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RESEARCH ARTICLE

STRUCTURAL EVALUATION AND INSILICO STUDY OF PROTEINS OF *ASTERIAS RUBENS* - "STARFISH AS NEW SOURCE TO MARINE PROTEINS"

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ABSTRACT

Marine sources have received great attention recently; research on marine-derived molecules has discovered new bioactive compounds with vital properties increasing their applicability as nutraceuticals in the food and supplement industries. Most notably Hippocrates, the "father of modern medicine", is recorded as describing the therapeutic effects of various marine invertebrates and their constituents on human health. Astreias is an important marine of the family Asteriidae known for its variety of medicinal properties. Functional characterization of a protein sequence is one of the most frequent problems in biology. This task is usually facilitated by accurate three-dimensional (3-D) structure of the protein. The number of protein sequences that can be modeled, as well as the accuracy of the prediction, is increasing steadily because of the growth and number of known protein sequences and structures as well as improvements in the modeling software. It is currently possible to model, with useful accuracy. Significant parts of approximately one half of all known protein sequences. This research report deliver an innovative summary of bioinformatics study of *Asterias rubens* with emphasis on the current development and future directions, which shall provide tools and resources necessary to understand and uphold advances in this important field. The aim of the present study, 10 proteins of *Asterias rubens* were analysed using bioinformatics tools. Structural prediction and functional characterization of proteins of *Asterias rubens* were done using Expasy Protparm server, 3D structure was done using SWISS MODEL. The important enzymes present in *Asterias rubens* involved Reproductive function and proper growth and development of human body.

Keywords: *Asterias rubens,* Computational tools, Algae, Therapeutic effects, Bioinformatics

1. INTRODUCTION

Pharmaceutical market is growing rapidly and continuously. But, still the demand for new drug discovery is encouraged. The reason behind this motivation can be the growing numbers of drug–resistant infectious disease and more and more upcoming disorders. The terrestrial resources have been greatly explored and thus academic and industry researchers are striving to get lead molecules from the inner space of oceans. The marine resources are nowadays widely studied because of numerous reasons. One of the reason is as the oceans cover more than 70% of the world surface and among 36 known living phyla, 34 of them are found in marine environments with more than 300000+ known species of fauna and flora The rationale of searching for drugs from marine environment stem from the fact that marine plants and animals have adapted to all sorts of marine environments and these creatures are constantly under tremendous selection pressure including space competition, predation, surface fouling and reproduction [1].

Starfish (*Asterias rubens*) are probably the single most significant predator of blue mussels living on the seabed in natural mussel beds, bottom cultures or relaying areas. In areas like these, starfish can cause significant decreases in the number of live mussels that are otherwise fished and used for human consumption [2]. The occurrence of starfish in the inner Danish waters has been increasing and still is [3]. Hence, the mussel fishermen in Limfjorden, Lillebælt and Isefjorden, have reported that the mussel fishery has been plagued by substantial amounts of starfish that negatively affect the fishery. The fishermen have suggested that if they fish the starfish, they could be of benefit elsewhere [4].

Determinations of certain biologically important elements have been made on different species of the genus *Asterias*, found in their respective area in the world. Starfish contains various secondary metabolites including steroids, storoidal glycosides, anthraquinones, alkaloids, phospholipids, peptides, and fatty acids (FA). These chemical constituents exhibit cytotoxic, hemolytic, antiviral, antifungal and antimicrobial activities. Therefore, starfish is of great interest as a natural bioactive marine product and presumably in a wide variety of pharmacological activities [5].

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The sea stars are members of the phylum echinodermata, and belong to the deuterostomes that also include the vertebrates. The echinoderms have no heart, no centralized nervous system, no respiratory organs and no specialized excretory organs. They are exclusively marine and most species are unisexual [6]. *Asterias rubens* is native to the north-east of Atlantic Ocean, and is the most common starfish found in Danish waters. *Asterias rubens* produces the secondary metabolite asterosaponins, that are pentaglycoside or hexaglycoside sulfated steroids. These molecules are interesting because of their hemolytic, cytotoxic, anti-bacterial, anti-fungal, anti-viral and anti-tumor properties [7].

