European Journal of Medicinal Chemistry 154 (2018) 133–143

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Modulating the degree of fucosylation of fucosylated chondroitin sulfate enhances heparin cofactor II-dependent thrombin inhibition

Li Xu [a, 1], Na Gao [a, b, 1], Chuang Xiao [a], Lisha Lin [a], Steven W. Purcell [c], Mingyi Wu [a, *], Jinhua Zhao [a, *]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, China
[b] School of Pharmaceutical Sciences, South-Central University for Nationalities, Wuhan, 430074, China
[c] National Marine Science Centre, Southern Cross University, Coffs Harbour, NSW, 2450, Australia

ARTICLE INFO

Article history:
Received 7 March 2018
Received in revised form
30 April 2018
Accepted 15 May 2018
Available online 15 May 2018

Keywords:
Fucosylated chondroitin sulfate
Glycosaminoglycan
Anticoagulant activity
Heparin cofactor II
Intrinsic factor Xase complex

ABSTRACT

Fucosylated chondroitin sulfate (FCS), an unusual glycosaminoglycan with fucose side chains, is a promising anticoagulant agent. To assess the effect of its structure on anticoagulant activity, its derivatives with various degrees of fucosylation (DF), molecular weights (Mw) and sulfation patterns were prepared and characterized. Biological tests showed that their APTT (activated partial thromboplastin time) prolonging activity and intrinsic factor Xase complex (factor IXa-VIIIa-Ca^{2+}-PL complex) inhibitory activity were both reduced in FCS derivatives with lower Mw and DF. However, FCSs with DF at least 16% resulted in greater heparin cofactor II (HCII)-dependent thrombin inhibitory activity in response to decreasing DF, and these activities did not depend on Mw (Mw > 5.2 kDa). Solution competition binding assay further suggested that modulating the DF of FCS derivatives might enhance inhibition of thrombin by activating HCII. These findings imply that FCS derivatives with suitable chain length and DF value may be novel anticoagulants by activating HCII.

1. Introduction

Heparin cofactor II (HCII) is a plasma serpin that can inactivate thrombin (factor IIa, FIIa), the final protease in the blood coagulation pathway, by forming a bimolecular complex to slow down the coagulation process [1]. This serine protease is involved in the regulation of blood coagulation, atherogenesis and neointima formation. Furthermore, it may help to down-regulate the *in vivo* pathological process of atherosclerosis and thrombosis after vascular injury [2,3]. Unlike antithrombin, HCII-deficient mice develop normally, and no spontaneous thrombosis or other morphological abnormalities have been observed [4,5]. But such mice are more prone to vascular occlusion after arterial injury, suggesting that HCII may have a more important role in response to vascular injury [6].

Some glycosaminoglycans (GAGs), such as dermatan sulfate (DS), heparin and chondroitin sulfate, can accelerate the HCII-thrombin reaction and potentiate the activity of HCII by 1000 times [7,8]. Binding of the GAG to HCII induces its allosteric activation, which increases the accessibility of both the reactive site and an N-terminal acidic domain of the inhibitor to thrombin [9]. Subsequently, DS has been developed as a clinically effective antithrombotic agent owing to its ability to enhance the *in vivo* anticoagulant activity of HCII [10,11].

Fucosylated chondroitin sulfate (FCS) is an unusual GAG that can be extracted from widely traded sea cucumbers (Echinodermata, Holothuroidea). It also exhibits strong thrombin inhibitory activity by heparin cofactor II [7,12]. FCS possesses a chondroitin sulfate-like backbone, but is markedly different from the typical mammalian GAGs because of its unique sulfated fucose side chains (Fig. 1) [12,13]. FCS is a potent anticoagulant through multiple mechanisms. Some mechanisms are well documented such as factor Xa and thrombin inhibition by antithrombin (AT) (AT-dependent anti-FXa and anti-thrombin), inhibition of thrombin by heparin cofactor II (HCII), and inhibition of the intrinsic factor Xase complex (FXase) [14–18]. Previous investigations indicated that the

* Corresponding authors.
E-mail addresses: wumingyi@mail.kib.ac.cn (M. Wu), zhaojinhua@mail.kib.ac.cn (J. Zhao).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ejmech.2018.05.024
0223-5234/

