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Current Comments
Synthetic heparin☆

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Current Comments are a rapid outlet for scientific opinions on a topic of general interest.

Heparin is a complex polysaccharide-based anticoagulant drug that is essential for the practice of modern medicine [1]. Used in extracorporeal therapies, such as kidney dialysis, in the treatment of coagulation disorders, such as deep vein thrombosis (DVT), and to passivate the surfaces of indwelling devices, such as catheters, a heparin-based product is the first choice whenever blood clotting needs to be prevented or controlled.

There are three forms of heparin: unfractionated heparin (molecular weight average $MW_{avg} \sim 15\,000$), low molecular weight heparin (LMWH, $MW_{avg} \sim 6000$), and ultra low molecular weight heparin (ULMWH, $MW_{avg} < 2000$) [1–3]. The most widely used form is unfractionated heparin, a century-old drug prepared from animal tissues. Despite its widespread use it has several limitations including required *intravenous* administration and side effects such as heparin induced thrombocytopenia (HIT) and bleeding. In the 1990s, LMWHs were introduced and they have successfully captured a large share of the market becoming the anticoagulant of choice to treat DVTs. LMWHs are prepared from animal-sourced unfractionated heparin through controlled depolymerization. These have the major advantage of being *subcutaneously* bioavailable and have a longer half-life allowing their outpatient use and/or self-administration. A major disadvantage of LMWHs is that, unlike unfractionated heparin, their anticoagulant effect is not readily reversible, thus increasing the risk for bleeding due to overdosing. The last decade has brought us ULMWHs (i.e., fondaparinux), first synthesized by Sinaÿ *et al.* [4], which have well controlled pharmacokinetics/pharmacodynamics, have no viral or prion impurities (possible in animal-sourced materials), are *subcutaneously* bioavailable, and are manufactured under current good manufacturing process (cGMP). Unfortunately, these agents are very expensive and like LMWHs are not readily reversible.

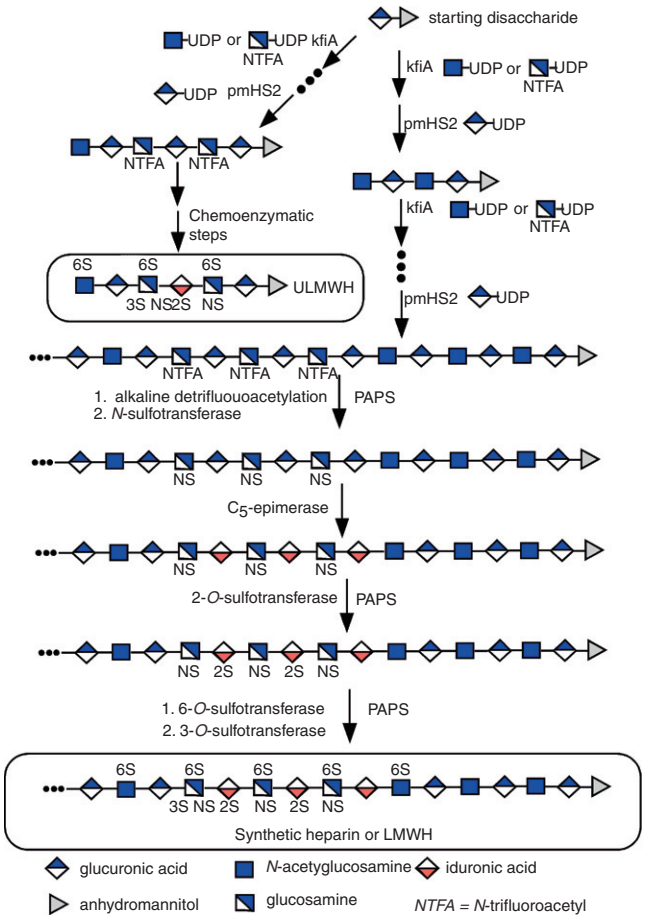
In 2007–2008, there was a heparin crisis that resulted from contaminated batches of heparin and LMWH entering the marketplace [5]. Severe side effects (i.e., a rapid drop in blood pressure), some leading to death, were ascribed to batches of contaminated unfractionated heparin imported from China. This crisis led to the recall of much of the heparin on the market and could have been a much greater problem had there not been sufficient amounts of non-contaminated product to meet the needs of dialysis and surgery patients. The contamination was traced to an adulteration of the crude heparin precursor with an oversulfated chondroitin sulfate somewhere in the process between the slaughterhouse where heparin was collected from pig intestines and the pharmaceutical manufacturing site. While ULMWH was not contaminated in the US, it could not be relied on to alleviate this crisis because of its high cost, difficulty to produce in sufficient quantities to meet worldwide needs, and limited utility for kidney dialysis. The inspection of

