of the intrinsic pathway of blood coagulation (7, 10-13). Several families (e.g. Fitzgerald, Williams, and Fauche) have been found with a blood-clotting deficiency in vitro, which can be corrected by adding purified HMW kininogen to the plasma of the afflicted persons (11-13). The mechanisms by which HMW kininogen contributes to contact activation reactions have been demonstrated (7, 14). Thus, kinin and kininogen play important roles in many pathophysiological conditions, especially in inflammation.

We have recently reported the cloning and sequence analysis of the cDNAs for bovine HMW and LMW kininogen precursors (prekininogens) (8, 9). Bovine HMW and LMW prekininogen mRNAs share an identical sequence throughout the 5' untranslated region and the protein-coding region up to the sequence encoding the first 12 amino acid residues of the L chains, and the two mRNAs diverge from the 3' sequences that follow. Examination of a genomic clone containing the 3'-terminal portion of the bovine kininogen gene has revealed that the divergent 3'-terminal region of LMW prekininogen mRNA is specified by a discrete genomic segment and that this segment is linked with a small intervening sequence to the exon specifying the 3'-untranslated region of HMW prekininogen mRNA. Thus, it is concluded that bovine HMW and LMW prekininogen mRNAs are transcribed from the same gene.

Human HMW and LMW kininogens have recently been purified to homogeneity in several laboratories (15-19), and the amino acid sequences have been reported for the amino-terminal portions of the L chains adjacent to the kinin moiety in both HMW and LMW kininogens (17, 19). However, the primary structures of the large portions of the two kininogens remain to be determined. The elucidation of the whole primary structures of human HMW and LMW kininogens provide much insight into the mechanisms involved in kinin generation and in the contact activation of the coagulation reaction. Furthermore, the analysis of the structural basis for the relationship between the two human kininogens is important for understanding the molecular mechanisms underlying kininogen deficiency. In the present study, we have constructed and sequenced cDNAs for human HMW and LMW prekininogens to disclose their complete amino acid sequences and to examine the structural relationship between the two prekininogen mRNAs. We report here the whole primary structures of human HMW and LMW prekininogens and the interrelationship between the structures of the two prekininogen mRNAs. The sequence comparison of human HMW prekininogen with the bovine counterpart allows us to explain the structural basis for the different modes of processing of the human and bovine HMW kininogens as well as for the
different kinetics of the actions of the two HMW kininogens in the contact activation reactions. In the accompanying paper, we describe the structural organization of the human kininogen gene (20).

**EXPERIMENTAL PROCEDURES**

**Materials**—Reagents were obtained as follows: [α-32P]dCTP (3000 Ci/mmol) and [γ-32P]ATP (5000 Ci/mmol) from Amersham Corp.; oligo(dT)-cellulose (Type 7) and Escherichia coli ribonuclease H from New England Biolabs; SI nucleases from Sunkyo Co.; the Klenow fragment of E. coli DNA polymerase I and E. coli DNA ligase from New England Biolabs; avian myeloblastosis virus reverse transcriptase from Life Sciences Inc.; aminobenzyloxymethyl paper from Schleicher & Schuell; terminal deoxynucleotidyltransferase, bacterial alkaline phosphatase, and T4 polynucleotide kinase from Takara Shuzo Co.; restriction endonucleases from Takara Shuzo Co., Bethesda Research Laboratories, and New England Biolabs. The oligodeoxynucleotide was synthesized by the modified triester methods (21).

**Cloning Procedures**—Isolation of cloned cDNAs for human prekininogens was performed according to the procedure described previously (8, 9). A human liver cDNA library constructed by the method of Berg (22) was described previously (8, 9). Total RNA was extracted from a human liver (23), and poly(A) RNA was isolated by oligo(dT)-cellulose chromatography (24). For the isolation of cDNAs for human HMW prekininogen, about 70,000 transformants were screened by hybridization (25) with the T3- and T7-cDNA fragment of 401 bp containing nucleotide residues 75–475 of bovine HMW prekininogen (8). For the isolation of cDNAs for human HMW prekininogen, about 250,000 transformants were screened by hybridization with the RsaI-EcoRI fragment of 812 bp containing nucleotide residues 1233–2044 of bovine HMW prekininogen cDNA, which was excised from clone pKG294 (9). The DNA probes were labeled with nick translation with [α-32P]dCTP, and hybridization and filter washing were carried out at 55 °C. The primer-extended cDNA was cloned according to the procedures described previously (26). In brief, a synthetic oligodeoxynucleotide primer specified in the text was elongated by reverse transcriptase, with the use of 200 μg of poly(A) RNA as template and 5 nmol of the synthetic oligodeoxynucleotide as a primer. The cDNA synthesized was converted to double-stranded cDNA, which was cloned in plasmid pBR322 by inserting it into the PstI site using poly(dG)-poly(dC) homopolymeric extension. The isolation and the sequence determination of the genomic clones covering the bradykinin region are described in the accompanying paper (20). All of the cloning procedures were conducted in accordance with the guidelines for research involving recombinant DNA molecules issued by the Ministry of Education, Science and Culture of Japan.

