MODIFICATION AND POTENTIAL USES OF HEPARIN AND OTHER ACIDIC POLYSACCHARIDES

by
R.J. Linhardt
Division of Medicinal and Natural Products Chemistry,
College of Pharmacy,
University of Iowa, Iowa City, Iowa 52242

BACKGROUND

Heparin is a polydisperse, highly sulfated, linear polysaccharide of repeating uronic acid and glucosamine residues (Figure 1). Although heparin has been used clinically as an anticoagulant for the past 50 years, its precise structure remains unknown [1]. Heparin is biosynthesized as a proteoglycan (PG, Mr~1 million) consisting of a central core protein from which 10-11 long, linear polysaccharide chains extend [2]. The drug heparin is recovered from porcine intestinal mucosa or bovine lung as a glycosaminoglycan (GAG), a linear polysaccharide chain without any associated protein. GAG heparin has a Mr ranging from 5,000-40,000 (degree of polymerization (dp) 10-80) with an Mr(avg) of 13,000. Even the heparin chain corresponding to the most prevalent dp represents a mere 5 mol% of a typical GAG heparin preparation [3]. GAG heparin has a second level of structural complexity associated with its primary structure or sequence [4].

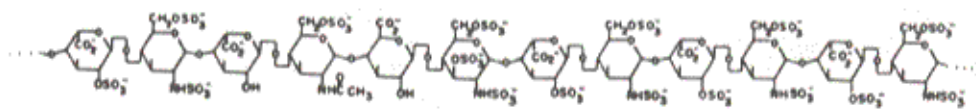

Figure 1

Heparin and heparan sulfate are structurally similar macromolecules with polysaccharide side chains comprised of alternating 1->4 linked sulfated and or acetylated glucosamine and sulfated or nonsulfated iduronic or glucuronic acid residues. They can be distinguished from one another by their ratio of N-acetylation to O-sulfation [5]. Chondroitin sulfates keratan sulfate, and hyaluronic acid are other classes of GAGs which are structurally similar to heparin and have been used as heparin substitutes [6].

Heparin's primary application is as an anticoagulant, however, it is more appropriate to consider heparin in a polyelectrolytic drug having a multiplicity of biological activities [7]. Virtually any cationic protein (pI>7) and many anionic proteins are capable of binding to heparin under physiological conditions. Within the past decade a growing number of biological activities [8] have been demonstrated to be regulated by heparin ranging from its effect on angiogenesis [9] to the regulation of the immune response [10]. Low molecular weight heparins and heparinoids have been undergoing clinical trials as anticoagulant/antithrombotic agents [11] for use in a wide variety of disease states ranging from deep vein thrombosis [12] to non-hemmorrhagic stroke [13]. These agents include both natural products chemically or enzymatically derived from heparin as well as fully synthetic acidic oligosaccharides and polysaccharides.

RESULTS AND DISCUSSION

Heparin's biological activities are primarily mediated through its binding to proteins and its regulation of their activities. Substitutes for heparin must bind to these proteins and regulate these same activities. Heparin's binding is primarily through electrostatic interactions and depends on its high charge density. Most heparin substitutes which have been proposed are highly sulfated polyanions, prepared enzymatically or chemically, and can substitute for heparin by the positioning of these charged groups. Ideally these heparin substitutes should exhibit tighter binding to these proteins in order to have higher potency. Only the antithrombin (ATIII) binding site in heparin has been sufficiently studied to develop a well defined structure activity relationship. A synthetic heparin pentasaccharide containing this binding site has ATIII binding avidity comparble to heparin (heparin-ATIII, Kdiss= 10^{-7}[14].

Although heparin's major activity as an anticoagulant is widely exploited it is also associated with its most common side-effect. Bleeding

