Inhibition of jack bean urease by amphiphilic peptides

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Abstract

In the current study, amphiphilic peptides were designed and screened against Jack bean urease by using computer aided drug discovery approach. The result showed that out of thirty eight amphiphilic peptides **1, 3, 12, 18, 30**, and **33** exhibit stronger binding affinity with the active site of the enzyme and establish hydrogen bonds with the nickel ions i.e. Ni 841 and Ni 842 as well as make non-polar and hydrophobic contacts with the nonpolar residues in the active site. The selected amphiphilic peptides were synthesized by solid-phase peptide synthesis strategy, characterized by Fast atomic bombardment mass spectroscopy (FAB-MS) and Nuclear magnetic resonance spectroscopy (^1H and ^{13}C -NMR) and performed *in vitro* urease inhibitory activity. Amphiphilic peptides **12** and **33** showed excellent urease inhibitory activity, ($p < 0.001$) with IC_{50} values $20.5 \pm 0.01 \mu\text{M}$, and $28.1 \pm 0.03 \mu\text{M}$ respectively, which was considerably better than thiourea used as positive control. We suggest that these amphiphilic could be potent urease inhibitors and serve as the starting point for effective interventions.

Introduction

Nitrogen is essential component for the growth of pathogenic bacteria's and plants, which is produced by urease enzyme from urea [1]. The pathogens in large intestine of human being and animals hydrolyze urea into ammonia and carbamate by urease enzyme, which may cause adverse effects such as infectious stones, stomach ulcer, and peptic ulcer. It also causes the pathogenesis of hepatic encephalopathy, pyelonephritis, hepatic coma, urolithiasis, ammonia, and urinary catheter encrustation [2–4]. The presence of this enzyme is an important indicative for bacterial infections [5].

The structure of urease isolated from *Klebsiella aerogenes* was first determined in 1995. The active site of this enzyme consists of two nickel ions (Ni 841 and Ni 842) having inter atomic distance of about $3.5 \text{ \AA}$, which are joined together by carbamylated lysine (KCX 490) and an oxygen donor. Both Ni ions also coordinated with two histidines (His 409) and a water molecule, while Ni2 has an additional coordination with aspartate (Asp 494) as shown in Fig. 1 [6].

Various urease inhibitory synthetic compounds like quinolones [7], oxadiazole and benzimidazole [8], ethyl thiazolidine-4-carboxylate [9], and dihydro pyridone [10], have been reported. Among natural products, flavonoids and terpenoids also showed urease inhibitory activity [11]. Recently Svane et al. studied 71 commercially available compounds for their anti-ureolytic properties against both the ureolytic bacterium *Klebsiella pneumoniae* and purified jack bean urease. Of the tested compounds, 30 showed more than 25% inhibition of the ureolytic activity of *Klebsiella pneumoniae* or jack bean urease [12].

Currently available data in this field represents irrefutable progress in the development of drugs for the treatment of infections associated with *ureolytic bacteria*. Therefore, structural activity relationship of organic compounds with the urease enzyme of different sources is necessary to identify selective sites (allosteric and catalytic site), due to less conserved nature of allosteric site as compared to catalytic site [13, 14].

It has been reported that compounds having amphiphilic nature showed potent activity due to strong dipole-dipole interaction of the ligands with the nickel metallocentre and polar amino acids and hydrophobic interaction with non-polar amino acids in the vicinity of active sites [15].

Amphiphilic peptides (AP) have wide range of applications i.e., translational research [16–18], cancer research [19, 20], drug and gene delivery [21–23], and nanotechnology [24].

Keeping in view the need of more potent urease inhibitors and inspired from natural biomolecules such as lipids, and proteins having wide range of applications, we first time intended amphiphilic peptides to check urease inhibition potential.

Material And Methods

All chemicals, i.e., Wang resin Fmoc-protected L-amino acids, coupling reagents and solvents were purchased from Novabiochem, and Sigma-Aldrich. Bruker (Switzerland) ^1H -NMR (400 MHz), ^{13}C -NMR (125 MHz), and Fast atomic bombardment mass spectrometry (FAB-MS) were used for structure determination of amphiphilic peptides and purification of peptides were performed by reverse-phase recycling HPLC (Shimadzu).

Amphiphilic peptides (1–38) scan by molecular docking

Thirty eight peptides having hydrophobic chain (**1–38**) were docked against Jak bean urease enzyme (PDB ID: 4H9M) by using MOE software (2019.0102). The 2D structure of the peptides were generated in ChemSketch package and converted into 3D form in MOE packages. Thiourea (PubChem-2723790) was used as reference molecules for the comparative binding affinity of the peptides. Water and co-crystallized non-protein molecules were removed, from the enzyme, except the nickel ions and the ligand near nickel ions. The default parameters of the MOE package, i.e., Placement: Triangle Matcher, rescoring 1: London dG, Refinement: Forcefield, rescoring 2: GBVI/WSA, were used for docking study. For each ligand total twenty conformations were set to generate, and the top-ranked conformations based on docking score were selected for additional analysis [25].

