Poster # PT223

Elucidation of structure and functional characteristics of OBI-1, a recombinant porcine sequence FVIII

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Introduction

OBI-1 is a low antigenic recombinant, B-domain deleted, porcine sequence FVIII developed for the treatment of acquired hemophilia A. It provides optimal clotting by re-enabling the intrinsic pathway and restoring normal physiological coagulation processes by substituting for inhibited human FVIII[1,2,3,4]. Management of rational dosing schedule can be monitored using normal laboratory FVIII One Stage or Chromogenic Coagulation assays.

Objective

This study investigates the structure and function of OBI-1 and confirms its suitability as a factor VIII product.

Methods

OBI-1 samples were obtained from manufacturing scale batches used in clinical trials. Amino acid composition analysis, rp-HPLC, and peptide mapping determined the primary structure and post translation modifications. Higher order structure was elucidated using circular dichroism, fluorescence emission spectrum, and mass spectrometry, and assessment of intra-molecular bonds. Functional characterization included an evaluation of OBI-1 thrombin digestion by SDS-PAGE, binding affinity to von Willebrand factor (VWF) by SEC-HPLC, and one-stage coagulation assay.

Results

- OBI-1 is a 1448 amino acid heterodimer with a molecular mass of ~ 170 kDa, composed of a ~ 90 kDa heavy chain and ~ 80 kDa light chain (see Table 1).
- The B-domain normally present in native FVIII is replaced in OBI-1 with a 24 amino acid linker, 12 of each is derived from both the N-terminal and C-terminal ends of the native porcine B-domain respectively.
- Tryptic peptide maps and LCMS (Figure 1) determined the intra-peptide disulfide bonds in each of the light and heavy chains (Table 2).
- The amino acid sequences determine by LCMS are consistent with the expectation that the heterodimer is held together by non-covalent, metal ion interactions, as seen in native, human FVIII (see Table 3).
- Characterization of post-translational modifications identified 4 N-linked glycosylation sites, 4 O-linked glycosylation peptides, and 7 sulfated tyrosines (see Table 2 & 4).
- Approximately 36% of OBI-1 protein is formed of β-sheets and 1% as α-helices, with the remainder in random coil form (see Figure 2).
- Thrombin activation generates cleavage products, A1, A2 and A3C1C2 fragments comparable to human FVIII (see Figure 3).
- Functional characterization showed that OBI-1 binds to VWF with high affinity in a 1:1 ratio (Figure 4).
- The pair-wise sequence homology is 86% when compared with similar B-domain deleted human FVIII. Some amino acid sequences at known human FVIII immunological epitopes at the A2 and C2 domains are comparatively different in OBI-1 (see Table 5, 6 & 7). These differences provide the basis for reduced inactivation by circulating inhibitory human FVIII antibodies.

Graphs and Tables

Table 1: Amino Acid & Domain Sequence of OBI-1

Name	B-Domain Deleted Recombinant Porcine Factor VIII is also known as OBI-1					
Number of Amino Acids:	1448					
Amino Acid Distributions:	ALA	A	82	LEU	L	132
	ARG	R	83	LYS	K	66
	ASN	N	55	MET	M	37
	ASP	D	83	PHE	F	69
	CYS	C	19	PRO	P	81
	GLN	Q	63	SER	S	123
	GLU	E	82	THR	T	76
	GLY	G	89	TRP	W	29
	HIS	H	53	TYR	Y	66
	ILE	I	68	VAL	V	92
Formula:	$C_{7427}H_{11320}N_{2016}O_{2162}S_{56}$					
Molecular Weight (AA):	~ 170kDa					

Heavy Chain Domains	Amino Acid Sequence	No. of Residues	Light Chain Domains	Amino Acid Sequence	No. of Residues
A1 Domain	1-372	372	A3 Domain	807-1135	329
A2 Domain	373-740	368	C1 Domain	1136-1288	153
			C2 Domain	1289-1448	160
Molecular Weight ~ 90 kDa			Molecular Weight ~ 80 kDa		

Table 2: Comparison of Tyrosine Sulfation & Disulfide Crosslinked Sites in Human FVIII with OBI-1 determined from LCMS

