Knowledge of the organization of the kininogen gene and protein structure and function correlations has allowed the development of a model of high molecular weight kininogen. Domains 1–3 on the heavy chain are evolutionarily related to cystatin and the latter two are inhibitors of cysteine proteases. Proteolytic cleavage in domain 4 to release bradykinin causes a conformational change, exposing a surface-binding region (domain 5) on the disulfide-linked light chain. The carboxyl-terminal domain 6 contains a zymogen binding sequence for factor XI and prekallikrein which, with domain 5, accounts for its cofactor activity. To explore further the domain structure, we have determined the shapes of high molecular weight kininogen and prekallikrein by electron microscopy of rotary shadowed preparations and computer image processing. High molecular weight kininogen appears to be a linear array of three linked globular regions about 16 nm long, with the two ends also connected by another thin strand. Both prekallikrein and kallikrein have a compact globular shape, with a subdivision that is sometimes visible. Different functional domains of high molecular weight kininogen were identified by monoclonal antibodies against these regions, as well as ligand binding of prekallikrein. These studies indicate that one end globular region is the prekallikrein-binding domain, the other comprises the cysteine protease inhibitor domains and the smaller central nodule is the surface-binding domain. Cleavage of high molecular weight kininogen with plasma kallikrein yields two-chain high molecular weight kininogen resulting in a striking change in conformation: the central surface-binding domain swings out so that it is still adjacent to the prekallikrein-binding domain but no longer in the middle. These structural studies provide insight into the interactions of these proteins and aspects of the mechanisms of their actions.

Human high molecular weight kininogen (HK) is a single chain glycoprotein with a molecular mass of 120 kDa (1), first identified as a precursor of the vasoactive nonapeptide bradykinin. HK, however, also has important functions in the contact system of blood coagulation by serving as a cofactor for several enzymatic reactions in the contact phase. Normally, both prekallikrein (PK) (2, 3) and Factor XI (4) cleave the blood as binary complexes with HK. Like many of the reactions in the blood clotting cascade, contact phase reactions are greatly accelerated by interaction with a negatively charged surface in vitro. Putative in vivo anionic surfaces are the altered endothelial cell surface or the subendothelial matrix exposed in a damaged vessel. Factor XII binds to negatively charged surfaces in the absence of other proteins, but HK is necessary for efficient binding of both PK and Factor XI (5). Bound Factor XII can autocatalytically activate Factor XII to XIIa, but this is a slow reaction. On the surface, PK and Factor XII are involved in a rapid reciprocal activation reaction (6), such that Factor XIIa activates prekallikrein (bound to HK) to kallikrein, which in turn activates Factor XII to Factor XIIa. Factor XIIa then converts Factor XI (bound to HK) to Factor XIa, which in turn activates Factor IX to IXa. HK contains a large amount of O-linked carbohydrate in its light chain; there is also N-linked carbohydrate in the heavy chain. Many of the oligosaccharide chains are located in a stretch of the light chain beyond the highly positively charged region, closer to the carboxyl-terminal end of the molecule, but these are not required for cofactor activity (7).

Although PK and Factor XI are normally bound to HK, intact HK is not an efficient cofactor for the enzymatic reactions just described. HK is cleaved by kallikrein, producing two-chain HK (HKa), sequentially releasing bradykinin and another peptide (5). The remaining 65-kDa heavy chain is linked to the 45-kDa light chain by a single disulfide bond. This cleavage of HK appears to involve an increase in the binding of HK to negatively charged surfaces (8). Cleavage of HK by Factor XIa, in contrast, leads to inactivation of the cofactor function of the molecule (9). The carboxyl-terminal portions (D6) of the light chain contain overlapping sequences responsible for the binding of the heavy chain of PK and factor XIa. The amino-terminal region of the light chain (D5) contains a histidine-glycine-rich and histidine-glycine-lysine-rich regions which appear to be responsible for binding to negatively charged surfaces (10).

Kininogen molecules can also act as inhibitors of cysteine proteases, such as papain, calpain, and cathepsins H and L. HK and low molecular weight kininogen are the major cysteine protease inhibitors of plasma. This protease inhibitory activity resides on the identical heavy chains of both kininogens. The cloning of cDNAs for human high and low molecular weight

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The abbreviations used are: HK, high molecular weight kininogen; HKa, cleaved high molecular weight kininogen; D1, domain 1 of HK; D2, domain 2 of HK; D3, domain 3 of HK; D4, domain 4 of HK; D5, domain 5 of HK; D6, domain 6 of HK; PK, prekallikrein.

