The Coagulation-Kinin Pathway of Human Plasma

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INITIATION of the intrinsic coagulation-kinin pathway requires the interaction of certain negatively charged surfaces with three plasma proteins: factor XII (Hageman factor [HF]), prekallikrein, and high-molecular weight (HMW) kininogen. Prekallikrein and HMW kininogen circulate in plasma as a complex with a 1:1 molar stoichiometry. These proteins bind to initiating surfaces, HF becomes activated, prekallikrein is converted to kallikrein, and HMW kininogen is digested to release the vasoactive peptide bradykinin (Fig 1). A second major plasma substrate of activated HF (HFa), factor XI, also circulates as a complex with HMW kininogen and is converted to factor Xla, which continues the intrinsic coagulation cascade. During the past 15 years, considerable progress has been made in elucidating the molecular details of these reactions. The aforementioned factors also interact with other plasma proteins, the peripheral vasculature, and with a variety of cell types to contribute to the body’s inflammatory response. In addition, new methods have been developed to assess the contribution of this portion of the coagulation cascade to human disease. This review will include progress made in each of these areas, controversies that exist, and directions for future investigation.

INITIATION OF CONTACT ACTIVATION

Human HF, coagulation factor XII, is a single-chain β globulin of molecular weight 80,000. During contact activation, HF becomes cleaved and then fragmented. The structural changes are accompanied by the development of enzymatic activity expressed against low-molecular weight (LMW) peptide derivatives as well as a number of plasma proteins. One paradox of contact activation is that kallikrein, the major plasma protease capable of activating HF (Fig 1) and other weaker activators (factor Xla and plasmin), are themselves formed in HF-dependent reactions. Purified HF and prekallikrein show minimal enzymatic activity if precautions are taken to eliminate contaminating active enzyme. However, when mixtures of HF and prekallikrein are exposed to an “activating” macromolecular anionic material (surface), rapid activation of both proteins takes place.

Any mechanism proposed for the initiation of contact activation must explain the behavior of normal and deficient plasmas. An important example is Fletcher trait plasma, which is deficient in prekallikrein and has a coagulation defect that is corrected upon reconstitution with prekallikrein. If the time of incubation with surfaces is increased, the clotting time in a two-stage assay approaches that of normal plasma; thus, surface-dependent activation of HF can occur in the absence of prekallikrein, and HFa can in turn convert factor XIa to factor XIa, so that coagulation can proceed. Little kinin is formed, however, because prekallikrein is absent (Fig 1). Small amounts of kinin may be made relatively slowly by direct cleavage of HMW kininogen by HFa or factor Xla, but the amounts are unlikely to be physiologically significant. In contrast, HF-deficient plasma does not activate upon contact with a surface. These observations draw attention to the central role of HF in contact activation; prekallikrein apparently plays an accelerating role rather than a required role. This concept is supported by the discovery that human HF is able to autoactivate, a feature shared with the rabbit contact system but apparently not the bovine system. The autoactivation of HF may explain its activation in prekallikrein-deficient plasma and the consequent correction of the clotting defect.

The ability of HFa to cleave and activate native HF has been demonstrated directly for both rabbit and human proteins. In addition, the kinetics of the appearance of activity in purified HF exposed to a surface has been analyzed by an iterative computer model, an explicit second-order kinetic model, and by demonstration of exponential initial rates. All of these analyses have yielded data consistent with a simple autoactivation scheme in which HFa digests HF to produce more HFa.

The autoactivation of HF focuses our attention on the interaction of HF with a surface. This reaction poses the same dilemma as does the complete contact activation reaction in plasma; namely, how does one or more species of zymogen develop enzymatic activity when exposed to a...
HF activation by kallikrein is less clear and varies with the surface XI, and the generation of bradykinin. The major cofactor role of depicting HF autoactivation, activation of prekallikrein and factor (5 activations of HF and prekallikrein create a system with al29 and by Kurachi et al3 who observed rapid activation of development of this hypothesis was proposed by Heimark et al.28 The cleavage of a low level of enzymatic activity precedes the observable activating prekallikrein.27'28 In this hypothesis, the induction of a low level of enzymatic activity precedes the observable cleavage of HF to make recognizable HFa.28 The cleavage step occurs subsequent to kallikrein formation, and the latter cleavage and that this augmentation is one component of the cleavage leading to HFa formation. This observation suggested that the initial rate of kallikrein formation was similar inhtio or that it functions in some manner to induce activity in the surface amplifies traces of activity present in zymogen ab initio or that it functions in some manner to induce activity in previously inactive molecules. McMillin et al and Ratnoff and Saito originally proposed that HF undergoes a conformational change upon binding to a surface whereby it exposes or creates an active site that is then capable of activating prekallikrein. In this hypothesis, the induction of a low level of enzymatic activity precedes the observable cleavage of HF to make recognizable HFa. The cleavage step occurs subsequent to kallikrein formation, and the latter enzyme acts upon HF in a feedback reaction. A further development of this hypothesis was proposed by Heimark et al29 and by Kurachi et al30 who observed rapid activation of prekallikrein and of factor XI by bovine HF and suggested that the initial rate of kallikrein formation was similar whether zymogen HF or HFa was used. These authors postulated that surface-bound HF was capable of a substrate-induced conformational change that created an efficient prekallikrein activator. This activity would not be detected by LMW peptide substrates that lack the macromolecular structures necessary to induce the conformational change.