Modification Types	Primary Locations	
	Heavy Chain	Light Chain (human sequence numbers in Light Chain are different from OBI-1 due to B-domain)
Tyrosine Sulfation 7 of 66 Tyrosine are sulfated	Y^{346}, Y^{351}, Y^{718}, Y^{719}, Y^{723} [hY^{347}, hT^{352}, hY^{719}, hY^{720}, hY^{724}]	Y^{780}, Y^{796} [hY^{1705}, hY^{1721}]
Disulfide Crosslinks 19 cysteines 4X, 2 Free in heavy chain 4X, 1 Free in light chain	$C^{154} \rightarrow C^{180}$ [$hC^{154} \rightarrow hC^{180}$] $C^{249} \rightarrow C^{330}$ [$hC^{249} \rightarrow hC^{330}$] $C^{528} \rightarrow C^{554}$ [$hC^{529} \rightarrow hC^{555}$] $C^{630} \rightarrow C^{711}$ [$hC^{631} \rightarrow hC^{712}$] Free C^{311} [hC^{311}] Free C^{692} [hC^{693}]	$C^{948} \rightarrow C^{974}$ [$hC^{1873} \rightarrow hC^{1899}$] $C^{1015} \rightarrow C^{1019}$ [$hC^{1940} \rightarrow hC^{1944}$] $C^{1137} \rightarrow C^{1285}$ [$hC^{2062} \rightarrow hC^{2210}$] $C^{1290} \rightarrow C^{1442}$ [$hC^{2215} \rightarrow hC^{2367}$] Free C^{1116} [hC^{2000}]

Figure 1: Tryptic Peptide Maps of 3 Batches of OBI-1

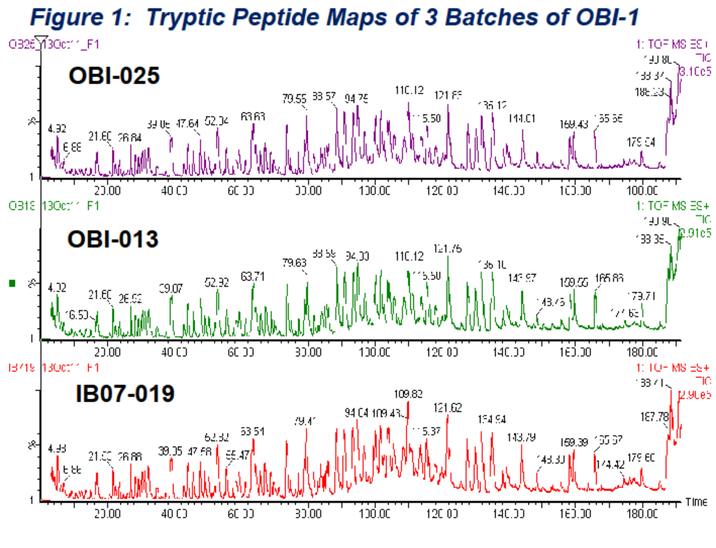


Figure 2: Far-UV CD Spectra of OBI-1 (batch IB07-020 & OBI-007)

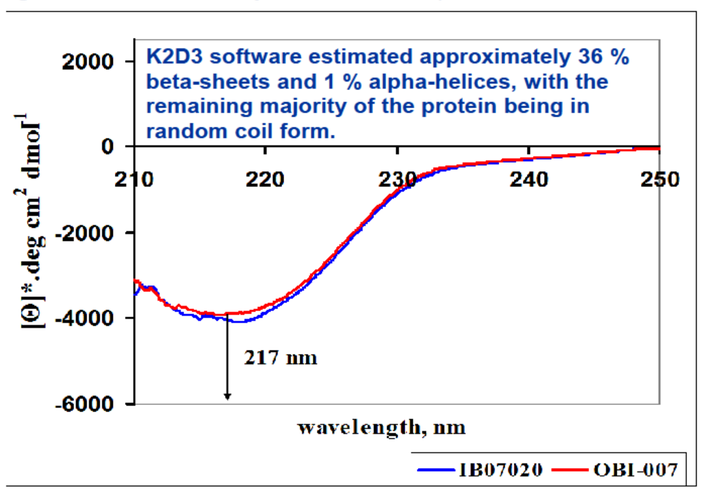


Table 3: Comparing Metal Binding Sites in Human FVIII & OBI-1

Calcium Binding Site in the A1 Domain of Heavy Chain [1-366]

hFVIII ...SYWKASEGAEYDDQTSQRE KEDDKVF...
OBI-1 ...SFWKSSEGAEYEDHTSQRE KEDDKVL...

