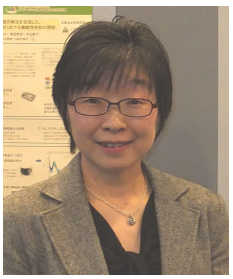
Principal Investigator: Ikuko Kakizaki
Grant Title: Enzymatic remodeling of glycosaminoglycan polysaccharides
Abstract

Background and Objective:



Testicular hyaluronidase (EC3.2.1.35) is a hydrolase that acts on hyaluronan (HA) and chondroitin sulfates (CSs) at the β1, 4-*N*-acetylhexosaminide bonds and liberates their disaccharide units from the non-reducing ends. We have succeeded in synthesizing remodeled oligosaccharides of HA and CSs using the transglycosylation activity of bovine testicular hyaluronidase[1]. However, in order to apply as research tools, we need remodeled *polysaccharides* to have lengths sufficient to interact with other molecules. Remodeling polysaccharides is though difficult because the testicular hyaluronidase simultaneously catalyzes hydrolysis and transglycosylation and the reaction products can act as substrates for both reactions. In this study, we aimed to establish a technique to elongate the desired structure at the non-reducing ends of HA and CS polysaccharides using the bovine testicular hyaluronidase.

Methods:

The transglycosylation reaction by bovine testicular hyaluronidase was performed using pyridylaminated HA polysaccharides or pyridylaminated CS polysaccharides (CSC, CSE) as acceptors and 20-fold molar of HA polysaccharides or CS polysaccharides as donors. Reaction products were analyzed by gel filtration HPLC by monitoring both UV and fluorescence. Fractions were collected using a micro-fraction collector and analyzed by dot-blotting using HA binding protein and antibodies recognizing CSs, and by cellulose acetate membrane electrophoresis.

Results:

The optimal conditions of transglycosylation reaction to obtain longer products than the acceptor control (no donor) were shown to be 48 h at 4°C for HA transferred pyridylaminated CSC, and 3 h at 4°C for CSC transferred pyridylaminated HA. Based on these conditions, transglycosylation using CSE, which is the most challenging structure in remodeling, as an acceptor or a donor, was performed at 72 h at 4°C and 3 h at 4°C, respectively, to synthesize hybrid polysaccharides of HA and CSE (Figure 1). It is difficult to completely separate transglycosylated products from the donor-derived carbohydrate chains, so the products were roughly purified by gel filtration HPLC. Dot blotting conditions applied to the structural analysis of the products were established. Size analysis by HPLC, dot-blotting and cellulose acetate electrophoresis of collected fractions suggested that the donor-derived structures were transferred to the non-reducing end of the acceptor polysaccharides. Therefore, we were able to clear the first step in establishing methods of remodeling, rough purification, and analysis the structure, of hybrid polysaccharides of HA and CSs.

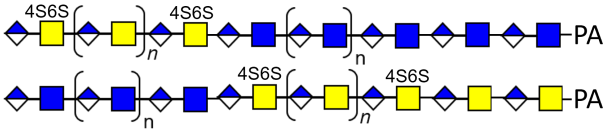


Figure 1. Examples of hybrid polysaccharide consisting of hyaluronan and chondroitin sulfate having E-units at the non-reducing end (upper) or at the reducing end (lower) enzymatically synthesized using bovine testicular hyaluronidase.

1) Endo, M., & Kakizaki, I. (2012). *Proceedings of the Japan Academy, Ser. B*, 88, 327-344.