Definitive proof of the creation of active sites prior to polypeptide chain cleavage is difficult to obtain. The reciprocal activations of HF and prekallikrein create a system with positive feedback that is therefore subject to rapid acceleration. Tankersley and Finlayson25 estimated the kinetic constants for the component reactions and calculated that, at plasma concentrations of HF and prekallikrein, the introduction of a surface into the mixture where one molecule of active enzyme is present per milliliter (5 x 10^{-13}g of total protein) will result in 50% activation of either protein in 13 seconds. Such a low level of contamination of native HF with HFa has not been achieved; a level of 0.02% is the lowest thus far reported.31 Therefore, if native HF had no activity, rapid activation of prekallikrein would be observed due to the HFa that is necessarily present.

Because human HF is able to autoactivate, low levels of HFa can accumulate in preparations of HF, even in the absence of a surface. Addition of irreversible inhibitors of active enzyme (eg, diisopropyl fluorophosphate, DFP) does not deplete active enzyme in a preparation much below 0.1%. We used a rapidly acting chloromethyl ketone inhibitor to try to destroy all traces of HFa in preparations of HF and thereby reduced enzymatic activity to less than 1/4,200 of that of HFa.31 Even after such treatment, HF was able to autoactivate and activate prekallikrein, albeit with very low initial rates. These experiments led to a proposal that the enzymatic activity of the zymogen (if any) is too low to measure and that the mechanism of contact activation of human plasma involved extremely low levels of active enzyme that are continuously present. That this might be possible in vivo is suggested by experiments in which prolonged incubation of purified HF with C1 esterase inhibitor (C1 INH) failed to prevent the conversion of HF to HFa or HF fragment (HFF).32 Therefore, the contact of plasma with a surface may induce activation by accelerating many 1,000-fold a normally occurring slow turnover of HF and prekallikrein. The effect of trace amounts of activity already present is amplified by the surface. The possibility that native HF does have trace activity analogous to the activity present in trypsinogen33 is not excluded, although it is subject to the constraints of the aforementioned experiments. It should also be considered that the functioning of the contact system in vivo might require formation of trace quantities of HFa by other plasma enzymes, perhaps derived from cells.

There appear to be two components to the mechanism by which surface accelerates the reactions of contact activation. Griffin34 demonstrated that the activation of HF by a series of different proteases was accelerated in the presence of a surface. He proposed that HF underwent a conformational change upon binding that increased its susceptibility to the cleavage leading to HFa formation. This observation suggests that surface-bound HF is a better substrate regardless of whether HFa (autoactivation) or kallikrein effects its cleavage and that this augmentation is one component of the catalytic action of the surface.

It is not known whether the cleavage and activation of surface-bound HF is accomplished by an enzyme molecule bound to a neighboring surface site or by one that remains free in solution. In the latter case, the catalytic effect of the surface would be due in large part to the creation of a local milieu in which the reversible binding of the protein components results in their increased concentration. Alternatively, the surface might physically locate the enzyme and the substrate in neighboring binding sites just as an enzyme does for its substrates. Another way to phrase this question is to ask whether both components of the Michaelis complex interact with the surface or only the substrate. Different mechanisms may be important for activation by HFa or kallikrein and with different surfaces.

Once a small amount of HFa has been produced, it converts prekallikrein to kallikrein, the major plasma enzyme that activates HF. There is, therefore, reciprocal
activation in which these two proteins digest each other. Because the rate of HF activation by kallikrein is much more rapid than HF activation by HFa, this pathway should be kinetically dominant. This conclusion is supported by studies of normal and prekallikrein-deficient plasma (Fig 1).

Recent evidence indicates that kallikrein also has to bind to the surface for maximum efficiency of HF activation. Thus, kallikrein cleaves surface-bound HF 3,000 times faster than fluid-phase HF, but cleavage by an equimolar quantity of the isolated (reduced and alkylated) light chain of kallikrein, which lacks the surface binding site, is accelerated only 30-fold. The activation of prekallikrein by HFa is also facilitated by surface, but to a lesser degree; in one study, dextran sulfate gave a 60-fold increase in cleavage by HFa and had no effect on the rate of cleavage by HFa.

It is pertinent at this point to consider the variety of compounds that have been used as surfaces. Much of the original work delineating the pathways of contact activation was done with kaolin. In terms of contact activation, however, kaolin is an ill-defined substance that is capable of binding many plasma proteins. More recently, dextran sulfate and sulfatide have been used as surfaces in studies of contact activation. These materials have the advantage of being much more homogeneous and, in the case of dextran sulfate, truly soluble. Using these activators, it has been possible to determine the rates of appearance of enzymatic activity and thereby to derive kinetic constants for the component reactions.

The idea of a reciprocal reaction between the zymogen and activated forms of HF and prekallikrein was complicated by the realization that there was yet another component required for optimal contact activation. Persons were studied whose plasma was not deficient in HF or prekallikrein, but their plasma gave very long kaolin-induced partial thromboplastin times (PTT) and generated no bradykinin. This led to the identification of HMW kininogen as a nonenzymatic cofactor that augments reciprocal activation. Initial experiments conducted with kaolin demonstrated that the presence of HMW kininogen increases the rate of conversion of prekallikrein to kallikrein by HFa and also increases the rate of activation of HF by kallikrein. HMW kininogen also increases the rate of factor XI activation by HFa.

