Venom-Sweet-Venom: *N*-Linked Glycosylation in Snake Venom Toxins

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Abstract: Protein glycosylation represents one of the most important post-translational events, and is a mean of diversifying a protein without recourse to the genome. The venoms produced by snakes contain an abundance of glycoproteins with *N*-linked carbohydrates. *N*-linked glycosylation can ensure the correct folding of important functional domains. Characterization of carbohydrates structures aids in development of human therapeutics by snake venom toxins.

Keywords: N-linked oligosaccharides, snake venom, enzyme activity, serine protease.

INTRODUCTION

The post-translational events are a mean of diversifying a protein without recourse to the genome. These modifications include phosphorylation, sulphation, processing by proteolysis, lipid attachment and glycosylation. Glycosylation is one of the most complex and ubiquitous post-translational modifications of proteins in eukaryotic cells. Protein glycosylation promotes the maturation and quality control of proteins in the lumen of the secretory pathway. This event plays an important role in biological processes ranging from protein folding and subcellular localization, to ligand recognition and cell-cell interactions. They can play important structural and functional roles for a protein, as well as acting to sterically protect a protein from proteolysis or antigenic recognition [1].

PROTEIN GLYCOSYLATION

Glycosylation can be classified into four types based on the nature of chemical linkage between specific acceptor residues in the protein and sugar: *N*-linked and *O*-linked glycosylation, *C*-mannosylation, and GPI (glycosylphosphatidylinositol) anchors (reviewed in [2]). The glycosylation of a protein is a complex biological pathway which is ordered and non-random. Glycan processing is a central part of the quality control mechanisms of glycoprotein folding. This event involves a particular amino acid sequence, called sequon. However, not all sequons are occupied and, of those that are occupied, not all are necessary for folding.

In *N*-glycosylation process, this site is a consensus sequence of Asn-X-Ser/Thr, where X is any amino acid except proline [3]. *N*-linked glycans are large, flexible and hydrophilic modifications that can extend ~3 nm away from the glycoprotein [1]. The *N*-glycosylation happens specifically for proteins that are being moved through the compartments of the cellular secretory pathway, from the endoplasmic reticulum (ER) to the Golgi and onwards (Fig. **1**).

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N-linked glycosylation begins with the assembly of the complete dolichol-linked oligosaccharide donor [4]. Thus, two *N*-acetylglucosamine (GlcNAc) are attached to lipid dolichol phosphate on the external side of the ER membrane. The sequential addition of single monosaccharides results in the formation of the mature lipid-linked oligosaccharide, $Glc_3Man_9GlcNAc_2$-PP-dolichol. At this point, the partially finished core glycan is flipped across the endoplasmic reticulum membrane, so that it is now located within the reticular lumen [5]. In the lumen, the glycan is transferred *en bloc* by the oligosaccharyltransferase to an Asn residue in the consensus sequence Asn-X-Ser/Thr of the polypeptides that emerge from the ribosome. Once transferred, *N*-linked glycans generally undergo extensive processing reactions (trimming), whereby the glucose residues are removed, as well as several mannose residues. These trimming occur initially in the ER, and go on in the Golgi apparatus [6]. Their oligosaccharide attachments are substantially modified by the removal and addition of new sugars, such as neuralminic acid. This processing and modification of N-linked glycans results in many different variations glycoproteins structures.

Studies on the structure-function relationship of the glycoproteins have shown that the carbohydrate part of a glycoprotein confers important and specific biological roles: immunogenicity [7, 8], solubility [9], recognition [10], protection from proteolytic attack [11], induction and maintenance of the protein conformation in a biologically active form [12, 13]. In really, there are four major ways in which glycosylation affects the structure or function of a protein. The first two involve intramolecular interactions between sugar and protein, either directly or within a ternary complex (e.g., CD2 molecule) [12]. The others depend mainly on intermolecular interactions. First, oligosaccharides modify local structure and overall dynamics of the protein to which they are attached (e.g., Tissue plasminogen activator) [14]. Second, oligosaccharides may modify the functional activity of a protein (e.g., glycoprotein hormones) [15, 16]. In general, oligosaccharides may be involved in molecular (cell-cell and cell-protein) recognition events and in signal transduction after receptor binding.

