Marine Sponge Derived Cyclic Peptides for Removal of Mercury

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This study deals with heavy metal toxicity removal in particular mercury and methyl mercury ions in human. Mercury toxicity in the form of thimerosal used as preservative in vaccines has been linked to autism in children and constitutes an important research topic. This study deals with possible removal of Hg^{2+} and CH_3Hg^+ ions from human utilizing marine sponge derived cyclic peptides and their analogs. *Ab initio* molecular orbital calculations with complete optimization and intermolecular interaction calculations have been utilized for the purpose. A number of marine sponge derived cyclic peptides have been shown to be suitable for non-covalent carriage of mercury ions without premature expulsion probability. Stylistatin and its analogs with enhanced 'drug-like features' have been recommended as lead compounds for the development of non-toxic biocompatible drugs for mercury toxicity removal in human. These findings remain to be verified by *in vitro* or *in vivo* pharmacological studies.

Keywords: Marine sponge, Cyclic peptide, Stylistatin, Methyl mercury, Druggability.

INTRODUCTION

Heavy metal toxicity in human, whether intentional or coincidental, is one of the most challenging tasks to deal with medically [1-3]. The commercially available drugs in market are the limited number of chelating agents like dimercaprol, British antilewisite (BAL) DMSA, DMPS. This work is focussed at understanding the sources, treatment options available and further line of action to deal with mercury toxicity issues in human.

There are different sources of mercury toxicity in human:

- Dental amalgams which continuously release mercury that is absorbed by human body tissues.
- By eating fish, seafood contaminated with methyl mercury (MeHg^+). Methyl mercury toxicity has been associated with neurological disorder in adults and impaired neurological development in children [4].
- Use of mercury as a fungicide, mildewcide or pesticide, as latex paint preservative and in outdoor fabric treatment [5].
- Thimerosal used as a preservative in vaccines. Children with autism, a significant number in North America, have been correlated with mercury overload in their body [6].

All these uses of mercury have been banned in USA for quite some time now. They lead to incorporation of Hg in the food chain one way or the other resulting in eventual mercury toxicity in human.

Mercury has profound cellular, cardiovascular, haematological, pulmonary, renal, immunological, neurological, endocrine, reproductive and embryonic toxicological effects [7-10]. To understand removal techniques we also need to know the poisonous forms of mercury. Poisoning can result from Hg vapour inhalation, Hg ingestion and Hg injection. Absorption can also be through the skin. Mercury has three forms: elemental Hg, inorganic salts and organic compounds. The deadliest form of Hg poisoning results from methyl- Hg^+ as it is easily bioaccumulated [11]. Elemental Hg is poorly absorbed, hence the least toxic of all. Methyl- Hg^+ poisoning increases as we move up in the food chain specially in fish. Chelation with DMSA or DMPS reduces Hg load when administered within few hours of exposure [12]. In communities that totally survive on fishing, the rate of mercury poisoning in children is high. In Japan the famous Minamata disease has also been associated with Hg toxicity.

The risks associated with Hg poisoning being high, WHO has set acceptable level as low as 0.006 mg/L for drinking water [13]. However, the question is when exposed to Hg in any form due to one of the above reasons; what is the best and safest treatment option available to us. In an emergency chelation (orally administered) with 2,3-dimercapto succinic acid results in enhanced Hg excretion [14]. Likewise other chelators *e.g.*, DMPS, α -lipoic acid, EDTA *etc.* may also be used orally or through intravenous administration. However, DMPS is

about three times more toxic as compared to DMSA. DMSA being less toxic is administered orally but is not devoid of side effects. Its side effects include diarrhoea, nausea, vomiting, appetite loss and rashes. α -Lipoic acid (ALA) is often used as dietary supplement due to its oxidative stress reducing ability [15,16]. But, it also has special affinity for mercury ions [4] and helps remove heavy metal ions from our body *via* kidneys acting as detoxifying agent. However, α -lipoic acid has a very short half-life of 3-4 h [17] in our body and can therefore, redistribute these heavy metals and leave them all over again unattended in our blood stream to become all the more dangerous. We are thus in constant lookout for better chelating agents. Some marine microorganism derived compounds have been utilized for bioremediation purposes and waste water management due to their metal ion removal property [18]. Additional benefit of using these naturally occurring compounds is their less toxic nature and that they do not cause any secondary pollution. These compounds have not been used as a treatment option in human yet. For that matter, many antimicrobial peptides have been investigated but not reached the market due to their haemolytic activity, proteolytic issues and as a result poor pharmacokinetics [19].