One critical observation regarding the mechanism by which HMW kininogen functions as a cofactor was the demonstration that in plasma both prekallikrein and factor XI circulate bound to HMW kininogen and that these complexes bind to initiating surfaces via the HMW kininogen moiety. In plasma, HMW kininogen augments the attachment of prekallikrein and factor XI to surfaces such as kaolin, when the HMW kininogen is cleaved, this effect is augmented. Thus, HMW kininogen may be an activatable cofactor, although the magnitude of this effect is uncertain. Adhesive glycoproteins such as fibrinogen may compete with HMW kininogen for binding to initiating surfaces. Studies have shown the presence of antigenic fibrinogen on clot-promoting surfaces that becomes undetectable after ten minutes of contact when HMW kininogen is present, an effect not observed in HMW kininogen-deficient plasma. It will be of interest to determine whether cleaved and uncleaved HMW kininogen behave differently in such assays.

In buffer systems, prekallikrein and factor XI bind to kaolin equally well in the presence or absence of HMW kininogen, but activation of each by HFa is dependent upon the presence of HMW kininogen. Aside from the quantitative binding effects seen in plasma, therefore, HMW kininogen must place the substrates of HF in a conformation that facilitates their cleavage (Fig 2). More difficult to explain is the ability of HMW kininogen to augment the cleavage and activation of HF by kallikrein since it has no demonstrable effect upon the enzymatic activity of kallikrein (using synthetic substrates) and no known interaction with HF. The dissociation constant for the prekallikrein (kallikrein)–HMW kininogen complex is 15 nmol/L and at plasma concentrations, about 10% to 20% of prekallikrein circulates free. Thus, kallikrein that is formed by activation of surface-bound prekallikrein–kininogen complexes can dissociate from the surface. It is then able to digest adjacent HF molecules on the same or other particles. In this fashion contact activation can be disseminated along the surface. Thus, the effective concentration ratio of kallikrein/HF is increased in the presence of HMW kininogen, which may account for the augmentation.

In contrast to these observations are studies of the human contact activation system that have been done with purified HF and prekallikrein by using dextran sulfate or sulfatide as activators. Under these conditions, both HF and prekallikrein are rapidly activated in the absence of HMW kininogen. The cofactor produces a small acceleration in the activation of HF by kallikrein, whereas its effect on the reverse reaction has not been reported. This contrasts with activation in the presence of kaolin in which activation of prekallikrein by HFa is strongly dependent on HMW kininogen. When dextran sulfate is added to plasma, however, almost no kallikrein is produced if HMW kininogen is absent. Thus, the effects of a surface vis-à-vis the role of HMW kininogen differ somewhat depending upon the nature of the surface used and whether the reaction is studied in plasma or with purified components.

One way in which a plasma milieu might be different relates to observations that HMW kininogen may protect
kallikrein and factor Xla from inhibition by C1 INH, α2 macroglobulin, and αt antitrypsin and thereby augment the plasma half-life of each enzyme. The light chain of HMW kininogen, the portion of the protein that binds to prekallikrein and factor XI, was reported to be as effective in preventing the inhibition as intact HMW kininogen. This result suggested that the phenomenon cannot be explained by substrate competition between HMW kininogen and C1 INH at the active site of kallikrein. This is a controversial point, and Van der Graaf et al observed no effect of HMW kininogen upon inhibition of kallikrein by C1 INH. We found the second-order rate constant for the reaction of kallikrein with Cl INH to be unchanged in the presence of a saturating concentration of kinin-free HMW kininogen. Therefore, we are unsure whether HMW kininogen protection of enzyme inactivation contributes significantly to its augmentation of contact activation.

CLEAVAGE OF PROTEINS DURING CONTACT ACTIVATION

Figures 3 to 5 diagram the cleavages and fragments resulting from activation of each component of the system and indicate functional domains where they have been defined. HF (Fig 3) is initially cleaved within a disulfide bridge so that a single chain of 80,000 is converted to a two-chain enzyme in which a heavy chain of 50,000 is disulfide linked to a light chain of 28,000. No loss in size results, and the enzyme is called HFa (or α HFa). The binding site for the surface is on the heavy chain, and the light chain contains the active enzymatic site. Further cleavage by kallikrein digests HFa at two sites (2 and 3) in sequence to form HFF (or β HFa) in which a heavy chain of 28,000 is disulfide linked to a light chain of either 2,500 or 500. A mixture of these two forms accounts for the doublet seen in alkaline disk gels or unreduced sodium dodecyl sulfate (SDS) gels.

Enzymes other than kallikrein are able to cleave and activate HF, including plasmin and factor Xla, but these reactions are less efficient. The cleavage of HF by HFa during autoactivation is of potentially greater significance than these reactions. Autoactivation produces, in addition to HFs and HFF, a third form in which the serine protease domain is linked to a 12,000-MW fragment of heavy chain to give a molecule of MW 40,000 before reduction (Fig 3), but kallikrein rapidly converts it to HFF.