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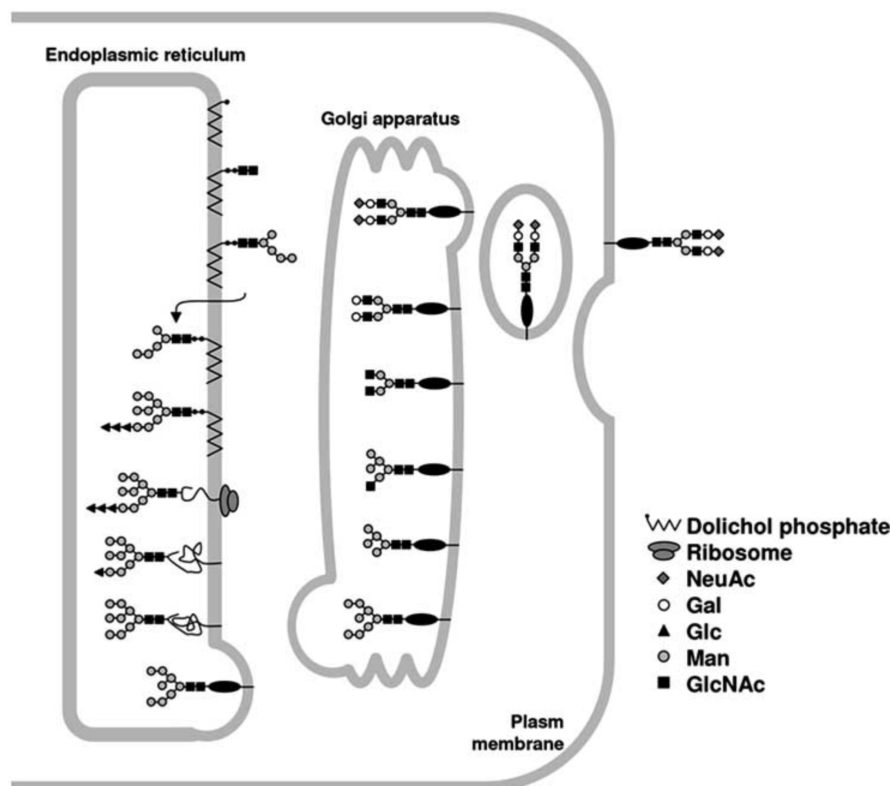

Figure 1. Understanding the biology of protein glycosylation. Synthesis of the glycan core precursor begins in the cytoplasm; the core oligosaccharide structure is flipped into the lumen of the ER and further glycosylated. The core structure is transferred in the ER to a nascent protein and further processed. Diversification then occurs in the Golgi resulting in various glycoproteins structures.

SWEET-VENOM

Snake venoms are complex mixtures of proteins including phospholipases A2, myotoxins, hemorrhagic metalloproteases and other proteolytic enzymes, cytotoxins, cardiotoxins and others [17]. In spite of the fact that viperid venoms may contain well over 100 protein components, venom proteins belong to only a few major protein families, including enzymes (serine proteinases, Zn_2C-metalloproteases, L-amino acid oxidase, group II PLA2) and proteins without enzymatic activity (ohanin, disintegrins, C-type lectins, natriuretic peptides, myotoxins, cysteine-rich secretory protein (CRISP) toxins, nerve and vascular endothelium growth factors, cystatin, and Kunitz-type protease inhibitors) (reviewed in [18]). These complex mixtures of multiple biologically active components contain an abundance of glycoproteins with *N*-linked carbohydrates that can influence activity of the toxins.

Tan *et al.* [19] showed that proteolytic specificity of deglycosylated rhodostoxin, the major hemorrhagin isolated from crude *Calloselasma rhodostoma* (Malayan pit viper) venom, is substantially different from that of native rhodostoxin. The changes in proteolytic specificity of rhodostoxin upon deglycosylation suggest that deglycosylation presumably causes changes in the interactions between the rhodostoxin and the relevant substrates in the membranes. The observation that hemorrhagic properties of snake venom hemorrhagins are dependent on post-translational modifications was confirmed by Garcia *et al.* [20]. Expression of recombinant ACLH zymogen and catalytic domain of jararhagin, hemorrhagic snake venom metalloproteinases from *Agkistrodon contortrix laticinctus* and *Bothrops jararaca* venom, respectively, in *E. coli* resulted in proteins without post-translational modifications. Both recombinant metalloproteinases were active on fibrinogen and fibronectin but they did not show any hemorrhagic activity. Li *et al.* [21] also observed that treatment of two highly hemorrhagic metalloproteinases of 68 and 62 kDa purified from *Crotalus viridis viridis* venom with *N*-glycosidase F blocked their hemorrhagic activity.