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kininogens, and consequent analysis of the gene structure has revealed more about the structures and relationships of these proteins. In the identical heavy chains, there are three tandemly repeated groups homologous to cystatin (called D1, D2, and D3) coded for by three exons. Only D2 and D3 contain a critical binding sequence, QVVG, common to other cysteine protease inhibitors. Only D2 inhibits calpain since, in contrast to D3, it contains a specific inhibitory region distinct from QVVG which directly inhibits calpain (11).

Recently, it has been discovered that cleaved HK (HKα) is a potent anti-adhesive protein which can displace fibrinogen from neutrophils and platelets (12). It inhibits the spreading of osteosarcoma and melanoma cells on vitronectin and of endothelial cells, platelets, and mononuclear blood cells on vitronectin or fibrinogen (13). The histidine-glycine-lysine-rich domain is critical for this function. It appears that these reactions could be mediated through binding of cleaved HK (HKα) to thrombospondin, since complex formation has been shown recently between the light and heavy chain of HK and thrombospondin (14).

Many of the experimental results, including the gene structure, suggest that this multifunctional protein is made up of multiple domains or independently folded regions of protein. The kininogens, with such a multiplicity of diverse functions, appear to be a good example of the evolution of complex proteins by duplication and fusion of various genes. However, there has been no direct evidence for a simple correspondence between the exons and domains.

Electron microscopy of HK, HKα, and PK and two antibodies directed against specific domains was carried out to reveal the shapes of these proteins and allow identification of functional regions. Knowledge of the shapes of these proteins and localization of the structural and functional domains could provide insight into mechanisms of their actions.

EXPERIMENTAL PROCEDURES

High molecular weight kininogen (1) and prekallikrein (15) were purified as described previously. The concentrations of purifiedHK (16) and PK (17) were determined as described elsewhere. Kallikrein-cleaved HK was generated as described previously (18). Polyacrylamide gel electrophoresis patterns of prekallikrein, kallikrein-cleaved HK (HKα), and HK under reducing and nonreducing conditions are shown in Fig. 1 to demonstrate the purity of these preparations. The preparation of Fab' fragments were by a slight modification (19) of a previously described method (20).

HK and PK samples for electron microscopy were initially at a concentration of about 2 mg/ml in 0.15 M NaCl, 0.02 M sodium acetate, pH 5.3; antibodies were at a concentration of about 1 mg/ml in 0.15 M NaCl, 0.02 M Tris-HCl, pH 7.4. In several experiments, HK was incubated with 6 mM dithiothreitol for 30–60 min at 37 °C. PK was incubated with different molar ratios of HK (usually 1:1, 2:1, or 1:2) for 1–2 h at 37 °C or 18 h at 4 °C. In the longer experiments, sometimes 0.1 M phenylmethylsulfonyl fluoride was used to inhibit possible proteolysis. Similarly, antibodies were also incubated with different molar ratios of HK (usually 1:1, 2:1, or 1:2). Samples were then diluted to a concentration of about ~25 μg/ml into a volatile buffer (usually 0.05 M ammonium formate at pH 7.4) and 30–70% glycerol.

Rotary-shadowed specimens were prepared by quickly spraying this dilute solution of molecules onto freshly cleaved mica and shadowing with tungsten or platinum in a vacuum evaporator (Denton Vacuum Co., Cherry Hill, NJ) (21–23). Most of the images shown here were from specimens sprayed and evaporated from 70% glycerol and shadowed with a light coat of tungsten, since this technique gave the best resolution results, as described below. All of the specimens were examined in a Philips 400 electron microscope (Philips Electronic Instruments Co., Mahwah, NJ), usually operating at 80 kV and a magnification of × 60,000. All experiments were repeated many times and large numbers of specimens were examined and photographed randomly to ensure that the results were reproducible and to minimize the selection of nonrepresentative images. Measurements of the sizes of observed structural features are reported as mean ± standard deviation, including the number of particles measured (n), and have been corrected for the layer of metal shadow.

