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2294 Human kallikrein 5 (hK5): Autoactivation, substrate specificity, regulation by inhibitors and cleavage of extracellular matrix and basement membrane components.

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Human kallikrein 5 (KLK5) is a member of the human kallikrein gene family of serine proteases that map to locus 19q13.4. Preliminary results indicate that the protein, hK5, may be a potential serological tumor marker for breast and ovarian cancer. Here, we describe production of pro-hK5 in Pichia pastoris. After a two-step purification procedure, we show that the protein is enzymatically active, indicating that it can autoactivate. The enzyme is differentially glycosylated (~44kDa, ~40kDa, ~35kDa, ~30kDa). We obtained a single band (~28kDa) after treatment with the enzyme N-glycosidase F. Mass-Spectrometric analysis indicated that all bands represent hK5. Casein and gelatin zymographs showed that all four glycosylated forms are enzymatically active. We tested catalytic activity of hK5 against the fluorogenic substrates Gly-Pro-Arg-AMC and Gly-Pro-Lys-AMC. The Kcat/Km was 12 mM-1* min-1 and the Vmax 249 nM/min for the first substrate while no reaction was detected with the second substrate indicating that the hK5 prefers Arg but not Lys in the P1 position. The substrates Val-Pro-Arg-AMC and Phe-Ser-Arg-AMC had the highest Vmax (2361 and 2034 nM/min respectively) and Kcat/Km ratio (946 and 877 mM-1* min-1 respectively). By SDS Page, we have shown that hK5 forms stable complexes with the inhibitors a2-antiplasmin (a2-AP) and antithrombin III (ATIII). Furthermore, we determined the enzyme inhibition constant (Ki) for the aforementioned and benzamidine (53nM, 69nM and 188nM respectively). The inhibitors a1-antirtypsin (AAT) and a1-antichymotrypsin (ACT) did not inhibit hK5, while the high molecular weight inhibitor a2-magroclobulin (a2M) inhibited hK5 at high concentrations. Finally, we showed that the hK5 can digest the fluorogenic conjugates of collagen type I, collagen type II and fibrinogen.This study shows that the serine protease hK5 is enzymatically active, has trypsin-like catalytic activity and is inhibited by plasma serine protease inhibitors. Furthermore, this serine protease is able to digest components of the extracellular matrix (ECM) and the basement membrane, thus allowing us to speculate that it may be involved in tumor progression and metastasis.

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