ARTICLE IN PRESS

Carbohydrate Research xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Minireview

Re-visiting the structure of heparin

Benito Casu *, Annamaria Naggi, Giangiacomo Torri

G. Ronzoni Institute for Chemical and Biochemical Research, via G. Colombo, 81 20133 Milan, Italy

ARTICLE INFO

Article history:
Received 13 June 2014
Accepted 22 June 2014
Available online xxxx

Keywords:
Heparin structure
Active site for antithrombin
Low-molecular-weight heparins
Heparin oligosaccharides
Protein binding
Conformations

ABSTRACT

The sulfated polysaccharide heparin has been used as a life-saving anticoagulant in clinics well before its detailed structure was known. This mini-review is a survey of the evolution in the discovery of the primary and secondary structure of heparin. Highlights in this history include elucidation and synthesis of the specific sequence that binds to antithrombin, the development of low-molecular-weight heparins currently used as antithrombotic drugs, and the most promising start of chemo-enzymatic synthesis. Special emphasis is given to peculiar conformational properties contributing to interaction with proteins that modulate different biological properties.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Heparin is a well-known sulfated polysaccharide widely used as an anticoagulant and antithrombotic drug and with perspective uses in other therapeutic fields. This paper is an overview of advances in the knowledge of structure and structure-activity relationships of heparin as evolved over the past few decades, with glimpses on its early history. To meet with the space constraint of mini-reviews, the authors referred to previous extended reviews and books,[1–7] to some landmark papers and to most recent articles of groups active in the field for recognizing contributions that could not be specifically mentioned in the present context. Such an exercise implied some arbitrary choices: this overview follows the strategy of reporting 'case studies' involving the research groups of the authors. It was also meant as a tribute to *Carbohydrate Research* for its steadily contributing, since its early issues, to the progresses in heparin research.

2. From 'mysterious heparin' to Wolfrom's structure

Heparin and its anticoagulant activity were 'discovered' in Toronto about 100 years ago by McLean and Howell, who were actually looking for a pro-coagulant substance extracted from tissues and thought to be a phospholipid. It had taken some time to the scientific community to realize that heparin is in fact a sulfated polysaccharide belonging to the class of glycosaminoglycans (GAG), constituted by alternating disaccharide sequences of an uronic acid and a hexosamine. It is noteworthy that, while still being a 'mysterious' entity, that is, before the exact nature of the component carbohydrate residues and location of sulfate groups along the polysaccharide chains were established, heparin started to be successfully used as an anticoagulant in clinics, arising enthusiasm especially as a life-saving drug that permitted open-heart surgery.[8]

Reviews on the history of heparin have been published by Rodén[9] and Barrowcliffe.[10] Hereinafter some milestones of the early history are mentioned. In 1935, Jorpes established that the uronic acid and aminosugar ratio of heparin was 1:1 as in chondroitin sulfates. Since the sulfur content of heparin corresponded to about 2.5 sulfate groups per disaccharide unit and on the assumption of a regular structure of its polysaccharide backbone, heparin was erroneously thought to be an oversulfated chondroitin sulfate.[†] Some years later Wolfrom, who thought that D-glucuronic acid (GlcA) was the only uronic acid component of heparin, proposed

Abbreviations: GAG, glycosaminoglycan; GlcA, D-glucuronic acid; $GlcA2SO_3$, D-glucuronic acid 2-sulfate; GlcN, D-glucosamine; $GlcNSO_36SO_3$, D-glucosamine N,6-sulfated; IdoA, L-iduronic acid; $IdoA2SO_3$, L-iduronic acid 2-sulfate; TSD, trisulfated disaccharide; AT, antithrombin; HA, high affinity for AT; LA, low-affinity for AT; ATBR, antithrombin-binding region; LMWH, low-molecular-weight heparin; $GlcNSO_33,6SO_3$, 3,6-O-sulfated D-glucosamine; $ManNSO_36SO_3$, D-mannosamine 6-sulfate; 1,6-anhydro-$GlcNSO_3$, 1,6-anhydro-D-glucosamine N-sulfate; 1,6-anhydro-$ManNSO_3$, 1,6-anhydro-D-mannosamine N-sulfate; ULMWH, ultra-low-molecular-weight heparin; FGFs, fibroblast growth factors; FGF1, fibroblast growth factor-1; FGF2, fibroblast growth factor-2; FGFR, fibroblast growth factor receptor; 3OST-1 and 3OST-3, 3-O-sulfotranferases 1 and 3; K5, *E. coli* K5 polysaccharide; HQSCcos, statistical correlation two-dimensional NMR spectroscopy.

