

GLYCOSAMINOGLYCANS MODIFY THE INTERACTION OF HUMAN NEUTROPHIL AND PANCREATIC ELASTASES ON THEIR SPECIFIC CHROMOGENIC SUBSTRATES

Oliveira-Santos J., Natuline, J., Cruz-Silva, I., Praxedes-Garcia, P., Gozzo, A.J., Nunes, V.A., Nader, H.B., Araujo M.S.

Departamento de Bioquímica–UNIFESP/EPM, São Paulo, Brazil.

Human neutrophil (HNE) and porcine pancreatic (PPE) elastases are the two major serine proteases that cleave elastin. HNE is found in dense granules of polymorphonuclear leukocytes and it is essential for phagocytosis and defense against infection by microorganisms. Otherwise, PPE is stored as an inactive zymogen in pancreas and is secreted into the intestines where it is activated by trypsin and, then participates in digestion. Enzymes can be influenced by some factors including glycosaminoglycans, as demonstrated by our group. The objective of this work was to study the effect of glycosaminoglycans on the hydrolysis of MeO-Suc-Ala-Ala-Pro-Val-pNan and Suc-Ala-Ala-Ala-pNan by HNE and PPE, respectively. The values of $K_m=1.00\text{mM}$, $k_{cat}=10.2\text{s}^{-1}$ and $k_{cat}/K_m=10.2\times 10^3\text{M}^{-1}\text{s}^{-1}$ for HNE, and $K_m=4.53\text{mM}$, $k_{cat}=11.9\text{s}^{-1}$ and $k_{cat}/K_m=26.4\times 10^3\text{M}^{-1}\text{s}^{-1}$ for PPE were determined in triplicate and the standard errors were lower than 10%. Glycosaminoglycans decreased until 3.3-fold the catalytic efficiency of HNE and this effect seems to be stereo specific and not purely due to the charge content. Indeed, the iduronic acid can be important for this effect, considering that chondroitin sulfate, which not contain iduronic acid, didn't modify the catalytic efficiency of the enzyme. Besides, k_{cat}/K_m of PPE was not changed by chondroitin sulfate, but differently of HNE, heparin and heparan sulfate improved the k_{cat}/K_m of PPE, probably due to lots of negative charges in these glycosaminoglycans.

(CAPES, CNPq, FADA/UNIFESP, FAPESP).

Key words: glycosaminoglycans, human neutrophil elastase, pancreatic porcine elastase, interaction