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**SHORT COMMUNICATION**

**Disaccharide analysis of chondroitin and heparin from farmed Atlantic salmon**

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**Abstract**

The heparin disaccharides detected in farmed Atlantic salmon (*Salmo salar*) gills and intestines have, with one exception, been reported in porcine heparin. The relative amounts of disaccharides appear to be very different in the two species.

Two chondroitin disaccharides with a proposed essential role in the zebrafish (*Danio rerio*) development and differentiation are detected in farmed Atlantic salmon. In addition, most of the chondroitin/dermatan sulfate and heparin disaccharides detected here have been reported in zebrafish, in support of the claims of the heparin presence in fish. The same chondroitin/dermatan disaccharides were detected in the bones of bony fishes. The rare heparin disaccharide UA2S-GlcN was in relative highest amounts in both gills and intestines. In addition, it is shown that salmon heparin contains the trisulfated disaccharide that is a crucial part of the pentasaccharide in high affinity heparin that binds and activates antithrombin. This trisulfated disaccharide was also reported in zebrafish heparin. In context with our previous reports, this communication suggests that structures similar to the antithrombin-binding pentasaccharide are present in farmed Atlantic salmon heparin. This could enhance the potential of marine sources for use in antithrombosis.

**Introduction**

Marine organisms appear to be a source of medicinal compounds that is only at the start of exploitation. Mourão [1] reviewed the perspective on the use of sulfated polysaccharides from marine organisms in the antithrombotic treatment where some important reasons for this use were given. It is also referred to skepticism of this potential use due to the lack of the pentasaccharide binding and the resultant conformational activation of antithrombin.

A review on anticoagulant marine sulfated glycans, claimed that the specific heparin pentasaccharide is not present there [2].

The existence of heparin in fish has been disputed, as discussed by Zhang et al. [3] and was countered by their findings. The results suggested that heparin participates in the development of zebrafish. Heparin/heparin sulfate has been shown to interact with a high number of human proteins and have been attributed to a number of networks (interactomes), such as regulation of cell proliferation, inflammatory response, blood cell development and system development [4]. The system development could therefore be a possible biological role for heparin/heparan sulfate in fish. Isolation and characterization of chondroitin sulfate from bony fishes [5] showed the presence of nonsulfated, monosulfated and disulfated chondroitin disaccharides with quantitative results. The authors suggested that the disulfated disaccharides could be a useful marker for the marine origin of chondroitin sulfate [5].

Previously, we have described heparin from farmed Atlantic salmon with a 136.8 U/mg antifactor Xa activity and a disaccharide that is a part of the pentasaccharide that binds the basic fibroblast growth factor and with antithrombin activation activity [6]. Affinity chromatography of salmon heparin using an immobilized heparin pentasaccharide-binding pentapeptide gave an activity of 169 U/mg antifactor Xa [7].

**Purification of salmon heparin**

Materials

Sartobind Anion Direct was from Sartorius Stedim Biotech, Göttingen, Germany.

The Mono Q anion exchanger was run on a FPLC system, both were from GE Healthcare, Oslo, Norway.

Methods

Glycosaminoglycan detection and analysis

Glycosaminoglycan detection had been described previously [6].The analysis was performed at the Glycotechnology Core Resource, University of California, San Diego, USA. Following lyase digestion, the profile of the derived disaccharides was determined by anion exchange HPLC with UV and fluorescent detection.

Purification of heparin

The preparation of crude heparin has been described [6]. Briefly, the homogenate was treated with protease, followed by incubation at 80 oC for 1 h and tangential flow filtration and concentration. Further purification applied the membrane absorber Sartobind Anion Direct (250 mL). The pH of salmon heparin filtrated supernatant was adjusted to 5.5 by adding 0.5 M ammonium acetate, acetic acid pH 5.5 to 25 mM (final concentration of acetate) and NaCl to a final concentration of 10 mM. The solution was recirculated on the membrane absorber at around 25 mL/min for 60 min and then washed with 150-200 mL of the equilibration buffer (25 mM ammonium acetate, acetic acid, pH 5.5 in 10 mM NaCl). Elution was carried out using 20 mL 3 M NaCl in 5 mM ammonium acetate, acetic acid, pH 5.5. The eluates were desalted, concentrated and freezedried [6]. These samples (gills, 4.5 mg; intestines, 16.7 mg) were further purified using anion exchange (Mono Q, 3.9 mL) chromatography on a FPLC system with the same solutions and as described for the Dowex exchange chromatography [6] with the exception that a 0-4 M NaCl gradient (100 mL) was used. The eluates were desalted and freeze-dried. Heparin from gills and intestines was purified. Both samples were divided in two equal parts prior to glycosaminoglycan analysis.