The complete amino acid sequences of the heavy chain of HFa and both chains of HFF are now known. Fujikawa and McMullen found nine amino acids in the light chain of HFa, two short domains resemble fibronectin, two growth factor regions are homologous with epidermal growth factor, and a kringle structure is seen, as has been reported in plasminogen, tissue plasminogen activator, urokinase, and prothrombin. The light chain of HFa (or heavy chain of HFF) containing the active site has a high degree of homology with tissue plasminogen activator and progressively less homology.

![Figure 3](image)

Fig 3. Sites of cleavage of HF during contact activation. Site 1, within a disulfide bridge, converts HF to HFa. Cleavage external to the disulfide bond at sites 2 and then 3 converts HFa to the two forms of HF. The light chain of HFa (residues 334 to 353 or 343 to 353) is derived from the C-terminal end of the heavy chain of HFa. Since the surface binding site is at the amino terminal end of HF or HFa, formation of HFF leads to dissociation from the surface. The active-site charge-relay complex (His, Asp, Ser) is at the C-terminal end.

![Figure 4](image)

Fig 4. Diagrammatic representation of the activation of prekallikrein and factor XI by HFa. Cleavages occur within disulfide bridges in each case, and the active site is in the light chain(s). Because factor XI is dimeric, factor Xla has two identical active sites.
with urokinase, plasmin, trypsin, factor X, or factor IX. Strong homology with proteins involved in fibrinolysis is apparent. More recently, the complete amino acid sequence has been deduced by using factor XII cDNA isolated from a cDNA library prepared from human liver mRNA. HFF possesses only 2% to 5% of the coagulant activity of HFa. HFF is an effective prekallikrein activator, however, and can lead to bradykinin formation until C1 INH binds and inactivates it.

Prekallikrein is a single chain γ globulin (isoelectric point IEP 8.9) with two forms at MW 85,000 and 88,000, both of which are present in all plasmas tested. As many as seven forms can be identified by isoelectric focusing. Prekallikrein is converted to kallikrein (Fig 4) by cleavage within a disulfide bridge such that a heavy chain of 52,000 is disulfide linked to light chains of either 33,000 or 36,000 (one for each molecular form). The heavy chain interacts with the surface and binds to HMW kininogen, whereas the light chain has the enzymatic active site. Purified light chain retains enzymatic activity on synthetic substrates or in fluid-phase kinin generation but is ineffective as a surface-dependent coagulation factor. During lengthy incubations, the heavy chain of kallikrein is further cleaved so that a kallikrein of lower MW results (fl-kallikrein), with little loss of kinin-forming ability; but activation of other proinflammatory actions (discussed subsequently) are altered.

Factor XI is unusual among coagulation factors because it consists of two identical disulfide-linked chains (Fig 4). Upon activation each chain is cleaved to yield disulfide-linked heavy and light chains; therefore, the active enzyme has four chains. The heavy chains bind to the surface. Each light chain has an active site and, when inactivated, can bind 1 mol of inhibitor. Also, it has recently been shown that the molar ratio of binding of HMW kininogen to factor XI is 2:1.

HMW kininogen is cleaved by plasma kallikrein at two sites (Lys-Arg and Arg-Ser) to liberate the nonapeptide bradykinin. Both cleavages occur within a disulfide bridge so that kinin-free HMW kininogen consists of a heavy chain of MW 62,000 disulfide linked to a light chain variously reported as having an MW of 56,000 to 62,000. It is unclear whether release of bradykinin results in chains that are equal in size (by SDS–polyacrylamide gel electrophoresis) and that another cleavage follows that forms the 56,000 band or whether the size differences reported in these studies are due to differences in technique. Further proteolysis of the light chain by kallikrein converts it to a light chain of 45 to 47,000. The heavy chain of HMW kininogen, which is identical to the heavy chain of LMW kininogen, has been shown to inhibit cysteine proteases such as cathepsin L, papain, or platelet calpain 2. In fact, cDNA analysis of α1 thiol proteinase inhibitor demonstrates that it is identical to LMW kininogen. The light chain of HMW kininogen is the coagulant portion of the molecule and possesses a binding site for the surface as well as a binding site for the heavy chain of prekallikrein or factor XI (Fig 5). These binding constants are estimated to be 3.4 × 10⁷ mol/L⁻¹ and 4.2 × 10⁶ mol/L⁻¹, respectively.

Human LMW kininogen is identical to HMW kininogen from the amino-terminus to 12 residues beyond the bradykinin moiety. During transcription of kininogens the DNA corresponding to this segment can be spliced into two different DNA segments, which correspond to the kininogen light chains (Fig 6). Those latter segments are completely different in terms of size, amino acid sequence, and function. LMW kininogen has no procoagulant activity and no known interaction with other proteins of the contact activation system, and the function of its light chain (if any) is unknown.

Bovine HMW kininogen has an additional kallikrein cleavage site located beyond the bradykinin moiety in a region that would correspond to the amino-terminal segment for the human HMW kininogen light chain. Cleavage at this site results in the loss of a fragment (termed fragment 1.2) which appears to contain the binding site for the surface. Its removal thus destroys the coagulant cofactor activity. The species difference may be attributable to the presence of a 22-residue sequence in the human but not the bovine protein and the presence of Lys rather than Arg at the site of the human protein analogous to the carboxy-terminus of bovine fragment 1.2. In humans, factor XIa performs a similar cleavage in the HMW kininogen light chain, which likewise inactivates it and may represent an intrinsic control mechanism.