Copper Binding Site in the A1 Domain of Heavy Chain [1-366]

hFVIII ... PEVHSIFLEGHTFLVRNHRQASLEISPITFLTAQTLLMDLGQFLLFCHISSHQHD...
OBI-1 ...PEVHSIFLEGHTFLVRHHRQASLEISPLTFLTAQTFLMDLGQFLLFCHISSHHHG...

Copper Binding Site in the A3 Domain of Light Chain [807-1135]

hFVIII ...ENIHSIHFSGHVFGIWRVECLIGEHLHA...
OBI-1 ...ENIHSIHFSGHVFGIWRIECLIGEHLQA...

Table 4: N-Glycan Distribution in OBI-1

Location	Peptide	AA Residue	Glycan Type
Heavy Chain	T21	Asn^{214}	Predominantly bi-antennary, complex-type glycans that are core fucosylated. Contain 0, 1 or 2 sialic acids.
Heavy Chain	T23	Asn^{240}	Major N-linked glycans observed at this site were bi-antennary oligosaccharides. Uncharged, but a small portion contained sialic acid. Do not contain core fucoslyation.
Light Chain	T94	Asn^{926}	Predominantly fucosylated, bi-antennary complex-type oligosaccharides. Majority are sialylated.
Light Chain	T124	Asn^{1234}	Contained neutral, high mannose type oligosaccharides having a range of structures. Predominantly Man5 – Man8, with smaller amounts of Man9.

Table 5: Comparing Human FVIII A2 Domain Immunological Epitopes with OBI-1[5]

hFVIII	R^{484}	P	L	Y^{487}	S^{488}	R^{489}	R	L	P^{492}	K	G	V^{495}	K
OBI-1	S^{484}	A	L	H^{487}	P^{488}	G^{489}	R	L	L^{492}	K	G	W^{495}	K
hFVIII	H	L	K	D	F^{501}	P	I	L	P	G	E	I^{508}	
OBI-1	H	L	K	D	M^{501}	P	I	L	P	G	E	T^{508}	

Table 6: Comparing Human FVIII C2 Domain Immunological 3E6 Epitopes (impacts binding to vWF & phospholipid surfaces)[6]

hFVIII	E^{2181}	S^{2182}	K	A	I	S	D	A^{2188}					
OBI-1	Q^{1297}	N^{1298}	K	A	I	S	D	S^{1304}					
hFVIII	T^{2202}	W	S	P	S	K^{2227}	A	R	L	H	L	Q	G	R^{2215}
OBI-1	T^{1318}	W	S	P	S	Q^{1323}	A	R	L	H	L	Q	G	R^{1331}

Table 7: Comparing Human FVIII C2 Domain Immunological G99 Epitopes (impacts proteolytic activation of Factor VIII)[6]

hFVIII	Q^{2222}	V	N^{2224}	N^{2225}	P^{2226}	K^{2227}	E	W^{2229}						
OBI-1	R^{1338}	V	S^{1340}	S^{1341}	A^{1342}	E^{1343}	E	W^{1345}						
hFVIII	H^{2269}	Q^{2270}	W	T	L	F	F	Q	N^{2277}	G	K^{2279}	V^{2280}	K	V^{2282}
OBI-1	R^{1385}	R^{1386}	W	T	L	F	L	Q	D^{1393}	G	H^{1395}	T^{1396}	K	V^{1398}
hFVIII	R^{2307}	I	H	P	Q^{2311}	S	W	V^{2314}	H^{2315}	Q^{2316}	I^{2317}			
OBI-1	R^{1423}	I	H	P	T^{1427}	S	W	A^{1430}	Q^{1431}	H^{1432}	I^{1433}			