**RNA Blot-Hybridization Analysis**—RNA was denatured with 1 M guanidin/50% (v/v) dimethyl sulfoxide (27), electrophoresed on a 1.0% agarose gel, dialyzed against an alkaline solution (31), and transferred to diazobenzyloxymethyl paper. Hybridization and washing were carried out according to the procedures described by Alwine et al. (28). Human and E. coli RNAs were used as size markers.

**S1 Nuclease Mapping Analysis**—HMW and LMW prekininogen mRNAs were separated from each other by sucrose density gradient centrifugation; human liver poly(A) RNA (200 μg) was centrifuged through 11.5 ml of a 5–25% linear sucrose gradient containing 20 mM Tris-HCl, pH 7.8, 2 mM EDTA, and 0.5% sodium dodecyl sulfate in a Beckman SW-41 rotor at 40,000 rpm and 20 °C for 6.5 h. Fractions containing HMW prekininogen mRNA or LMW prekininogen mRNA were identified by RNA blot-hybridization analysis. The total poly(A) RNA and the fractions containing either HMW or LMW prekininogen mRNA were hybridized to appropriate probes in a solution containing 50 mM Tris-HCl, pH 8.0, 1 mg/ml salmon sperm DNA, 20% formamide, 8 M urea, and 0.1% sodium dodecyl sulfate in a microwell plate, denatured at 65 °C, and allowed to hybridize at 42 °C for 24 h. The hybridization was washed at 65 °C for 2 h in a buffer containing 0.1% SDS, 0.1× SSC (8602 (32)). Nick translation was conducted with the use of [α-32P]dCTP (33). DNA sequence analysis was carried out by the procedure of Maxam and Gilbert (34).

**RESULTS AND DISCUSSION**

**Isolation and Nucleotide Sequence of cDNA Clone for Human HMW Prekininogen and Assignment of Its Amino Acid Sequence**—A human cDNA library was constructed with the plasmid DNA vector of Okayama and Berg (22) by using poly(A) RNA extracted from a human liver. This library was screened by hybridization with a cDNA fragment specifying the common protein-coding region of bovine HMW and LMW prekininogen mRNAs. Three hybridization-positive clones were isolated from approximately 70,000 transformants. Upon restriction enzyme analysis, the three clones were all found to share common restriction sites. Clone pKG36, which carried the largest cDNA insert of the three, was subjected to nucleotide sequence analysis according to the strategy indicated in Fig. 1a. The primary structure of human HMW prekininogen mRNA together with the amino acid sequence was deduced from the 1,531-nucleotide sequence determined for the cDNA insert of clone pKG36. The result is summarized in Fig. 2. A nonpeptide bradykinin was encoded by nucleotide residues 1,141–1,167. The deduced amino acid sequence of the 11 residues directly distal to the bradykinin sequence also agreed with the corresponding sequence reported for the purified protein (19). The remaining amino acid sequence of human HMW prekininogen was deduced from the reading frame corresponding to the amino acid sequence of bradykinin and its adjacent sequence. The translational initiation site was assigned to the first methionine codon AUG at nucleotide residues 1–3, because this is the first AUG codon that appears downstream from the nonsense codon UAG (residues -6 to -4) found in frame. It has recently been reported that the amino terminus of mature bovine kininogens starts at position 19 of their precursor proteins (prekininogens) (35), indicating that the 18 amino-terminal amino acid residues of bovine prekininogens serve as a signal peptide (36). Because human and bovine prekininogens are well conserved in their amino-terminal sequences, including the cleavage site of the signal peptide of bovine prekininogens, we assume that the mature human kininogens are preceded by the signal peptide of the 18 amino acid residues, thus starting with the glutamine residue at position 19.

