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Unexpected exosites on the surface of matrix metalloproteinase-12 that fine-tune specificity for elastin and collagen V

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MMP-12 hydrolyzes collagens and elastin in normal physiological processes of development, wound repair, and reconstruction of the extracellular matrix. However, chronically elevated levels of MMP-12 activity can sustain disease states in certain tissues. Excessive MMP-12 activity upon elastic fibrils of lungs and arteries causes inflammation at those sites, resulting in emphysema, while excessive activity upon collagens triggers rupture of atheromatous plaques, which can lead to heart attack or stroke. Thus, research into the binding site interactions between MMP-12 and macromolecular substrates provides surface characterization of the enzyme and expedites the process of designing artificial inhibitor molecules to selectively inactivate MMP-12. The catalytic domain of MMP-12 spans amino acid residue 100 to 263, and this region is particularly important during hydrolysis of substrates. Three secondary binding sites within the catalytic domain were examined: phenylalanine 202 (F202), threonine 205 (T205), and histidine 206 (H206). Site-directed mutagenesis was performed at the aforementioned exosites to obtain single point mutations glutamine 202 (F202Q), lysine 205 (T205K), and histidine 206 (H206D). The MMP-12 mutants were expressed as the 18.2kDa catalytic domain in BL21 derived *E. coli* Rosetta™ 2 host strains. The mutated MMP-12 enzymes were isolated as insoluble inclusion bodies after lysis of *E. coli* cells using the French press. The inclusion bodies were solubilized in neutral 6M urea buffer, and preliminary purification with cation-exchange chromatography yielded a nucleic acid-free fraction of denatured enzyme. Refolding of the MMP-12 mutants was done by dialysis with serially diluted urea solution containing Tris-HCl buffer and $CaCl_2$ at pH 7.5 without Zn++. $ZnCl_2$ was added to the final dialysis buffer to complete the refolding process. Final purification of active renatured enzyme was achieved by another cation exchange chromatography run. Then, a Bio-Rad protein assay determined the concentration of the three MMP-12 mutants. Two types of flurometric-based kinetic experiments were performed to study changes in catalytic activity of the three MMP-12 mutants versus wild-type MMP-12: active site titration and substrate activity. Active site titration quantified the concentration of active sites for each MMP-12 mutant. The substrate runs for the substrates FS-6, fluorescently labeled elastin, and a triple helical peptide mimic of collagen V (THP-V) produced raw kinetics data, in the form of progress curves. Microcal Origin Pro 7.5 was used to analyze the data to give the kcat, the number of times each enzyme site converts substrate to product per second, and the Km, the concentration needed to achieve one half V_{max}. Furthermore, dividing K_{cat} by K_m revealed the amount of activity upon FS-6, elastin, and THP-V for each MMP-12 mutant with FS-6 as the control for mutated and wild-type MMP-12. Since the k_{cat}/K_m for wild-type MMP-12 was already known, comparison of activity upon THP-V and elastin between wild type MMP-12 and each MMP-12 mutant was achieved. Ongoing data collection suggests diminished catalytic activity upon elastin and THP-V in all three MMP-12 mutants (F202Q, T205K, and H206D) when compared to wild-type MMP-12, while showing no decrease in activity upon the general MMP substrate, FS-6. To date, data suggests that all three exosites are individually involved in fine-tuning MMP-12 specificity for elastin and triple helical peptide mimics of collagen V.