Research Article

In Silico Analysis of *Mytilus Galloprovincialis* Proximal Thread Matrix Protein with Synthetic and Phyto Inhibitors

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ABSTRACT

The adhesive protein helps aquatic organisms to attach to hard and wet substratum resulting in micro and macro biofouling which reduce the performance of ships and also increase their fuel requirements. To prevent biofouling, the shipping industries use antifouling paints which contain many chemical inhibitors but these inhibitors are toxic and harmful to the environment and hence environmental friendly biocides are being investigated. Bioinformatics tools are helpful to identify new and novel compounds for human health problems. These tools can also be used to explore the possibility of proposing novel eco-friendly compounds for the prevention of biofouling and biofilm formation. An attempt has been made to study the interaction of known inhibitors and the proximal thread matrix protein and the results were presented in this paper. By comparing the binding energy values of different inhibitors, the strength of binding of different chemicals with the protein and thus the chemical having better inhibiting property were described.

Keywords: Environmental biofilms, biofouling, antifouling paints, matrix protein, synthetic and phytochemicals

INTRODUCTION

Biofouling is the settlement of living plants and animals on the surface which is having continuous contact with water of any type. Microfouling and macrofouling are of two successive stages of fouling process. Microfouling refers to the formation of viscous and syrupy bacteriological layer by adhesive microorganisms whereas macrofouling refers to the attachment of larvae of larger sessile organisms like barnacles, mussels, polychaete worms, hydroids, bryozoans, and seaweeds. One of the most common biofouling sites in the environment is on the hulls of ships, where organisms are often found attached and it has been periodically removed manually during docking of the ships.

Biofouling leads to many significant problems like increased fuel requirements, problems related to propulsion system, introduction of invasive species etc. To prevent the attachment of the sessile organism, organometallic compounds are being applied on the underwater hulls of ships and boats.

Macro-foulers cause additional and even more severe problems for subsea operators. This grouping includes many larger animals and plants. Among them various species of mussels and barnacles which adhere to the surfaces permanently to underwater substratum. Blue mussel, *Mytilus edulis* and *M. galloprovincialis* attach to a variety of surfaces in an aqueous environment by using natural glues secreted from byssus which is composed of threads that is incredibly strong, water soluble and durable and provides adhesiveness to the mussel's foot.

M. galloprovincialis is an invasive, biofouling species that adheres to a variety of substrates underwater, using a proteinaceous anchor called the byssus. Approximately 70% of proximal and 90% distal threads of the dry weight of thread comprises of important molecular components of collagenous proteins (pre COLs).[1] Proteins (preCOLs), generally dictate the higher order self-assembly and mechanical properties of byssal and the threads contain additional matrix components that separate and lubricate the collagenous microfibrils during deformation in tension the byssus.

Prediction of the functional properties of protein is possible due to the availability of a large number of online tools and servers from different sources. Computational tools and databases of the protein and genome sequences are considerably helpful for the researchers to understand physicochemical and structural properties of protein that are available in those databases.

M. galloprovincialis's proximal thread matrix protein 1 (PTMP1) has deposited in PDB with an accession number 4CN8. Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other. When the protein binds with the ligand, there is some energy released which is called binding energy and it is represented by E-value. This value helps to know the strength of binding.

This matrix protein present in byssus of organisms can be inhibited by a chemical present in antifouling paints. Here, the matrix protein which helps in formation of byssus and adhesion acts as a receptor and the inhibiting chemical acts as a ligand. Both of these bind with each other and this binding is known as protein ligand binding. Hence thirteen synthetic and phyto-compounds were

selected as ligands in this study to determine the binding activity with the *M. galloprovincialis* matrix protein.

MATERIALS AND METHODS

Matrix Protein

The proximal thread matrix protein from the mussel byssus was taken as the receptor. The PDB format of the matrix protein, 4CN8 was obtained from protein data bank (http://www.rcsb.org).