**Isolation and Nucleotide Sequence of cDNA Clone for Human LMW Prekininogen and Assignment of Its Amino Acid Sequence**—The three clones described above were found to contain the cDNA sequence for LMW prekininogen.
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Therefore, a further attempt was made to isolate cDNA clones for human HMW prekininogen by screening the human cDNA library with a cDNA probe specifying the bovine HMW prekininogen mRNA sequence. Forty-five hybridization-positive clones were isolated from approximately 250,000 transformants. Clone pHKG6, which carried the largest cDNA insert in the 45 hybridization-positive clones, was subjected to nucleotide sequence analysis according to the strategy indicated in Fig. 1b. The 1,935-nucleotide sequence determined (residues 1,284-3,218) was found to contain a large reading frame, which corresponded well to the carboxyl-terminal sequence of bovine HMW prekininogen. However, this clone contained neither the sequence encoding the bradykinin sequence nor the sequence corresponding to the 37 amino acid residues reported for the amino-terminal portion of the L chain (17). Therefore, the following alternative approach was attempted to isolate the cDNA sequence containing the upstream region of the human HMW prekininogen mRNA. A synthetic oligodeoxyribonucleotide primer, 5'-CCATGCCCCTT-3', which was complementary to the residues 1,352-1,361 of human HMW prekininogen mRNA, was elongated by reverse transcription, and the resultant primer-extended cDNAs were cloned and screened. The hybridization probe used for screening was a cDNA fragment corresponding to a portion of the H chain-coding region of the human LMW prekininogen mRNA (the DdeI-Sau3A1 fragment containing residues 479-1,124), because the DNA fragment corresponding to the H chain-coding region was found to hybridize to both HMW and LMW prekininogen mRNAs (see below). However, this approach again turned out to be unsuccessful, despite the fact that about 200,000 transformants were screened in this experiment. This failure is probably due to a very low content of intact human HMW prekininogen mRNA in the human liver poly(A) RNA preparation which was made available to us. The nucleotide sequence encoding the bradykinin sequence and its surrounding region was finally elucidated by the isolation and sequence determination of a genomic segment encoding the bradykinin region (20). Thus, based upon the data combining the cDNA sequence of clone pHKG6 and the genomic sequence encoding the bradykinin region, the 3' nucleotide sequence of human HMW prekininogen mRNA and its corresponding amino acid sequence up to the bradykinin sequence are presented in Fig. 3.

The open reading frame encoding the bradykinin sequence terminates at residues 1,932 with the termination codon UAA. The L chain of human HMW kininogen consists of 255 amino acid residues with a calculated molecular weight of 28,248. This value is considerably smaller than those reported for the purified HMW kininogen L chain (15-17), which range between 44,000 and 65,000, depending on the evaluation meth-
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The nucleotide sequence of the mRNA was deduced from that of the cDNA insert of clone phKG36. Nucleotide residues are numbered in the 5' to 3' direction, beginning with the first residue of the initiator methionine codon AUG. The sequences of the putative signal peptide and kininogens are indicated in single letter code.

The possible glycosylation sites conforming to the Asn-X-Ser/Thr sequence are underlined.

The arrow indicates the point of divergence of the LMW and HMW prekininogens. Because none of the cDNAs isolated for human HMW prekininogen in this study contained the upstream sequence encoding the 12 amino acid residues of the L chain at position 626. However, it was previously reported that O-glycosidic carbohydrates are present in the L chain (7, 17), and this glycosylation may account for the difference in the molecular weight described above.