Urease inhibition assay

Modified Berthelot assay was used for urease activity determination [26]. Mixture of 25 μL Jack bean urease enzyme (0.015 unite), 10 μL of phosphate buffer (50 mM) having neutral pH, were incubated at 30 °C for 15 min in 96-well plates with **1, 3, 12, 18** and **33** (5 μL of 0.5 mM). 40 μL of urea (20 mM) solution was added to each well plate. Ammonia production was quantified by the reaction with phenol-hypochlorite, which produce light blue colored complex measured at 625 nm. Percent inhibitions were calculated by using the following formula.

$$\text{Inhibition (\%)} = 100 - (\text{abs. of test well} / \text{abs. of control}) \times 100$$

IC₅₀ values of active peptides were determined by measuring activities at further dilutions and the data was computed by using EZ- 11 Fit Enzyme software (Perrella Inc, USA).

Results And Discussion

Peptide design rationale and screening simulations

Thirty-eight amphiphilic peptides were designed by selecting amino acid from each group, i.e., group 1= (RHK), group 2 = (DE) and group 3= (STNQ) and hydrocarbons chain (Table 1). The binding affinity of all amphiphilic peptides (**1–38**) with active site of urease were investigated through docking and it was observed that **1, 3, 18, 30** has good binding affinity with active site of enzyme, while **12** and **33** has excellent binding affinity as shown in Table 2. The high potential of **12**, and **33** might be due to carboxylic and guanidino group, respectively, at the side chain of second amino acid, which donates some of its electron density via inductive effects to nickel ions and London dispersion forces between hydrophobic tail of AP and nonpolar amino acids in the vicinity of active site. The molecular interaction of the peptides **1, 3, 12, 18, 30**, and **33** are shown in Fig. 2.

Table 1
Designed amphiphilic peptides (1–38)

No.	Sequence	No.	Sequence
1	N-Dodecanoyl-Arg-Ser-Ser	20	N-Dodecanoyl-Arg-Val-Ser
2	N-Dodecanoyl-Arg-Asp-Ser	21	N-Dodecanoyl-Arg-Ala-Ser
3	N-Dodecanoyl-Arg-Thr-Ser	22	N-Dodecanoyl-His-Met-Ser
4	N-Dodecanoyl-Arg-Gln-Ser	23	N-Dodecanoyl-His-Ile-Ser
5	N-Dodecanoyl-His-Ser-Ser	24	N-Dodecanoyl-His-Val-Ser
6	N-Dodecanoyl-His-Asn-Ser	25	N-Dodecanoyl-His-Ala-Ser
7	N-Dodecanoyl-His-Thr-Ser	26	N-Dodecanoyl-Lys-Met-Ser
8	N-Dodecanoyl-His-Gln-Ser	27	N-Dodecanoyl-Lys-Ile-Ser
9	N-Dodecanoyl-Lys-Asn-Ser	28	N-Dodecanoyl-Lys-Val-Ser
10	N-Dodecanoyl-Lys-Thr-Ser	29	N-Dodecanoyl-Lys-Ala-Ser
11	N-Dodecanoyl-Lys-Gln-Ser	30	N-Dodecanoyl-Arg-Lys-Ser
12	N-Dodecanoyl-Arg-Asp-Ser	31	N-Dodecanoyl-His-Lys-Ser
13	N-Dodecanoyl-Arg-Glu-Ser	32	N-Dodecanoyl-Lys-Lys-Ser
14	N-Dodecanoyl-His-Asp-Ser	33	N-Dodecanoyl-Arg-Arg-Ser
15	N-Dodecanoyl-Glu-His-Ser	34	N-Dodecanoyl-His-His-Ser
16	N-Dodecanoyl-Lys-Asp-Ser	35	N-Dodecanoyl-Lys-Arg-Ser
17	N-Dodecanoyl-Lys-Glu-Ser	36	N-Dodecanoyl-His-His-Ser
18	N-Dodecanoyl-Arg-Met-Ser	37	N-Dodecanoyl-Lys-His-Ser
19	N-Dodecanoyl-Arg-Ile-Ser	38	N-Dodecanoyl-Arg-His-Ser

Table 2
Inhibition constant (k_i) and Gibb's free binding energy (ΔG) of amphiphilic peptides calculated through MOE

No.	ΔG (kcal mol ⁻¹)	k_i (μ M)
1	-6.31	36.12
3	-6.65	20.80
12	-7.34	5.00
18	-5.58	64.74
30	-5.12	256.60
33	-7.63	4.25
Std = Thiourea	-0.89	236287.03

Synthesis

The targeted amphiphilic peptides (Fig. 3) were synthesized by Fmoc protocol (Scheme 1). In brief, Fmoc-Serine (3 equiv.) was treated with Wang resin (2 gm) in presence of tripyrrolidino phosphonium hexafluoro phosph- 77 as a coupling reagent (3 equiv.) and *N, N*-diisopropylethylamine base (3 equiv.). Similarly, second and third amino acids were coupled. After the formation of polar head by Fmoc protection and deprotection strategy [27], peptide was further treated with decanoic acid to introduce nonpolar nature. 95% trifluoro acetic acid was used for the cleavage of AP from Wang resin.