Examples of each of the micrographs of individual molecules or complexes were filtered, using a gaussian low pass filter, to remove noise and enhance structural features of the images. The electron microscope negatives were digitized on a microcomputer-driven Optronics P1000 rotating drum microdensitometer (Optronics, Chelmsford, MA) using a 25-μm spot size. Image processing was carried out as described below on a Silicon Graphics Iris Indigo X24Z workstation (Silicon Graphics, Mountain View, CA) using the Semper 6.3 Plus image processing language (Synoptics, Newton, MA).

RESULTS

Electron Microscopy of High Molecular Weight Kininogen and Changes with Proteolytic Activation—Rotary shadowing of proteins dried in the presence of glycerol has allowed the visualization of a wide variety of proteins with relatively few artifacts, since glycerol appears to protect against distortion during drying (24). However, visualization by electron microscopy of proteins with molecular mass as low as that of HK requires careful attention to the details of specimen preparation technique. Rotary shadowing of HK with platinum, which provides excellent contrast, revealed elongated rod-like structures that were sometimes bent (results not shown). Since little substructure was visible, a light shadow of tungsten, which has a smaller grain size, was used for most of these experiments.

In addition, it was found that fields of molecules more uniform in appearance and with higher contrast could be produced by increasing the percentage of glycerol in the spray buffer. This result is probably accounted for by the surface binding properties of HK and/or the small size of this protein. The surface-binding domain of HK may bind to patches of negative charge from the silicates making up the mica. This conclusion was reinforced by the finding that the most variable feature of images of the HK molecule at low glycerol concentrations was the surface-binding domain (see below). Examination of rotary-shadowed samples of HK that had been freeze-dried in the absence of glycerol produced images similar to those with high
glycerol, indicating that there are no apparent artifacts introduced by the glycerol (24).

Examples of each class of images of the proteins or complexes were filtered to enhance structural features and reduce noise. Tungsten particles in the background of shadowed images of proteins contribute heavily to the high frequency content of their Fourier transforms. Elimination or smoothing of this noise can be achieved in the frequency domain by the use of a low pass filter, which retains low frequency information and eliminates or attenuates a specified range of high frequency noise components in the image transform. A circular mask was applied to each digitized image to exclude as much of the background as possible. Then, the masked image was floated to the average image density, and the Fourier transform was calculated. A considerable amount of the intensity information in the transform was located at spatial frequencies greater than 0.067 Å⁻¹ (15 Å resolution), which is approximately the expected limit of resolution of the rotary shadowing technique arising from the size of the tungsten grains. A gaussian low pass filter was applied to the image transform, extending over the spatial frequency range of 0.002 to about 0.067 Å⁻¹, with the midpoint of the range at 0.033 Å⁻¹. With such a filter, the intensities associated with spatial frequencies above 0.067 Å⁻¹ (15 Å) were excluded, whereas those located at 0.033 Å⁻¹ (30 Å) were reduced to ~37% of their original value. As illustrated in the figures, each low pass filtering produced a considerable reduction in the density of the background noise in the resultant filtered image, with a minimum of blurring or distortion in the appearance of the shadowed molecule itself. All molecular features in the filtered images were also present in the original micrographs, another indication that artifacts were not introduced.

Some typical images of HK are shown in Fig. 2, a and b. The gallery of images here and in the other figures illustrates the variety of appearances. The most commonly observed structure was a linear array of three globular nodules, with the middle one usually appearing slightly smaller than those at the ends, but often being variable in size and sometimes not being visible. The average length of the molecules was 16 ± 3 nm (n = 360), after correction for the layer of metal. The end globular regions had a diameter of 4.5 ± 1.5 nm. Some molecules appeared to be curved or bent. Although the appearance of the molecules was somewhat variable because they appear to be flexible, about 95% of the images were consistent with the features described. In some cases, additional substructure could be seen. For example, occasionally the end regions appeared to be subdivided into several smaller domains. Sometimes a thin strand arising from each end appeared to join each other alongside the rest of the molecule (four images on the left side of Fig. 2a). In some of these images, the most obvious indication of the presence of such a junction was a small shadow-casting structure adjacent to the center of the molecule. It should be emphasized that nearly all molecules had the appearance of a linear or curved array of beads with a length of ~16 nm, but some of these other features were only seen occasionally. Examples of images of HK molecules that have been enhanced by low pass filtering are shown in Fig. 2e.

In the presence of a reducing agent, such as dithiothreitol, there was little change in appearance of the molecules. However, when the thin strands just described were occasionally seen, with dithiothreitol they appeared to be no longer touching each other (Fig. 2a, right two images and filtered image on the right in Fig. 2e).