Characterization of Human HMW and LMW Prekininogen mRNAs and Their Structural Relationship—In the previous study (6, 9), we have shown that bovine HMW and LMW prekininogen mRNAs share an identical sequence throughout the 5' sequence up to the sequence encoding the 12 amino acid residues of the L chains and that the two mRNAs then encode the unique amino acid sequences for the respective kininogens. Because none of the cDNAs isolated for human HMW prekininogen in this study contained the upstream region of the mRNA sequence, the structural relationship between the human HMW and LMW prekininogen mRNAs was investigated by RNA blotting and S1 nuclease mapping analyses. The RNA blotting analysis was conducted by using three different cDNA probes (Fig. 4). Two cDNA probes, specific for either the LMW or the HMW prekininogen...
Furthermore, the observed difference in the lengths of HMW representing the LMW and HMW prekininogen mRNAs, indicated that LMW prekininogen mRNA gave rise to the two bands representing the specific regions of the two prekininogen mRNAs (residues 1,204–1,451 in LMW prekininogen mRNA and residues 1,204–3,218 in HMW prekininogen mRNA). In order to investigate the homology in the 5′ sequences of the two prekininogen mRNAs in more detail, RNA fractions containing either LMW or HMW prekininogen mRNA were obtained by sucrose density gradient centrifugation of total human liver unfractionated poly(A) RNA were analyzed by S1 nuclease protection. The nucleotide sequence of the mRNA was deduced by combining the sequence of the cDNA insert of clone phKG6 and that of the genomic subclone derived from XhKG-D described in the accompanying paper (20). The cDNA insert of clone phKG6 included the sequence up to the residue 1284. Because the upstream sequence of the bradykinin-coding region was used as a hybridization probe, the numbering of the nucleotide residues follows that of the sequence of the cDNAs for Two Human Prekininogens family sequence and its flanking direct repeat sequences are marked with double underlines, respectively. Other details are the same as those described in Fig. 2.
the three RNA preparations all gave rise to an identical single band upon alkaline agarose gel electrophoresis after S1 nuclease digestion. The observed size (approximately 1,220 nucleotides) corresponded to the distance between the 5' end-labeled StuI site and the 3' end of the antimesage strand of the cDNA probe. In contrast, when the cDNA probe containing a part of the H chain-coding region and its distal region of prekininogen mRNA was used, the RNA fractions containing LMW and HMW prekininogen mRNAs yielded different bands with mobilities corresponding to sizes of approximately 770 and 525 nucleotides, respectively. The unfraccionated RNA preparation naturally gave rise to both of the bands. The estimated size of the approximately 770 nucleotides corresponded to the sequence extending from the 3' end-labeled HpaII site to the 5' end of the antimesage strand of the cDNA probe, while the estimated size of the approximately 525 nucleotides accorded with the sequence ending at the point of divergence between the LMW and HMW prekininogen mRNA sequences. Thus, the results obtained by the S1 nuclease analysis demonstrated that human HMW and LMW prekininogen mRNAs share an identical sequence throughout the 5'-untranslated region and the protein-coding region up to the region preceding the sequences specifying the divergent carboxyl-terminal amino acid sequences of the two prekininogens. Furthermore, the observed identity in the 5' sequence of the two prekininogen mRNAs indicates that HMW prekininogen shares a common signal peptide, H chain, and bradykinin moiety with LMW prekininogen. Thus, it can be concluded that human HMW kininogen consists of 626 amino acid residues with a calculated molecular weight of 69,896. In the accompanying paper (20), we discuss the molecular basis for the generation of the overlapping sequence of the two human prekininogen mRNAs on the basis of the structural analysis of the human kininogen gene.

The 3'-untranslated regions of HMW and LMW prekininogen mRNAs are 1286 and 170 nucleotides long, respectively. The sequence AAUAAA is found 18 and 25 nucleotides upstream from the polyadenylation sites of HMW and LMW prekininogen mRNAs, respectively. It has been suggested that this sequence AAUAAA present at similar positions of other eukaryotic mRNAs serves as a signal for polyadenylation after transcription (38). A peculiar feature of the 3'-untranslated region of HMW prekininogen mRNA is the presence of the Alu family sequence, which is widely distributed in the intervening sequences of the human genomic DNA but rarely observed in its mRNA-coding sequences. The 123-nucleotide sequence (residues 2385–2507) homologous to the 3' one-third of Alu family sequence is flanked on both sides by short direct repeats, as reported for other Alu family sequences (39).

Comparisons of Amino Acid Sequences of Human and Bovine Prekininogens—The amino acid sequences of human HMW and LMW prekininogens were compared with those of the bovine counterparts reported previously (8, 9), and the comparison is schematically illustrated in Fig. 6. In this figure, regions that markedly differ in the lengths of the protein sequences are indicated by dotted marks, and positions showing the amino acid replacements are displayed by vertical lines. Two regions in the human and bovine prekininogens differ markedly in the lengths of the protein sequences. One such region is the carboxyl end of the LMW prekininogens,
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Fig. 6. Comparison of the amino acid sequences of human and bovine prekininogens. Vertical lines indicate positions in which the two sequences have different amino acids. Dotted boxes indicate the regions in which the two sequences show insertion/deletion of the amino acid sequence; the nucleotide and amino acid sequences in these regions are indicated above or below the kininogen structures; hyphens show the positions which lack the corresponding sequences; a direct repeat at the end points of the extra human sequence is underlined. Boxes shown by wavy lines represent the putative signal peptide and bradykinin (BK). The white and the black triangles indicate the positions in which the human sequence lacks or possesses one amino acid residue as compared with the bovine sequence, respectively. Possible disulfide bonds of kininogens are shown by the lines above the prekininogen structures; disulfide bonds connecting the H chain and the L chain are shown by arrows.

