In-silico characterization of hyaluronidase enzyme of Ovophis okinavensis using computational tools and servers

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***Abstract*- The Ovophis okinavensis is a highly posisonous snake belonging to the family viperidae. The venom of this snake is hemotoxic and neurotoxic leading to death of the organism without quick treatment. The anti-venoms which are available at market may or may not show successfull action to the venom. The death may occurs due to late response or due to the use of wrong anti-venoms. However lots of researches are going on to find out the exact anti-venoms for venoms. The anti-venoms are mainly prepared in-vitro using snake venoms. The main objective of this study is to analyse the hyaluronidase venom of Ovophis okinavensis by in-silico method and to find out its active site or binding site from where the researchers can have a brief idea about finding new anti-venoms.**

I. INTRODUCTION

Ovophis okinavensis is otherwise known as Hime habu or Okinawa pitviper. Okinawa pitviper belongs to kingdom animalia, phylum chordata, class reptilia, family Viperidae, order Squamata, genus *Ovophis*, *species O. okinavensis*. This species is commonly found in japan. The body is generally pale greenish-brown, or yellowish-olive colour with darker brownish or greenish dorsal blotches, each bordered with yellowish scales. They are more commonly found in woodland, forests, mountains, fields, in farming areas with nearby streams, ponds, and other water sources, mainly feed on rodents and other vertebrates. The reproduction is Both oviparous and ovoviviparous. These species are highly poisonous and the venom is hemotoxic [1], [2]. The venoms which found in snakes are mainly enzymes and they are i.e, Cholinesterase, Amino acid oxidase, Adenosine triphosphatase, Peptide bradykinin potentiators, Polypeptide toxins, Proteolytic enzymes, Hyluronidases, Proteases, Phospholipases, Thrombin like enzymes, Nerve growth factor, Glycoproteins, lactate dehydrogenases, acidic and basic phosphatases. Among of these hyaluronidase is found in *O. okinavensis [3]*. Hyaluronidase is responsible for the inflammatory response of venom by softening of tissue. HA is present everywhere in human body and mostly found in soft connective tissue. HA provied elasticity to the joints and rigidity to the vertebrate disks, found in the vitreous body of the eye. HA is a linear polysaccharide made up of repeating disaccharide units with glucosamine links. Here the study involves the physico-chemical analysis, secondary and tertiary structure, backbone confirmation, model validation, active site prediction etc. The main objective of this study is to find out the structure of the protein along with its physico-chemical properties and finding the active sites. From avtive site prediction it will be easy to have an idea that at what sites the inhibitor can bind to hyaluronidase enzyme to inhibit its toxic effect in living beings.

II. MATERIALS AND METHODS

Sequence retrieval

The amino acid sequence of hyaluronidase enzyme was retrived from uniprot with the uniprotID of U3TBU1. The informations which were retrived from uniprot are given in table.1.

Physico-chemical characterization of hyaluronidase

The physico-chemical properties of the enzyme was studied by using protparam tool [4]. From this analysis the theoretical PI, molecular weight, aliphatic index, extinction coefficient, number of amino acids, total number of positively and negatively charged residues, atomic position, chemical formula, instability index and GRAVY (Grand average of hydropathicity) of the enzyme

were found. The detail information is given in table.2-table.4.

Prediction of templates

The similarity search is generally done by using BLAST tool and the protein-protein similarity search was carried out by using the blastP tool. So the retrived amino acid sequence was subjected to blastP against PDB. From the result the suitable templates were found for further study. The selected templates for model building are given in table.5.

Secondary structure prediction of protein

The secondary structure of protein was predicted by using CFFSP server from where the percentage of helices, sheets and turns were found (fig.1). The detail information is given in table.6.

Homology modelling

The 3D structures of the hyaluronidase enzyme was generated by using homology modelling concept, in which four different templates were selected for model building [5]-[10] . The models were generated by using modeller9.12 tool. The align2d.py, model-single.py and evaluate-model.py files were rum on the python script by setting the target, template and number of models to be generated. Here 5 models for the selected template were generated and the best model was selected on the basis of lowest DOPE score. The model is given in gif.2. The properties which were found after structure visualisation is given in table.7 and table.8.

Model validation

The final model was further subjected to Rampage server for the analysis of backbone confirmation of protein [11]. The backbone confirmation was generated which showed the number of residues lying in allowed region, favoured region and in outlier regions. The result of Rampage server is given in fig.3. Depending upon these characters the best model is selected. The ANOLEA server was used to find out Z-score and Q-mean score. Least Z-score indicates the best model. The validated models information and backbone confirmation is given in table.9.

Active site prediction

The active site or binding site plays an imporatnt role where the substrate binds to the enzyme. This study is quite helpful in the field of drug designing and drug discovery. The active sites were predicted by using castP server. The active sites are shown in table.10 and fig. 4.

