ABSTRACT

Cathepsin K is a papain-like cysteine peptidase that is expressed and secreted from osteoclasts, and is a crucial enzyme in bone resorption in vertebrates. It can cleave the triple-helical region of type I collagen, the major organic component of bone. The collagen triple helix is inherently resistant to proteolytic cleavage and can be cleaved only by a handful of endogenous peptidases, called collagenases. Cathepsin K has a unique collagen-degrading activity and is capable of cleaving at multiple sites within the triple-helical region of the collagen molecule. However, the mechanism of cathepsin K catalysed collagen degradation remains largely unknown. In this research, we were interested how collagen triple helix binds into enzyme active site, using methods like enzyme kinetics, computer modelling and structure determination. To understand potent collagenolytic activity of cathepsin K, a part of research was done on mammalian cathepsin L, which is known to exhibit weak collagenolytic activity, but lacks proline specificity at the residue P2 position, which is usual for collagenases. Herein we compare the collagenolytic activity of cathepsins K and L, and show that both enzymes have similar affinity towards soluble type I collagen and produce similar fragmentation patterns of this substrate. We identify a cathepsin L cleavage site after position Gln^{24} in type I collagen, which is in the immediate vicinity of a cathepsin K cleavage site after Gln^{21}. Both enzymes are also able to release soluble fragments from insoluble type I collagen. Using FITC-labelled collagen, we determine a four-fold higher release of soluble fragments for cathepsin K in comparison with cathepsin L. In the continuation, we substitute active center residues of cathepsin L with those found in cathepsin K and discover that three substitutions surrounding the S2 pocket ($Leu^{69}Tyr$, $Ala^{214}Leu$ and $Gly^{164}Ala$) suffice to increase the collagenolytic activity of cathepsin L to a level comparable with cathepsin K. Furthermore, these substitutions also switch the cleavage site to the one cleaved by cathepsin K (after Gln^{21}). Computational analyses show that the collagen triple helix also fits into the active site of cathepsin L and one chain adopts a conformation suitable for catalysis. Potent collagenolytic papain-like peptidases are known in certain plants and non-vertebrate parasites. Emergence of papain-like collagenases in such evolutionarily diverse groups indicates that the ability to cleave the collagen triple helix is an inherent property and limited only by its ability to form catalytically competent complex which depends on its substrate specificity. In this work we also determine the three-dimensional structure of a complex between an inactive mutant cathepsin K^{C25S} and a synthetic collagen 17-peptide with the sequence P-P-$(G\text{-}P\text{-}Hyp)_4$-G-P-P. Against expectation, electron density was observed in a well-known allosteric site, which corresponds to a Pro-Hyp fragment of the peptide. This opens a new possibility that short fragments produced by collagen degradation can regulate the collagenolytic activity of cathepsin K thru allosteric mechanisms. Results presented here will help us further understand cathepsin K activity in bone remodelling proses at the molecular level and in drug design that will inhibit only collagenolytic activity of cathepsin K.

Keywords: cathepsin K, cathepsin L, collagen, collagenolytic activity, allosteric regulation