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Prostase/PRSS17/human kallikrein 4 (hK4) is a member of the human kallikrein family of serine proteases. It has its highest expression in the human prostate with very low expression levels in other normal tissues. In the LNCaP prostate cancer cell line, its expression is mainly under the control of androgen. KLK4 expression is an indicator of poor prognostic outcome in ovarian carcinoma. In addition, hK4-specific autoantibodies were demonstrated in serum of prostate cancer patients but not in healthy individuals suggesting that hK4 may play a role in the diagnosis and monitoring of prostate cancer. Initial enzymatic study on E. coli derived, non-glycosylated, chimeric recombinant hK4 has confirmed trypsin-like activity with preference for Arg over Lys at the P1 site and has shown degradation of the seminal plasma protein prostatic acid phosphatase, and activation of two well-known seminal plasma proteins pro-prostate specific antigen and pro-urokinase-type plasminogen activator. In this study, we have undertaken to further characterize the substrate preferences of hK4 and determine possible cleavage of extracellular matrix proteins in hopes of gaining more insight into its possible role in normal physiology and cancer progression. We have expressed the mature form of hK4 in the eukaryotic expression system P. pastoris, and purified the recombinant protein from the supernatant with anion exchange and benzamidine affinity chromatography. Mass spectrometry and N-terminal sequencing confirmed the identity of recombinant hK4, which has an apparent mass of 25 kDa on SDS-PAGE gel under non-reducing conditions. Optimal buffer conditions were determined, followed by test of enzymatic activity of 55 nM hK4 on 9 fluorogenic tripeptide substrates (VPR, VLK, GPR, QGR, EKK, PFR, FSR, GGR, GPK) each conjugated to amino-methyl-coumarin (AMC). Enzyme kinetic parameters such as Km, Kcat and Vmax were determined for the best substrates, as well as digestion of the fluorogenic conjugates of the extracellular matrix (ECM) proteins collagen I, collagen IV and fibronectin. Our results confirm trypsin-like activity of hK4 with a preference for Arg over Lys at the P1 site and show no hK4 activity on the chymotrypsin-specific substrate AAPF. VPR proved to be the most preferred substrate, with Vmax of 109 nmol/min/L and Kcat/Km ratio of 38.7 mM/min. With Arg at the P1 site, hK4 showed preference for Pro at the P2 site over Gly, Ser or Phe. hK4 was also able to degrade fibronectin and to a lesser extent collagen. These results indicate that the trypsin-like enzyme hK4, with preference for Arg at the P1, is able to cleave the ECM proteins collagen and fibronectin, giving further evidence of its possible role in prostate cancer progression and metastasis.

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