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CONFORMATION OF HIGH MOLECULAR WEIGHT KININOGEN: EFFECTS OF KALLIKREIN AND FACTOR XIa CLEAVAGE *

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Summary. The effect of kallikrein and factor XIa proteolysis of high molecular weight kininogen (HK) was investigated. Circular dichroism (CD) spectroscopy showed that cleavage of HK by plasma kallikrein or urinary kallikrein, both of which result in an active cofactor (HK_a), results in conformational change that is characterized by increase in CD ellipticity at 222 nm. This suggests an increase in organized secondary structures. By contrast, cleavage of HK by factor XIa which results in an inactive cofactor (HK_i) is characterized by a dramatic decrease in CD ellipticity at 222 nm suggesting an entirely different type of conformational change. The intrinsic fluorescence of HK is enhanced after cleavage by all three proteases. These conformational changes may play a role in determining the structure and function of HK_a and HK_i.

Contact activation of plasma initiates a series of enzymatic steps leading to blood coagulation, liberation of vasoactive peptide bradykinin and conversion of plasminogen to plasmin (1-3). Initiation of each of these functional activities depends upon a complex interaction between a negative surface and four plasma proteins: factor XII, prekallikrein, factor XI and HK. HK is a single chain glycoprotein with an M_r of 120,000 (4). Cleavage of HK by plasma kallikrein liberates bradykinin as well as a 8 kDa peptide to give a molecule consisting of 65 kDa heavy chain and a 45 kDa light chain joined together by a disulfide bond (5,6). This kinin-free molecule (HK_a) exhibits procoagulant activity and binds to negatively charged surfaces in the presence of plasma concentrations of fibrinogen to a much greater extent than HK (7). In contrast, proteolysis of HK by factor XIa to HK_i results in loss of procoagulant activity

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concomitant with more extensive cleavage of the light chain (8). In the present studies, circular dichroism and fluorescence spectroscopy were used to investigate the conformations of cleaved and uncleaved HK in order to understand what specific structural alterations (in addition to the proteolytic cleavage) occur in the procofactor HK which might account for its conversion to an active and inactive cofactor.

MATERIALS AND METHODS

MATERIALS. Human HK was purified from fresh frozen plasma by a modified procedure (9) of Kerbiriou and Griffin (5). This preparation of HK on reduced SDS PAGE is essentially a single band of 120 kDa with greater than 98% purity and has a specific activity of 12.5 U/mg. Human plasma kallikrein was prepared by activation of purified prekallikrein by factor XII fragments (10) and was a doublet of 88 and 85 kDa on unreduced SDS gel eclectrophoesis. The specific activity was 14.5 μmol min^{-1} mg^{-1} with H-D-Pro-Phe-Arg-P-Nitroanilide as a substrate. Factor XIa was prepared from factor XI activated by factor XIIa (11) and was a single band of 160 kDa on unreduced SDS gel electrophoresis with a specific activity of 203 coagulant units/mg. Human urinary kallikrein was a gift of Dr. Julie Chao, Univ. of South Carolina, and was a single component of 25 kDa on unreduced SDS gel electrophoresis.

CLEAVAGE OF HK BY KALLIKREIN AND FACTOR XIa. Catalytic amount of the protease was added to HK and incubated at 37°C. HK was incubated with plasma and urinary kallikrein for 24 hr at molar ratios of 1:10 and 1:25, respectively. A 1:72 molar ratio of factor XIa to HK was used and incubation was for 96 hr to insure complete proteolysis. Soybean trypsin inhibitor (STI) was added at one-tenth plasma kallikrein concentration at the end of the incubation period to stop the reaction. Portions of these reaction mixtures were then analyzed in 10% SDS PAGE and the remaining solutions were used for circular dichroism measurements.

