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1807 Enzymatic action, substrate specificity and regulation of human kallikrein 14 (hK14).

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Human kallikrein 14 (hK14; encoded by the *KLK14* gene) is a member of the human kallikrein family of secreted serine proteases. Initial mRNA studies reported steroid hormone regulation of *KLK14*, its differential expression in hormone-related malignancies and the association of tissue *KLK14* mRNA levels with the prognosis of breast and ovarian cancer patients. Recent evidence at the protein level suggests that hK14 may also be a potential screening/diagnostic marker due to its elevation in the sera of breast and ovarian cancer patients. In this study, our aim was to delineate the kinetics, substrate specificity and regulation of the hK14 protein as a first step in understanding its physiological relevance. Mature hK14 was expressed in *P. pastoris* and purified from the culture supernatant by cation exchange chromatography, after which the expected 25-kDa protein, along with two major degraded forms (~22kDa and ~13kDa) were observed. All forms were identified as hK14 by mass spectrometry and N-terminal sequence analysis, suggesting that recombinant hK14 is enzymatically active and capable of proteolysing itself, with P1-Arg preference, which may serve as a regulatory mechanism. The substrate specificity of hK14 was studied with fluorogenic 7-amino-4-methylcourmain (AMC) synthetic peptides specific for either trypsin-like or chymotrypsin-like proteases and combinatorial fluorogenic 7-amino-4-carbamoylmethylcoumarin (ACC) synthetic substrate libraries with amino acid diversity at the P1, P2, P3 and P4 positions. Our collective results indicate that hK14 has a highly specific P1 preference for Arg and to a much lesser extent for Lys, confirming its predicted trypsin-like substrate specificity. Furthermore, while hK14 exhibits broad specificity at P2 and P3, it shows preference for aromatic amino acids (Tyr, Trp and Phe) at P4. Out of the various AMC peptides studied, hK14 hydrolysis of VPR-AMC and FSR-AMC resulted in the highest Vmax (2783 and 2995 nM/min, respectively) and Kcat/Km (5409 and 897 mM/min, respectively) values. Kinetic analysis of hK14 inhibition by various plasma serine protease inhibitors was also investigated. Our findings show that hK14 activity is inhibited most efficiently by a_1-antichymotrypsin followed by anti-thrombin III, a_2-antiplasmin and a_1-antitrypsin. Lastly, *in vitro* experiments indicate that hK14 can digest: 1) bovine serum albumin after a P1-Arg residue, as determined by N-terminal sequencing, 2) casein and gelatin, as indicated by zymography and 3) several basement membrane/extracellular matrix proteins including collagen I, II and III, fibronectin, laminin and vitronectin by Western blot analysis, implicating hK14 in tissue remodelling and/or tumor invasion and metstasis. In conclusion, we confirm the trypsin-like P1-Arg specificity of hK14, identify regulatory mechanisms and potential biological substrates and physiological roles..

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