complications can range from mild mucosal oozing to intracranial hemorrhaging. Low molecular weight (LMW) heparins represent a new class of therapeutic agents called antithrombotics [15]. These LMW heparins offer several advantages over the anticoagulant heparin. These include a reduced effect on platelets and increased specificity of action. Not all of heparin's side-effects are undesirable. Some of these side-effects could be exploited and new pharmacologically active agents prepared if only these activities could be enhanced. For example, heparin releases and activates lipoprotein lipase (LPL) but does so only at concentrations which fully anticoagulate [16]. If a heparin could be prepared which was devoid of anticoagulant activity with high LPL releasing activity it might represent a useful agent in the treatment of atherosclerosis. Heparin inhibits complement activation but only at concentrations ten-times those required for full anticoagulation [10]. Recent results in our laboratory have demonstrated that it is possible to prepare heparin-oligosaccharides equipotent with heparin (on a weight basis) towards complement but without anticoagulant activity [10]. Such a drug or one which contained equal potency as an anticoagulant and as a complement activation inhibitor might be useful in preventing both coagulation and complement activation in extracorporeal therapy. There are scores of other heparin side-effects which might be usefully exploited resulting in the preparation of new classes of drugs. One major problem with heparin is its poor bioavailability when administered by certain routes [17]. Also there has been has been the lack of understanding of its metabolism and it demonstrates an eratic and sometimes unpredictable rate of clearance [18]. The preparation of homogeneous heparins which are pure single entities would go a long way towards solving these problems in facilitating the development of sensitive chemical assays.

Part of the problem associated with the preparation of blood compatable surfaces is the lack of a complete understanding of coagulation and thrombosis. Ideally, one would like to mimic blood's natural container, a vessel lined with endothelial cells, as closely as possible. The surface must be stable and survive enzymatic and chemical attack from the components present in the circulation. Simple adsorption of heparin onto a polymer produces a blood compatable surface which only last a short period of time until the heparin leaches from the surface [19,20]. Covalent immobilization offers an alternative in that the linkage is chemically stable but these surfaces also have a short lifetime possibly due to the enzymatic stripping of heparin from the surface. Enzymes which act on heparin are present in the circulation such as exo- and endoglycuronidases. A precise understanding of the nature of surface-heparin linkages as well as their control on the enzyme accessibility of the immobilized heparin chain are required to design a stable heparinized surface. Our laboratory is currently examining the question of how the orientation of a heparin chain immobilized to a surface ie., coupled through either its reducing-end or its non-reducing end, effects linkage stabitity when a heparinzed surface is exposed to enzymes in the circulation. Work along these lines is required to intelligently design blood compatable surfaces.

Heparin substitutes have been prepared by modification of naturally occurring polysaccharides, by the total synthesis of heparin-like polymers, and most recently by the synthesis of small sulfated heparin-like oligomers. The synthetic 3-O-sulfated pentasaccharide, representing heparin's ATIII binding site was first prepared by Choay et.al. in a multi-step synthesis in less than 5% yield [21], but the cost of synthesis may also preclude its used as a therapeutic agent. Heparin-oligosaccharides of defined structure have been prepared from heparin in our laboratory using enzymatic methods [22]. These heparin oligosaccharides possess a number of important biological activities including their capacity to inhibit complement activation in vitro with nearly equipotency to heparin on a weight basis [10]. Further studies will be required to demonstrate this activity in vivo as well as to develop large-scale inexpensive methods to prepare these heparin oligosaccharides [22]. Synthetic, highly sulfated, lactobionic acids have recently been prepared and might represent in interesting new class of antithrombotic agents [23]. Synthetic analogs of heparin have a great advantage in that they can permit access to unusual structures which do not occur in the nature and thus are not found in natural products [24]. On the other hand carbohydrate synthesis is

extremely complex and tedious involving many blocking and deblocking steps required to protect sensitive functionality. Thus it is difficult to predict when structurally complex pentasaccharide to decasaccharide sized structures will be preparable in a cost effective manner by the synthetic chemist. Ultimately, the biotechnologist might be able to displace both the synthetic and the natural product routes if the desired sequence can be prepared using recombinant genetics.

Whether or not structurally defined, homogeneous heparin-oligosaccharides will ever be used as a therapeutic agents depends on several factors. First, it will be important to demonstrate improved anticoagulant properties including better bioavailability, pharmacokinetics, dose control and dose monitoring, reduced side-effects, easier reversal and higher specificity. Secondly, it will be necessary to exploit heparin's other activities such as complement regulatory activity, angiogenesis regulatory activity, smooth muscle proliferative regulation, lipoprotein lipase release and activation activities, and antiviral activity. However, before these activities can be exploited they must be separated and the absence of side-effects as well as high potency (a high therapeutic index) must be demonstrated. A third potential route to better exploit heparin is one based on understanding how heparin acts and what its natural physiological roles are. Once known this may result in previously unforseen applications for heparin oligosaccharides.

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