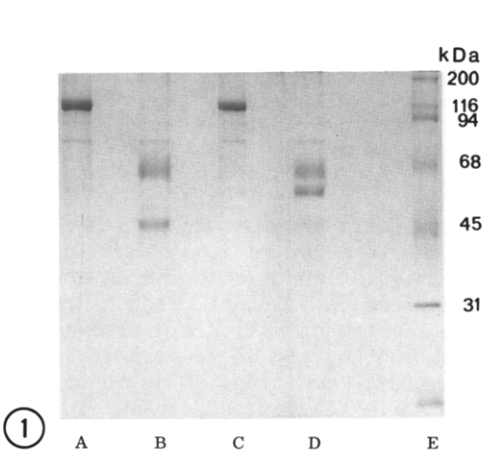
CIRCULAR DICHROISM MEASUREMENTS. A Jasco 500C recording spectropolarimeter equipped with a DP 500 data processor and a microcell adapter was used. The machine was routinely calibrated using ammonium-d-camphor-10-sulfonate. The CD spectra from 200 nm to 270 nm were measured at room temperature in a 90 μL microcell with a pathlength of 1.0 cm at a sensitivity of 2 millidegrees, time constant of 4 s, scan speed of 2 cm/min and wavelength expansion of 5 nm/cm. The protein concentration ranged from 116 to 128 μg/ml using a solvent, 0.1M NaF, 0.002 M sodium phosphate buffer, pH 7.5. Sodium chloride was replaced by sodium fluoride in order to improve the sensitivity and signal to noise ratio at lower wavelength. Nearly identical CD spectra were obtained in 0.15 M NaCl, 0.02 M Tris, pH 7.5 (except for higher signal to noise ratio). This indicates that sodium fluoride does not affect the conformation of HK. The data were converted to mean residue ellipticity in degrees cm^2 $dmole^{-1}$ using a mean residue weight, M_o = 112. The reported CD spectra of kallikrein-cleaved and factor XIa-cleaved HK were corrected by subtracting the CD spectra of identical amounts of the proteases and STI under identical conditions. The contribution of kallikrein plus STI did not exceed 7.5% while that of factor XIa plus STI did not exceed 5%. In order to prevent adsorption to quartz cells the following steps were taken: (i) the microcell was cleaned with 9:1 mixture of H_2SO_4 and HNO_3 and rinsed exhaustively with distilled water; (ii) it was dried with a steady stream of nitrogen and filled with a 1 mg/ml polybrene solution. The cell was again washed with water and dried in nitrogen before the sample was added. When compared to the uncoated cell, the polybrene-coated cell showed no CD spectrum at the wavelength region of interest.

Fluorescence was measured using a Perkin Elmer LS 5B Fluorometer. The excitation wavelength was set at 290 nm and the emission spectra were scanned from 300 nm to 500 nm. All fluorescence measurements were performed at room temperature in polybrene-coated microcell with a pathlength of 3 mm and utilizing a slit width of 3 nm. The protein absorbance at 280 nm did not exceed 0.1.

RESULTS

The SDS-PAGE patterns of intact HK (120 kDa) and kallikrein-cleaved HK are shown in Figure 1. Plasma kallikrein cleaved HK into two prominent polypeptides corresponding to the heavy chain, 65 kDa and the light chain, 45 kDa. Urinary kallikrein cleaved HK into two components corresponding to the heavy chain, 65 kDa and an intermediate light chain, 56 kDa. Factor XIa cleaved HK (Figure 2) into two major polypeptides, the heavy chain, 65 kDa and a degraded light chain, 40 kDa. Minor bands below 36 kDa, are also light chain fragments (8).

The circular dichroism of intact HK and cleaved HK are shown in Figure 3A. The ellipticity of intact HK is characterized by a single band having a minimum at 214-215 nm corresponding to 2,100 ± 100 degrees cm^2 $dmole^{-1}$. Compared to intact HK, the CD spectrum of HK cleaved by plasma kallikrein is characterized by a small decrease in amplitude and a 4 nm 'red shift' from 214 nm to 218 nm. The CD spectrum of HK proteolyzed by urinary kallikrein is almost



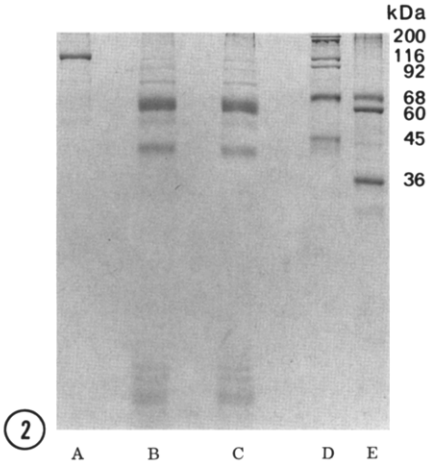


Figure 1. SDS-PAGE Patterns of Kallikrein-cleaved HK. (A) and (C) are HK alone; (B) HK in plasma kallikrein; (D) HK in urinary kallikrein; and (E) Standards.