HK was treated with kallikrein to release bradykinin (Fig. 1) prior to rotary shadowing and electron microscopy. Images of the resulting HKa (Fig. 2, c and d)) were distinctly different than those of molecules before proteolysis. The molecules were usually still trinodular, but the three globules were no longer arranged linearly, but often as the vertices of a triangle. In many images, it was roughly a right triangle with the smaller
The antibody is specific for cysteine protease-binding domains, D2 and D3, of high molecular weight kininogen. Rotary-shadowed antibodies have a characteristic three-lobed shape. Antibody 2B5 appears to bind to one of the large globular regions at one end of HK, with HKa, the antibody is associated with the globular region of the molecule that is separated by a short distance from the other large nodule and the small nodule that is usually nearby. To aid in the interpretation of the electron micrographs, a tracing of the shadowed complex is included below each image. Bar, 50 nm. c, examples of low-pass-filtered images of molecules of HK with 2B5 bound, corresponding to those first and last from the left in row a. d, examples of low-pass-filtered images of HKa with 2B5 bound, corresponding to molecules that are first and fourth from the left in b.

Examples of filtered HKa molecules are shown in Fig. 2f. Cleaved HK was more variable in appearance than before activation, with both the distances and angles of the three globules changing from one molecule to the next. However, more than 89% of the images displayed three nodules near each other but no longer constrained to be arrayed linearly in a row. In overall summary, the change in conformation upon cleavage for 1-2 h at 37 °C or 18 h at 4 °C, and the complexes were shadowed with tungsten. 2B5 is an IgG-class antibody that has the typical three-lobed or Y-shaped appearance by electron microscopy of rotary-shadowed preparations. Nearly all interpretable images of 2B5 with HK appeared to show one lobe of an antibody binding to one of the large globular regions at one end of HK (Fig. 3a). Low pass-filtered images of HK with 2B5 bound are shown in Fig. 3c. In some cases, a single antibody binding two molecules of HK could be seen, but these images were often more difficult or confusing to interpret.

Complexes of 2B5 with HKa often had the appearance of the three-lobed antibody with a paired large and small nodule nearby (Fig. 3b). On comparison of these images with those of HKa by itself, it became apparent that the antibody was attached to one of the three nodules that were arranged in a roughly triangular manner. Specifically, the antibody was bound to the large nodule that was not associated with the small nodule. Examples of micrographs of complexes of HKa with 2B5 bound with the noise reduced by low pass filtering are in Fig. 3d.

Monoclonal antibody C11C1 binds specifically to a repeating sequence (HGLGHGHEQQHGLGHGH) in the surface-binding domain (18). Since the number of amino acids in the surface-binding domain suggested that it would be small, we used a preparation of Fab from C11C1 in these experiments. Electron microscopy of complexes of C11C1 Fab incubated with HK for 1-2 h at 37 °C or 18 h at 4 °C showed extra mass associated with the central domain of HK (Fig. 4a). The size of the extra nodule in this region was about the same as one of the three arms or lobes of an IgG molecule. Again, nearly all interpretable images with extra mass apparent showed an extra nodule near the central region of the HK. Images that were low pass-filtered are shown in Fig. 4c.

Complexes of C11C1 Fab with HKa revealed an extra nodule associated with the smallest of the three domains that was usually near one of the larger domains (Fig. 4b). These images were somewhat variable or complex in appearance, probably because of the apparent flexibility of the HKa. Different types of images are included in Fig. 4b to illustrate the variety observed. However, in each case there is extra mass associated with one of the three nodules, usually the smallest. Occasionally, as in the first image on the left in Fig. 4b, thin strands connecting the two larger nodules can be seen, with the Fab
orientations of molecules on the surface giving rise to different views, more than 96% of molecules were globular with a diameter of 9 ± 2 nm ($n = 270$). Sometimes a cleft was visible, dividing the molecule into unequal portions. Images of prekallikrein filtered to reduce background noise are shown in Fig. 5e. Kallikrein, produced by cleavage of prekallikrein by activated factor XII, appeared to be similar in appearance to PK at the resolution afforded by these techniques of electron microscopy.

