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Presentation Title:	**Purification and characterization of human kallikrein 11, a candidate prostate cancer biomarker, from seminal plasma**
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Human kallikrein 11 (hK11) is a secreted serine protease that belongs to the human tissue kallikrein family. It is most highly expressed in the prostate tissue and found to be abundant in seminal plasma. The physiological substrates and functions of hK11 are still unknown. Preliminary experiments showed that hK11 had value as a serum diagnostic biomarker for prostate cancer. To understand the enzymatic characteristics of hK11 and its possible roles in prostate cancer, we purified and functionally characterized native hK11 from seminal plasma. We found that hK11 was present in seminal fluids in the range of 2 to 37 µg/ml, with a mean of 15 µg/ml and a median of 11 µg/ml. It appears that hK11 is the most abundant tissue kallikrein in the seminal plasma after prostate specific antigen. With immunoaffinity purification and reverse phase HPLC, we purified hK11 from seminal plasma to homogeneity. In seminal plasma, hK11 is present as a free unbound enzyme with 40 kDa molecular weight. About 50% of it is in the active form. Another 50% of it is internally cleaved. The cleavage occurs after Arg156 (Genbank accession number AF164623), generating two 20 kDa peptides that are connected by internal disulfite bonds. The purified hK11 displays trypsin-like enzymatic activity. It can cleave some synthetic peptides after arginine residues. It does not cleave peptide bonds after lysine residues and those substrates for chymotrypsin. The cleavage of hK11 at Arg156 results in inactivation of the enzyme. We also examined the effect of some common serine protease inhibitors on hK11 enzymatic activity. We found that anti-thrombin III, a_1-antichymotrypsin, a_2-antiplasmin, and a_1-antitrypsin have no effect on hK11 enzymatic activity and do not form complexes with hK11 in *in vitro* experiments. The strongest inhibitor that we found was APMSF. It can completely inhibit hK11 enzymatic activity at a concentration 100-fold higher than that of hK11. Aprotinin and an hK11 specific monoclonal antibody can inhibit hK11 enzymatic activity up to 40%. In our effort to further identify the enzyme that can cleave hK11 at Arg156 in seminal plasma, we isolated plasmin as a strong candidate. It can partially cleave native hK11 at Arg156. This is the first report on purification and characterization of native hK11. Our results provide experimental evidence of the protease characteristics of hK11 and suggest that the major physiological substrates of hK11 are most likely to be present in the seminal plasma. Its role in prostate cancer pathogenesis needs to be further elucidated.

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