* Corresponding author.
E-mail address: casu@ronzoni.it (B. Casu).

[†] Curiously, a major crisis in the use of heparin in clinics was caused in the late 2007 and early 2008 by contamination with an unnatural oversulfated chondroitin sulfate (see Section 11).

http://dx.doi.org/10.1016/j.carres.2014.06.023
0008-6215/© 2014 Elsevier Ltd. All rights reserved.

ARTICLE IN PRESS

the heparin structure to be represented by repeating units of alternating 1,4-linked $GlcA2SO_3$ and $GlcNSO_36SO_3$ residues, where the configuration of glycosidic bonds was proposed to be α for both GlcA and GlcN on the basis of optical rotation data.[11] The configuration of GlcA in heparin was subsequently demonstrated to be β.[12]

3. First re-visitation: L-iduronic acid enters into the picture

Wolfrom's structure for heparin remained undisputed for several years. Although in the meantime L-iduronic acid (IdoA) had been detected in some heparins, it was generally thought that these findings were reflecting residual contents of IdoA-containing GAGs such as dermatan sulfate, which is difficult to totally remove from heparin preparations. Undoubtedly, acceptance of Wolfrom's structure has been also a tribute to his outstanding reputation as a carbohydrate chemist. However, such a consent was mainly due to serious problems facing everybody who attempted to tackle the heparin structure using methods available at the time for obtaining di- and oligosaccharide fragments to reconstruct the structure of the polysaccharide. In the late 1960s and early 1970s the application of milder methods of acid hydrolysis and the advent of non-destructive analytical techniques such as NMR spectroscopy rapidly changed the picture. In 1962, Cifonelli and Dorfman found IdoA to be a major uronic acid component of heparin,[13] and in 1968 preliminary NMR studies by Perlin indicated that the structure of heparin was not as simple as depicted by Wolfrom's formula, and that IdoA residues substantially contributed to the structure of the polysaccharide.[14] Analysis of higher resolution (220 MHz) spectra[15] and chemical studies[16] together with independent structural studies by Lindahl on nitrous-acid generated fragments[17] finally provided clear evidence that IdoA (mostly as $IdoA2SO_3$) was the prominent uronic acid of heparin. In the meantime, Wolfrom had admitted that his earlier results had led to an incorrect conclusion.[18] As reviewed in Refs. 1,10, the hydrolytic procedures previously applied were leading to the loss of IdoA-containing fragments; as a consequence, Wolfrom's analysis was based on the minor GlcA-containing ones. In a study exploiting for the first time NMR spectroscopic analysis of enzymatic digests (provided by Dietrich), Perlin also established the α-L-configuration of the $IdoA2SO_3$ residues of heparin.[19] The optical rotation of heparin was rationalized by studies with model synthetic iduronates.[20] A complete analysis of ^1H and ^{13}C NMR spectra of heparin, highlighting its major components, was published in 1979.[21] These findings led to establish the structure of the major disaccharide repeating units of heparin as shown in (Fig. 1A). The trisulfated disaccharide (TSD) repeating sequences $–IdoA2SO_3–GlcNSO_3,6SO_3–$were found to be especially prominent (up to 90%) in preparations from beef lung, an organ that for some time was the principal source of heparin, and somewhat less represented (about 75%) in heparins from porcine mucosa, which largely replaced beef lung as an animal tissue for preparation of clinical heparins. The complement to 100% of uronic acids was GlcA, usually associated also with N-acetylated (instead of N-sulfated) glucosamine residues. Biosynthetic studies, especially by the Uppsala group (reviewed in Ref. 22) definitively proved that GlcA- and GlcNAc-containing sequences were actual constituents of the heparin chains, their presence being the result of incomplete modification of the biosynthetic precursor chains constituted by repeating –GlcA–GlcNAc–sequences. These studies, together with structural analysis of extensively purified heparins (see Section 11) definitively established the concept that heparin was structurally microheterogeneous. Heparins from different tissues and animal species were found to have different contents of $IdoA2SO_3$ and IdoA, and to be heterogeneous also in terms of size, being composed of chains of different length, their mean MW ranging from 10 to 20 KDa depending also on the method of preparation.[23,24]

