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Review

Perspectives on progressive strategies and recent trends in the production of recombinant human factor VIII

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ABSTRACT

Factor VIII (fVIII), a glycoprotein cofactor, plays a crucial role in the intrinsic blood coagulation pathway. As one of the most essential blood clotting factors known today, fVIII is the largest and most complex commercialized therapeutic protein used in the treatment of hemophilia A, an X-linked recessive disorder. Two lyophilized fVIII concentrates (viz., plasma fractionated and recombinant) are in use to treat hemorrhagic episodes in patients suffering with hemophilia A. Recombinant fVIII (rfVIII) products that are devoid of human and animal protein expressed in mammalian cells can be used as an alternative to plasma derived (pd) products fractionated from human blood. Although effective, the expression of rfVIII in heterologous mammalian expression systems at an industrial scale is complicated due to complex fVIII structure and non-human pattern of post-translational modifications, particularly glycosylation. Chinese hamster ovary (CHO) is the most commonly used host cell line for the production of various biotherapeutics. Product safety and adaptability to grow in suspension is the most desirable feature that makes CHO, a suitable host for rfVIII production. Even though the therapeutic and commercial application of rfVIII protein from CHO has increased extensively, further studies are required at cellular to bioprocess level to overcome the challenges in production, purification and processing. Efficient strategies are required to attain better products pertaining to the glycosylation path, productivity, stability, etc., to bring down the cost of expensive therapeutics like rfVIII that obviates these biotherapeutics affordable to common man. This review summarizes the various approaches and developments that have been in practice in fVIII production.

Contents

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1. Introduction

Factor VIII (fVIII), one of the most complex trace plasma glycoprotein, plays a key role in the intrinsic pathway of blood coagulation [1,2]. Deficiency of the circulating functional blood coagulation fVIII results in hemophilia A, a recessive X-linked bleeding disorder that occurs in males and in homozygous females [3,4]. Around 10,000 new cases of hemophilia A are reported every year worldwide with a prevalence of 1 in 5000 males [5]. Hemophilia A patients experience prolonged bleeding after trauma and in severe cases, spontaneous recurrent bleeding with permanent joint damage results in chronic arthritis and severe disability [3,6,7]. Diagnosis of hemophilia A is done based on bleeding propensity which is related to the measured concentration of the active fVIII detected in the normal healthy blood. Due to the qualitative deficiency of fVIII protein, 5–10% patients are affected whereas the remainder is affected due to the reasons of quantitative deficiency. Keeping in view about the limitations of existing methods and the need for an infection free fVIII concentrates, the development of first rfVIII became a major breakthrough approved by food and drug administration (FDA) (Kogenate by Miles-Cutter/Bayer Corporation, and Recombinate by Baxter Healthcare) in 1992 [8–10]. Recombinant human fVIII (rhfVIII) that are produced from either Chinese hamster ovary (CHO) or Baby hamster kidney cells (BHK) has been available in the market for more than 25 years [10–12].

The major concern in hemophilia treatment is the affordability of rfVIII and shortage of fVIII supply [8,13–16]. The titers of rhfVIII achieved in production are less than 1 unit/10^6 cells/day and so failed to satisfy the demands [1,17]. A prophylactic infusion of 2000 units of fVIII three times a week is required to prevent hemorrhages or haemarthrosis, and the treatment costs approximately $ 150,000 to $ 300,000 per year [6,7,18,19]. Additional follow up treatments and frequent doses adopted to maintain fVIII level in circulation due to its short half-life (~12–14 h) is not possible because of further rise in treatment cost [17,20,21]. Even though rhfVIII carry a very low risk of infectious agent transmission, the randomized clinical trial conducted by French observational studies showed 2.4 higher risk of fVIII inhibitors associated with rfVIII products compared to plasma derived (pd) von Willebrand factor (vWF) containing fVIII [9,22]. About, 25 to 30% hemophiliac patients are subjected to higher bleeding due to inhibitor development which necessitates costly treatments to induce immune tolerance [23,24]. To enhance half-life, different strategies like recombinant Fc fusion proteins, PEGylation of B domain truncated as well as full length rfVIII, development of single chain construct has been employed where, rFc fusion proteins had shown 1.5 to 1.7 fold increase in half-life (~18 h) [25–27]. However, hemophilia A patients will have more benefits if there is a unique balance in increase in the half-life of rfVIII along with the increase in production levels [28,29]. Developing a process for the commercial production of fVIII like complex glycoprotein is an imposing challenge. Many biopharmaceutical companies have applied various strategies for large scale production of fVIII based on increasing fVIII titers. This review intends to cover comprehensive information on fVIII structure, function, as well as progressive strategies and recent trends that could be accomplished for cost effective fVIII production.

