

THE INFLUENCE OF NON-PROTEIN AMINO ACIDS AND PEPTIDES ON BACTERIAL COLLAGENASE

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Bacterial collagenolytic proteases have drawn increasing attention due to their essential role in the global nitrogen cycling and their virulent role in some diseases. The inhibition of these enzymes is attractive, as it does not attack the pathogen directly but rather blocks the colonization and infiltration of the host by the clostridia.

Optically active non-protein α -amino acids have been screened for their ability to inhibit collagenase of *Clostridium histolyticum*. Both structure-based drug design approach (modeling) and that of determining enzyme activity in the presence of amino acids have been used to identify low molecular weight inhibitors of collagenase. The compounds able to inhibit collagenase activity have been revealed and IC₅₀ values have been estimated.

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Keywords: non-protein amino acid, peptide, collagenase, inhibition.

Introduction. Bacterial collagenases play an essential role in the global nitrogen cycling. These enzymes are also considered as a virulence factor in some diseases. Inhibition of these enzymes aimed to stop disease development is quite attractive, as inhibitors do not attack the pathogen directly, but rather block infiltration of infection into the host cells. Targeting extracellular enzymes provide a substantial benefit, because inhibitors do not need to cross the bacterial cell wall, which has turned out to be challenging in many cases [1].

There are two *Clostridium histolyticum* collagenase classes encoding by the genes *colG* and *colH*. *Clostridium* collagenase molecule is composed of two parts: the N-terminal collagenase unit (or module) and the C-terminal recruitment domains [2]. The collagenase unit contains an N-terminal activator domain and a C-terminal peptidase/catalytic domain with a conserved zinc-binding motif in the latter. In addition to the active zinc ion, a calcium ion is also required for the full enzymatic activity. The known synthetic inhibitors of metalloproteases ordinarily are zinc-binding compounds demonstrating nonspecific action [3]. It is anticipated that the

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development of highly selective inhibitors of clostridial collagenase would be effective to combat these bacteria. The influence of non-protein amino acids and peptides on the collagenase activity has been studied. The compounds inhibiting collagenase activity have been revealed and half-maximal inhibitory concentration (IC_{50}) values have been estimated.

Materials and Methods. Non-protein amino acids and peptides used in this study were synthesized at the Scientific and Production Center “Armbiotechnology” NAS RA and Institute of Pharmacy of Yerevan State University [4–7]. Collagenase G from *Clostridium (Hatheway) histolyticum* (EC 3.4.24.3) and miscellaneous reagents were purchased from “Sigma-Aldrich” (now “Merck”).

Docking of ligand to enzyme was done by AutoGrid 4, AutoDock Vina software [8]. Crystallographic structure of collagenase G was taken from the Protein Data Bank of Research Collaboratory for Structural Bioinformatics (PDB ID: 2Y50).

Collagenase activity was measured by the method used for determination of free amino group [9]. The reaction mixture contained 0.05 M HEPES buffer, pH 7.2, 10 mg/mL gelatin and 0.025 mg/mL collagenase. The concentration of free amino groups in the reaction mixture was determined by ortho-phthalaldehyde (OPA) reagent containing 0.2 M borate buffer, pH 9.7, 0.1667 mg/mL OPA and 1.18 mM mercaptoethanol. The reaction mixture (50 μ L) was added to OPA reagent (1.5 mL) and H₂O (1.5 mL). A_{340} was recorded after 5 min incubation at room temperature.

Results and Discussion. The interaction of collagenase with non-protein amino acids and peptides has been studied by AutoGrid 4, AutoDock Vina. Gibbs energy (ΔG) and dissociation constant (K_D) values have been calculated. The collagenase activity in the presence of studied compounds was determined and IC_{50} values were calculated. The data on compounds that demonstrated inhibitory activity are presented in the Table.

Interaction of the studied compounds with collagenase

Compounds	ΔG , kcal/mol	K_D , μ M	IC_{50} , mM
(S)-2-Amino-3-methylbut-3-enoic acid	-4.6	424.77	5.30
(S)-2-Amino-2-(4-bromo-benzyl)-pent-4-ynoic acid	-5.5	92.99	2.89
(S)-2-Amino-2-(2,4-dichlorobenzyl)pent-4-ynoic acid	-6.4	20.36	1.48
(S)-2-Amino-2-(2,6-dichlorobenzyl)pent-4-ynoic acid	-6.5	17.19	1.31
Alanyl-(S)- β -[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]- α -alanine	-6.2	28.53	2.38
Alanyl-glycyl-(S)- β -[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]- α -alanine	-6.1	33.78	2.12
2-Aminohex-5-enoic acid	-5.1	182.66	3.18
2-Aminopent-4-enoic acid	-5.0	216.25	4.26

The non-protein amino acids and peptides, listed in the Table, have demonstrated ability to interact with collagenase, according to the docking analysis. The results on enzyme activity measurements have indicated that these compounds inhibited collagenase activity but demonstrated the various IC_{50} values. It was shown previously that heterocycle-substituted non-protein amino acid (S)- β -[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]- α -alanine inhibited collagenase activity ($IC_{50}=2.1$ mM) [10]. According to the docking analysis, di-peptide alanyl-(S)- β -[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]- α -alanine and tripeptide alanyl-

-glycyl-(*S*)- β -[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]- α -alanine interacts with collagenase. The measurement of enzyme activity indicates that both peptides demonstrate inhibitory features; nevertheless, IC_{50} values do not exceed the IC_{50} value determined for (*S*)- β -[4-allyl-3-(pyridin-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]- α -alanine. Thus it could be confirmed that inclusion of this amino acid into the peptides structures does not enforce inhibition of collagenase activity.