For some echinoderms, as well as in other animal groups, the sacrificing of body parts is a way of escape from predators. The sea star has an autotomy plane, close to the central disc, where it can "release" the attacked arm. *Asterias* has a single autotomy plane for each arm, while other echinoderms have the capacity to autotomize at various points along the whole arm length [8].

In this study ten protein sequences of *Asterias rubens* were selected and analyzed with the help of computational tools. In silico approach provide useful information by identifying the primary, secondary, tertiary structure predictions and ten proteins role in human reproductive system which can be used for further analysis.

Fig. 1. The common sea star *Asterias rubens* with three regenerating arms.

2. MATERIALS AND METHODS

2.1. Se*quence Retrieval*

The FASTA sequence of the proteins [TABLE: 1] were retrieved from Genbank database hosted by the NCBI (http://www.ncbi.nlm.nih.gov) [9].

2.2. Primary Structure Prediction

For Physio-chemical characterization, theoretical Isoelectric Point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average of hydropathy (GRAVY) were computed using the Expasy Protparm server[10] (http://us.expasy.org/tools/protparam.html).

2.3. Secondary structure prediction

SOPMA (Self Optimized Prediction Method with Alignment) was used for the secondary structure prediction.

2.4. Functional characterization

SOSUI and TMHMM v.2.0 tools were used to characterize whether the protein is soluble or trans membrane in nature. Inter Pro is an integrated resource for protein families, domains and functional sites. Inter Pro incorporates the major protein signature databases into a single resource. These include: PROSITE, which uses regular expressions and profiles, PRINTS, which uses Position Specific Scoring Matrix-based (PSSM-based) fingerprints, ProDom, which uses automatic sequence clustering, and Pfam, SMART, TIGRFAMs, PIRSF, SUPERFAMILY, Gene3D and PANTHER, all of which use hidden Markov models (HMMs). Superfamily and molecular function were predicted by Inter pro protein sequencing and classification [11]. (http://www.ebi.ac.uk/interpro/).

2.5. Sequence Alignment

Sequence alignment of Maturase K (ALN49191.1.) was performed using pair wise sequence alignment tool (NCBI- BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and multiple sequence alignment was done using the EBI-CLUSTAL OMEGA (http://www.ebi.ac.uk/Tools/msa/clustalo/) tool. Clustal Omega also has powerful features for adding sequences to and exploiting information in existing alignments, making use of the vast amount of precomputed information in public databases like Pfam [12]. The emphasis of this work was to find the regions of sequence similarity, which in other words allows us to yield functional and evolutionary relationships among the proteins considered in this study.

3. RESULTS AND DISCUSSION

Asterias rubens (family Asteriidae) It has been well-known for decades and around the world, that starfish are a serious pest of both

mussels and oyster-bed [13]. Reported that starfish was a serious pest of oyster-beds in the coastal waters of the north-eastern United States, and were captured in considerable quantities by fishermen engaged in oyster culture. The starfish were here grounded into meal and sold for either food for farm animals or used as fertilizer.

Table 1. Proteins of *Asterias rubens*

S. No.	Accession Number	Protein	Length
1	Q9SV11	Cytidine Mono phosphate-N-acetylene –N-acetyl neuraminic acid hydroxylase	653
2	PO2286	Histone H2B gonadal	122
3	A8DX82	Glyceraldehyde -3 –po4-Dehydrogenase	312
4	Q8IA45	Alpha Amylase	492
5	AOAOU2J6Z0	Cholecytpkinin type	`163
6	AOAOU2PX76	Tyrotrophin – releasing hormone	225
7	AOAOU2Q685	Corticotrophin releasing hormone type	130
8	AOAOU2NQTS	Gonadotropin releasing hormone	121
9	Q38JJ7	Peptidoglycon recognition Protein	195
10	AOAODMEJ9	NPS/CCAP type receptor	449