Prekallikrein was incubated with HK for 1–2 h at 37 °C or 18 h at 4 °C prior to spraying and shadowing. Observation of these complexes indicated that PK binds to one end of the HK molecule, i.e. to one of the large nodules (Fig. 5, b and c). Several different types of images were seen. Images in which the PK was lateral to the end globular region of HK are the simplest to interpret (Fig. 5b and filtered images in f). Often the molecules lie on top of each other so that only one end of HK protrudes (Fig. 5c and filtered images in g). At least 90% of all images could be put into one of these two categories. Examination of electron micrographs of complexes of PK bound to HK revealed PK associated with one end of the HK molecules such that a single globular domain was still visible, but usually not the large and small nodules that are near each other (Fig. 5d). In other words, the PK binds to the end of HK consisting of the large nodule associated with the small domain, so that they can no longer be seen.

**D I S C U S S I O N**

**The Shape of High Molecular Weight Kininogen and Identification of Domains**—In the electron microscope studies presented here, we have visualized HK and PK and correlated some of the observed structural features with known functional domains. Computer image processing was used to filter out some of the background noise in the micrographs. HK is an elongated trinodular molecule with a length of about 16 nm. Such an elongated structure is consistent with gel filtration results, in which HK elutes as if it were a higher molecular mass protein (2). Although most molecules have three globular domains arranged linearly in a row, the variety of curved configurations observed indicates that HK is flexible. This conclusion is consistent with predictions of secondary structure from the amino acid sequence, suggesting the presence of many turns but little α-helix, indicating a flexible molecule (26).

Monoclonal antibodies have been used to localize specific portions of the polypeptide chain in the observed structural features. Antibody 2B5, which binds specifically to cysteine protease inhibitor domains D2 and D3 (25), attaches to one of the end globular regions. Observations of 2B5 bound to HK showed that this antibody binds to the end region not associated with the smaller nodule. The three cystatin-like domains together comprise ~323 amino acids, the mass of which would be consistent with the size of the observed globular region.

Monoclonal antibody C11C1, which is specific for the sequence HGLGHDHEQQHGLGHH in the surface-binding domain (18), binds to the small central region of HK. There are ~91 amino acids in the surface-binding domain, consistent with the considerably smaller size of the central nodule. This nodule is not visible in all images of HK molecules, probably because a structure of this size is at the limit of visualization by these techniques of electron microscopy. This domain may also be variable in size, since it can bind so strongly to charged patches on the mica surface that it flattens and so cannot produce much of a shadow.

We have shown that the globular domain at the other end of HK binds PK (see below for more on PK); presumably, it also binds Factor XI, portions of which are homologous to PK. This domain is made up of ~80 amino acids. Although this is considerably fewer amino acids than in the cysteine protease in-

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**Fig. 5.** Electron microscopy of rotary-shadowed prekallikrein (a) and prekallikrein with high molecular weight kininogen (b and c) or cleaved high molecular weight kininogen (d). a, prekallikrein has the appearance of a globular protein, although in many images a cleft, corresponding to the division into heavy and light chains, is visible. b and c, PK binds to one of the large nodules at the end of the HK molecule. In d, the PK is lateral to the end globular region of HK, whereas in c the molecules lie on top of each other so that only one end of HK protrudes. d, PK binds to one end of HKs such that a single globular domain is still visible, but not the large and small nodules that are near each other. To aid in the interpretation of the electron micrographs, a tracing of the shadowed molecule or complex is included below each image. Bar, 50 nm. e, examples of low pass-filtered images of prekallikrein, corresponding to the second and third molecules in a, f, low pass-filtered images of HK with PK bound, corresponding to the first and fourth images in row b, g, other filtered images of HK with PK bound, corresponding to the first and last molecules in d.

fragment binding to the small nodule adjacent to one of the end globular regions.

**Electron Microscopy of Prekallikrein and Its Binding to High Molecular Weight Kininogen**—Rotary-shadowed prekallikrein appeared to be globular in shape (Fig. 5a). Although there was some variability in appearance, probably because of different
The carboxyl- and amino-terminal ends of HK are tied together by a disulfide bond between amino acids 10 and 596. Occasionally, in the electron micrographs thin strands are seen as projections from each end of the molecule. In preparations of HK molecules, a small shadow-casting structure opposite the central domain that is occasionally observed may be the site at which the two thin strands are tied together by this disulfide bond. In electron microscope images of HK in the presence of reducing agents such as dithiothreitol, occasionally thin strands that are not connected to each other can be observed to be extending from each end. Thus, it appears that the thin strands may be the amino- and carboxyl-terminal portions of polypeptide chains that connect the heavy and light chains. Of course, such structures would normally be beyond the resolution of these techniques of electron microscopy, but it may be that the strands are sometimes slightly above the surface during shadowing and thus produce shadows more prominent than expected.