The major kininogen of rodents is T kininogen from which a kinin (Ile-Ser-bradykinin) is cleaved by high concentrations of trypsin or cathepsin D but not by kallikreins. The proteins corresponding to HMW and LMW kininogens are present but are minor components; all three kininogens are strongly homologous at the DNA and amino acid sequence level.

**THE INTRINSIC FIBRINOLYTIC PATHWAY**

An HF-dependent pathway leading to the conversion of plasminogen to plasmin was described in the 1960s and early 1970s. A defect in this pathway has been observed in plasma deficient in either HF, prekallikrein, or HMW kininogen. This is a relatively weak fibrinolytic pathway, and it is difficult to demonstrate in whole plasma because it does not lead to formation of large quantities of a potent plasminogen activator. Relatively small quantities of plasmin are generated, and the plasmin is then rapidly inactivi-
vated by plasma inhibitors. Therefore, most of the aforementioned studies utilized diluted acidified plasma in which inhibition of both contact activation and plasmin are minimized and fibrinolytic activity can be measured. Although the identification of the plasminogen activator in this pathway has proved to be particularly difficult, certain aspects of the abnormal behavior of various deficient plasmas are predictable, given that HF is required. Prekallikrein and HMW kininogen are needed for optimal HF activation, and HMW kininogen is required for activation of both prekallikrein and factor XI; therefore, a deficiency of any of these proteins will be associated with abnormal fibrinolysis.

By utilizing purified components, kallikrein was first shown to be a plasminogen activator \(^{108}\). A plasminogen proactivator was described subsequently that appeared to differ from prekallikrein \(^{109}\), the activity was demonstrable in prekallikrein-deficient plasma. \(^{108}\) Others disagreed, reporting that plasminogen proactivator and prekallikrein were identical. \(^{111,112}\) Later studies, however, demonstrated that a plasminogen proactivator could be identified in the \(\gamma\) globulin fraction of prekallikrein-deficient plasma and was identified as factor XI. \(^{73}\) When compared, kallikrein and factor XIa were found to be equipotent as plasminogen activators. \(^{113}\) The concentration of prekallikrein, however, is much greater than factor XI (six- to tenfold); kallikrein can dissociate from surfaces into the fluid phase, whereas factor XIa does not, and prekallikrein can be activated in the fluid phase by HF. Therefore, kallikrein appears to be more important than factor XIa as a plasminogen activator although one can demonstrate a fibrinolytic abnormality in factor XI-deficient plasma. \(^{114}\) The potency of both kallikrein and factor XIa on a molar basis is, however, many thousandfold less than urokinase. \(^{113,113,114}\) Activated HF (HFa or HFf) also can convert plasminogen to plasmin directly, \(^{115}\) but it is only about 5% as potent as kallikrein. \(^{111}\) These are all weak reactions, and one can readily argue that plasminogen is not a significant substrate for any of these enzymes. It is interesting to note, however, that direct incubation of factors VIIa, IXa, Xa, or thrombin with plasmin fails to generate plasmin at any concentration tested. Of the coagulation reactions, only proteins of the contact activation system catalyze the conversion of plasminogen to plasmin. More recently, there have been reports that kallikrein can activate the trace of prourokinase present in plasma \(^{118}\) and urokinase might then be the major plasminogen activator. \(^{119}\) Inhibition by antiurokinase antibodies supports this notion, \(^{120}\) whereas others disagree. \(^{120a}\) Nevertheless, the quantity of urokinase generated is very small. The effects of both C1 INH and \(\alpha\)-antiplasmin may be abrogated by the addition of certain organic compounds (eg, flufenamic acid derivatives) to plasma. \(^{121,122}\) Contact activation of plasma treated in this way results in the formation of only about 35 ng/mL of plasmin \(^{121}\) (Fig 7).

**INTERACTION WITH OTHER PLASMA PROTEASES AND WITH CELLS**

HFa has been reported to activate factor VII \(^{123,124}\) and thereby initiate the extrinsic coagulation pathway. This

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**Fig 7.** Interaction of proteins of contact activation with other plasma proteolytic cascades. HFf can initiate activation of the classic complement pathway, whereas kallikrein can convert prorenin to rennin and participate in intrinsic fibrinolysis. Kallikrein, factor XIa, and HFa can all directly convert plasminogen to plasmin. Kallikrein can also convert single-chain urokinase (termed prourokinase; this is an active enzyme that is normally inhibited in plasma by a competitive inhibitor) to two-chain urokinase (which transiently escapes inhibition). Whether this urokinase is the major plasminogen activator or whether it is the direct effect of kallikrein is uncertain.

**Contributions minimally to the kaolin-activated PTT as usually performed, but it is responsible for the shortened clotting time seen when plasma is exposed to the cold.** \(^{125,126}\) This is accentuated in women who use oral contraceptives containing estrogen, apparently due to an increased concentration of HF. \(^{127-129}\) It is theorized that this pathway might contribute to the increased incidence of thrombosis reported as a complication of oral contraceptive use.

