



INHIBITION OF KALLIKREINS BY FUKUGETIN

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Abstract

The family of the tissue kallikrein (KLK1) and kallikrein-related peptidases (KLKs), encoded by the largest contiguous cluster of protease genes in the human genome, consists of 15 homologous, single-chain, secreted serine proteases [1-2]. The expression of KLKs occurs in many organs including skin, pancreas, colon, brain, breast and prostate, where they are mainly secreted by epithelial cells. In addition to the so far described physiological roles of KLKs, their unusual expression and activity have been reported in various pathological conditions such as Alzheimer's disease and in many cancer-related processes, such as cell growth regulation, angiogenesis, invasion, and metastasis, being KLK3/PSA one of the most important biomarkers for prostate cancer. In this study, we report the inhibition of KLKs 1, 2, 3, 5, 6 and 7 activities by fukugetin, compost isolated from *Garcinia brasiliensis*. Whereas KLKs 1, 2, 5 and 6 present mainly trypsin-like specificity, KLKs 3 and 7 are known for their chymotrypsin-like specificity. Fukugetin was extracted and isolated from *G. Brasiliensis* fruit as previously described [3]. The fruits were collected at the herbarium of the University of Viçosa, Minas Gerais, Brazil. Mature kallikreins were expressed and purified from a baculovirus/insect cell line system. Enzymes concentrations were determined by the method of Bradford. Purity and characterization of the enzymes were confirmed by SDS/PAGE, N-terminal sequencing, and mass spectrometry. Kallikrein proteolytic activity was monitored on the fluorescence resonance energy transfer (FRET) peptides, Abz-KLRSSQ-EDDnp (KLK1, KLK2, KLK5 and KLK6) and Abz-KLYSSQ-EDDnp (KLK3 and KLK7). KLK2 and KLK1 showed the least values of IC₅₀, 3,2 uM and 5,7 uM, respectively. To better understand the interaction between KLK1 and KLK2 with fukugetin, we performed a detailed kinetic study to determine the mechanism of inhibition. These results suggest that fukugetin acts as a mixed-type inhibitor of KLK1 and KLK2, called linear partial competitive inhibition. In order to rationalize the observed mechanism of inhibition, we performed molecular docking combined with molecular dynamics simulations to identify the putative binding site of fukugetin bound to KLK1. The resulting 300 conformations of the ligand were processed by the built-in clustering analysis. Our findings provide additional insight into the possible binding mode of fukugetin, and might have implications for further optimization of this inhibitor.

References:

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