4. Discovery and synthesis of the binding site for antithrombin Mechanism of anticoagulation

Soon after Rosenberg's milestone finding that the anticoagulant activity of heparin was mainly based on its ability to bind antithrombin (AT), thus accelerating by several orders of magnitude the AT-mediated inhibition of coagulation factors, in 1976 Rosenberg's, Lindahl's and Andersson's groups independently discovered that heparin was heterogeneous in terms of its interaction with AT. In fact, they separated chains with high affinity (HA) from those with low affinity (LA) for AT, and found that the anticoagulant activity was largely associated with the HA chains, these latter constituting only about one third of those of the unfractionated polysaccharide (UFH). That also was a landmark finding and a surprising one, since the compositional differences between HA and LA chains were subtle and not easily detectable. Rosenberg observed that nitrous acid-generated fragments obtained from HA heparin contained significantly more unsulfated IdoA than the LA species. Similarly the Ronzoni and Choay groups noticed marked differences in the NMR spectra of HA and LA oligosaccharides. However, the structure of the antithrombin-binding region (ATBR) remained elusive for some more time, until Lindahl's group established that the ATBR was a pentasaccharide (structure shown in Fig. 1B). This pentasaccharide contains the typical 3-O-sulfated GlcN, optionally also 6-O-sulfated, $GlcNSO_33,6SO_3$ residue.[25] Noteworthy, the unsulfated IdoA residue preceding this pentasaccharide sequence, systematically found in HA species from porcine mucosal heparin, did not significantly contribute to the affinity for AT. Identification of sulfate groups essential for high-affinity binding to AT (as depicted in Fig. 1B) was completed by the combined application of classical methods for sulfated oligosaccharide analysis; this latter work, which appeared in n. 100 of *Carbohydrate Research*,[26] remains an important reference in the field. All sulfate groups essential and/or important for high-affinity binding to AT (as depicted in Fig. 1B) were identified.[27] The 3-O-sulfate group taken as a marker of the ATBR was also characterized by a specific signal in the ^{13}C NMR spectra of HA heparin oligosaccharides obtained with different methods.[28] In variants of ATBR found in other heparin sources, especially from bovine lung, the 3-O-sulfated GlcN residue is prevalently N-sulfated and the IdoA residue preceding the active pentasaccharide is $IdoA2SO_3$.[29]

Even before the structure of the ATBR was fully elucidated, the Choay group started an ambitious program of chemical synthesis of the active pentasaccharide, reaching its goal in 1983.[30] (For detailed coverage of discovery and synthesis of ATBR see Refs. 31–33) In a collaboration between Sanofi and Organon, the pentasaccharide was later developed as an antithrombotic drug (fondaparinux, Arixtra®).[33]

Regarding the structure of heparin with respect to thrombin inhibition it was shown, using chemical synthesis, that a hexadecasaccharide displaying an ATBR at the reducing end is required.[33] The basis for the specificity of AT-mediated inhibition of thrombin was later established by crystallographic studies using a synthetic hexadecasaccharide.[34]

5. Minor sequences. Updated view of the heparin structure

Minor sequences, contributing to the structural microheterogeneity of heparin, include internal $GlcA2SO_3$ residues, first discovered by Conrad in liver HS (see Ref. 3) and identified in heparin lyase I-generated fragments of porcine mucosal heparin.[35,36] N-unsubstituted GlcN residues are among the very minor sequences of heparin (see Section 11). Some of the heparin chains terminate, at their reducing end, with a 'linkage region' (LR) reminiscent of the sequence –GlcA–Gal–Gal–Xyl–Ser linking the carbohydrate

Download English Version:

https://daneshyari.com/en/article/7794249

Download Persian Version:

https://daneshyari.com/article/7794249

Daneshyari.com