There are ~22 residues in the sequence between the first cysteine residue and the cysteine protease inhibitor domains and another ~23 amino acids between the PK-binding domain and the last cysteine, for a total of ~45 amino acids. If both of these polypeptides were random coils, they would have a total length of ~16.2 nm, which would be just long enough to connect across the 16 nm length between the ends of the molecule. However, the first cystatin-like domain is least homologous to the others and does not bind cysteine proteases, so it is possible that part of this portion of the polypeptide chain also contributes to the connector.

HK is a multifunctional protein in which specific portions of the amino acid sequence have been identified with certain functions. It has been suggested that these functional regions are organized into distinct structural domains. The shape of the molecule and the localization of domains determined by electron microscopy confirm this hypothesis. A schematic representation of the shape of HK and its organization into domains is shown in Fig. 6a. The HK molecule after reduction of the disulfide bond linking the carboxyl- and amino-terminal ends is shown schematically in Fig. 6b.

**Prekallikrein and Its Interactions with High Molecular Weight Kinogens**—PK appears to be a globular protein with a diameter of about 9 nm. The gel filtration behavior of PK (27), when compared with polyacrylamide gel electrophoresis in SDS (28, 29), is consistent with the observed globular structure. Sometimes a cleft is observed which may represent the boundary between the heavy and light chains.

Observations of PK-HK complexes showed that the PK-binding domain is at one end of HK. Electron microscopy of complexes of PK with HKa revealed that PK binds to the end nodule that has the smaller globular domain nearby. This conclusion concerning the location of the binding site is most obvious in images in which most of the PK molecule lies lateral to one end of HK. However, other complexes, in which PK lies on top of one end of HK, show the flexibility of the HK. More importantly, in this configuration the (pre)kallikrein could reach the cleavage sites to release bradykinin. The proposed interaction between HK and PK is shown schematically in Fig. 6c.

**Observations of Changes in Conformation on Cleavage of HK by Plasma Kallikrein**—Plasma kallikrein cleaves HK in a three-step sequential manner (30). The first cleavage yields a "nicked" kinogen composed of two disulfide-linked chains with molecular masses of 64 and 56 kDa. The second cleavage yields bradykinin and an intermediate kinin-free protein. The third cleavage results in a stable kinin-free protein composed of two disulfide-linked 64- and 45-kDa chains and liberates a small 7-kDa peptide. There is considerable evidence from circular dichroism spectroscopy for a conformational change accompanying these cleavage steps (31). These studies showed that cleavage by kallikrein to produce HKa was accompanied by a conformational change characterized by an increase in ellipticity at 222 nm, suggesting an increase in organized structure. Circular dichroism spectra of HK suggest that there is
little α or β secondary structure, but the increase in ellipticity indicates an apparent increase in α-helical content on conversion to HKa. The amino acid sequences on either side of the cleavage sites have high α-helical potential as determined by the method of Chou and Fasman (32). The cleavage of HK is also accompanied by enhancement of the intrinsic fluorescence, suggesting that the environment of aromatic residues is modified by the proteolysis.

Such a conformational change is readily evident in images of HKa as compared with HK. In HK the three nodules are arranged linearly, whereas they roughly form a triangle in HKa. With the identification of domains just described, it is apparent that the small central surface-binding domain swings out upon activation. These observations are consistent with a change in conformation of HKa. The amino acid sequences on either side of the cleavage sites. Constraints imposed by the linear arrangement of domains in HK or other environmental factors may also account for the apparent increase in α-helical content. It may be that there is a strained configuration of the linearly arranged domains in HK that is maintained by the amino- to carboxyl-terminal disulfide, since the predicted length of portions of polypeptide chain making up this connector is about the same as the measured molecular length.

Furthermore, the observed change in conformation of HKa could account for the increase in its tendency to bind to surfaces. It could be that the surface-binding sequences are partially blocked in native HK but are exposed by this change in conformation. With the triangular configuration of the cleaved molecule, the surface-binding domain would be in a position to interact with the vessel surface while the other domains were extended above the surface, free to interact with other proteins, as shown schematically in Fig. 6d.

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