FEBS Letters 585 (2011) 2461–2466

FEBS Letters

journal homepage: www.FEBSLetters.org

Asparagine 405 of heparin lyase II prevents the cleavage of glycosidic linkages proximate to a 3-*O*-sulfoglucosamine residue

Wenjing Zhao [a], Marie-Line Garron [b], Bo Yang [c], Zhongping Xiao [c], Jeffrey D. Esko [d], Miroslaw Cygler [b,*], Robert J. Linhardt [a,c,*]

[a] Department of Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA
[b] Department of Biochemistry, McGill University, Montreal, Quebec, Canada H3G 1Y6
[c] Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA
[d] Department of Cellular and Molecular Medicine, Biomedical Sciences Graduate Program, University of California, San Diego, La Jolla, CA, USA

ARTICLE INFO

Article history:
Received 4 June 2011
Revised 20 June 2011
Accepted 21 June 2011
Available online 6 July 2011

Edited by Stuart Ferguson

Keywords:
Heparin lyase II
Heparinase
Site-directed mutagenesis
Protein engineering
Heparin
3-*O*-Sulfoglucosamine
Substrate specificity

ABSTRACT

Heparin and heparan sulfate contain a rare 3-*O*-sulfoglucosamine residue critical for anticoagulation and virus recognition, respectively. The glycosidic linkage proximate to this 3-*O*-sulfoglucosamine is resistant to cleavage by all heparin lyases (Heps). HepII has a broad specificity. The crystal structure of the wild type HepII identified its active site and showed a close spatial proximity between Asn405 and the 3-OH group of the bound glucosamine residue. In this study, we mutated Asn405 to the less sterically demanding Ala405 or Gly405, which broadened the substrate specificity of HepII and caused it to cleave the resistant linkage proximate to the 3-*O*-sulfoglucosamine residue.
© 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

Heparin and heparan sulfate (HS) are negatively charged, linear polysaccharides consisting of repeating uronic acid-glucosamine disaccharide units. The major uronic acid components in heparin and HS are iduronic acid (IdoA) and glucuronic acid (GlcA), respectively. Heparin (Fig. 1A) is more highly sulfated than HS, with its uronic acid residues commonly modified with 2-*O*-sulfo groups and its glucosamine residues predominantly substituted with *N*-sulfo and 6-*O*-sulfo groups and in rare cases with 3-*O*-sulfo groups. The signature 3-*O*-sulfo group containing glucosamine residue found within the antithrombin (AT) binding sequence of heparin plays an essential role in binding and activating AT [1–3] (Fig. 1A). In HS a 3-*O*-sulfo group containing glucosamine residue is also contained in the glycoprotein (gD) binding site required for Herpes simplex virus type 1 (HSV-1) entry to cells [4–6].

Heparin and HS can be depolymerized through a β-elimination mechanism by bacterial heparin lyases (Heps) (or heparinases), a group of polysaccharide cleaving enzymes commonly expressed and serving nutritional purposes in various microorganisms [7]. Among the identified three Heps, HepII from *Pedobacter heparinus* (previously known as *Flavobacterium heparinum*) displays a uniquely wide range of substrate specificity, cleaving both heparin and HS at the linkage between glucosamine residue and either IdoA or GlcA residue having various sulfation patterns [8–10]. Despite this broad specificity, neither HepII nor any of the other currently known Heps are capable of cleaving the glycosidic linkage (−1, +1) when the proximate glucosamine residue at the reducing end of the linkage (+2 subsite) contains a 3-*O*-sulfo group (Fig. 1A) [11]. Recent studies on HepII in our laboratory have determined the structure of HepII with and without complexed HS oligosaccharide substrate or a disaccharide product [12,13].

Abbreviations: HS, heparan sulfate; GlcA, glucuronic acid; IdoA, iduronic acid; GlcNS6S, 6-*O*-sulfo-*N*-sulfoglucosamine; GlcNAc6S, 6-*O*-sulfo-*N*-acetylglucosamine; GlcNS3S6S, 3,6-*O*-sulfo-*N*-sulfoglucosamine; AT, antithrombin; Hep, heparin lyase; PAGE, polyacrylamide gel electrophoresis; LC, liquid chromatography; MS, mass spectrometry

* Corresponding authors. Address: Department of Biology, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA. Fax: +1 518 276 3405 (R.L. Linhardt), +1 514 496 5143 (M. Cygler).

E-mail addresses: mirek.cygler@bri.nrc.ca (M. Cygler), linhar@rpi.edu (R.J. Linhardt).

0014-5793/$36.00 © 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2011.06.023