HF (but not HFa) has been shown to activate enzymatically the first component of complement \(^{130}\) when it is incubated with purified Cl or added to plasma (Fig 7). Cl activation is due to cleavage of the C1r subcomponent \(^{131}\) by HF. Little complement activation is seen, however, when kaolin is incubated with whole plasma. Significant complement activation may be seen only in conditions that result in substantial conversion of HFa to HFf. One such circumstance is C1 INH deficiency (ie, hereditary angioedema) in which HF activation may contribute to complement consumption. \(^{132,133}\) Kallikrein can also cleave C1 subcomponents, but the net result is destruction rather than activation. On the other hand, kallikrein can activate factor B of the alternative complement pathway and thereby substitute for factor D. \(^{134}\)

Kallikrein has been reported to interact with human leukocytes in a variety of ways. It is a chemotactic factor for neutrophils \(^{135}\) and monocytes, \(^{136}\) and it has been shown to cause neutrophil aggregation \(^{137}\) and release of elastase. In a rabbit model, kallikrein stimulation of chemotaxis appeared to require cleavage of C5 and release of C5a chemotactic factor. \(^{138}\) It is therefore possible that C5 bound to the surface of neutrophils is being cleaved in the aforementioned reactions. However, antikallikrein serum was inhibitory whereas anti-C5 serum had no effect; the authors therefore concluded that the effect of kallikrein on human neutrophils is independent of complement. Further, a degraded form of kallikrein (\(\beta\)-kallikrein) in which the heavy
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Chain is partially digested is enzymatically active on kininogen to form kinin, but its reactivity with neutrophils is markedly attenuated. HFa (but not HFF) has also been shown to stimulate neutrophils; a requirement for a binding site in the heavy chain appeared to be required for the reaction to proceed. There are no studies demonstrating either a cell surface receptor for these enzymes or required cleavage of surface components. In each instance, however, the active site of the enzyme is required so that the proenzyme or DFP-treated enzyme is inactive.

The components of the contact activation system also appear to interact with platelets. There are apparently separate platelet receptors for plasma factor XI and factor Xla. Platelets also have intrinsic factor XI activity that differs from plasma factor XI in MW and isolectric point but that cross-reacts with it immunologically. This activity is present in patients who are deficient in plasma factor XI. Platelet-associated factor XI can be activated by both HF-dependent and -independent mechanisms. The membrane of activated platelets also appears to provide a surface on which HF can be activated, a process dependent on prekallikrein and HMW kininogen, so that bound factor XI is then activated. HMW kininogen demonstrates binding to platelets that is zinc dependent. It has been shown to be present within the alpha granules of platelets, becomes available during platelet activation, and augments binding of factor Xla. Yet it is clear that platelets are not requisite for contact activation as they are for later steps in coagulation. Therefore, contact activation proceeds normally in platelet-poor plasma, and the addition of platelets provides little or no augmentation. In fact, there is evidence that platelets may actually inhibit prekallikrein and factor XII activation by secretion of β thromboglobulin; however, platelet activation in peripheral tissues due to injury and/or localized thrombosis may contribute to contact activation and local kinin formation. In some circumstances the platelets may also provide a factor XII bypass in contact activation.

CONTROL MECHANISMS

Regulation of the HF pathways occurs by both intrinsic and extrinsic mechanisms. Cleavage of HFa to HFF is one example of an intrinsic control in which coagulation via the surface-dependent activation of factor XI is limited whereas bradykinin generation via fluid-phase activation of prekallikrein continues. Likewise, the digestion of kinin-free HMW kininogen light chain by factor Xla limits its coagulant activity. Extrinsic controls are provided by plasma inhibitors of each enzyme (Table 1). The only major plasma inhibitor of HFa or HFF is C1 INH. There are two main kallikrein inhibitors in plasma: α2 macroglobulin and C1 INH. When kallikrein is added to plasma, approximately half is bound to each inhibitor at equilibrium; When a surface such as kaolin is added to plasma, however, close to 70% or 80% of the kallikrein formed is bound to C1 INH; the mechanism responsible for this change in inhibition ratio is unknown. Factor Xla is inhibited primarily by α1 antitrypsin and C1 INH, whereas plasmin is inhibited by α2 antiplasmin and α2 macroglobulin. Anti-thrombin III (AT III) accounts for only a few percent of plasma inhibition of each of these factors, but its contribution in the presence of heparin is uncertain. There is clearly less augmentation than is seen with thrombin. Some studies have revealed little augmentation of AT III inhibition of HFa but kallikrein or factor Xla in the presence of heparin, whereas others find that heparin has a prominent effect upon the rate of AT III inhibition of each of them. These reactions need to be reassessed with purified components and the rate of inhibition of each enzyme carefully determined in heparinized plasma. For such studies the dose of heparin and definition of the activity of the fraction used will be critical.

