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Crystal Structures of Human Tissue Kallikrein 4: Activity Modulation by a Specific Zinc Binding Site

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Human tissue kallikrein 4 (hK4) belongs to a 15-member family of closely related serine proteinases. hK4 is predominantly expressed in prostate, activates hK3/PSA, and is up-regulated in prostate and ovarian cancer. We have identified active monomers of recombinant hK4 besides inactive oligomers in solution. hK4 crystallised in the presence of zinc, nickel, and cobalt ions in three crystal forms containing cyclic tetramers and octamers. These structures display a novel metal site between His25 and Glu77 that links the 70–80 loop with the N-terminal segment. Micromolar zinc as present in prostatic fluid inhibits the enzymatic activity of hK4 against fluorogenic substrates. In our measurements, wild-type hK4 exhibited a zinc inhibition constant (IC_{50}) of 16 μM including a permanent residual activity, in contrast to the zinc-independent mutants H25A and E77A. Since the Ile16 N terminus of wild-type hK4 becomes more accessible for acetylating agents in the presence of zinc, we propose that zinc affects the hK4 active site *via* the salt-bridge formed between the N terminus and Asp194 required for a functional active site. hK4 possesses an unusual 99-loop that creates a groove-like acidic S2 subsite. These findings explain the observed specificity of hK4 for the P1 to P4 substrate residues. Moreover, hK4 shows a negatively charged surface patch, which may represent an exosite for prime-side substrate recognition.

Keywords: inhibitor; loop; complex; prostase; X-ray

Introduction

When the human tissue kallikrein gene family was discovered in the 1980s, it was initially concluded that the entire family comprises three genes, namely *KLK1*, coding for pancreatic/renal kallikrein (hK1), *KLK2*, coding for human glandular kallikrein 2 (hK2), and *KLK3*, coding for prostate-specific antigen, PSA (hK3).[1] Recently, their gene products were designated as classical human tissue kallikreins that belong to a family of 15 human enzymes with highly conserved gene structure, colocalizing on the chromosomal locus 19q13.3–q13.4. Of these 15 homologous serine proteinases, only hK1 displays an activity similar to plasma kallikrein, i.e. release of a

Abbreviations used: hK4, human kallikrein 4; PSA, prostate specific antigen; PABA, *p*-amino-benzamidine; uPA, urokinase-type plasminogen activator.

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vasoactive peptide (lysyl-bradykinin) upon cleavage of low-molecular weight kininogen.[2,3]

Human kallikrein 4 was discovered in prostate and consequently called prostase.[4,5] hK4, also known as PRSS17, KLK-L1, KLK4, and peptidase S01.251 from clan PA(S) according to the MEROPS database,[6] is highly expressed in the prostate and has been suggested as a potential prognostic marker for prostate cancer.[7–9] Also, the presence of hK4 in seminal and urine fluid has been reported.[10] Moreover, this kallikrein was observed in ovarian tissue under healthy conditions, but was found to be significantly up-regulated in corresponding cancer cells.[11] Another crucial function of hK4 is the enamel mineralization during dental development, as demonstrated by a mutation of hK4, which causes *amelogenesis imperfecta*.[12] Nevertheless, the distribution of hK4 seems to be more widespread, as indicated by expression in mammary and salivary glands, testis,[13] or skin.[14] Even the intranuclear localization has recently been reported for hK4.[8] The latter finding has been controversially discussed, since hK4 is predominantly a secreted protein.[15] However, the occurrence of a truncated splice version lacking a complete catalytic triad may account for the intracellular location.[16,17] hK4 efficiently activates PSA and another tumor-associated serine proteinase, the urokinase-type plasminogen activator (uPA).[18] hK4 is most likely expressed as a preproprotein of 254 amino acid residues, converted by a signal peptidase to a 228 residue proprotein, and activated by an unknown enzyme to the 224 amino acid mature proteinase.[19,20] The enzymatic characterisation and specificity profiling of hK4 revealed a trypsin-like specificity.[19,21]

Only recently, the first X-ray structures of human tissue kallikreins, namely hK6,[22] a pro-hK6 mutant,[23] and hK1[24] have been released. Previously, insights into the tissue kallikrein topology had been gained by the porcine pancreatic kallikrein A structure.[25,26] Meanwhile, structures of other mammalian kallikreins have been deposited, such as the Zn^{2+}-inactivated hK2 ortholog rat tonin,[27] mouse kallikrein-13,[28] the hK8 ortholog mouse neuropsin,[29] and horse kallikrein 3.[30]