In this work we have considered some marine sponges derived cyclic peptides as an alternative to metal chelator drugs like DMSA, DMPS to treat mercury toxicity in human.

EXPERIMENTAL

Ab initio molecular orbital calculations have been performed at the Hartree-Fock level with the Steven's CEP-31G [20] basis set. Complete geometry optimizations are performed with the Berny's gradient method [21,22]. Calculations have been performed twice on each compound, once in absence of metal ion and then in presence of ion. Choice of basis set is due to the availability of basis set for heavy metal. Geometry relaxation allows the peptide to reorganize maximizing interactions with it. The energy of the reorganized compound and metal ion were calculated separately to yield the interaction energy as follows:

$$E_{\text{int}} = E_{\text{complex}} - (E_{\text{reorganized peptide}} + E_{\text{ion}})$$

Energy required for reorganization of peptide in presence of ion has been calculated as:

$$E_{\text{reorganization}} = E_{\text{reorganized peptide}} - E_{\text{peptide in absence of metal ion}}$$

$$\text{Overall stabilization} = E_{\text{int}} + E_{\text{reorganization}}$$

To address druggability issues, ADME properties of these compounds have been estimated using QikProp module of

Schrodinger software [23]. This module predicts properties as proposed by Jorgensen and other researchers in the field [24-26].

RESULTS AND DISCUSSION

In this work we have investigated several cyclic amphipathic peptides derived from various marine sponges for their ion carriage properties and possible usage in metal toxicity removal. These are largely hydrophobic hepta or octapeptides with antimicrobial activity and other pharmacological properties as well. Their sequence and antitumor activity data are given in Table-1 [27-32].

As mentioned in the methodology part these peptides were first optimized without heavy metal and then with the metal ion. The overall stabilization was calculated after considering reorganization required to capture the ion and the results are collected in Table-2.

The optimized structures of cyclic peptides in absence and in presence of heavy metal ion are shown in Figs. 1-4. Results for axinastatin 4 and axinellin A are shown in (Fig. 1). Both the cyclic peptides utilize backbone carbonyl oxygens to hold Hg^{2+} ion non-covalently after little reorganization to properly hold Hg^{2+} ions. Fig. 2 shows Hg^{2+} ion carriage by dominicin and eurijanicin C. So far dominicin seems to be the best for mercury ion carriage. The peptides being of comparable size; the disposition of the residues dictates as to what extent the backbone carbonyls be reorganized to maximize stabilization of cation. Similarly, Figs. 3 and 4 show results for more marine sponge derived cyclic peptides. All the cyclic peptides considered in this study show efficient carriage of Hg^{2+} ion. Best stabilization is obtained when all the backbone carbonyls reorganize to interact symmetrically and efficiently with the ion like in dominicin and sylissatin A. These compounds being highly hydrophobic are predicted to be cell and tissue permeable. It implies that they have the capacity to capture mercuric ions from inside cells and tissues. The marine sponges thus act as biofilters for contaminated sea water and help marine animals survive. However, sea animals thriving on these sponges allow them to enter the food chain eventually affecting human metabolism. Instead, they may be intelligently utilized in combination with DMSA/DMPS to reduce heavy metal toxicity in human. The most poisonous form of mercury is the methyl mercury ion due to its tendency to bioaccumulate by attaching itself to any thiol group present in many proteins. We have studied methyl mercury ion capture by stylissatin A, which was shown to be the best for Hg^{2+} ion capture. These results are depicted in Fig. 5. The presence of electron releasing

TABLE-1
MARINE MICROORGANISM DERIVED CYCLIC PEPTIDES

Cyclic peptide	Resource	Sequence	MIC value ($\mu\text{g/mL}$)	Ref.
Axinellin A	<i>Axinella carteri</i>	cAsn-Pro-Phe-Thr-Ile-Phe-Pro	3.0	[27]
Axinastatin 4	<i>Axinella cf. carteri</i>	cLeu-Thr-Pro-Leu-Trp-Val-Pro	0.057	[28]
Dominicin	<i>Eurypon laughlini</i>	cIle-Ile-Ile-Leu-Pro-Pro-aThr-Pro		
Eurijanicin C	<i>Prosuberites laughlini</i>	cLeu-Phe-Pro-alle-Ser-alle-Pro	49	[29]
Hymenistatin 1	<i>Phakellia fusca</i>	cIle-Ile-Ile-Pro-Pro-Tyr-Val-Pro	3.5	[30]
Phakellistatin 1	<i>Phakellia fusca</i> Thiele	cIle-Phe-Pro-Tyr-Pro-Ile-Pro	7.5	[31]
Stylissatin A	<i>Stylissa massa</i>	cIle-Phe-Pro-Ile-Pro-Phe-Tyr		
Shearamide A	<i>Eupenicillium shearii</i>	cGly-Phe-Pro-Val-Thr-Pro-Ile-Trp	0.0011	[32]