Bradykinin is an exceedingly potent vasoactive peptide that can cause venular dilatation, increased vascular permeability, hypotension, constriction of uterine and gastrointestinal (GI) smooth muscle, constriction of coronary and pulmonary vasculature, bronchoconstriction, and activation of phospholipase A2 to augment arachidonic acid mobilization. Its regulation is of prime importance, and plasma contains a variety of enzymes that serve this function. Recent studies of kinin degradation from our laboratory are summarized in Fig 8. First, the C-terminal arginine is removed by carboxypeptidase N, thereby leaving the residual octapeptide. The des-Arg9-bradykinin formed is inactive in the skin or GI tract, but it is equipotent to bradykinin in causing hypotension. Separate receptors for these two moieties have been proposed and are called B1 (des-Arg9-bradykinin) and B2 (bradykinin). The rate of Arg removal in serum, however, far exceeds the rate in plasma, whereas the level of carboxypeptidase N is unaltered. It appears that a carboxypeptidase B-like enzyme is generated during coagulation that is secreted, perhaps, by platelets or neutrophils. Des-Arg4-bradykinin is then degraded by angiotensin-converting enzyme to the pentapeptide Arg-Pro-Pro-Gly-Phe plus the tripeptide Ser-Pro-Phe. The C-terminal phenylalanine is then cleaved from each peptide by enzymes possessing

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inhibitor</th>
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<td>HFa (HFF)</td>
<td>C1 INH</td>
</tr>
<tr>
<td>Factor Xla</td>
<td>α1 Antitrypsin, C1 INH</td>
</tr>
<tr>
<td>Kallikrein</td>
<td>C1 INH, α2 macroglobulin</td>
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<tr>
<td>Plasmin</td>
<td>α2 Antiplasmin, α2 macroglobulin</td>
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Table 1. Major Inhibitors of Active Proteases of the Contact Activation System

Arg Pro Pro Gly Phe Ser Pro Phe Arg or CP8 Arg Pro Pro Gly Phe Ser Pro Phe Arg or CP8 des-Arg4-Brdykinin ACE

Fig 8. Pathway for degradation of bradykinin in human plasma. One or more carboxypeptidases remove the C-terminal arginine, and des-Arg4-bradykinin is cleaved by angiotensin-converting enzyme (ACE). The pentapeptide and tripeptide products are further degraded by enzymes (not yet characterized) with activities resembling carboxypeptidase A and/or a prolylcarboxypeptidase.
activities consistent with carboxypeptidase A and prolylcarboxypeptidase, respectively. These enzymes have not yet been isolated or characterized. Not shown in Fig 8 is the further clearance of Arg-Pro-Pro-Gly and Ser-Pro so that the final degradation products of bradykinin digestion are Arg-Pro-Pro-Gly and free amino acids.

Because bradykinin is a peripheral vasodilator, it has been considered to act as a potential “counterbalance” to the vasopressant effects of angiotensin II. From the previous discussion, it is clear that these two peptides are also related in terms of metabolic control since angiotensin-converting enzyme cleaves the decapeptide angiotensin I to generate angiotensin II and also degrades and inactivates bradykinin or des-Arg⁵-bradykinin. It is important to point out that although angiotensin-converting enzyme readily degrades bradykinin in physiological buffers, one bradykinin is first converted to des-Arg⁵-bradykinin in plasma or serum and the latter peptide is actually the substrate for the converting enzyme.

There are other interactions of the kallikrein/kinin system and the renin/angiotensin system in plasma and serum that have been described. For example, plasma kallikrein has been shown to convert prorenin to renin (Fig. 7) when plasma is acidified and then neutralized, prorenin is converted to renin, and the latter alkaline phase is an HF-dependent reaction. It appears that acidification negates the effects of plasma inhibitors and affects prorenin susceptibility to activation. The importance of this reaction under normal physiological conditions is uncertain.

**ASSESSMENT OF CONTACT ACTIVATION IN HUMAN DISEASE**

The HF-dependent pathways described herein are not likely to contribute to thrombosis and hemostasis in a major way. Thus, although activation of HF leads to in vitro coagulation, in most thrombotic disorders there is activation of the extrinsic pathway occurring either alone or in addition to HF activation, and the effects of tissue factor predominates. Certainly, there is no thrombosis associated with hereditary angioedema, a disorder in which contact activation is prominent. Likewise, patients deficient in HF, prekallikrein, and HMW kininogen have no obvious bleeding diathesis, and factor XI–deficient patients have a bleeding disorder that is highly variable and often mild. Observations such as these have led to considerations of physiological mechanisms of factor XI activation that bypass the other contact activation components, but these remain unsettled. Most evidence suggests that the importance of the cascade relates to the pathogenesis of inflammatory reactions, the local control of blood flow (in which bradykinin functions as a hormone), and perhaps control of BP. To assess the HF-dependent pathways in human disease, we ideally would like to measure each active enzyme, demonstrate cleaved HMW kininogen, and assay bradykinin. These determinations are limited because the enzymes and the bradykinin are rapidly inactivated, and often one has to determine the level of residual proenzyme. One can quantitate HF, prekallikrein, or HMW kininogen in plasma by coagulant assay, immunologic determination of antigen levels, or by in vitro activation and evolution of enzymatic activity. Shifts in electrophoretic mobility are indicative of complex formation between enzymes and their inhibitors and provide qualitative evidence that activation has occurred. Thus far monoclonal antibodies that react solely with the active site of enzymes have not been developed, although De Agostini et al have described a monoclonal antibody that reacts with a neoantigen in the kallikrein–C1 INH complex.

The previously described assays are useful if sufficient activation has occurred to cause significant depletion of substrate. But if only a small amount of each factor is converted, it may be missed. Assays have recently been developed for enzyme-inhibitor complexes based on the assay for plasmin–α₂ antiplasmin complexes by Harpel. These include double-antibody enzyme-linked immunosorbent assay methods for HFa–C1 INH, kallikrein–C1 INH, and kallikrein–α₂ macroglobulin complexes, which can detect as little as 1% activation of any of the components. Bradykinin and des-Arg⁵-bradykinin can be determined by radioimmunoassay.