Figure 2. SDS-PAGE Patterns of Factor XIa-cleaved HK. (A) HK alone; (B) and (C) HK plus in factor XIa; and (D) and (E) Standards.

identical with that of plasma kallikrein-cleaved HK (data not shown). In HK hydrolyzed by factor XIa no spectral shift was observed. However, the amplitude of the CD spectrum was decreased by about 20% relative to intact HK. The apparent differential effect of kallikrein and factor XIa on HK is clearly demonstrated by taking the difference CD as shown in Figures 3B and 3C. This is done by subtracting the CD spectrum of intact HK from protease-cleaved HK at identical concentration of HK. The significant feature of the difference CD spectrum of HK cleaved by kallikrein is the negative trough at 222nm (Figure 3B) indicating that its CD spectrum is more negative (increase ellipticity) than intact HK in this region. This small but significant difference CD is highly reproducible and corresponds to 150 ± 20 degrees cm^2 $dmole^{-1}$. The difference CD of HK hydrolyzed by factor Xla is also presented (Figure 3 C). The difference CD is positive above 210 nm suggesting a decreased ellipticity relative to intact HK in this region which is maximal at 215 nm and corresponds to 400 ± 75 degress cm^2 $dmole^{-1}$.

The intrinsic fluorescence of intact HK was compared with kallikrein-cleaved and factor XIa-cleaved HK (Figure 4). With the exception of urinary kallikrein, it was found that the relative intrinsic fluorescence of HK at 340 nm was enhanced indiscriminately by both kallikrein and factor XIa cleavage. Urinary kallikrein cleavage exhibited a 20% enhancement while plasma kallikrein and factor XIa cleavage produced a 40% to 50% enhancement. Since the excitation wavelength was 290 nm, it can be inferred that this fluorescence is mostly due to changes in the microenvironment of tryptophan residues and to a lesser extent to tyrosine residues.

DISCUSSION

Circular dichroism is a useful tool for studying protein conformation and conformational changes in solution. The circular dichroism of some specific polypeptide conformations are known. Within the wavelength region used in these studies (200 nm to 250 nm), the CD spectrum of α-helical structure is generally characterized by two overlapping negative troughs at 210 nm and 222 nm with ellipticity near 30,000 degrees cm^2 $dmole^{-1}$ and the β-sheet structure is