The primary structure prediction was done with the help of protparam tool (Table 2). The parameters were computed using Expasy's protparam tool which revealed that the molecular weights for two different proteins as 26249.79 (Ribulose bisphosphate carboxylase), 2590.85 (PsbA). The pI of two protein was less than 7 which indicated that they are acidic in character. The proteins are found to be compact and stable at their pI [14]. Among the two proteins one is showed instability index lesser than 40, indicating that the protein are stable.

Aliphatic index of the proteins ranged between 73.74-90.78. The computed extinction coefficients help in the quantitative study of protein–protein and protein–ligand interactions in solution. The range of GRAVY (Grand Average of Hydropathicity) of *Asterias rubens* proteins was found to be -0.324 to -0.534. The lowest value of GRAVY indicates the possibility of better interaction with water and only one protein indicates the less interaction with water. (NPS/CCAP type receptor 0.229) [15].

The secondary structure prediction of *Asterias rubens* proteins (Table-3) was analyzed by SOPMA which revealed that alpha helix, extended strand, beta turn and random coil, were more predominant. In all the two proteins alpha helix dominates which is followed by random coil, extended strand and beta turn. The secondary structure were predicted by using default parameters (Window width: 17, similarity threshold: 8 and number of states: 4). TMHMM v.2.0 and SOSUI predicted that 2 proteins were soluble protein.

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Secondary structure prediction of proteins by SOPMA revealed that α – helix, random coil, β – turn and extended strand were more prevalent. In ten protein of *Asterias rubens*, α – helix predominates, random coil region was frequent (Table: 3). In proteins, extended strand dominates followed by random coil and α – helix. Domains are evolutionary units, often identified as recurring sequence or 3D structure. Inter pro tool analysis of proteins of *Asterias rubens* revealed its super family, molecular function (Table 4).

Table 2. Parameters computed using expasy's protparam tool

S. No.	Protein	Accession number	Length	Mol.wt	PI	-R	+R	EC	II	AI	GRAVY
1	Cytidine Mono phosphate-N-acetylene –N-acetyl neuraminic acid hydroxylase	Q9SV11	653	75335.51	5.32	98	75	138240	45.96	73.74	-0.534
2	Histone H2B gonadal	PO2286	122	13591.82	10.52	9	28	7450	40.13	64.84	-0.795
3	Glyceraldehyde -3 –po4-Dehydrogenase	A8DX82	312	33628.57	7.84	35	36	31525	24.97	90.32	-0.054
4	Alpha mylase	Q8IA45	492	55186.24	5.89	51	40	112800	34.48	77.24	-0.279
5	Cholecytpkinin type	AOAOU2J6Z0	`163	18340.28	5.51	26	21	28085	36.49	72.39	-0.726
6	Tyrotrophin – releasing hormone	AOAOU2PX76	225	26645.23	8.75	38	41	90870	54.27	47.29	-1.395
7	Corticotrophin releasing hormone type	AOAOU2Q685	130	14792.01	9.57	15	19	3105	65.72	97.62	-0.419
8	Gonadotropin releasing hormone	AOAOU2NQTS	121	13872.77	5.88	16	14	23490	46.99	78.10	-0.618
9	Peptidoglycon recognition Protein	Q38JJ7	195	21339.01	9.20	12	20	32930	42.33	69.95	-0.324
10	NPS/CCAP type receptor	AOAODMEJ9	449	50056.29	9.58	25	44	75790	27.27	90.78	0.229

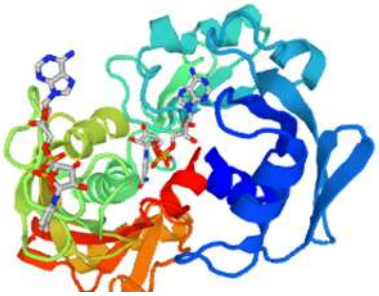
Mol. Wt – molecular weight (Daltons), pI – Isoelectric point, -R - Number of negative residues, +R – Number of Positive residues, EC – Extinction Coefficient at 280 nm, II – Instability Index, AI – Aliphatic Index, GRAVY – Grand Average Hydropathicity.

