Contact Factors and Kinins: Experimental

Waterloo Room

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4.00

0595 ACTIVATION OF PURIFIED C1, THE FIRST COMPLEMENT COMPONENT, BY PURIFIED PLASMA KALLIKREIN AND PLASMIN

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Many similarities exist between the proteins, activation mechanisms and control processes of the complement (C) system and the Hageman factor pathways (the coagulation, kinin-forming and fibrinolytic systems). Earlier studies have presented evidence both in favor of and against activation of the classical C pathway by plasmin at the level of the C1s subcomponent of C1. To study this problem the effects of purified plasma kallikrein and plasmin on isolated C1r, C1s, the calcium-dependent C1rs complex, and macromolecular C1 were studied. Kallikrein activated C1r as shown by its increased ability to cleave ^{125}I-C1s. Kallikrein activated C1s as assessed by hydrolysis of synthetic substrates. This activation was associated with cleavage of ^{125}I-C1s into several fragments with the largest being 29,000 daltons; the usual $\overline{C1s}$ heavy chain of 59,000 daltons generated by $\overline{C1r}$ was not evident. Kallikrein also cleaved ^{125}I-C1s in C1rs and in macromolecular C1. Plasmin activated C1r and cleaved ^{125}I-C1r into fragments of 35,000 daltons and smaller; the usual $\overline{C1r}$ heavy chain was not evident. Plasmin also activated C1s and cleaved it into fragments similar in size to those generated by kallikrein. Plasmin activated C1rs and C1, apparently via its action on C1r. Thus, kallikrein and plasmin both directly activate C1. These studies suggest that activation of prekallikrein or plasminogen in plasma may lead directly to C triggering via activation of C1.

4.15

0596 DFP (DIISOPROPYLFLUOROPHOSPHATE) STUDIES ON MOLECULAR MECHANISMS OF SURFACE-DEPENDENT ACTIVATION OF PREKALLIKREIN (PK) AND FACTOR XII (HAGEMAN FACTOR, HF).

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Previous ^{3}H-DFP studies showed that HF binding to kaolin does not result in formation of new active sites. New studies show that single chain zymogen HF, in solution or surface-bound, reacts slowly with DFP causing loss of coagulant activity and uptake of 1 mol DIP/mol HF with a 2nd order rate constant, k_2=0.4 $M^{-1}min^{-1}$. High MW kininogen (HMWK) did not affect the rate constant for this reaction. PK reacts with DFP causing loss of coagulant activity with k_2=0.6 $M^{-1}min^{-1}$. These values are $\sim 10^3$ lower than for the activated enzymes (160 and 500 $M^{-1}min^{-1}$ for HF_a and kallikrein respectively) and are similar to values for trypsinogen that exhibits weak enzymatic activity. HF, PK, HMWK, and kaolin were separately preincubated with 40mM DFP for 5 min to inhibit traces of active enzymes. Then, mixing of these reagents in the presence of 40mM DFP caused a burst of cleavage of ^{131}I-HF and ^{125}I-PK, the extent of which depended on the amount of HMWK. To avoid reciprocal proteolysis of HF and PK, "killed" zymogens (DIP-HF or DIP-PK formed by 48 hr incubation with 40mM DFP) were used in place of HF or PK. Controls showed that PK and DIP-PK were similarly cleaved by purified HF_a. If DIP-^{131}I-HF or DIP-^{125}I-PK was substituted for HF or PK in mixtures of HF, PK, HMWK, and kaolin, no detectable cleavage occurred. These data allow that inherent activity of single chain forms of HF or PK may trigger surface dependent reactions, but the vast majority of molecules are activated by reciprocal proteolysis between HF and PK with HMWK as a cofactor.