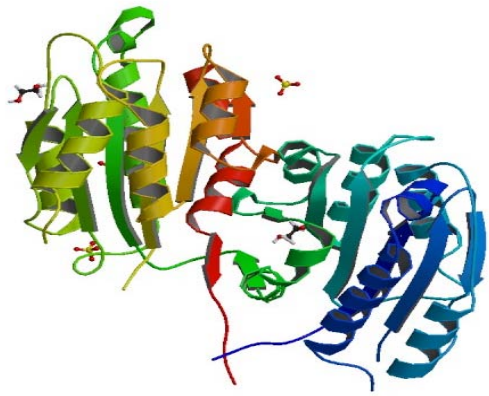

Figure 1: Structure of proximal thread matrix protein 1 (PTMP1) from the mussel byssus

(PDB ID: 4CN8)

Ligand Compounds

Silver ion, polyvinyl alcohol, eucalyptol, carvacrol, triphenyltin chloride, thymol, lead acetate trihydrate, benzoyl peroxide, bis (tri-n-butyltin) oxide, benzoic anhydride (benzoate), dibutyltin, gallotannic acid and tributyltin were the compounds used for docking studies. The SDF files of the ligands were obtained from the PubChem compound database. (http://pubchem.ncbi.nlm.nih.gov/)

Validation of the Structure of Adhesive Protein using Ramachandran Plot

A Ramachandran plot was used to validate the protein structure. It was a way to visualize the backbone of dihedral angles ψ against ϕ of amino acid residues. For validating the structure of matrix adhesive and collagen protein 4CN8, SAVS server was used. SAVS was a server used for analysing protein structures and also assessing their validation of proteins. The PDB format of the protein was uploaded and the program was run. The stereo-chemical quality of the modelled protein structures were then checked and validated using PROCHECK. The Ramachandran plot for the adhesive protein was obtained and thus it was validated as the residues in the most favoured region was found to be 91.3%.

Docking of Synthetic and Phytochemical Compounds with Adhesive Protein

The docking between the collagen matrix protein that acts as a receptor and the phyto chemicals (carvacrol, thymol, eucalyptol) and synthetic compounds (silver ion, polyvinyl alcohol, triphenyltin chloride, lead acetate trihydrate, benzoyl peroxide, bis (tri-n-butyltin) oxide, benzoic anhydride (benzoate), dibutyltin, gallotannic acid and tributyltin) as ligands were done using the HEX protein docking software. HEX is software which is used to dock the proteins with ligands to form docked complexes. The E-values for each of the ligands were obtained after the docking process. The E-value is the binding energy in KJ/mol, which is liberated when the ligand binds to the receptor.

RESULTS AND DISCUSSION

Marine mussel's byssus thread secretes specialized protein adhesives that allow them to attach themselves to various substrates in aquatic and coastal environments. The protein adhesives secreted by marine mussels overcome the harsh conditions that are prevailing in the aquatic environments and adhere tightly to wet surfaces by using the byssus (which consists of a bundle of threads) secreted from the foot of the mussel.[2,3] Byssus thread matrix proteins (TMPs) which are rich in amino acids glycine, tyrosine and asparagine, were shown to own distinctive repeated sequence motifs, noteworthy transcriptional heterogeneity and were scattered throughout the byssal thread.[4] Additionally, deamidation was shown to occur at a significant rate in a recombinant TMP and in the byssal thread as a function of time. Furthermore, charge heterogeneity presumably due to deamidation was observed in TMPs extracted from threads and that has a potential to alter the functions of protein. The TMPs were localized to the preCOL-containing secretory granules in the collagen gland of the foot and are assumed to provide a viscoelastic matrix around the collagenous fibersin byssal threads.[5,6] Matrix protein which helps to attain the mechanical properties of byssus thread and it helps the marine organisms to adhere to the substratum leading to biofouling. Figure 1 refers to the structure of collagenous proximal matrix protein isolated from *M. galloprovincialis* which has lot of resemblance to *M. edulis.*

