

STRUCTURAL INTERACTIONS SITES OF MATRIX METALLOPROTEINASE-12 WITH COLLAGEN TRIPLE HELIX, MICELLES, AND A NATURAL PRODUCT FROM GREEN TEA

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ABSTRACT

Matrix metalloproteinase (MMP-12) is associated with many costly, life-threatening diseases, such as atherosclerosis, pulmonary emphysema, asthma, and multiple sclerosis. Therefore, macrophage secreted MMP-12 is likely found in many parts of the body likely interacting with extracellular components as have been shown and previously investigated by in vitro studies. MMP-12 is capable of cleaving elastin and may biologically cleave other extracellular components, such as collagen. This article seeks to investigate interactions of MMP-12 with a collagen-mimicking triple helical peptide (THP). The methods employed for this study involved paramagnetic relaxation enhancement (PRE). A THP paramagnetically labeled with the artificial amino acid TOAC was used to investigate the interaction between MMP-12 and a THP. This study aims to answer the question of how a THP binds to MMP-12. Preliminary structures of the complex presented here have converged. The THP binds to MMP-12 in a specific manner in one type of angle of inclination. Whereby, the orientations of the THP appear to have the N-terminus along the unprimed side of MMP-12. These results may also further hint at an exosite near the C2 calcium binding site on MMP-12.

The inhibition of MMP-12 by a natural polyphenol, (-)-Epigallocatechin gallate (EGCG) was investigated. Also, structural information concerning the binding sites of EGCG to MMP-12 was obtained. The methods employed involve paramagnetic relaxation enhancement, inhibition of enzyme activity, and stability by urea denaturation. A paramagnetic ECG, an analog of EGCG, was generated by incubating briefly in the presence of peroxide and horse radish peroxidase. The radical was used in an attempt to characterize the interaction between EGCG

and MMP-12. The initial NMR investigations involving paramagnetic relaxation enhancement (PRE) or/and NOE experiments with EGCG suggest two to three binding sites on MMP-12, including one near a calcium binding site. Inhibition of enzyme kinetics and denaturation melts were also carried out with MMP-12 in the presence of EGCG to better ascertain the most significant binding site of EGCG to MMP-12. The kinetic experiments indicate that inhibition appears to be non-competitive, and there is a calcium dependence for the EGCG inhibition of MMP-12. The calcium dependent inhibition suggests that the calcium site (C2 site or Ca 403) indicated in the PRE and NOE experiments could be the key site of allosteric inhibition of MMP-12 by EGCG.

This study investigates the interaction of MMP-12 with dodecyl phosphatidylcholine (DPC) micelles and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) micelles. The composition of the micelles has been varied. For example, DPC/CHAPS micelles and DPC/CHAPS doped with cholesterol sulfate micelles have been investigated. Chemical shift perturbations and line broadening analysis has been conducted, and the initial results suggest a KD of physiological importance. Cross-correlation rates and the extent of chemical shift perturbation between the DPC/CHAPS and DPC/CHAPS/cholesterol sulfate experiments suggest that the negatively charged cholesterol sulfate micelles may alter its association with MMP-12. The binding locations for the DPC micelles appear to near the unprimed side of MMP-12, and along the upper portion of the active site. Also, the saturable binding location of the DPC micelles on MMP-12 may be in a similar binding location than that of the DHPC type micelle, whereby some residues above the active site are affected. However, the greatest CSPs for the DHPC micelle were observed along the unprimed subsites. These results are suggestive of potential interaction between MMP-12 and lipid rafts or cell membranes, which could be relevant to MMP-12 pathophysiology.