III. RESULT

Sequence retrieval result

The amino acid sequence of hyaluronidase was retrived which showed that it has 449 number of amino acids.

Table.1 the information which retrived from uniprot

Amino acid length	organism	function
449	*Ovophis okinavensis*	Random hydrolysis of (1->4)-linkages between N-acetyl-beta-D-glucosamine and D-glucuronate residues in hyaluronate

Physico-chemical analysis result

The structure and function of an enzyme is based upon its physical and chemical properties. The molecular weight, theoretical pI, total number of negatively charged residues, total number of positively charged residues, total number of atoms, aliphatic index and grand average of hydropathicity of hyaluronidase were found.

Table.2 Physico-chemical properties of hyaluronidase

Molecular weight	Theoretical pI	Total number of negatively charged residues	Total number of positively charged residues	Total number of atoms	Aliphatic index	Grand average of hydropathicity (GRAVY)
52464.4	9.07	45	58	7324	78.86	-0.382

Table.3 Atomic composition of hyaluronidase enzyme

atom	symbol	Number of atoms
Carbon	C	2383
Hydrogen	H	3624
Nitrogen	N	644
Oxygen	O	648
Sulfur	S	25

Table.4 Amino acid composition result

Amino acids	symbols	Number of residues	In percentage
Ala	A	29	6.5%
Arg	R	26	5.8%
Asn	N	26	5.8%
Asp	D	24	5.3%
Cys	C	11	2.4%
Gln	Q	13	2.9%
Glu	E	21	4.7%
Gly	G	23	5.1%
His	H	17	3.8%
Ile	I	27	6.0%
Leu	L	40	8.9%
Lys	K	32	7.1%
Met	M	14	3.1%
Phe	F	23	5.1%
Pro	P	20	4.5%
Ser	S	25	5.6%
Thr	T	19	4.2%
Trp	W	12	2.7%
Tyr	Y	25	5.6%
Val	V	22	4.9%
Pyl	O	0	0.0%
Sec	U	0	0.0%

Selected templates

2PE4 template of hyaluronidase-1 (Homo sapiens) was selected for hyaluronidase after getting the result from blastp. The templates are generally selected on the basis of more identity, query coverage and with less e-value.

Table.5 properties of the selected template

template	Chain	identity	Query cover	e-value	organism	molecule
2PE4	A	41%	92%	1e-112	Homo sapiens	Hyaluronidase-1

Secondary structure analysis result

From the secondary structure analysis the helices, turns and sheets were found which gave an idea about the structure of protein. The helices are denoted with red lines, sheets with green lines and turns with blue lines respectively.

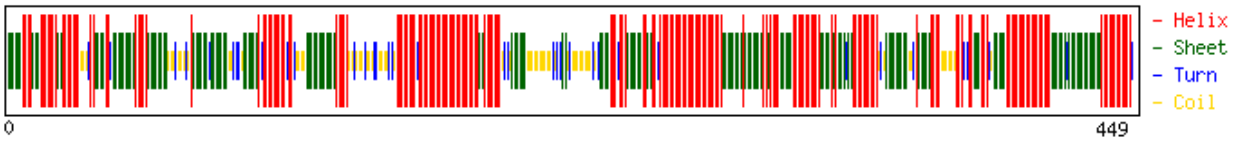

Fig.2 secondary structure of hyaluronidase enzyme

Table.6 composition of helices, sheets, turns

templates	helices	sheets	turns
2PE4	292 65.0%	196 43.7%	55 12.2%

Homology modelling result

The finally generated model was visualised using discovery studio visualiser. The helices were denoted with red colurs, sheets with sky blue colours and the loops were denoted with white colours respectively. The atom count, formal charge sum, molecular surface area, solvent accessible surface area of the models were generated from PyMol and beta factor, stability of the models, VDW radius, minimized enegy were generated from Yasara tool.

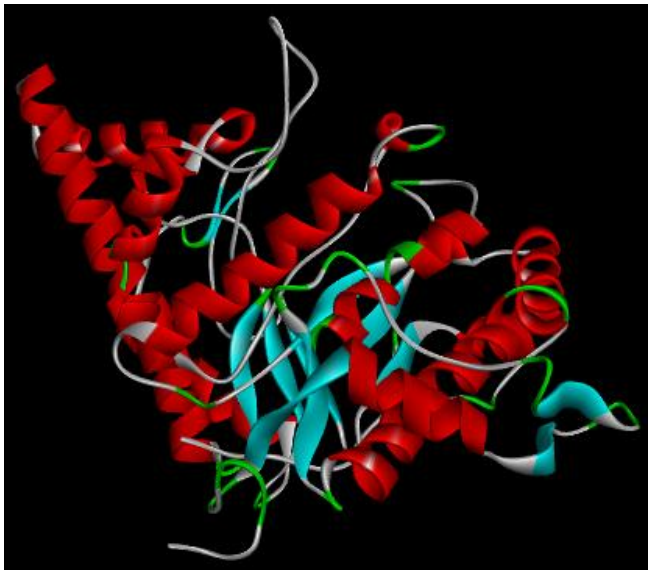

Fig.2 tertiary structure of hyaluronidase

Table.7 Result obtained from yasara tool

template	Beta factor	Stability of object	Minimized energy	VDW radius
2PE4	0.0	582.14kcal/mol	-1.31587	50.756 A