The snake venom forms the most abundant family of three-fingered proteins, relatively small water-soluble toxins with different biological activities. Osipov *et al.* [22] described the first indication of the presence of a glycosylated three-fingered toxin in snake venom. The authors showed that glycosylation leads to a substantial decrease in cytotoxicity and *in vivo* toxicity.

Ande *et al.* [23] report the effects of L-amino acid oxidase (LAAO) from the venom of Malayan Pit viper (*Calloselasma rhodostoma*) on the viability of Jurkat cells. LAAO was found to be *N*-glycosylated at Asn361 and Asn172. The two glycan residues are remarkably homogeneous, consisting of a bis-sialylated, biantennary, corefucosylated dodecasaccharide [24]. Ande *et al.* [23] hypothesize that the proapoptotic effect of LAAO may involve interaction with the cell surface via sialic acid binding immunoglobulin-like lectins (siglecs).

Many of these glycoproteins of snake venoms are enzymes which belong to serine proteases group and affect the hemostatic system through several mechanisms [25, 26, 27, 28]. There are several serine proteases such as the kallikrein-like, with hypotensive action releasing bradykinin, and the thrombin-like serine proteases, responsible for the fibrin clot formation at the end of the blood clotting cascade, among others. Enzymes able to affect the hemostatic system are divided into four groups: (i) coagulant and procoagulant, (ii) anticoagulant, (iii) inhibitors of platelet function, and (iv) activators of the fibrinolytic system. A survey of the literature indicates the striking biochemical diversity of snake venom serine proteases. In particular, the reported molecular weights range from 25 to 28 kDa to values as high as 65–70 kDa, although primary structures of many serine proteases indicate a molecular weight of <28 kDa. This discrepancy suggests a major role for post-translational modifications (PTMs) in the molecular diversity of serine proteases, among which glycosylation could be the most prominent [29]. Murayama *et al.* [30] described a trypsine-like serine peptidase, *Bothrops* protease A, with molecular mass of the predicted mature protein of 25,409 Da, but with 67,400 Da obtained in SDS-polyacrylamide gel electrophoresis, implying that ~62% of the total molecular mass is due to carbohydrate moieties.

Snake venom serine proteases present *N*- or *O*-glycosylation sites that differ from one enzyme to the other [31]. According to Zhu *et al.* [32], the slight difference between the catalytic efficiency of AaV-SP-I and AaV-SP-II - two serine proteases from the *Agkistrodon acutus* venom - might be related to distinct glycans linked to residues close to active sites of these molecules. In BjussuSP-I, an analysis by the NetNGlyc Prediction Server showed two *N*-glycosylation sites localized on residues Asn77 and Asn129 [26]. These glycosylation sites improve the catalytic activity of BjussuSP-I, since the formation of fibrin clot was reduced in 50% by deglycosylation of the native toxin [33]. The study conducted by Zhu *et al.* [32] showed that the presence of glycosylation sites next to the active sites of AaV-SPs is a convincing explanation for their altered catalytic activities. Other serine proteases with glycans interfering activity were described. Silva-Junior *et al.* [29] showed that deglycosylated forms of BJ-48 thrombin-like of *Bothrops jararacussu* were more susceptible to inhibition of SBTI than native BJ-48. These results suggest that *N*-linked glycans may be involved in steric blockage of the access of specific macromolecular inhibitors to the enzyme active site.

