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 through chelation of charged amino acids with the nickel ions i.e., Ni²⁺ 841 and Ni²⁺ 842 as well as hydrophobic contacts of the nonpolar tail 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 ¹³C-NMR) and in vitro urease inhibitory activity of amphiphilic peptides was studied. Amphiphilic peptides 12 and 33 showed excellent urease inhibitory activity, (p < 0.001) with IC₅₀ values 20.5 ± 0.01, and 28.1 ± 0.03 µM respectively, which was considerably better than thiourea used as positive control.

Graphical Abstract

Keywords Docking · Synthesis · Amphiphilic Peptides · Jack Bean Urease · Inhibition

Highlights
• Molecular docking.
• Solid-phase synthesis of amphiphilic peptides.
• FAB MS-MS and ¹H and ¹³C NMR study of amphiphilic peptides.
• Urease inhibitory activity.

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Introduction

Nitrogen is essential component for the growth of pathogenic bacteria 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, pylonephritis 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$^{+2}$ 841 and Ni$^{+2}$ 842) having inter atomic distance of about 3.5 Å, which are joined together by carbamylated lysine (KCX 490) and an oxygen donor. Both Ni$^{+2}$ ions also coordinated with two histidines (His 409) and a water molecule, while Ni$_3$ has an addition coordination with aspartate (Asp 494) as shown in Fig. 1 [6].

Various urease inhibitory synthetic compounds like quinolones, oxadiazole and benzimidazol, ethyl thiazolidine-4-carboxylate, and dihydro pyridone, have been reported [7–10]. Recently Svane et al. studied seventy-one commercially available compounds for their anti-ureolytic properties against both the ureolytic bacterium (*Klebsiella pneumoniae*) and purified jack bean urease. Out of which thirty compounds showed more than 25% inhibition of the ureolytic activity of *Klebsiella pneumoniae* and jack bean urease [11]. Among natural products, flavonoids and terpenoids also showed urease inhibitory activity [7]. Currently available data represents irrefutable progress in the development of drugs due to their toxicity or their instability for the treatment of infections associated with *ureolytic bacteriai*. Therefore, structural activity relationship of organic compounds with the urease enzyme of different sources is necessary to identify selective sites (allosteric and catalytic site) [12, 13].

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 [14]. Amphiphilic peptides (AP) have wide range of applications i.e., translational research [15–17], cancer research [18, 19], drug and gene delivery [20–22], and nanotechnology [23].

| 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-Lys-Arg-Ser |
| 12  | N-Dodecanoyl-Lys-Arg-Ser  | 31  | N-Dodecanoyl-Lys-His-Ser |
| 13  | N-Dodecanoyl-Lys-Glu-Ser  | 32  | N-Dodecanoyl-Lys-Lys-Ser |
| 14  | N-Dodecanoyl-Lys-Asp-Ser  | 33  | N-Dodecanoyl-Arg-Arg-Ser |
| 15  | N-Dodecanoyl-Lys-Asp-Ser  | 34  | N-Dodecanoyl-Lys-His-Ser |
| 16  | N-Dodecanoyl-Lys-Asp-Ser  | 35  | N-Dodecanoyl-Lys-Arg-Ser |
| 17  | N-Dodecanoyl-Lys-Asp-Ser  | 36  | N-Dodecanoyl-Lys-His-Ser |
| 18  | N-Dodecanoyl-Lys-Asp-Ser  | 37  | N-Dodecanoyl-Lys-His-Ser |
| 19  | N-Dodecanoyl-Lys-Asp-Ser  | 38  | N-Dodecanoyl-Arg-Asp-Ser |

| Table 2 Inhibition constant (ki) and Gibb’s free binding energy ($\Delta G$) of amphiphilic peptides calculated through MOE |
| No. | $\Delta G$ (kcal mol$^{-1}$) | ki ($\mu$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

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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.

**Results and discussion**

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.
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 chelates with nickel ions and hydrophobic interaction with 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.

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 benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBop) as a coupling reagent (3 equiv.) and N, N-diisopropylethylamine base (3 equiv.). In the same way, second and third amino acids were coupled. After the formation of polar head by Fmoc protection and deprotection strategy [24], peptides were further treated with decanoic acid to introduce nonpolar nature. 95% trifluoroacetic acid was used for the cleavage of amphiphilic peptides from Wang resin. Reverse-phase HPLC was used for purification of amphiphilic peptides to get 39% yield for 1, 12 and 33, while for 3, 18 and 30 yield was 56%.

The FAB MS spectra for 1, 3, 12, 18, 30, and 33 shows molecular ion [M + H]+ peak at 503.2, 517.3, 544.1, 547.3, 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 Supplementary Information) of amphiphilic peptides 1, 3, 12, 30 and 33 respectively showed the c1 cleavage i.e., between alpha carbon and amine groups of serine, while the peaks at 328 showed c2

Fig. 3 Structures of amphiphilic peptides (1, 3, 12, 18, 30, 33)
cleavage (between alpha carbon and amine groups of second amino acid). Similarly, the peaks at 311 in FAB MS spectra of all the amphiphilic peptides are due to the formation of b2 ion, i.e., loss of arginine + decanoic acid (Fig. 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 multiplet 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 spectra of 1, 3, 12, 18, 30 and 33 showed carbonyl carbons between δ 170.0–174.0. (for detail 1H-NMR and 13C-NMR data see Supplementary Information Table 4).

Structure–activity relationships (SAR)

Structure–activity relationship (SAR) relates the chemical structure of a compound and its biological activity. In this study, inhibition of urease depends on the nature of the amphiphilic peptides. Structure displayed in Fig. 1 show that the first amino acid (L-serine) and third amino acid (L-Arginine) in all amphiphilic peptides are same, while nature of second amino acid, which played an important role in the inhibition of urease enzyme. The bioactive data presented in Table 3 showed that charged amino acid at second position i.e., L-Aspartic acid in 12 (IC50 = 20.5 ± 0.01 µM), and L-Arginine in 33 (IC50 = 28.1 ± 0.03 µM) showed good activity as compared to other amphiphilic peptides having uncharged amino acids.

Experimental

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), 13C-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 1–38 having hydrophobic chain 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 Chem sketch 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.

\[
\text{Inhibition (\%)} = 100 - \left( \frac{\text{abs. of test well}}{\text{abs. of control}} \right) 
\]

IC$_{50}$ 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).

**Conclusion**

In conclusion, result obtained from the docking study showed that out of thirty-eight amphiphilic peptides, only 1, 3, 12, 18, 30, and 33 have strong interaction with the active site of enzyme by chelation charged amino acids with nickel ions and hydrophobic interaction with nonpolar amino acids in the vicinity of active site. Top-ranked amphiphilic peptides confirmed from the docking study were synthesized and evaluated for urease inhibition. All synthesized amphiphilic peptides showed some degree of inhibition (20.5–76 µM), but 12 and 33 showed excellent urease inhibition activity with IC₅₀ value 20.5 ± 0.01 µM, and 28.1 ± 0.03 µM, respectively.

Results suggest the contact of hydrophilic and hydrophobic part of the amphiphilic peptides with enzyme played an important role in inhibition. These discoveries create a scaffold of effective compounds with optimized pharmacological profile for the treatment of urease related diseases. Docking and in vitro studies six amphiphilic peptides agreed with experimental results related to inhibition of urease. More work, however, is needed to establish the efficacy and safety of these peptides prior to further steps. Animal studies are also recommended to get more information about the inhibitory and cytotoxicity of these compounds.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare no competing interests.

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