Table.8 Result obtained from pymol tool

template	Atom count	Formal Charge sum	Molecular surface area	Solvent accessible surface area
2PE4	3423	9.0	43146.234 A^2	18945.334A^2

Table.9 Result obtained from ANOLEA-SWISS SERVER

template	QMEAN score	Z-score
2PE4	0.71	-0.672

Backbone confirmation analysis result

The backbone confirmation shows that 92.8% residues lies in most favoured region, 2.2% of residues lies in outlier region. From this it is clear that the enzymes is suitable for better analysis.

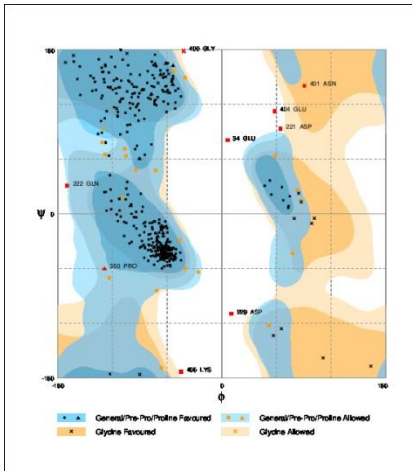

Fig.3 backbone confirmation of hyaluronidase

Active site prediction result

From the castp server the active sites of the enzyme were found which are denoted with green balls. These are sites at which the ligands or the inhibitors of hyaluronidase bind to reduce the toxic effects.

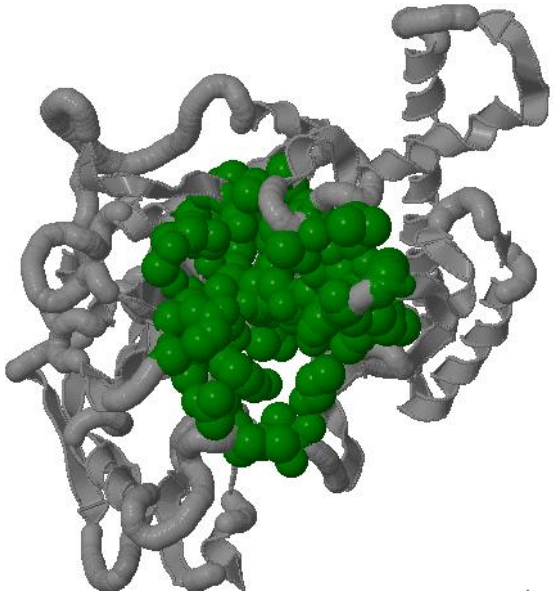

Fig.4 active sites of hyaluronidase

Table.10 list of the amino acids found at active sites along with their position

Binding positions	Amino acids	Nature of amino acid
41	N	hydrophilic
43	P	hydrophobic
66	A	hydrophobic
67	N	hydrophilic
77	I	hydrophobic
70	Y	polar
80	P	hydrophobic
88	Y	polar
89	I	hydrophobic
90	D	hydrophilic
91	D	hydrophilic
93	G	hydrophobic
132	V	hydrophobic
134	D	hydrophilic
135	W	polar
136	E	Charged
137	N	hydrophilic
138	W	polar
139	R	hydrophilic
144	R	hydrophilic
148	S	hydrophilic
149	K	hydrophilic
151	V	hydrophobic
152	Y	polar
207	Y	polar
208	L	hydrophobic
209	Y	polar
210	P	hydrophobic
211	D	hydrophilic
212	C	polar
213	H	hydrophilic
215	Y	polar
251	P	hydrophobic
252	N	hydrophilic
254	Y	polar
256	E	Charged
268	F	hydrophobic
272	R	hydrophilic
275	E	Charged
293	Y	polar
294	R	hydrophilic
298	A	hydrophobic
299	Y	polar

329	W	polar
330	G	hydrophobic
331	S	hydrophilic
332	M	polar
333	Q	hydrophilic

IV. DISCUSSION

From the above analysis the physico-chemical properties, secondary structures, homology model, active sites of the enzyme were found. The backbone confirmation of enzyme shows that most of the residues lies in favoured region and the active sites which were found can be used for inhibitor binding analysis to reduce the poisonous effect.

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