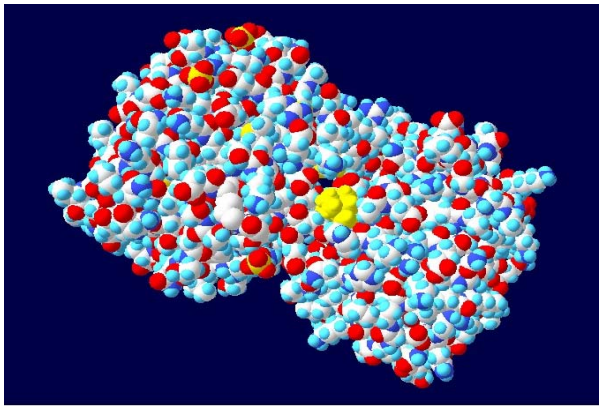

Figure 2: Docked complex of receptor 4CN8 and ligand eucalyptol (centred in yellow)

Effective nontoxic agents showing antifouling activity against fouling organisms are of great interest. Phytochemical compounds such as eucalyptol, carvacrol,

thymolare reported to be environment friendly compounds and also possess antimicrobial properties.[7-9] Carvacol and thymolexibit antifouling properties against pathogens *Salmonella aureus, S. epidermidis* and the reference strain *S. epidermidis* ATCC 35984 and they were also considered as harmless phytochemical compounds.[10-12] Furthermore, acute and short-term toxicity studies suggested that they might not create any risk for human and animal health.[13,14] Hendry has proved significant antimicrobial efficacy of eucalyptus oil against microorganisms grown in planktonic and biofilm cultures. To determine the binding effectiveness of phytochemical compounds with matrix protein the docking analysis was performed. The results demonstrated that they have considerable amount of binding. Phytocompounds such as eucalyptol, carvacrol and thymol have considerable binding energy and the E-values of -145.47, -151.67 and -165.36 KJ/mol, respectively, were obtained as illustrated in Figure 3.

Some of the known synthetic inhibitors which are used as the ingredients of antifouling paints like tributyltin, dibutyltin, silver ion, polyvinyl alcohol, triphenyltin chloride, lead acetate trihydrate, benzoyl peroxide, bis(tri-n-butyltin) oxide, benzoic anhydride (benzoate) and gallotannic acid were used as ligand against matrix protein in docking studies and the results were shown in Figure 3. They are the main active ingredients in certain biocides which are used to control the proliferation of a broad spectrum of microorganisms.[15] Tributyltin (TBT) was a chemical present in antifouling paint which prevents the adherence of microorganisms which causes biofouling. It was used in wood preservation, antifouling pesticide in marine paints, antifungal action in textiles and industrial water systems, wood pulp and paper mill systems and breweries. TBT was found to be extremely toxic to marine organisms and caused imposex in marine gastropods. Thus it is considered as a marine pollutant and has been banned worldwide.

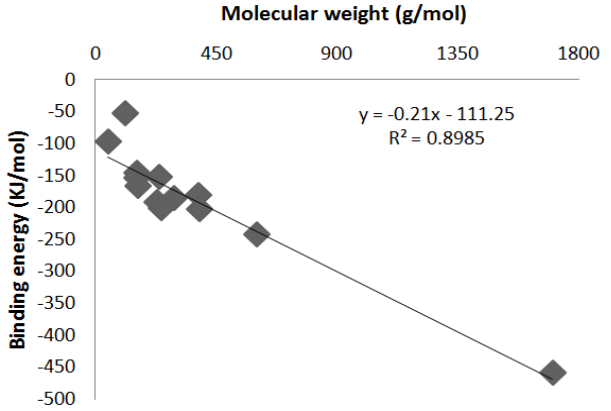

Figure 3: Relation between the molecular weights and binding energies of 4CN8 receptor with selected synthetic and phyto-compounds using Hex Docking Software

The docking between proximal thread matrix protein 4CN8 with the synthetic chemical exhibited a low binding energy of -96.73 KJ/mol for polyvinyl alcohol and that also has the lowest molecular weight (Table 1).