Table 3. Secondary structure results of proteins of *Asterias rubens*

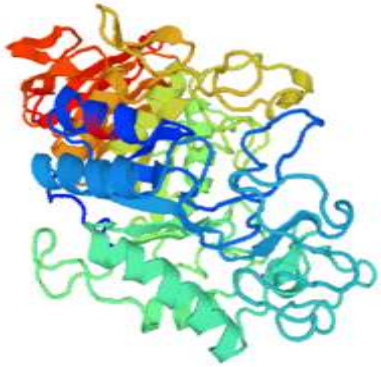
S. No.	Secondary structure	Q9SV11	PO2286	A8DX82	Q8IA45	AOAOU2J6Z0	AOAOU2PX76	AOAOU2Q685	AOAOU2NQTS	Q38JJ7	AOAOD5MEJ9
1	Alpha helix	23.74%	43.44%	32.05%	25.61%	28.22%	33.78%	28.46%	55.37%	28.72%	30.51%
2	Extended strand	24.04%	6.56%	24.36%	26.42%	21.47%	15.11%	19.23%	10.74%	17.95%	28.06%
3	Bend turn	13.02%	9.02%	12.82%	12.80%	12.88%	7.11%	43.08%	29.75%	43.08%	31.18%
4	Random coil	39.02	40.98%	30.77%	35.16%	37.42%	44.00%	43.08%	29.75%	43.08%	31.18%

1) Cytidine Mono phosphate-N-acetylene –N-acetyl neuraminic acid hydroxylase

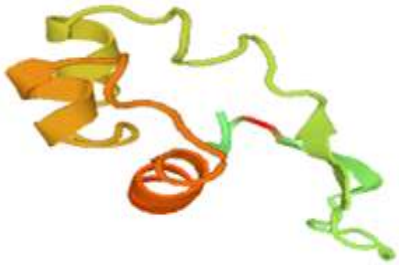
2) Histone H2B gonadal0



3) Glyceraldehyde -3 –po4-Dehydrogenase

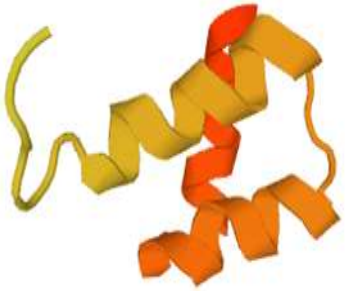


4) Alpha Amylase



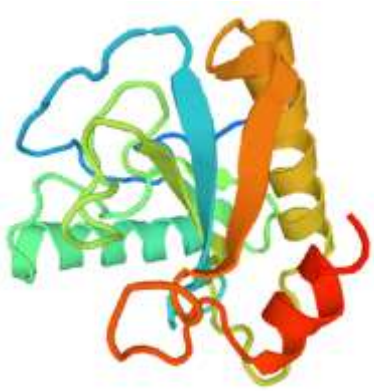
5) Cholecytpkinin type

6) Tyrotrophin – releasing hormone



7) Corticotrophin releasing hormone type

8) Gonadotropin releasing hormone



9) Peptidoglycon recognition Protein

10) NPS/CCAP type receptor

ENZYMES OF *ASTREIAS RUBENS* AND THEIR ROLE IN HUMAN

Cytidine Mono phosphate-N-acetylene –N-acetyl neuraminic acid hydroxylase:

Sialic acids are a family of more than 50 naturally occurring acidic nine-carbon backbone monosaccharides. The predominant sialic acids in mammals are N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc), which commonly occupy the terminal positions of various glycan chains. The only known biosynthetic pathway for generation of Neu5Gc takes place in the cytosol and is catalyzed by the cytidine monophospho- N-acetylneuraminic acid hydroxylase (Cmah). The Cmah enzyme is the only known biosynthetic pathway to generate the major mammalian sialic acid Neu5Gc. Besides namH, a bacterial hydroxylase, it is the only known enzyme capable of catalyzing the generation of an Nglycolyl group in nature [16].

HISTONE H2B GONADAL:

Posttranslational histone modifications are essential for proper cell function. The N-termini of histone tails contain amino acid residues that are affected by methylation, acetylation, phosphory-lation, ubiquitylation and sumoylation. The sum of these modifications and the information they communicate is referred to as the histone code. Methylation is one of the most prevalent histone posttranslational modifications. It is monitored by histone methyltransferases [HMTases] and is generally associated with gene silencing. Methylation of H3K9, for example, is a classic indication of gene silencing and is commonly found in heterochro- matin, as well as silenced promoters. In some cases, however, methylation of arginine and lysine residues can lead to gene activation. For example, methylation of histone H3K4 is implicated in gene expression. More than one methyl group may be transferred to a single amino acid residue [17]. The location and number of methyl groups in a region of DNA convey a specific epigenetic signal Histone acetylation is associated with increased levels of transcription and is modulated by both histone acetyl transferases (HATs) and histone deacetylases (HDACs). HATs activate gene expression, while HDACs inhibit gene expression. Acetylated lysines are specifically recognized by bromodomain-containing proteins and act to enhance chromatin remodeling. Phos- phorylation of histones occurs on serine residues and generally leads to gene activation [18].

GLYCERALDEHYDE 3-PHOSPHATE:

Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme which catalyzes the conversion of glyceraldehyde 3-phosphate to 1, 3-diphosphoglycerate. The most common form is the NAD+-dependent enzyme found in all organisms studied so far and which is usually located in the cytoplasm. In addition to its metabolic function, studies have demonstrated that GAPDH is present on the surface of several microbial pathogens and may facilitate their colonization and invasion of host tissues by interacting directly with host soluble proteins and surface ligands. Surface localization of GAPDH was first demonstrated in the Gram-positive pathogen, *Streptococcus pyogenes*. In this organism, surface-exposed GAPDH binds several mammalian proteins including the uPAR/CD87 membrane protein on pharyngeal cells], regulates intracellular host cell signalling events and contributes to host immune evasion. In addition, surface localization of GAPDH has been reported in enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli*; the

protein of these pathogens has been observed to bind to human plasminogen and fibrinogen, suggesting a role in pathogenesis. [19].

α –AMYLASE

α–Amylase are ubiquitous enzymes that catalyze the hydrolysis of internal α-1,4-glucosidic bonds in starch and related polysaccharides. They belong to the glycoside hydrolase family 13, one of the largest families of enzymes whose members are widespread in all three domains of life. The alpha-amylases are the calcium metalloenzymes which can't function in the absence of calcium. There are many digestive enzymes in humans and among them the most important one is pancreatic alpha-amylase, that act as a catalysis in the reaction which involves the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylopectin, amylose, glycogen, and numerous maltodextrins and is responsible for starch digestion. The other important enzyme is alpha-glucosidase or maltase hich catalyzes the final step of the digestive process of carbohydrates mainly starch by acting upon 1,4-alpha bonds and producing glucose as the final product. The large molecules like starch cannot cross the blood brain barrier as glucose has to reach the brain thus; to overcome this problem alpha-amylase cleaves the large starch molecules into smaller fragments of sugars in order to cross the blood brain barrier. If there will be excess conversion of starch to sugars, it will increase the sugar level in blood, then the role of insulin will come into action by ordering cells to metabolize the excess sugar moieties and store as energy sources i. e. glycogen. This cycle is endlessly happening in a healthy person. But in some cases, due to excess activity of amylase enzyme and insulin deficiency or resistance to insulin, level of blood glucose arises which might results in hyperglycaemia. To control hyperglycaemia several studies on inhibition of amylase enzyme activity is being studied. However, if there will be excessive inhibition of pancreatic alpha-amylase, it might cause abnormal bacterial fermentation of undigested carbohydrates in the colon resulting in flatulence and diarrhea [20].