TABLE-2
ION CARRIAGE PROPERTIES OF DIFFERENT MARINE SPONGE DERIVED CYCLIC PEPTIDES

Cyclic peptide	Sequence	Hg ²⁺ ion			CH ₃ Hg ⁺		
		Interaction energy (kcal/mol)	Reorganization energy (kcal/mol)	Overall interaction energy (kcal/mol)	Interaction energy (kcal/mol)	Reorganization energy (kcal/mol)	Overall interaction energy (kcal/mol)
Axinellin A	cAsn-Pro-Phe-Thr-Ile-Phe-Pro	-322.49	54.20	-268.29	—	—	—
Axinastatin 4	cLeu-Thr-Pro-Leu-Trp-Val-Pro	-297.53	57.13	-240.40	—	—	—
Dominicin	cIle-Ile-Ile-Leu-Pro-Pro-aThr-Pro	-368.83	71.04	-297.79	—	—	—
Euryjanicin C	cLeu-Phe-Pro-aIle-Ser-aIle-Pro	-342.67	80.09	-262.58	—	—	—
Hymenistatin 1	cIle-Ile-Ile-Pro-Pro-Tyr-Val-Pro	-311.94	53.55	-258.39	—	—	—
Phakellistatin 1	cIle-Phe-Pro-Tyr-Pro-Ile-Pro	-288.15	46.52	-241.63	—	—	—
Shearamide A	cGly-Phe-Pro-Val-Thr-Pro-Ile-Trp	-339.20	68.52	-270.68	—	—	—
Stylissatin A	cIle-Phe-Pro-Ile-Pro-Phe-Tyr	-369.36	61.87	-307.49	-100.36	18.95	-81.41
Stylissatin A analog 1	cIle-Ala-Pro-Ile-Pro-Ala-Ala	-392.07	81.64	-310.43	-101.13	19.28	-81.85
Stylissatin A analog 2	cAla-Phe-Pro-Ala-Pro-Phe-Phe	-397.01	81.92	-315.09	-98.06	17.92	-80.14
Stylissatin A analog 3	cGly-Phe-Pro-Gly-Pro-Phe-Phe	-394.83	82.75	-312.08	-99.08	21.04	-78.04

Fig. 1. Hg²⁺ ion carriage by cyclic peptides axinastatin 4 and axinellin A

Fig. 2. Hg^{2+} ion carriage by cyclic peptides dominicin and eurijanicin C

group on mercury partially alleviates the remainder charge on cation making the stabilization more difficult apart from its larger size and charge reduced to half as compared to mercuric ion.

It can be seen from Fig. 5 that methyl mercury cation (CH_3Hg^+) is held perpendicular to the plane of cyclic peptide and therefore very little reorganization is required. This implies that CH_3Hg^+ carriage is highly facilitated which is extremely encouraging as it is the most poisonous form of mercury. To understand drug ability prospects of these compounds we have evaluated ADME properties of these peptides which are shown in Table-3. The ADME features calculated indicate moderate drug ability. Out of the main five features based on Lipinski's

rule, the number of H-bond donors and acceptors are in the recommended range for moderate drug ability. The molecular weight needs to be cut down in a way that SASA is also reduced. If we can achieve this with retention of mercuric ion carriage characteristic; we can get an appropriate lead compound for the development of drug for mercury toxicity removal.

Three analogs of stylissatin A have been prepared and studied for their Hg^{2+} and CH_3Hg^+ ion carriage characteristics and ADME properties. Analog 1 has been prepared by mutating large aromatic amino acids with smaller ones. Analogs 2 and 3 are the result of different mutations of aliphatic hydrophobic amino acids with smaller ones. Ion carriage results are summarized in Table-2 and ADME properties are shown in Table-3.