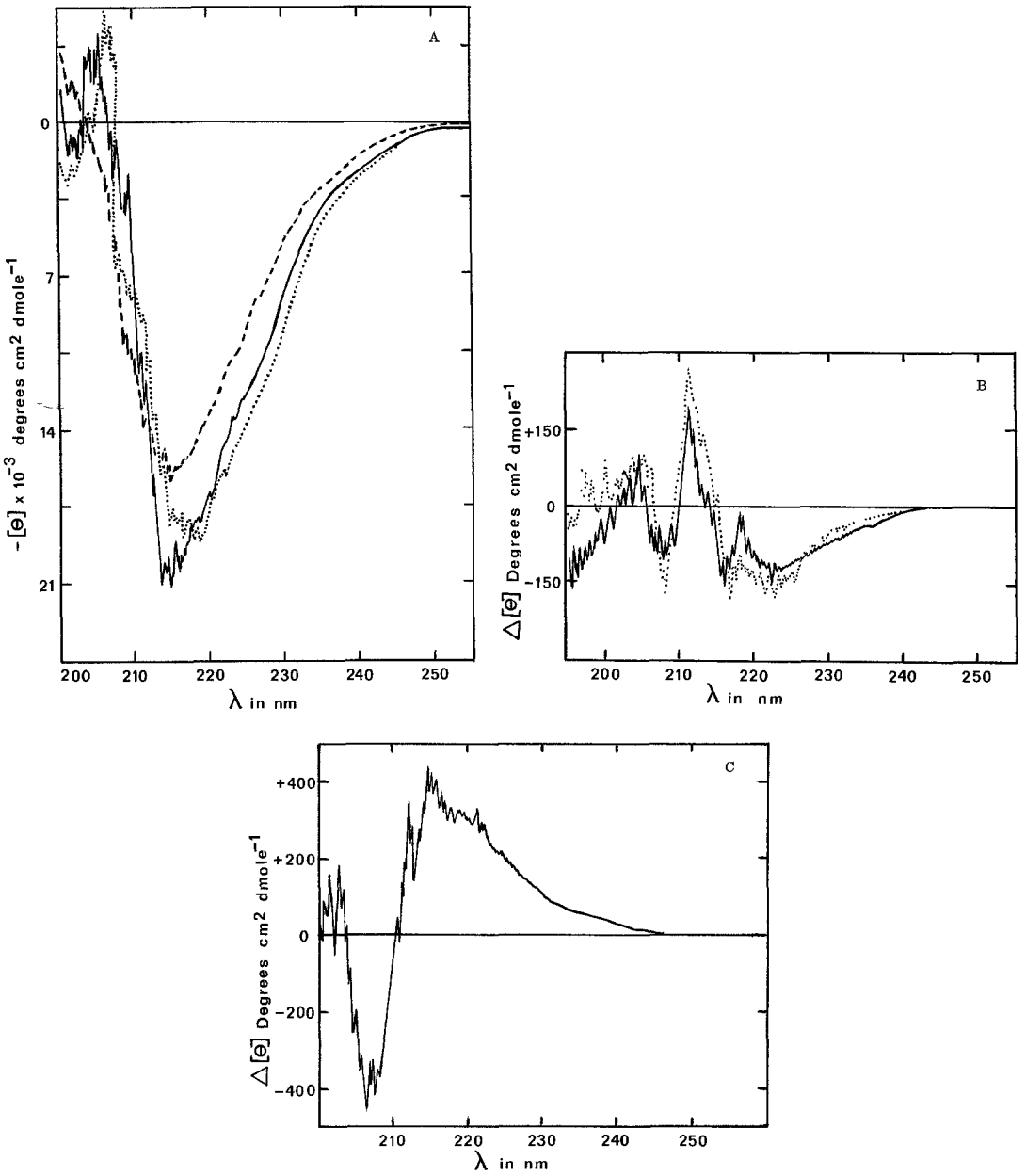


Figure 3. Circular Dichroism of HK. (A) Direct CD: (——), HK alone; (• • • •), HK plus plasma kallikrein; and (– – –), HK plus factor XIa. (B) Difference CD of HK plus plasma kallikrein (——) or urinary kallikrein (• • • •). (C) Difference CD of HK plus in factor XIa. Solvent: 0.1 M NaF, 0.002 M sodium phosphate, pH 7.5.

characterized by a negative trough at 216 nm with ellipticity of about 9,000 degrees cm^2 dmole^{-1} (12). The aperiodic, unordered secondary structure has low ellipticity of 2,000 degrees cm^2 dmole^{-1} at 210 nm and increases in intensity up to around 23,000 degrees cm^2 dmole^{-1} below 200 nm (12). Based on this