Potential glycosylation sites can be conserved among snake venom proteins into families. Most likely, we can observe glycosylation in loops proximal with catalytic sites. A search in NCBI protein database resulted in 74 serine peptidases S1 of Viperidae snake venoms containing the catalytic triad residues common to serine peptidases of the chymotrypsin family (corresponding to His57, Asp102 and Ser195 in chymotrypsinogen). Utilizing NetNGlyc Server we observed that only 8 sequences do not contain *N*-glycosylation predicted sites (Table **1**). In 59 sequences, the *N*-glycosylation predicted sites are localized close to His and Asp residues of the catalytic triad (Fig. **2**). Theoretical models obtained of 35 sequences (Table **1**) by homology modeling using Swiss-Model Server demonstrated that *N*-glycosylation predicted sites are localized on loops around of the groove containing the catalytic triad (Fig. **3**).

The articles showing the description of the carbohydrate moieties for snake venom glycoproteins demonstrate the existence of complex structures of glycans. Gowda and Davidson [34] showed the presence of complex-type oligosaccharides containing non-reducing terminal alpha-galactosyl residues and fucose residues linked to the proximal *N*-acetylglucosamine. The carbohydrate moieties are not involved in the functions of cobra venom factor (CVF) [35]; however, it was found that the oligosaccharides of CVF contain terminal alpha-galactosylated Le^X antigenic structures [36] and are reactive to the naturally occurring human anti-alpha-Gal antibody [35].

Structural analysis of the factor X-activating RVV-X, a glycoprotein of Russell`s viper venom, revealed that the majority of the oligosaccharides are complex bi-, tri-, and tetraantennary structures. Gowda *et al.* [37] demonstrated the presence of bisecting *N*-acetylglucosamine, an oligosaccharide found in a variety of animal glycoproteins, including of humans. In other snake venom glycoproteins envolved in coagulation activity, different structures are observed. The *N*-linked oligosaccharides of ancrod, a thrombin-like serine protease of *Agkistrodon rhodostoma*, contain predominantly NeuNAcalpha2,3Galbeta1-3GlcNAc [38]. In batroxobin, a thrombin-like serine protease of *Bothrops atrox moojeni*, contain exclusively terminal NeuAcalpha2,3GalNAcbeta1, 4GlcNAcbeta residues [39].

More recent studies using other techniques that lectin affinity methods, however the literature is limited yet. Geyer *et al.* [24] showed that major component of carbohydrate moiety of L-Amino-acid oxidase obtained from the venom of the Malayan pit viper (*Calloselasma rhodostoma*) is a bis-sialylated, biantennary, core-fucosylated dodecasaccharide. The dodecasaccharide observed is identical with one of the main glycan moieties identified in ancrod, isolated from the same snakes species. Fluorophore-assisted carbohydrate electrophoresis, MALDI-TOF mass spectrometry and NMR-spectroscopy indicate that the major oligosaccharide contributes ≈ 90% of the total glycan substituents. Zeng *et al.* [40] characterized a C-lectin snake venom glycoprotein from *Trimeresurus stejnegeri* using mass spectrometry and Edman degradation, coupled with protease and glycosidase digestion. The C-lectin and tryptic peptides treated or not with *N*-glycosidase F and Glu-C digest treated or not with alpha-mannosidase were characterized by mass spectrometry according to the mass difference.

Enzymes from snake venoms are being extensively explored as diagnostic reagents and therapeutic drugs. Ancrod (Arwin®) from *A. rodhostoma* and batroxobin (Defibrase®) from *B. moojeni* [41] are used in medical care for patients showing thrombosis, myocardium infarct, vascular peripheral diseases, acute ischemia and renal transplant rejection [26, 42]. Now, we have evidence for the important role played by the carbohydrates in the activity of recombinant snake venom enzymes. Thus, glycosylation may be an important feature for the optimization of the biological activities of these future drugs. Thus, post-translational modifications should be considered when produce recombinant proteins therapeutics. Several therapeutic proteins can be made

Table 1. Glycosylation Analysis in Viperidae Serine Peptidases Localized in NCBI Protein Database