Figure 3: SDS-PAGE & rpHPLC Profile of Thrombin Digested OBI-1

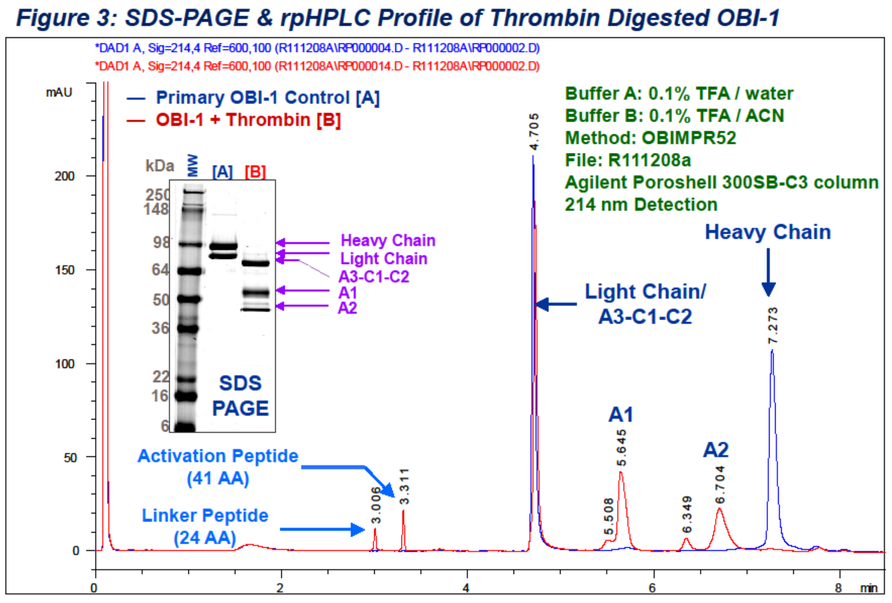
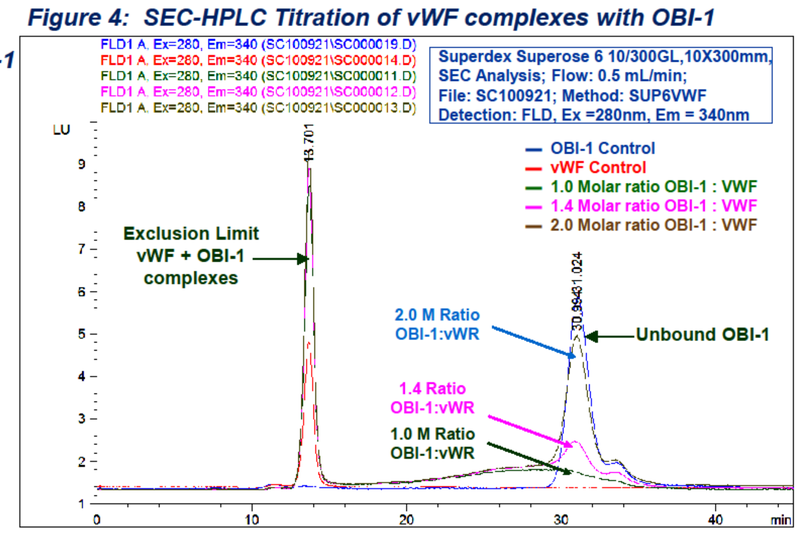


Figure 4: SEC-HPLC Titration of vWF complexes with OBI-1



Conclusions

The molecular structure of OBI-1 is consistent with the cDNA sequence for the OBI-1 expression construct. The higher order structure of OBI-1 shares similarities to that of human factor VIII. OBI-1 showed in vitro ability to be activated by thrombin to initiate the coagulation cascade, thus facilitating clot formation in FVIII-deficient plasma. Amino acid residues at known human factor VIII immunological sites at the A2 and C2 domains are sufficiently different in OBI-1 to reduce neutralization from human factor VIII antibodies in acquired hemophilia patients[4].

References

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If you have any additional questions, please feel free to contact Baxter Bioscience Medical Information at medinfo@baxter.com.

Conflicts of interest:

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Acquired Hemophilia

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