where the human sequence is 10 amino acid residues shorter than the bovine sequence. This difference is due to the presence of the termination codon UGA at positions 1282–1284 of the human mRNA in place of the leucine codon UUA present at the corresponding position of the bovine mRNA (8). Another divergent region is the middle portion of the L chains of HMW prekininogens, where 22 consecutive amino acid residues are inserted in the human sequence. This sequence is located in the histidine-rich sequence named Fragment 1-2 in the bovine sequence, which is characterized by the presence of repeating units composed of His-Gly, Gly-His, and His-Lys (7). A short direct repeat (CAUAAG) encoding one of these repeating units, His-Lys, is present at the end points of the extra human sequence, and this pattern is very similar to that observed in the length heterogeneity of the β-like globin genes in mammals (40). Thus, the deletion in the bovine L chain-coding region may be prompted by the presence of the direct repeat through the slipped mispairing mechanisms during DNA replication as proposed for the case of β-like globin genes by Efstratiadis et al. (40). Noteworthy also is that the extra human sequence present in the histidine-rich region contains large amounts of histidine and lysine residues including the repeating units of the His-Gly, Gly-His, and His-Lys sequences. This characteristic structure suggests that the extra human sequence also functions as a histidine-rich sequence to bind to a negatively charged surface in the contact activation reactions as discussed below.

Besides the above length heterogeneities observed in the L chains of the LMW and HMW prekininogens, two additions, one at amino acid residue 22 in the common H chain region and the other at residue 548 in the HMW prekininogen sequence, and one deletion between residues 438–439 in the HMW prekininogen sequence are noted in the human sequences when compared with the bovine counterparts. When additions/deletions are counted as one amino acid replacement regardless of their lengths, HMW and LMW prekininogens have an overall identity in 72.8 and 73.8% of the amino acid positions between the human and bovine sequences, respectively. Although amino acid replacements observed between the two sequences are apparently distributed throughout the protein sequences, several replacements present in the L chains of HMW kininogens may be crucial for the different processing patterns of human and bovine HMW kininogens. Such replacements will be discussed in more detail in the next section.

Some peculiar structural features observed in the H chain of the bovine kinogen are conserved in the human kinogen. We have previously found that the H chain of bovine prekininogen contains two internally homologous sequences, each of which consists of 122 amino acid residues and shares 6 cysteine residues at equivalent positions (8). Similar internal duplication of the amino acid sequences is observed in the H chain of the human sequence (Fig. 7). Each of the homologous regions is also composed of 122 amino acids (amino acid residues 103–224 and 225–346) and contains 6 cysteine residues located at exactly equivalent positions between the two homologous sequences. The two homologous sequences have 42 positions of identical amino acid residues, corresponding to about 34% identity. The internally homologous sequences of human kinogen can thus be aligned with those of the bovine counterpart, and this alignment indicates that the duplicated sequences of the two kinogens possess homologous amino acid residues at 36 out of 42 positions where the
two human homologous regions share common amino acid residues, including all six positions of the cysteine residues in the human duplicated sequences. Thus, the common amino acid residues between the two homologous regions have been highly conserved in the human and bovine H chains, suggesting that the regions may involve a specific but yet unidentified function of kininogen.

Sueyoshi et al. (41) have recently reported linkage patterns of disulfide bridges of bovine kininogens. They found that 16 out of the 17 cysteine residues in the H chain including the 12 residues in the duplicated regions are all involved in the formation of disulfide linkages connected by two adjacent cysteine residues and that the H chain and the L chain are linked by a single disulfide bond between the remaining one cysteine residue located at the amino terminus of the H chain and the cysteine residue of the L chain. The total 17 cysteine residues present in the human H chain are all located at positions exactly equivalent to those observed in the bovine H chain. Thus, the human H chain, like the bovine counterpart, can be organized to generate eight structural domains, each forming a loop structure connected by the adjacent Cys-Cys disulfide linkage.