Blast	*N*-glycan Prediction[1]	Homology Modeling[2]	Empirical Influence[3]	Blast	*N*-glycan Prediction	Homology Modeling	Empirical Influence
Q7SYF1	1	No		Q8UVX1	0	No	
O13063	6	No		P0C5B4	0	No	
Q71QI2	3	Yes	++	Q7T229	1	No	
O13058	4	Yes	++	P81176	1	Yes	+
Q802F0	4	No		Q6T5L0	1	No	
O42207	4	No		Q91053	1	Yes	+
Q2PQJ3	2	Yes	++	O93421	0	No	
1OP2	1	Yes	+	Q9YGI6	1	Yes	+
1OP0	1	Yes	+	Q9YGJ2	1	Yes	+
Q9I8X1	1	Yes	+	Q6IWF1	1	No	
O13059	1	Yes	+	P04971	1	No	
Q71QH7	1	Yes	+	AAA48553	1	No	
Q8AY79	1	Yes	+	P81661	1	No	
Q5W959	1	No		Q8QG86	3	No	
Q8QHK2	1	No		Q9PTU8	6	Yes	+
Q7SZE2	1	No		O13061	4	Yes	++
Q7SZE1	2	No		P33589	4	No	
P82981	0	No		Q9PSN3	6	Yes	++
P05620	0	No		Q8AY81	4	No	
Q91516	0	No		P85109	3	Yes	+
Q9DF67	2	Yes	+	P26324	4	Yes	++
Q9DF66	1	No		Q58G94	1	No	
Q27J47	1	Yes	+	O73800	3	Yes	++
P81824	1	No		Q9I8X2	4	Yes	++
P18965	1	Yes	+	Q9DG83	3	Yes	+
P18964	1	No		Q8UUJ1	1	Yes	-
Q9PT41	1	No		AAL48222	1	Yes	-
Q9YGJ8	0	No		Q9PTL3	1	No	
AAD01624	0	No		Q9PT51	3	No	
Q8QHK3	2	Yes	+	Q71QI3	5	No	
O13069	3	No		Q2QA04	2	Yes	-
Q71QH5	2	Yes	+	Q072L7	1	No	
Q71QJ3	2	Yes	+	2AIQ	3	Yes	++
Q71QI8	2	Yes	+	2AIP	3	Yes	++
Q71QJ1	2	Yes	+	Q8JH85	3	No	
Q8UUJ2	2	No		Q8JH62	4	Yes	++
AAL48221	2	No					

1- Predicted *N*-glycosylation sites with ≥ 50% probability.

2- Sequences with successfully modeling by Swiss-Model Server.

3- Empirical influence determined by catalytic triad proximity. ++, two or more *N*-glycans localized on loops with triad proximity; +, one *N*-glycan proximal; -, *N*- glycans opposite and/or remote of catalytic triad

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Q7SYF1 NINEHRSLVLLYN--SSRLFGGGTLINKEWVLSAAHCDGEN 70
O13063 NINEHRSLVVLFN--SSGVLCGGTLINQEYVLTAAHCDMPN 70
Q71QI2 NINEHRSLVVLFN--SSGALCGGTLINQEYVLAAAHCDMPN 70
Q802F0 NINEHRFLALVFN--SSGFLCSGTLINQEWVLTAAHCDMEN 70
O42207 NINEHRFLALVFN--SSGFLCSGTLINQEWVLTAAHCDMEN 70
1OP2   DINEHRFLVAFFNTTG--FFCGGTLINPEWVVTAAHCDSTN 46
1OP0   DINEHRFLVAFFNTTG--FFCGGTLINPEWVVTAAHCDSTD 46
Q9I8X1 DINEHRFLVAFFNTTG--FFCGGTLINPEWVVTAAHCDSTN 70
O13059 NINEHRSLVAIFNSTG--FFCSGTLINQEWVVTAAHCDSNN 70
Q71QH7 NINEHRSLVAIFNSTG--FFCSGTLINQEWVVTAAHCDSNN 70
Q8AY79 NINEHRSLVAIFNSTG--FFCSGTLINQEWVVTAAHCDSKN 70
Q5W959 NINEHRSLVAIFNSTG--FFCSGILLNQEWVLTASHCDSTN 70
Q8QHK2 NINEHRSLVAIFNSTE--FFCSGTLINQEWVVTAAHCDSTN 70
Q7SZE2 NINEHRSLVAFFNSTG--FFCSGTLVNEEWVLSAAHCDSTN 46
Q7SZE1 NINEHRSLVAFFNSTG--FFCSGTLINEEWVLTAAHCDNTN 70
Q9DF67 NINEHRSLVVLFNSSG--FLCGGTLINQDWVVTAAHCDSEN 70
Q27J47 NINEHRSLVVLFNSSG--FLCAGTLINKEWVLTAAHCDSEN 70
P81824 KINVHRSLVLLYNSSS--LLCSGTLINQEWVLTAAHCDSKN 46
Q9PTU8 NITEHRFLVEIFN--SSGLFCGGTLIDQEWVLSAAHCDMRN 70
O13061 NINEHRFLVALYEYTSMTFICGGTLINQEWVLTAAHCDRDT 72
P33589 NINEHRFLVALYDGLSGTFLCGGTLINQEWVLTAQHCNRSL 48
Q9PSN3 NINEHRFLVALYDVWSGSFLCGGTLINQEWVLTAAHCNMSN 48
P26324 NINEHRFLVAVYEGTNWTFICGGVLIHPEWVITAEHCARRR 48
2AIQ   NINEHRFLALVYAN---GSLCGGTLINQEWVLTARHCDRGN 45
2AIP   NINEHRFLALVYAN---GSLCGGTLINQEWVLTARHCDRGN 45
Q8JH85 NINQHRSLALLYNSSG--FLCGGTLINQQWVLSAAHCDMEN 70
Q8JH62 NINEHRSLVFLYNSS---FGCGGTLINQQWVLSAAHCDMEN 69