These latter assays have just begun to be applied to disease states; most of the literature describes results obtained with earlier methods. Hereditary angioedema receives considerable attention because C1 INH is absent and there is simultaneous activation of the HF-dependent pathways and the classic complement pathways. Patients present with episodic swelling occurring virtually anywhere in the body, attacks of severe abdominal pain due to edema of the bowel wall, and laryngeal edema. Although a kinin derived from a cleavage product of the second complement component was thought to be responsible for the edema, other studies suggest that bradykinin is the critical peptide.

Aside from hereditary angioedema, there is a relatively large amount of literature demonstrating activation of the HF-dependent pathways in plasma or serum in a wide variety of inflammatory disorders; in some disorders, the activation is coincident with the patients’ symptoms. There are no data, however, that prove that the consequences of such activation lead to disease manifestations or the converse, that inhibition of the pathway will ameliorate symptoms. These should be viewed as promising associations and are important areas for future investigation. These will only be briefly described.

Contact activation may occur during allergic diseases, for example, nasal washings of patients with allergic rhinitis who have been challenged with antigen contain lysyl bradykinin, bradykinin, kininogen, and kallikreins derived from mast cells and plasma. The latter may represent contact activation on a surface altered by the initial inflammation.
THE COAGULATION-KININ PATHWAY OF PLASMA

Other possibilities for activation might include negatively charged macromolecules in mucosal secretions or mast cell heparin. Although we routinely utilize dextran sulfates as model compounds upon which HF activation can occur, other sulfated mucopolysaccharides may serve similar functions in vivo. The synovial fluid of patients with rheumatoid arthritis has been shown to contain plasma kallikrein, which has been shown to activate procoagglanase to collagenase.


The synovial fluid of patients with rheumatoid arthritis has been shown to contain plasma kallikrein, which has been shown to activate procoagglanase to collagenase. Uric acid and pyrophosphate crystals can act as surfaces for contact activation and may contribute to the inflammation of gout and pseudogout. A role for the contact activation system in inflammatory arthritis is therefore possible and may involve other functions of the enzymes formed in addition to any effects of kinins.

Activation of HF is also induced by the lipid A component of endotoxin. The pooling of fluid into body cavities, the intravascular volume depletion, and the hypotension seen may be caused by release of bradykinin. Likewise, other bacterial infections such as typhoid fever have been shown to be associated with prekallikrein depletion, formation of kallikrein–Cl INH complexes, and Cl INH depletion as evidence of contact activation.

In patients hospitalized because of trauma, the onset of sepsis has been shown to be associated with kininogen depletion, and determination of serial kininogen levels had prognostic value. An unfortunate circumstance has dramatized the kinin-forming capacity of HFF; trauma patients given plasma protein fractions (as plasma expanders) that were contaminated with HFF showed profound hypotension.

In patients with disseminated intravascular coagulation (DIC) due to endothelial injury and/or endotoxemia (including gram-negative sepsis, gram-positive sepsis, or viremia), decreased levels of plasma factor XII and prekallikrein, and kallikrein inhibitory activity are seen; these changes were not observed in DIC associated with leukemia, carcinoma, or abortion. The data suggest activation of the HF-dependent pathways. A similar pattern of protein depletion has been observed in some patients with polycythemia vera, type II hyperlipidemia (familial hypercholesterolemia), and Rocky Mountain spotted fever. In some patients with nephrotic syndrome, a similar diminution of contact activation factors has been reported that could not be explained simply by a loss of proteins in the urine. Cirrhosis, as might be anticipated, is associated with diminished levels of plasma prekallikrein, HMW kininogen, and to a lesser degree, HF, which seems to be due to a diminished rate of protein synthesis.

CONCLUDING COMMENTS

Considerable progress has been made in understanding the consequences of activation of the HF-dependent pathway of plasma. The various proteins and the functions of each have been characterized; yet we have little insight regarding the functions in normal hemostasis and are just beginning to appreciate the pathogenic roles in various inflammatory conditions and shock states.

The fact that patients deficient in HF, prekallikrein, or HMW kininogen do not bleed can be considered as evidence against the importance of contact activation in hemostasis. On the other hand, coagulation is one of the major consequences of HF activation and is the initiating stimulus every time a PTT is performed. Indeed, our concepts regarding bleeding per se are not well developed (particularly for spontaneous bleeding), and it is even conceivable that the absence of one or more contact activation factors (or plasma bradykinin) is protective against bleeding. Likewise, although factor XI deficiency is considered mild compared with factor VIII or IX deficiency, understanding why some patients do or do not bleed with very low levels of factor XI may provide an important piece of information. Areas in which bradykinin may be important in terms of normal physiology include control of the microcirculation in peripheral organs, particularly glandular tissue, and control of BP as a counterbalance to the renin-angiotensin system.

It appears likely that contact activation occurs in a variety of inflammatory conditions and may contribute to disease manifestations such as increased vascular permeability, angioedema, recruitment of polymorphonuclear leukocytes, and changes in BP. With new, more sensitive and specific assays for the components of contact activation, bradykinin, and their various inactivated and/or degradation products should come new insights regarding their roles in disease pathogenesis and new approaches to their inhibition.

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The coagulation-kinin pathway of human plasma

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