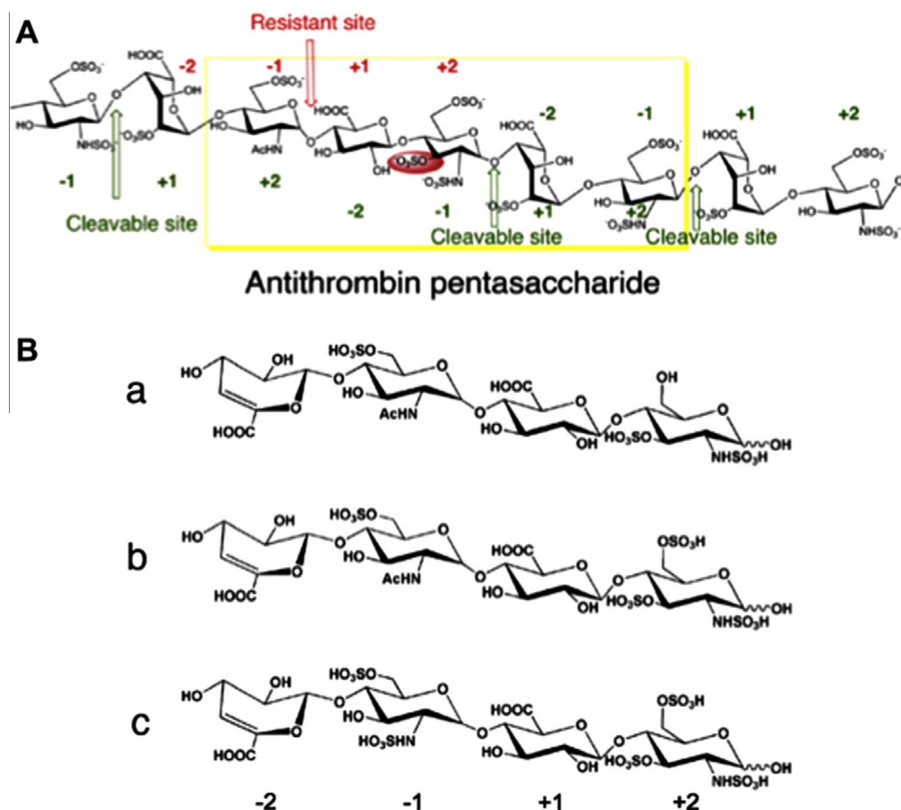

Fig. 1. HepII action on 3-*O*-sulfoglucosamine containing substrates. (A) Structure of a portion of the heparin chain containing an antithrombin binding site. Four sites are shown that should be HepII cleavable based on the residues at the −1 +1 sites. Three of these are HepII cleavable (shown in green) and one, containing a 3-*O*-sulfoglucosamine residue at subsite +2 is resistant (shown in red). (B) Chemical structures of used tetrasaccharide substrates. Tetrasaccharides **a**, **b** and **c** were prepared by exhaustively digesting heparin with HepI. The four monosaccharides in each structure are labeled as −2 to +2 from non-reducing end to reducing end. The 3-*O*-sulfoglucosamine residue is at subsite +2 and the putative HepII cleavage site is −1 +1.

Cleavage of substrates containing both uronic acid epimers IdoA and GlcA occurred within the same catalytic site in HepII. Potential active site residues participating in the various mechanistic steps of β-elimination have been identified along with their suggested roles. Specifically, His406 was found to be responsible for charge neutralization of the carboxyl group of uronic acid at the cleavage site. Tyr257 and His202 serve as the catalytic bases in the subsequent step by abstracting the proton of the C5 carbon from GlcA-containing (HS) and IdoA-containing (heparin) substrate, respectively [12]. In the crystal structure of HepII complexed with oligosaccharides occupying +1 and +2 sites the 3-*O*-hydroxy group of the glucosamine unit at the +2 subsite is buried and making contacts with the protein sidechains. The present study uses site-directed mutagenesis to perform protein engineering on HepII to test our hypothesis that expanding the size of the substrate-binding site near glucosamine 3-*O*-hydroxy group will make this mutant able to cleave substrates containing a 3-*O*-sulfoglucosamine residues at the +2 subsite. Several residues were mutated and the ability of the resulting enzymes to act on resistant substrates containing a 3-*O*-sulfoglucosamine residue was examined.

2. Materials and methods

2.1. Chemicals

Heparin and HS sodium salts were purchased from Celsus Laboratories, Cincinnati, OH. Disaccharide standards of heparin and HS were obtained from Iduron Co. Other chemicals including chromatography and gel preparation reagents used in this work were the same as previously reported [12,13].

2.2. Preparation of recombinant wild type and mutant HepII

The mutants were prepared using the previously described expression plasmid containing HepII gene [13]. Site-directed mutagenesis was performed by the QuickChange method using Site-directed Mutagenesis Kit (Stratagene), according to the manufacturer's protocol. Mutations were confirmed by DNA sequencing. All mutants were expressed in *Escherichia coli* BL21(DE3).

2.3. Preparation of the heparin-derived tetrasaccharide substrates

Heparin-derived tetrasaccharides were prepared as described in previous literature [14]. Briefly, heparin was exhaustively digested by HepI in 50 mM sodium phosphate buffer, pH 7.4. The resulting disaccharide components were removed by chromatography on a 100 × 5.0 cm Bio-Gel P10 column eluted with 0.2 M NaCl and the oligosaccharide fractions were desalted on a 100 × 2.0 cm P2 column and lyophilized. The product mixture was fractionated on a 20 × 250 mm semipreparative strong anion exchange (SAX)-high performance liquid chromatography (HPLC) column eluted with a salt gradient with absorbance detected at 232 nm. Individual tetrasaccharide peaks were desalted and further purified by repeated separation on the same HPLC column. Purity of each tetrasaccharide was determined to be >95% by analytical HPLC. Structures of these tetrasaccharides were characterized by liquid chromatography (LC)–mass spectrometry (MS) and 1D and 2D NMR [14–16].

Download English Version:

https://daneshyari.com/en/article/10871750

Download Persian Version:

https://daneshyari.com/article/10871750

Daneshyari.com