Q7SYF1   KYFCRDRK--SIVDKDIMLIKLNKPVNNSTH 123
O13063   KYFCLSSNNDTEWDKDIMLIRLNRSVNNSVH 125
Q71QI2   KYFCLSSNNDTKWDKDIMLIRLNRPVNNSVH 125
BAA19978 KYFCLSSNNDTEWDKDIMLIRLNRSVRNSKH 125
O13058   KYFCLSSNNDTEWDKDIMLIRLNRSVRNSKH 125
Q802F0   KFFCLRSNNDTKWDKDIMLIRLDSPVNNSAH 125
O42207   KFFCLRSNNDTKWDKDIMLIRLDSPVNNSAH 125
Q2PQJ3   KFICPNKNMSEVLDKDIMLIKLDKPISNSKH 100
Q9DF67   KFFCPNRKNDDEVDKDIMLIKLDSSVSNSTH 125
Q8QHK3   KFFCVSSKNYTFWDKDIMLIRLDRPVSNSEH 124
O13069   KFFCLSSKNYTKWDKDIMLIRLDSPVKNSAH 124
Q71QH5   KYFFPCSKNFTKWDKDIMLIRLNHPVNNSTH 124
Q71QJ3   KYFFPCSKNFTKWDKDIMLIRLNHPVNNSTH 124
Q71QI8   KYFFPCSKNFTKWDKDIMLIRLNHPVNNSTH 120
Q71QJ1   KYFFPCSKNFTKWDKDIMLIRLNHPVNNSTH 120
Q8UUJ2   KFFCLSSKTYTKWDKDIMLMRLKRPVNNSTH 103
AAL48221 KFFCLSSKTYTKWDKDIMLMRLKRPVNNSTH 104
Q7T229   KFFCLSSKTYTKWDKDIMLIRLKRPVNDSPH 127
P81176   KFFCLSSKNYTLWDKDIMLIRLDSPVKNSTH 103
Q6T5L0   KFFCLSSKNYTLWDKDIMLIRLDSPVKNSTH 103
Q91053   KFFCLSSKNYTLWDKDIMLIRLDSPVSNSEH 127
Q9YGI6   KFFCLSSKNYTLWDKDIMLIRLDSPVKNSAH 125
Q9YGJ2   KFFCLSSKNYTLWDKDIMLIRLDSPVKNSAH 125
Q9PTU8   KFFCLSSRNYTKWDKDIMLIRLNRPVNNSEH 125
O13061   KYIFNCSNNFTKWDKDIMLIKLDYPVNYSEH 127
P33589   KYFFRCNKNFTKWDED---IRLNRPVRFSAH 100
Q9PSN3   KYLFRCSKNFTKWDKDIMLIRLNKPVRNSEH 103
Q8AY81   KYFFRCRNNFTRWDKDIMLIRLNRPVRNSAH 127
P85109   KYFFRCSNNFTKWDKDIMLIRLDSPVNNSAH 103
P26324   RYFIRCNKTRTSWDEDIMLIRLNKPVNNSEH 103
Q58G94   KYFFNCRNNFTKWDKDIMLIRLNKPVSYSEH 103
O73800   KYFFACSNNFTKWDKDIMLIRLNRPVNNSEH 127
Q9I8X2   KYFYNCSNNLTTRDKDIMLIRLDRPVDNSTH 127
Q9DG83   KYFFPCSKNYTKWDKDIMLIRLYSPVRNSKH 127
Q8UUJ1   KYFCLSSRNYNQWDKDIMLIRLNRPVRNSAH 100
AAL48222 KYFCLSSRNYNQWDKDIMLIRLNRPVRNSAH 101
Q9PTL3   KYFRLSSRNYNQWDKDIMLIRLNRPLRNSAH 124
Q9PT51   KYFCLSSRNYNQWDNDIMLIRLNRPVRNSAH 100
Q71QI3   KYFCLSSKNYTRWDKDIMLIRLNRPVRNSAH 124
Q2QA04   KFICPNRKKDDEKDKDIMLIRMDSPVNISTH 129
2AIQ     KYFCLNTRNDTIWDKDIMLIRLNRPVRNSAH 100
2AIP     KYFCLNTRNDTIWDKDIMLIRLNRPVRNSAH 100
Q8JH85   KFFCLSNKSYTKWDKDIMLIKLNRRVKTSTH 125
Q8JH62   KFFCLSNKSYTKWDKDIMLIRLNSSVTYNTH 124
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Figure 2. Comparative analysis of the fragments of sequences of Viperidae serine peptidases demonstrating that the *N*-glycosylation predicted sites are localized close to His and Asp residues of the catalytic triad. Multiple alignment was made by ClustalW2 and *N*-glycosylation prediction was made by NetNGlyc 1.0 Server. Residues composing the catalytic site are in box and Asn-Xaa-Ser/Thr sequons with *N*-glycosylation potential = or > that 50% are in gray. Enzymes for this analysis were obtained from NCBI database.