☆ Current Comments contain the personal views of the authors who, as experts, reflect on the direction of future research in their field.

foreign suppliers and upgrading the pharmacopeial monographs has reduced the likelihood of a similar crisis in the future but the increased demand on heparin, as modern medicine is applied to more of the world, and the limited number of pigs (1 pig provides ~3 doses of unfractionated heparin or ~1 dose of LMWH) pose constraints on the supply of this critical drug. Since the crisis, the cost of heparin active pharmaceutical ingredient (API) has increased 10-fold.

Heparin is biosynthesized within the Golgi organelles of mast cells that are most commonly found in intestine, lung, liver and skin of higher animals [1]. Heparin biosynthesis, elucidated through the elegant work of Lindahl *et al.* [6], starts with the building of its linear polysaccharide backbone (even some bacteria are capable of this step) through the synthase-catalyzed alternating addition of two UDP-sugars. Backbone sugars are enzymatically *N*-deacetylated, *N*-sulfonated, *O*-sulfonated and epimerized at selected locations, affording the highly sulfated heparin. The enzymes involved in heparin biosynthesis are known and many have been cloned in the past decade. These enzymes have been largely used as tools by biochemists studying heparin structure and biosynthesis [7]. While much is now understood about their *in vitro* activity and specificity, little is known about how they are regulated and controlled *in vivo* in the Golgi. It is clearly understood, however, that the control of these enzymes could offer a great potential in the preparation of synthetic unfractionated heparin, LMWH and ULMWH. Moreover, it is also possible to consider the preparation of designer heparins [8] with reduced side effects (i.e., HIT), more defined physical, chemical, biological, and pharmacological properties.

In 2007, our laboratories chemoenzymatically prepared a small amount of bioengineered heparin from an *Escherichia coli*-derived polysaccharide [9]. Others, including Kuberan and Rosenberg [10], Lindahl and Casu [11], and DeAngelis [12] had also used similar approaches to prepare other heparin-like polysaccharides and oligosaccharides. Over the past 4 years, we have been aggressively examining the possibility of preparing sufficient quantities of a bioengineered, unfractionated heparin to meet the global supply needs (~100 tons/yr.) as a generic version of heparin [5,13]. The process begins with an *E. coli* fermentation to prepare polysaccharide, chemical de-*N*-acetylation, *N*-sulfonation a process that is now moving to the kilogram scale. The use of recombinant *O*-sulfotransferases and C5-epimerase results in a bioengineered heparin that closely resembles the chemical and biological properties of heparin. One critical component of this process is to reduce the cost of the sulfotransferase cofactor and sulfo group-donor PAPS, making up half the product mass, is being addressed by improved PAPS production and cofactor recycling [14,15].



