Impact of chondroitin sulfate-4 on human and rat cathepsin K collagenolytic activity

Krzysztof K Bojarski[1], Thibaut Chazeirat[2], Gilles Lalmanach[2], Fabien Lecaille[2], Sergey A Samsonov[1]

1 Faculty of Chemistry, University of Gdańsk, Wita Stwosza 63, Gdańsk 80-308, Poland
2 INSERM, UMR 1100, Centre d'Etude des Pathologies Respiratoires, Université de Tours, F-37032 Tours cedex, France

Cathepsins are a family of a protein-degrading enzymes that can be found in many living organisms. Most of them are cysteine proteases (Enzyme Commision number - EC 3.4.22) but are also cathepsins A and G which are serine proteases (EC 3.4.21) or cathepsins D and E which are aspartyl proteases (EC 3.4.23). To date there are 15 members of the cathepsin family with most of their structures experimentally available. Regardless differences in their aminoacid sequences cathepsins share the same secondary structure pattern which is reflected in the same 3D fold. It was demonstrated that enzymatic activity of cathepsins can be modulated by glycosaminoglycans (GAGs) - a group of linear negatively charged polysaccharides made of repetitive disaccharide units. Each unit consist of one aminosugar and one uronic acid. GAGs are located in the extracellular matrix. They are involved in many cellular processes including cell proliferation, angiogenesis, anticoagulation, adhesion and signalling cascades.

Cathepsin K is an enzyme consisting of 215 aminoacid residues in its mature form. Human cathepsin K is able to degrade efficently type I and type II collagens and its irregular functioning leads to osteoporosis and rheumatoid arthritis. The collagenolytic activity of human cathepsin K in bone osteoclasts is mediated specifically by complex formation with chondroitin 4-sulfate (C4-S), a sulfated glycosaminoglycan [1]. Negatively charged C4-S forms multiple contacts with basic amino acid residues on the backside of the cathepsin K molecule. However, little is known whether these electrostatic interactions between C4-S and human cathepsin K exist also in other species. Rat (*Rattus norvegicus*) is one of the most frequently used model for the study of human pathological processes including osteoporosis and rheumatoid arthritis. We chose rat cathepsin K that shares high amino acid sequence identity (88.4%) with human cathepsin K to perform molecular docking and molecular dynamic simulations with C4-S of variable length.

Calculations were performed with use of such computational methods as PBSA electrostatic potential analysis, molecular docking, molecular dynamics and free energy calculations. Our studies predict similar interactions between C4-S hexasaccharides and rat cathepsin K with those found in human cathepsin K as well as similar binding affinity to the fluorogenic substrates of cathepsin K. Nevertheless, differences were observed with longer C4-S chains which could potentially explain diverse collagenolytic activity of aforementioned enzymes measured *in vitro*.

[1] A. H. Aguda, P. Panwar, X. Du, N. T. Nguyen, G. D. Brayer, D. Brömme, *PNAS*, **2014**, *111(49)*, 17474-17479