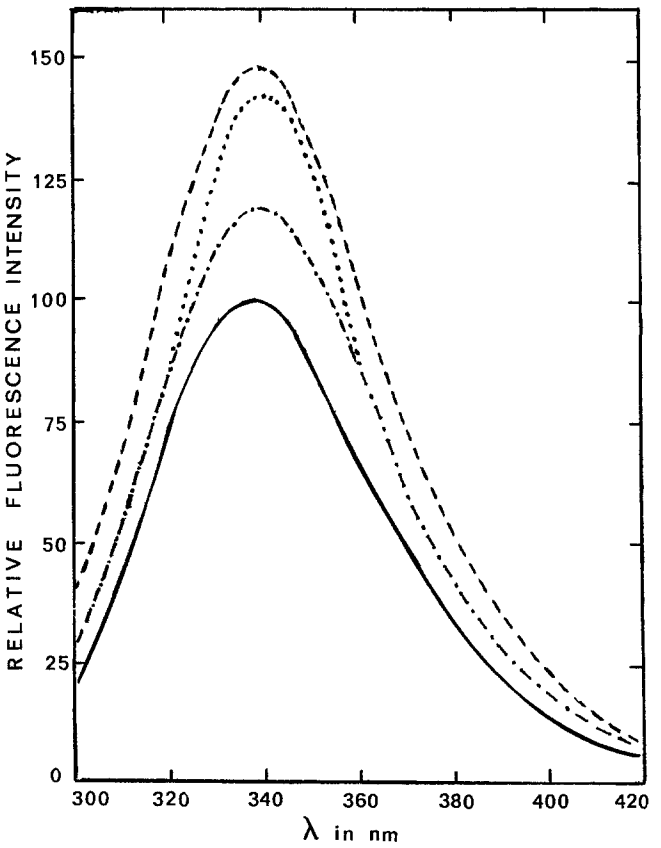


Figure 4. Intrinsic Fluorescence of HK. HK alone (———); HK in plasma kallikrein (- - - -); HK in urinary kallikrein (• - • -); and HK in factor XIa (• • • •). Excitation at 290 nm.

information and the intensity and position of the CD band of HK ($[\Theta]_{min}$ = 2,100 degrees cm^2 dmole^{-1} at 214 nm), it is easy to understand that there is very little organized secondary structure in HK (Figure 3). Most likely, the conformation consists of unordered structure with very little or no periodic elements of protein secondary structure. It should be noted that the magnitude of the CD spectrum seems too low even if one assumes a 100% unordered structure for HK. Aromatic amino acid residues, as well as disulfide bonds sometime give positive CD spectra in this region (13-15). In this case, a positive ellipticity originating from these non-peptide chromophores will tend to reduce the intensity of the negative ellipticity due to the amide transition. Further studies will be needed to prove if such is the case in HK.

The 'red shift' in the CD spectrum of HK after kallikrein cleavage is a clear indication of accompanying conformational changes. The maximum spectral shift at 222 nm ([Θ] = 150 degrees cm^2 $dmole^{-1}$) could be due to formation of some periodic secondary structure (i.e., α-helix) because apparent increase in elliplicity at 222 nm is generally observed when α-helical content of the protein is increased. Therefore, the conformational potentials of the amino acid sequence near the kallikrein cleavage sites were investigated. By using the prediction method of Fasman (16), it is found that the amino acid sequences close to either side of the cleavage sites in human HK have high α-helical potentials (Figure 5). These correspond to the amino acid segment 357-362 of the heavy chain and segment 6-10 of the light chain. It is possible that these segments are not entirely in their α-helical conformation in the native state of HK due to structural constraints within the molecule and the release of bradykinin allows these regions to realize their maximum helical potentials. This deduced structure is a likely possibility because CD measurement indicates that intact HK has very little or no α-helical structure. Cleavage by urinary kallikrein, which is restricted only to the bradykinin cleavage sites, shows also the same enhancement of the CD spectrum at 222 nm. By contrast, cleavage by factor XIa, which results in a more extensive proteolysis of the light chain of HK, indicates a decrease in ellipticity at 222 nm, suggesting an entirely different type of conformational change.

The cleavage of HK is accompanied by enhancement of the intrinsic fluorescence. This alteration suggests that the environment of aromatic amino acid residues is perturbed during proteolysis. Whatever are the exact molecular

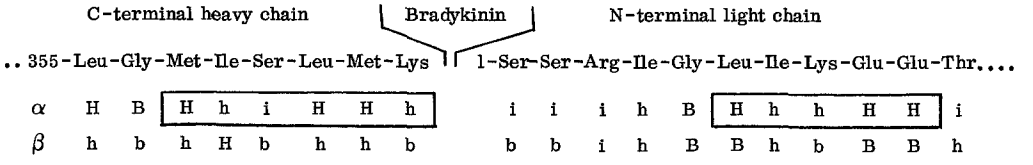


Figure 5. Conformational Potentials Around the Bradykinin Cleavage Site in HK. The last two lines represent the α-helical and β-sheet potentials of the amino acid residues as indicated: H, strong α or β former; h, moderate former; i, indifferent; B, strong α or β breaker; and b, moderate breaker (16). The segments of high α-helical potentials are boxed.

events responsible for these conformational changes, they may be important for the contrasting roles that HK_a and HK_i play after these proteolytic events. Further investigaton of the nature and origin of these changes could explain why kallikrein cleavage of HK results in increased affinity to negative surfaces and concomitant enhancement of coagulant activity while hydrolysis by factor XIa results in loss of coagulant activity.

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