Structure-function analyses of human kallikrein-related peptidase 2 establish the 99-loop as master regulator of activity.

Abstract:

Human kallikrein-related peptidase 2 (KLK2) is a trypsin-like serine protease predominantly expressed in prostatic tissue and secreted into prostatic fluid, a major component of seminal fluid. Most likely it activates and complements chymotryptic KLK3 (prostate-specific antigen) in cleaving seminal clotting proteins, resulting in sperm liquefaction. KLK2 belongs to the "classical" KLKs 1-3, which share an extended 99- or kallikrein loop near their non-primed substrate binding site. Here, we report the 1.9 Å crystal structures of two KLK2-small molecule inhibitor complexes. In both structures discontinuous electron density for the 99-loop indicates that this loop is largely disordered. We provide evidence that the 99-loop is responsible for two biochemical peculiarities of KLK2, i.e. reversible inhibition by micromolar Zn(2+) concentrations and permanent inactivation by autocatalytic cleavage. Indeed, several 99-loop mutants of KLK2 displayed an altered susceptibility to Zn(2+), which located the Zn(2+) binding site at the 99-loop/active site interface. In addition, we identified an autolysis site between residues 95e and 95f in the 99-loop, whose elimination prevented the mature enzyme from limited autolysis and irreversible inactivation. An exhaustive comparison of KLK2 with related structures revealed that in the KLK family the 99-, 148-, and
220-loop exist in open and closed conformations, allowing or preventing substrate access, which extends the concept of conformational selection in trypsin-related proteases. Taken together, our novel biochemical and structural data on KLK2 identify its 99-loop as a key player in activity regulation.