We have purified recombinant hK4, as well as two hK4 mutants, and have analysed the activity against two test substrates with a focus on the modulation by zinc. Besides, we have solved three X-ray crystal structures of the *p*-aminobenzamidine inhibited enzyme with cobalt, nickel, and zinc ions bound at a specific metal binding site (Figure 1). These structures reveal the structural determinants for the

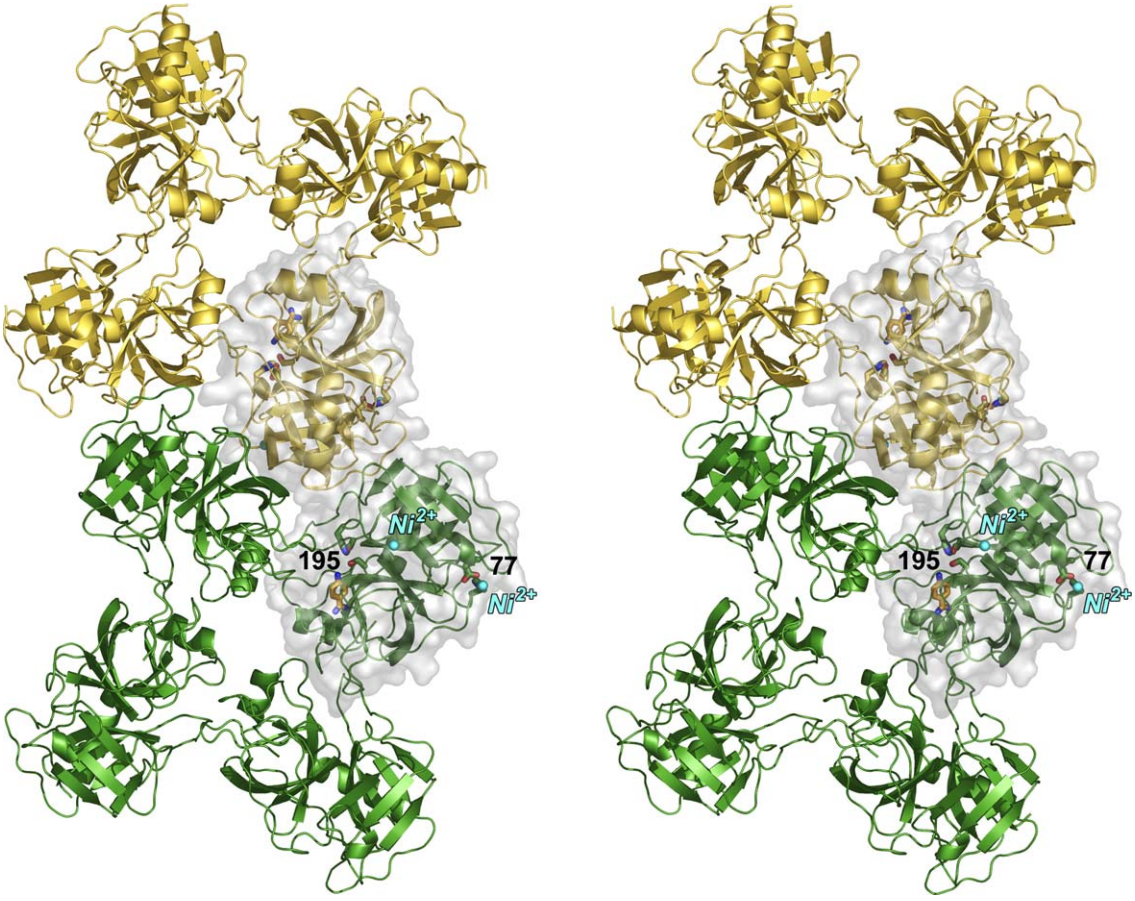

Figure 1. Stereo view of the molecular packing in hK4-Ni crystals of space group *P*4. The asymmetric unit consisting of two hK4 molecules is covered by a transparent surface, while symmetry-related molecules are depicted in green and yellow, respectively. The 4-fold symmetry axes (parallel to *c* of the unit cell) are perpendicular to the paper (*ab*) plane.