Eucalyptol is a natural organic compound which shows a good result and is a liquid with a cyclic ether and a monoterpenoid and is a common component seen in many mouthwash and tooth paste because it controls plaque and gingivitis and it also controls airway mucus hyper-secretion.[16,17] Carvacol and thymol are isomeric have the same molecular weight but they differ slightly in their binding energy.

Thymol is a known biocide and active fungicide whereas, carvacrolis found to inhibit the growth of *Pseudomonas aeruginosa* by inhibiting their proliferation.[18]

Table 1: Molecular weights of selected Synthetic and Phyto-compounds

SNo	Chemical formula	Compound	Pub Chem ID	Molecular wt (g/mol)
1	Ag^+	Silver ion	CID 104755	107.87
2	C_2H_4O	Polyvinyl alcohol	CID 11199	44.05
3	$C_{10}H_{18}O$	Eucalyptol	CID 2758	154.25
4	$C_{10}H_{14}O$	Carvacrol	CID 10364	150.22
5	$C_{18}H_{15}ClSn$	Triphenyltin chloride	CID 12540	385.47
6	$C_{10}H_{14}O$	Thymol	CID 6989	150.22
7	$C_4H_{12}O_7Pb$	Lead acetate trihydrate	CID 22456	379.33
8	$C_{14}H_{10}O_4$	Benzoyl peroxide	CID 7187	242.23
9	$C_{24}H_{54}OSn_2$	Bis(tributyltin) oxide	CID 16682746	596.10
10	$C_{14}H_{10}O_3$	Benzoic anhydride (Benzoate)	CID 7167	226.23
11	$C_8H_{18}Sn$	Dibutyltin	CID 6484	232.94
12	$C_{12}H_{27}Sn$	Tributyltin	CID 3032732	290.05
13	$C_{76}H_{52}O_{46}$	Gallotannic acid	CID 16134267	1701.20

Molecular Weight and Binding Energy

The correlation and regression analysis were carried out using SPSS v.16 software. It was observed that there was a strong correlation of r = -0.98, at 0.01 significance level, between the molecular weights (in g/mol) and the binding energies (in KJ/mol) of the docked complexes as given in Table 2. The negative binding energy values were obtained due to the representation of the binding affinities of docked complexes with a negative sign in HEX software. Therefore, the strong correlation indicated that as the molecular weight increases the binding energy of the docked complex also increases.

The regression coefficients relating the molecular weight and binding energy were given in Table 3. The relationship between the two parameters was defined by the equation below for the 13 synthetic and phytocompounds taken in the study,

Binding Energy = -0.210(Molecular Weight) – 111.255

Table 2: Strong correlation (r=-0.948) between molecular weight and binding energy

		Molecular weight	Binding energy
Molecular weight	Pearson Correlation	1	-.948**
	Sig. (2-tailed)		.000
	N	13	13

**. Correlation was significant at the 0.01 level (2-tailed).

Table 3: Regression coefficients relating molecular weight and binding energy

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-111.255	11.614		-9.579	.000
	Molecular weight	-.210	.021	-.948	-9.869	.000

a. Dependent variable: Binding energy

CONCLUSION

The matrix protein, which has adhesive and structural property, is present in organisms helping them to attach to any substratum causing biofouling. To prevent biofouling, antifouling paints are used which contains many toxic chemicals that directly has some adverse effect on the aquatic environment.

To overcome this, a better inhibitor that prevents adhesion, was needed and that too from a compound which has no effect on animal, human and environmental health. Thus docking studies were conducted using known phytocompounds and synthetic inhibitors by computational methods.

Further studies are needed to know the exact binding and active sites and also nature of inhibition etc. Such studies would allow us to predict different types of inhibitors which are less toxic or environmentally friendly.

Primary bioinformatics analysis reveals the binding nature of protein and inhibitor and also the extent of energy that is being produced.

This kind of characterization studies give good idea about the inhibition properties of known inhibitors and it would help us to look for novel inhibitors for the prevention of both micro biofouling and macro biofouling in the environment.

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