Fig. 3. Hg^{2+} ion carriage by cyclic peptides hymenistatin 1 and phakellistatin 1TABLE-3
PREDICTED ADME PROPERTIES OF MARINE SPONGE DERIVED CYCLIC PEPTIDES AND DERIVED ANALOGS

Cyclic peptide	Molecular weight (130.0-725.0)	Solvent accessible surface area (SASA) (300.0-1000.0 Å ²)	Volume (500.0-2000.0)	Number of H-bond donors (0.0-6.0)	Number of H-bond acceptor (2.0-20.0)	Globularity (0.75-0.95)	% Human oral absorption (>80 % is high <25 % is poor)	Rule of five (max is 4)
Axinellin A	816.95	1004.46	2210.15	3.00	17.70	0.8169	0.0	3
Axinastatin 4	807.00	1005.17	2212.51	2.25	15.45	0.8169	13.75	3
Dominicin	845.09	1021.86	2357.99	1.25	18.45	0.8384	33.51	3
Euryjanicin C	767.96	1020.08	2223.77	1.25	15.45	0.8077	33.68	3
Hymenistatin 1	893.13	1034.06	2430.12	2.25	18.50	0.8453	31.94	3
Phakellistatin 1	828.01	1030.53	2276.94	2.00	16.75	0.8122	47.64	2
Shearamide A	898.07	1023.68	2372.68	2.50	17.20	0.8404	22.76	3
Stylissatin A	878.07	1060.33	2412.02	2.25	15.50	0.8203	32.62	3
Stylissatin A analog 1	638.78	859.46	1836.80	1.25	14.75	0.8439	30.04	2
Stylissatin A analog 2	796.06	996.58	2208.57	1.25	14.75	0.8229	44.42	2
Stylissatin A analog 3	768.00	1004.62	2154.31	1.25	14.75	0.8029	40.66	2

Fig. 6 shows optimized conformation of anaog1 before and after ion carriage.

Results are comparable to stylissatin A indicating that the mutations do not affect the ion carriage properties and lead to significantly improved ADME properties (Table-3). Analogs 2 and 3 of stylissatin A also give similar results (Figs. 7 and 8;

Tables 2 and 3) indicating that it is possible to mutate some residues of these cyclic peptides with smaller hydrophobic residues with retention of mercury ion carriage characteristic and introduction of enhanced drug-like features. These marine sponges derived cyclic peptides may thus be tuned as per needs for heavy metal toxicity removal, in particular mercury and methyl mercury ion removal.

Shearamide A

Shearamide A carrying Hg^{2+} ion
 Interaction energy: -339.20 kcal/mol
 Overall Interaction Energy: -270.68 kcal/mol
 Reorganization Energy: 68.52 kcal/mol

Stylissatin A

Stylissatin A carrying Hg^{2+} ion
 Interaction energy: -369.36 kcal/mol
 Overall Interaction Energy: -307.49 kcal/mol
 Reorganization Energy: 61.87 kcal/mol

Fig. 4. Hg^{2+} ion carriage by cyclic peptides shearamide A and stylissatin A

Stylissatin A

Stylissatin A carrying CH_3Hg^+ ion
 Interaction energy: -100.36 kcal/mol
 Overall Interaction Energy: -81.41 kcal/mol
 Reorganization Energy: 18.95 kcal/mol

Fig. 5. Methyl mercury ion carriage by stylissatin A

Fig. 6. Mercury ion and methyl mercury ion carriage by stylistatin A analog 1

Fig. 7. Mercury ion and methyl mercury ion carriage by stylistatin A analog 2

Fig. 8. Mercury ion and methyl mercury ion carriage by stylistatin A analog 3

Conclusion

Several marine sponge derived cyclic peptides have been studied utilizing *ab initio* quantum mechanical molecular orbital calculations for their heavy metal toxicity removal properties. All the peptides showed good non covalent carriage of Hg^{2+} and CH_3Hg^+ ions. Predicted ADME characteristics indicate moderate druggability which can be further enhanced by tuning mutations as desired. The compounds being highly hydrophobic though overall amphipathic are expected to show good cell permeability. Thus they seem to be suitable for reducing mercury overload from intracellular accumulations as well. Being cyclic the chances of premature expulsion of heavy metal are close to negligible. Hence, there would not be complications of re-distribution of accumulated overload of mercury as in α -lipoic acid. Overall these compounds seem to be quite suitable for removing mercury ion toxicity and may very well be tuned for the removal of other heavy metals as well. Our findings remain to be endorsed by *in vitro* studies and animal model/clinical studies.

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