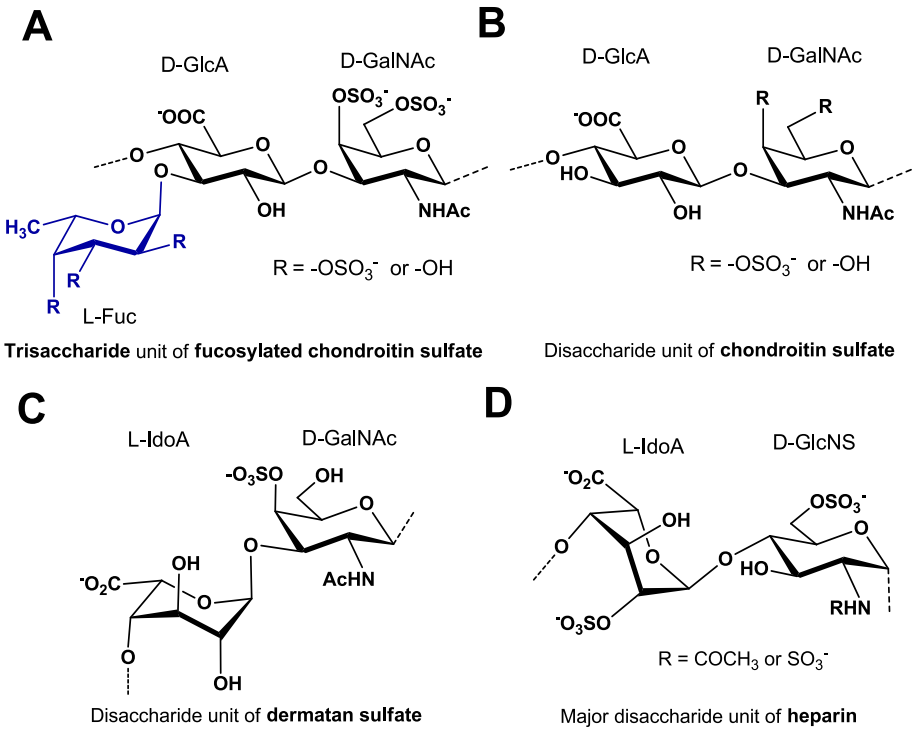

Fig. 1. Major repeating units of several glycosaminoglycans. Regular trisaccharide unit of fucosylated chondroitin sulfate (FCS) from sea cucumber (A) and major disaccharide unit of chondroitin sulfate (CS) (B), dermatan sulfate (DS) (C) and heparin (D).

fucose side chains or sulfate groups are required for the HCII-dependent thrombin inhibitory activity of FCS, since their removal by mild acid hydrolysis significantly weakened this activity [19,20]. Moreover, in the presence of HCII, the thrombin inhibitory activity of fucosylated chondroitin sulfate was about 100-fold higher than that of a chondroitin sulfate [7]. Since the structural backbone units of FCS are the same as those of chondroitin sulfates, the evidence implies that the fucose side chains of FCS are required for its potent thrombin inhibitory activity by HCII [20].

Previously, we studied the relationship between the structure and anticoagulant activity of the GAGs derived from sea cucumbers. We explored the structural modification of native FCS to improve its target selectivity and found that its AT-dependent anti-FXa and anti-thrombin activities could be eliminated by depolymerization, while the HCII-dependent anti-thrombin and the anti-FXase activities remained [12]. Our tests also found that when the molecular weight (Mw) of depolymerized FCS products was above 5 kDa, their HCII-mediated thrombin inhibitory activity increased significantly with the decrease of Mw [12]. The result was curiously inconsistent with other literature reports [21]. Apart from Mw, other structural differences between the FCS derivatives could also be factors contributing to the incongruent findings. Additionally, although research showed that the fucose side chains of FCS contribute to its HCII-dependent anticoagulant activity [19,20], the effect of its degree of fucosylation (DF) on this activity is still unclear. Therefore, its structure-activity relationship, especially the HCII-dependent inhibition activity of thrombin, remains to be clarified.

Factor IXa (FIXa), a serine protease, and factor VIIIa (FVIIIa), a protein cofactor, form a Ca^{2+}- and phospholipid surface-dependent complex referred to as the intrinsic factor Xase complex (FXase), which efficiently converts zymogen factor X to Xa [22,23]. The intrinsic FXase is the last rate-limiting step of the enzyme cascade in the intrinsic coagulation pathway. Consequently, it is becomingly recognized as a prime target for the development of safer anticoagulants [14,18]. Importantly, FCS also has potent intrinsic factor Xase complex inhibitory (anti-FXase) activity. And the depolymerized FCS has weak AT-dependent anti-FXa and anti-thrombin activities, and remains strong anti-FXase activity [12]. Its anticoagulant mechanisms are thus significantly different from those of heparin-like drugs, which have anti-FXa and/or anti-thrombin activities [11]. Hence the depolymerized FCS is becoming recognized as a prime candidate for safer anticoagulants with potential preventive and therapeutic applications [12]. Our previous study further suggested that a minimum of 6–8 trisaccharide units, free carboxyl groups and fucosylation of glucuronic acid (GlcA) residues may be required for potent anti-FXase activity of FCS [12]. However, there has been no detailed investigation about the effects of the degree of fucosylation of FCS on its activity to inhibit thrombin by HCII and the intrinsic factor Xase complex activity.

In this work, the relationships between structure and anticoagulant activity of FCS derivatives were studied to further search for FCS derivatives with selective anticoagulation activity. Specifically, we investigated the effects of degree of polymerization, degree of fucosylation and sulfate substitution types of the fucose side chains on their anticoagulant activities. These APTT (activated partial thromboplastin time) prolonging activities by using normal human plasma were carefully tested to illuminate these anticoagulant activities of FCS derivatives. Furthermore, their HCII mediated thrombin inhibitory activity and intrinsic factor Xase complex inhibitory activity were evaluated to clarify effects of these derivatives on their anticoagulant targets. This study contributes to understanding the structure-activity relationship of the sea cucumber GAG for anticoagulation and the findings are valuable for the development of new anticoagulant agents.