**Results and discussion**

The amount of chondroitin disaccharides found in the intestines is around 3.3 times higher than that of heparin disaccharides, in contrast to the results from gills while the amount of heparin disaccharides is more than 21 times that of chondroitin/dermatan disaccharides (Tables 1and 2). The chondroitin disaccharides UA-GalNAc4S and UA-GalNAc6S that may have an essential role in the development and differentiation in zebrafish [[3] are detected here (Table 2). In addition, UA-GalNAc, UA2S-GalNAc4S, UA2S-GalNAc6S (Table 2) is detected in zebrafish. All the chondroitin disaccharide reported from bones in bony fishes [5] is reported here (Table 1b). This includes the disulfated disaccharides have been suggested as useful markers for the marine origin of chondroitin [5]. Some of the heparin disaccharides were also previously detected by NMR analysis [6], UA2S-[1,4]-GlcNS from gills and UA-[1,4]-GlcNS from both tissues. All of the heparin disaccharides detected in zebrafish, except UA-GlcNAc6S [3], are reported here (Table 1). All the heparin disaccharides reported here have been described in porcine intestinal mucosal heparin with the exception of disaccharide UA2S-GlcN that was not in measurable amounts in porcine heparin [8] and appears to be a rare disaccharide [8] and was not detected in zebrafish [3], but is surprisingly found in the relatively highest amounts in salmon gills and intestines (Table 1). The disaccharide UA2S-[1,4]-GlcNS-6S is the last disaccharide (-g-h) in the heparin antithrombin binding pentasaccharide (d-e-f-g-h) and is the most common disaccharide in porcine heparin [9]. This disaccharide is in relatively small amounts in salmon (Table 1) and in relatively high amounts in zebrafish [3]. It should be emphasized that the tetrasulfated disaccharide g-f is not reported here, nor any disaccharide with the trisulfated monosaccharide (F). In sum, the previous reports [6, 7] and this communication suggest that the glycosaminoglycan effects and composition in salmon heparin, warrants further studies. Any nonanticoagulant fractions could also be of interest. The considerable amounts of farmed salmon waste (gurry) could be a valuable source that could enhance the potential of marine sources.

The author declares no conflict of interest.

## Electronic supplementary material

Electronic supplementary material The online version of this article

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**References**

1. Mourão, P.A.S.: Perspective on the Use of Sulfated Polysaccharides from Marine Organisms as a Source of New Antithrombotic Drugs. Mar. Drugs **13**(5), 2770-2784 (2015). doi:10.3390/md13052770

2. Pomin, V.H.: Anticoagulant motifs of marine sulfated glycans, Glycoconj. J. **31**(5), 341–344 (2014). doi:10.1007/s10719-014-9530-1

3. Zhang, F., Zhang, Z., Thistle, R., McKeen, L., Hosoyama, S., Toida, T., Linhardt, R.J., Page-McCaw, P.: Structural characterization of glycosaminoglycans from zebrafish in different ages. Glycoconj. J. 26(2), 211-218 (2009).doi:10.1007/s10719-008-9177-x

4. Ori, A., Wilkinson, M.C., Fernig, D.G.: A Systems Biology Approach for the Investigation of the Heparin/Heparan Sulfate Interactome. J. Biol. Chem. 286 (22), 19892-19904 (2011). doi: 10.1074/jbc.M111.228114

5. Maccari, F., Geleotti, F., Volpi, N.: Isolation and structural characterization of chondroitin sulfate from bony fishes. Carbohydr. Polym. 129, 143-147 (2015).

doi:10.1016/j.carbpol.2015.04.059

6. Flengsrud, R., Lie Larsen, M., Ødegaard, O.R.: Purification, characterization and in vivo studies of salmon heparin. Thromb. Res. **126**(6), e409-e417 (2010). doi:10.1016/j.thromres.2010.07.004

7. Flengsrud, R., Antonsen, S.G.: The binding of pentapeptides to biological and synthetic high affinity heparin. Bioorg. Med. Chem. Lett. **25**(21), 4774-4776 (2015). doi:10.1016/j.bmcl.2015.07.022

8. Lawrence, R., Olson, S.K., Steele, R.E., Wang, L., Warrior, R., Cummings, R.D., Esko, J.D.: Evolutionary Differences in Glycosaminoglycan Fine Structure Detected by Quantitative Glycan Reductive Isotope Labeling. J. Biol. Chem. 283(48), 33674-33684 (2008). doi:10.1074/jbc.M804288200

# 9. Capila, I., Linhardt, R.J.: Heparin-Protein Interactions. Angew. Chem. Int. Ed. 41(3), 390-412 (2002). doi:10.1002/1521-3773(20020201)41:3<390::AIDANIE390>3.0.CO;2-B

Table 1

Heparin disaccharides from salmon gills and intestines.

 Gills

DSCa Structure Amount % detected

 pmol

III-H UA2S-[1,4]-GlcN 275.05 60.1

IV-S UA-[1,4]-GlcNS 174.88 38.2

III-S UA2S-[1,4]-GlcNS 7.65 1.7

Total 457.58 100.0

 Intestines

IV-A UA-[1,4]-GlcNAc 173.13 21.4

III-H UA2S-[1,4]-GlcN 464.93 57.5

IV-S UA-[1,4]-GlcNS 146.33 18.1

II-S UA-[1,4]-GlcNS-6S 5.38 0.7

III-S UA2S-[1,4]-GlcNS 13.05 1.6

I-S UA2S-[1,4]-GlcNS-6S 5.50 0.7

Total 808.32 100.0

a DSC - disaccharide code

Table 2

Chondroitin/dermatan disaccharides from salmon gills and intestines.

 Gills

DSCa Structure Amount % detected

 pmol

Di-0S UA-GalNAc 5.66 26.6

Di-6S UA-GalNAc6S 9.01 42.4

Di-UA2S UA2S-GalNAc 6.59 31.0

Total 21.26 100.0

 Intestines

Di-0S UA-GalNAc 649.00 24.1

Di-4S UA-GalNAc4S 846.58 31.3

Di-6S UA-GalNAc6S 651.93 24.2

Di-UA2S UA2S-GalNAc 137.60 5.1

Di-diSE UA-GalNAc4S6S 48.35 1.8

Di-diSB UA2S-GalNAc4S 220.58 8.2

Di-diSD UA2S-GalNAc6S 144.34 5.3

Total 2698.37 100.0

a DSC - disaccharide code