CHOLECYSTOKININ:

Cholecystokinin is a hormone whose primary action is to stimulate the enzyme release from pancreatic acinar cells. After food is consumed and broken down in the stomach, chime then moves to the small intestine. There are varying amounts of fat in the chyme; this fat triggers the release of hormone Cholecystokinin. The peptide hormone CCK then sends chemical signals to the brain and pancreas where it reaches specific receptors. Cholecystokinin is a small peptide hormone that helps regulate many different functions in the body. CCK is located in the duodenum, the first part of the small intestine. CCK receptors however are located in various locations of the body, in order to conduct different biological functions in the body. CCK receptors are located on the brain, gallbladder, liver, pancreas acinar cells, and in the central nervous system. Cholecystokinin is very important for normal bodily functions. It is well known in the digestive system to help break down protein and fat in chyme. CCK is also linked to functions in the central nervous system. A major role that Cholecystokinin plays in the body is to trigger a feeling of satiety [21].

THYROTROPIN-RELEASING HORMONE (TRH):

Thyrotropin-releasing hormone (TRH) is a tripeptide (pGlu- His-Pro-NH2; where pGlu stands for pyroglutamic acid) that is released from the hypothalamus and transported via the portal vascular system to the anterior pituitary where it stimulates the release of thyroid-stimulating hormone (TSH) and prolactin from the anterior pituitary . In addition to its classical function as a releasing hormone, the distribution of TRH and its receptor indicates supplementary roles. TRH and TRH receptor (TRHR) are found distributed throughout the central and peripheral nervous systems as well as in other tissues including thymus and small intestine epithelial cells , consistent with its proposed role in the immune system [22].

CORTICOTROPIN-RELEASING HORMONE (CRH):

Corticotropin-releasing hormone (CRH), a 41 amino acid neuropeptide, is likely involved in all three types of stress-response. Behavioral responses may involve CRH present in the cerebral cortex and amygdala. Autonomic responses are controlled in part by brainstem fibers descending from the locus coeruleus, which receives CRH-containing fibers from the amygdala and paraventricular nucleus. Hormonal responses center on activation of the hypothalamic–pituitary–adrenal (HPA) axis, which is initiated by CRH present in the paraventricular nucleus of the hypothalamus (PVH) [23].

GONADOTROPIN-RELEASING HORMONE (GNRH)

The secretion of gonadotropin-releasing hormone (GnRH) by GnRH neurons is vital for reproductive competence in all mammalian species. GnRH neurons are unique in that they arise from progenitor cells located outside of the central

nervous system, in the olfactory placode. These neurons migrate through the nasal septum into the basal forebrain, where they establish their final

underlying the migration of the hypothalamic GnRH-producing neurons from the nasal region along the olfactory placode axons into the brain are still not fully understood. A detailed understanding of the process of GnRH migration clearly requires a complete cellular and molecular characterization of the GnRH neuron during early development.

4. CONCLUSION

The potential medicinal uses of *Asterias rubens* were supported by the presence bioinformatics activities. *Asterias rubens* has been exploited by the secondary compounds are further confirmed by bioinformatics studies, which conclusively and comprehensively validates its therapeutic potential. Hence, the need to exploit the potentials of these plants especially in areas of traditional medicine and pharmaceutical industries arises. Further analysis is required for drug target identification.

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