in a prokaryotic expression system such as *Escherichia coli* (e.g., insulin); however, the majority of therapeutic proteins

Figure 3. Theoretical models overlay of serine peptidases showing the disposition of *N*-glycosylation potential sites (dark) in loops close to catalytic triad (gray). The models were generated by Swiss-Model Server and cartoon was made using Swiss-PdbViewer version 3.7. Enzymes for this analysis are (Blast accession number): 1opO, 2aip, 2aiq, aa148222, o13058, o13059, o13061, o73800, p18965, p26324, p81176, p86109, q2pqj3, q2qa04, q7sze1, q8ay79, q8jh62, q8qhk3, q8uuj1, q9df67, q9dq83, q9i8x1, q9i8x2, q9psn3, q9vqi6, q9vqj2, q27j47, q71qh5, q71qh7, q71qi2, q71qi8, q71qj1.

require additional post-translational modifications to attain full biological function [43]. *N*-glycosylation in particular is essential for proper folding, pharmacokinetic stability, and efficacy for a large number of proteins [44]. Torii *et al.* [45] observed that apoxin I, an apoptosis-inducing factor with L-amino acid oxidase (LAAO) activity from Western diamondback rattlesnake venom, require post-translational modification to become active. Recombinant apoxin I protein expressed in 293T cells and secreted into the medium has an LAAO activity as does the protein purified from snake venom. Interestingly, the recombinant apoxin I protein kept in the transfected 293T cells showed only marginal oxidase activity against L-leucine, indicating that LAAO activity of apoxin I is suppressed in the cells. *N*-glycosylation is a possible modification involved in the maturation of the apoxin I protein and required for secretion and LAAO activity.

Although considerable attention has been given to glycosylation in therapeutic candidate proteins, further exploration of snake venom glycoproteins promises to yield new information about influence of glycans in enzymes activity. More attention should be spent to glycosylation determination in structural studies of snake venom glycoproteins.

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