Recently, our laboratories have announced a success in preparing an ULMWH, similar to fondaparinux, using a related chemoenzymatic process [3]. Instead of preparing the oligosaccharide backbone through fermentation, it is enzymatically synthesized by iterative addition of UDP-sugars. This is again followed by the use of recombinant *O*-sulfotransferases and C5-epimerase to afford a pure ULMWH in over a 100-fold higher yield than possible using chemical synthesis. The two ULMWH constructs prepared showed comparable pharmacological properties as fondaparinux. It remains to be seen whether this chemoenzymatic process can be commercialized to afford new ULMWHs for regulatory agency approval.In summary, heparin-based therapeutics are essential to modern medicine and will remain an important class of drugs for the foreseeable future. Modern biotechnological methods are now available to bring more advanced manufacturing process for heparins from the extractive methods developed in the early 20th century and the chemically synthetic methods of the late 20th century into the new millennium. The world is ready and waiting for the next generation of synthetic heparin.

References

1. Linhardt RJ: **Heparin: structure and activity**. *J Med Chem* 2003, **46**:2551-2554.
2. Linhardt RJ, Gunay NS: **Production and chemical processing of low molecular weight heparins**. *Semin Thrombos Hemostas* 1999, **25**:5-16.
3. Xu Y, Masuko S, Takieddin M, Xu H, Liu R, Jing J, Mousa S, Linhardt RJ, Liu J: **Chemoenzymatic synthesis of structurally homogeneous ultra-low molecular weight heparins**. *Science* 2011, **334**:498-501.
4. Sinaÿ P, Jacquinet J-C, Petitou M, Duchaussoy P, Lederman I, Choay J, Torri G: **Total synthesis of a heparin pentasaccharide fragment having high affinity for antithrombin III**. *Carbohydr Res* 1984, **132**:C5-C9.
5. Liu H, Zhang Z, Linhardt RJ: **Lessons learned from the contamination of heparin**. *Nat Prod Rep* 2009, **26**:313-321.
6. Lindahl U, Kusche-Gullberg M, Kjellén L: **Regulated diversity of heparan sulfate**. *J Biol Chem* 1998, **273**:24979-24982.
7. Esko JD, Selleck SB: **Order out of chaos: assembly of ligand binding sites in heparan sulfate**. *Annu Rev Biochem* 2002, **71**:435-471.
8. Peterson S, Frick A, Liu J: **Design of biologically active heparan sulfate and heparin using an enzyme-based approach**. *Nat Prod Rep* 2009, **26**:610-627.
9. Zhang Z, McCallum SA, Xie J, Nieto L, Corzana F, Jiménez-Barbero J, Chen M, Liu J, Linhardt RJ: **Solution structures of chemoenzymatically synthesized heparin and its precursors**. *J Am Chem Soc* 2008, **130**:12998-13007.
10. Kuberan B, Lech MZ, Beeler DL, Wu ZL, Rosenberg RD: **Enzymatic synthesis of antithrombin III-binding heparan sulfate pentasaccharide**. *Nat Biotechnol* 2003, **21**:1343-1346.
11. Lindahl U, Li JP, Kusche-Gullberg M, Salmivirta M, Alaranta S, Veromaa T, Emeis J, Roberts I, Taylor C, Oreste P *et al.*: **Generation of ''neoheparin'' from *E. coli* K5 capsular polysaccharide**. *J Med Chem* 2005, **48**:349-352.
12. Kane TA, White CL, DeAngelis PL: **Functional characterization of PmHS1, a *Pasteurella multocida* heparosan synthase**. *J Biol Chem* 2006, **281**:33192-33197.
13. Wang Z, Yang B, Zhang Z, Ly M, Takieddin M, Mousa S, Liu J, Dordick JS, Linhardt RJ: **Control of the heparosan *N*-deacetylation leads to an improved bioengineered heparin**. *Appl Microbiol Biotechnol* 2011, **91**:91-99.
14. Burkart MD, Izumi M, Chapman E, Lin CH, Wong CH: **Regeneration of PAPS for the enzymatic synthesis of sulfated oligosaccharides**. *J Org Chem* 2000, **65**:5565-5574.
15. Zhou X, Chandarajoti K, Pham TQ, Liu R, Liu J: **Expression of heparan sulfate sulfotransferases in *Kluyveromyces lactis* and preparation of 3′-phosphoadenosine-5′-phosphosulfate**. *Glycobiology* 2011, **21**:771-780.