Possible Mode of Processing of Human HMW Kininogen—Although human and bovine HMW kininogens possess a common biological function involved in contact activation reactions including blood coagulation and fibrinolysis pathways, they exhibit a marked difference in the mode of their cleavage by plasma kallikrein (7, 15, 16). As illustrated in Fig. 8, bovine HMW kininogen is cleaved by bovine plasma kallikrein into a kinin-free protein, bradykinin and Fragment 1-2. Upon prolonged incubation with kallikrein, Fragment 1-2 is further cleaved into Fragment 1 and Fragment 2. The former consists of the 69 amino acid residues starting with the amino terminus of the L chain, while the latter corresponds to the subsequent sequence of 41 amino acid residues, and both of the fragments contain a large amount of histidine residues as well as characteristic repeating units described above (7, 8). In the case of human HMW kininogen, human plasma kallikrein rapidly releases bradykinin and then slowly liberates the glycosylated form of the 8000-dalton fragment from the kinin-free kininogen (16). Although the 8000-dalton fragment is thought to be derived from the amino-terminal portion of the L chain, it does not correspond to either Fragment 1-2 or Fragment 1 with respect to molecular weight and amino acid compositions, particularly to the content of histidine residues (16). Thus, there is no release of any histidine-rich fragments from human HMW kininogen upon prolonged incubation with plasma kallikrein, and nothing is known about the molecular basis for the different mode of processing of human HMW kininogen.

A close examination of the L chain sequences of bovine and human HMW kininogens indicates that the amino-terminal portion of the human L chain possesses five arginine residues at positions 392, 427, 428, 437, and 449 in place of glutamine, histidine, glycine, isoleucine, and histidine residues observed at the corresponding positions of the bovine sequence, respectively (Fig. 8). A single arginine residue is known to serve as a proteolytic cleavage signal for kallikrein (42), as is observed at the carboxyl-terminal cleavage site of bradykinin. Thus, it is likely that at least some of the above arginine residues may serve as proteolytic cleavage sites for kallikrein and that the amino-terminal portion of the human L chain may be cleaved into several small fragments. If we take into account several O-glycosylations at the amino-terminal portion of the L chain (7, 17), it seems conceivable that the glycosylated 8000-dalton fragment represents the amino-terminal fragment starting with the serine residue directly adjacent to the bradykinin sequence (16) and ending with the arginine residue at position 427, 428, or 437. This assumption can be supported by a general agreement of the amino acid compositions of the amino-terminal fragment with those reported for the 8000-dalton fragment (16).

As described above, the human L chain contains an extra sequence of the 22 consecutive amino acid residues within the sequence corresponding to Fragment 2 of the bovine sequence. Furthermore, the arginine residue at the carboxyl end of Fragment 2 of the bovine sequence is converted to a lysine residue in the human sequence. The single lysine residue, instead of the arginine residue, is rarely used as a proteolytic signal in the processing of the precursor protein; although the amino-terminal processing point of bradykinin is located between the lysine and arginine residues, this cleavage probably occurs because the amino-terminal Arg-Pro bond of the bradykinin sequence is selectively resistant to serine-protease.

We assume that the change of the amino acid residue at the carboxyl end of Fragment 2 and possibly the presence of the extra human sequence present within Fragment 2 may affect the cleavage pattern of human kininogen, and Fragment 2 together with the extra human sequence, both consisting of the histidine-rich sequences, remain in the kinin-free kininogen after incubation with kallikrein (Fig. 8).

The above schema of the different processing of human and bovine HMW kininogens may also account for the different kinetics of contact activation reactions exhibited by human and bovine HMW kininogens. In kinetic studies of the contact activation reactions, it was shown that HMW kininogen and Factor XII associate with each other on a negatively charged surface to activate Factor XI as well as prekallikrein by surface-bound activated Factor XII (7, 14, 15). It was also reported that in the binding of HMW kininogen, the histidine-rich region and its following carboxyl-terminal region of the L chain are involved for the negatively charged surface and prekallikrein or Factor XI, respectively (7, 43, 44). Thus, it appears that HMW kininogen acts as a surface-bound recep-

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**Fig. 8. Possible difference in the modes of processing of human and bovine HMW kininogens.** The mode of processing of bovine HMW kininogen is taken from data reported by Iwanaga et al. (7). H, BK, L, F1-2, F1, and F2 represent the H chain, bradykinin, the L chain, Fragment 1-2, Fragment 1 and Fragment 2 respectively. Characteristic amino acid residues discussed in the text are described with the standard single-letter notation for amino acid residues; S-S indicates the disulfide bond connecting the H chain and the L chain. In the bovine sequence, the carboxyl-terminal region following Fragment 1-2 was named the L chain (7). However, because of the remarkable sequence homology of the human L chain with the bovine counterpart, we refer to the whole carboxyl-terminal sequence following bradykinin as the L chain in both human and bovine sequences. A dotted box indicates an extra sequence present in the human L chain. Plus signs stand for the histidine-rich regions.
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