Structural studies of **1, 3, 12, 18, 30, and 33**

The FAB MS spectra for **1, 3, 12, 18, 30, and 33** shows molecular ion $[M + H]^+$ peak at 503.2, 517.3, 547.3, 544.1, 572.1 and 544.2, respectively. The FAB MS-MS spectra further confirmed fragmentation pattern of the peptides. The fragment ion peak at 416, 430, 457, 460, 457, and 485 in FAB MS spectra (see supporting information) of amphiphilic peptides **1, 3, 12, 30** and **33** respectively showed the c_1 cleavage i.e., between alpha carbon and amine groups of serine, while the peaks at 328 showed c_2 cleavage (between alpha carbon and amine groups of second amino acid). Similarly, the peaks at 311 in FAB MS spectras of all the amphiphilic peptides are due to the formation of b_2 ion, i.e., loss of arginine + decanoic acid (Figure-4).

The amphiphilic peptides were further characterized by ¹H-NMR (400 MHz) and ¹³C- NMR (125 MHz, cryoprobe), by using CD₃OH as solvent to record the data. The ¹H-NMR spectra of amphiphilic peptides **1** showed three alpha protons in the region δ 3.90–4.50, Sixteen methylene protons of decanoic acid appeared as a multiplate at δ 1.39–2.06 and methyl proton between δ 0.90–1.15 (t). ¹³C-NMR data were recorded on 125 MHz (Cryoprobe) NMR machine, to obtained better ¹³C-NMR signals. ¹³C- NMR spectras

of **1, 3, 12, 18, 30** and **33** showed carbonyl carbons between δ 170.0-174.0. (for detail ^1H -NMR and ^{13}C -NMR data see supporting information Table 1).

In vitro urease inhibitory activity

Inhibition of urease by amphiphilic peptides (**1, 3, 12, 18, 30, 33**) at different concentrations were evaluated. It was observed that among the six amphiphilic peptides only **12** and **33** showed excellent activity having IC_{50} values $20.5 \pm 0.01 \mu\text{M}$, and $28.1 \pm 0.03 \mu\text{M}$ respectively, as compared to standard thiourea (IC_{50} $21.0 \pm 0.2 \mu\text{M}$) at ($p < 0.001$), which support MOE data (Table 3).

Table 3
In vitro urease inhibitory activity of Amphiphilic Peptides (**1, 3, 12, 18, 30, 33**)

AP. No.	$\text{IC}_{50} \mu\text{M} \pm \text{SEM}$
1	51.7 ± 0.01
3	48.4 ± 0.17
12	20.5 ± 0.02
18	58.0 ± 0.14
30	76.0 ± 0.03
33	28.1 ± 0.03
Std = Thiourea	21.0 ± 0.21
SEM standard error of the mean, Std standard inhibitor or urease enzyme	

Conclusion

In conclusion we designed amphiphilic peptides and screened against the Jak bean urease enzyme and found that **1, 3, 12, 18, 30**, and **33** have strong hydrogen bonding with the nickel ions of the active site of enzyme and hydrophobic interaction with non-polar amino acids in the vicinity of active site. Amphiphilic peptides **1, 3, 12, 18, 30**, and **33** were selected from the top ranked docking solutions, synthesized, and evaluated for urease inhibition. All synthesized amphiphilic peptides showed some degree of inhibition *i.e.*, $76 \mu\text{M}$ - $20.5 \mu\text{M}$, but **12** and **33** showed excellent urease inhibition activity with IC_{50} value $20.5 \pm 0.01 \mu\text{M}$, and $28.1 \pm 0.03 \mu\text{M}$ respectively.

Declarations

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Conflict of Interest:

All authors have no conflict of interest.

Supporting information

The supporting information contain ^1H , and ^{13}C -NMR data, FAB-MS spectra, and ^1H , ^{13}C -MR spectra.

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Figures

Figure 1

Schematic representation of the active site of urease enzyme [7].

Figure 2

The active site of the Jak bean urease enzyme and the binding of the top hit peptide at the active site. Schematics of the active site of urease enzyme, coordination of the nickel atoms and selected ligands. The key for interactions is given at the bottom.

Figure 3

Structures of amphiphilic peptides (1, 3, 12, 18, 30, 33)

Figure 4

FABMS-MS study of Amphiphilic Peptides (1, 3, 12, 18, 30, 33)

Supplementary Files

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