2. Structural and functional characteristics of fVIII: from biosynthesis to secretion level

fVIII was found to be synthesized and released into the bloodstream by vascular, glomerular, tubular endothelium and sinusoidal cells of liver [30]. The fVIII gene is one of the largest genes known with an extension of 186 kb situated at the end of the extended arm (Xq28) of the X-chromosome [28,29,31,32]. It constitutes approximately 0.1% of X-chromosome and structurally intricate with 26 exons and 25 introns with a size range from 69 to 3106 bp and 207 to 32,400 bp respectively [33,34]. The complete gene consists of approximately 177 kb of intron and 9 kb of exon encoding a polypeptide chain of 2351 amino acids, including 19 amino acids of N-terminal signal peptide [34].

The full-length unprocessed fVIII single-chain protein contains 6 domains, namely A1-A2-B-A3-C1-C2 which codes for a glycoprotein of molecular weight of ~300 kDa [7,35,36]. The synthesized single chain polypeptide of fVIII undergoes proteolytic processing into a two-chain molecule in the Golgi apparatus upon cellular secretion [37]. fVIII circulates as a divalent metal ion dependent heterodimer which exist as the heavy chain (HC) and light chain (LC) in plasma [38]. The A1-A2-B domains constituting the heavy chain and A3-C1-C2 constituting the light chain show non-covalent metal ion association [39]. The molecular weight of heavy chain spans between 90 and 210 kDa due to the limited processing of several proteolytic sites in the B domain and that of a light chain is 80 kDa, which do not undergo any processing [40]. At interdomain interfaces (A1-A2, A2-B, and B-A3), A domains are bordered by short spacers of 35 residues of 'a1' in between 337 and 372, 29 residues of 'a2' in between 711 and 740, 40 residues of 'a3' in between 1649 and 1689 containing clusters of Asp and Glu residues and are so-called acidic regions [36,41]. Each of the three A domains contains approximately 330 residues (A1-337, A2-338, A3-331) with 40% sequence identity to each other, and to the copper-binding protein ceruloplasmin [42]. The oxidase activity of ceruloplasmin supports the presence of one molecule of Cu^{2+} per molecule of fVIII [42]. Cu^{2+} binding sites were found at His 265, Cys 310, His 315 and Met 320 residues of A1 domain and His 1954, Cys 2000, His 2005, Met 2010 residues of A3 domain [28]. These Cu^{2+} binding sites allow the A1 domain to implement A3 domain binding conformation and helps in direct association of A1 and A3 domain [42]. The A domains further display approximately 40% of amino acid identity to factor V, the cofactor in the prothrombinase complex [35,37].

The C domains with approximately 160 residues (C1-152, C2-159) shows 20% sequence identity to discoidin protein fold family, such as galactose oxidase [42]. The C2 domain in fVIII helps in binding to vWF and platelet membrane surfaces [42,43]. Some reports suggest the role of C2 in binding to other proteins also, such as fXa and thrombin [41]. There are some reports about enhancing fVIII stability with modification in A1-C2 interface interactions [44]. There are 23 cysteine residues found in fVIII and among them 19 (3 free (Cys 310 in A1, Cys 692 in A2, Cys 2000 in A3) and 16 disulfide bonded) belong to A and C domains [39]. The cysteine residues (Cys 202, Cys 2169) near the amino-and carboxy terminal ends in fVIII C domains are connected by a single disulfide bond [41]. The largest domain in fVIII is B with 907 residues constituting 40% of entire sequence, encoded entirely by a single large incessant exon 14 that is highly conserved through evolution and is supposed to be an ancient insertion with no known function [6,7,45]. There is no structural homolog known to B domain and is relatively expendable for procoagulant activity [1,46]. It is unique; heavily glycosylated (especially N-linked glycosylation) as 19 out of 25 asparagine N-linked glycosylation attachment sites (Asn-X-Thr/Ser) and atleast 7 O-linked glycans of fVIII are present in B domain. It constitutes nearly one third of the molecular weight (~40%) of the fVIII molecule and have high carbohydrate content [39,47] (Fig. 2). The other 6 asparagine N-linked glycosylation sites found outside B domain are at 41 (A1), 239 (A1), 582 (A2), 1685 (a3), 1810 (A3) and 2118 (C1) residues [28]. The 19 asparagine N-linked glycosylation sites in the B domain show microheterogeneity owing to incomplete glycosylation which can lead to fVIII misfolding and its retainment in ER [48]. Several studies using gel systems under native and denaturing conditions show difficulty in focusing the 90–210 kDa heavy chain proteins of both rfVIII and plasma derived fVIII (pdfVIII) [26]. Inside the endoplasmic recticulum (ER), fVIII interacts with several chaperone proteins, such as calreticulin (CRT), calnexin (CNX), Immunoglobulin heavy chain-binding protein (BiP) or Glucose regulated protein 78 (GRP78), Glucose regulated protein 94 (GRP94), Protein disulfide isomerase (PDI), ERp57 disulfide isomerase which results in retaining of significant amounts of fVIII within the ER and limits the transport of non-native glycoproteins from ER to Golgi