2-Aminohex-5-enoic acid and 2-aminopent-4-enoic acid have similar structures. Evidently, the inhibition of collagenase by these amino acids rises with the side chain length. (*S*)-2-amino-2-(2,4-dichlorobenzyl)pent-4-ynoic acid and (*S*)-2-amino-2-(2,6-dichlorobenzyl)pent-4-ynoic acid have shown relatively low IC_{50} values, 1.48 mM and 1.31 mM, respectively (Fig.1).

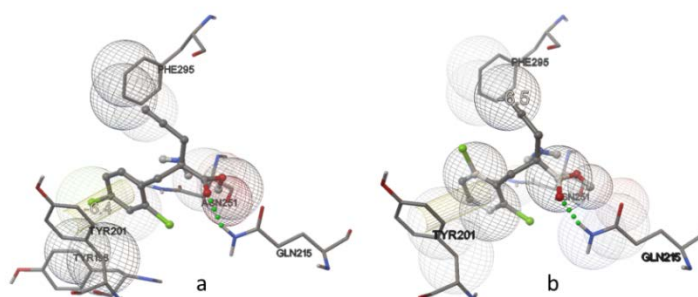


Fig 1. Interaction of (*S*)-2-amino-2-(2,4-dichlorobenzyl)pent-4-ynoic acid (a) and (*S*)-2-amino-2-(2,6-dichlorobenzyl)pent-4-ynoic acid (b) with amino acid residues of collagenase near the ligand.

There is similarity between the structures of these amino acids. The IC_{50} values estimated for these amino acids are almost the same. They differ from each other only by the position of the substituted chlorine.

According to the docking analysis, these compounds show the lowest level of Gibbs free energy, -6.4 and -6.5 kcal/mol respectively. Docking analysis also showed that carbonyl oxygen of (*S*)-2-amino-2-(2,4-dichlorobenzyl)pent-4-ynoic acid forms hydrogen bond with side chain amino group of Gln²¹⁵ (2.202 Å), and the phenyl ring of compound could form a π - π interaction with Tyr²⁰¹ (4.127 Å). The similar results have been obtained by the docking of (*S*)-2-amino-2-(2,6-dichlorobenzyl)pent-4-ynoic acid with collagenase, but in this case the length of hydrogen bond was 1.961 Å and the length of π - π interaction was 4.249 Å (Fig. 2).

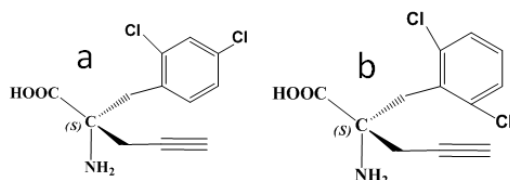


Fig. 2. Structures of

- a) (*S*)-2-amino-2-(2,4-dichlorobenzyl)pent-4-ynoic acid;
b) (*S*)-2-amino-2-(2,6-dichlorobenzyl)pent-4-ynoic acid.

It should be mentioned that both Gln²¹⁵ and Tyr²⁰¹ are included into activator domain of collagenase, which is required for complete activity [11]. Perhaps the inhibition of collagenase by these amino acids results from their ability to bind

activator domain of the enzyme. Evidently, the position of chlorine in these amino acids does not affect the ability to interact with collagenase and its inhibition.

Conclusion. Thus, non-protein amino acids and peptides able to inhibit clostridial collagenase activity have been revealed in this study. (*S*)-2-amino-2-(2,4-dichlorobenzyl)pent-4-ynoic acid and (*S*)-2-amino-2-(2,6-dichlorobenzyl)pent-4-ynoic acid are considered as the most effective collagenase inhibitors studied in this work.

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ՈՉ ՍՊԻՏԱԿՈՒՑԱՑԻՆ ԱՄԻՆՈԹՈՒՆԵՐԻ ԵՎ ՊԵՊՏԻԴՆԵՐԻ
ԱԶԴԵՑՈՒԹՅՈՒՆԸ ԲԱԿՏԵՐԻԱԼ ԿՈԼԱԳԵՆԱԶԻ ՎՐԱ

Բակտերիալ կոլագենոլիտիկ պրոտեազները մեծ դեր ունեն ազոտի գլոբալ շրջանառությունում և որոշ հիվանդություններ հարուցելու՝ շնորհիվ իրենց վիրուլենտության: Այս ֆերմենտների արգելակումը խոստումնալից է, քանի որ չունի անմիջական ազդեցություն պայթուցիկ վրա, այլ արգելափակում է կոստրիդիաների գաղութացումը և ներթափանցումը: Ստուգվել է օպտի-կապես ակտիվ որոշ ոչ սպիտակուցային α -ամինոթթուների են *Clostridium histolyticum*-ի կոլագենազի հետ փոխազդելու ունակությունը: Կոլագենազի ցածրամոլեկուլային արգելակիչներ հայտնաբերելու համար օգտագործվել են ինչպես կառուցվածքաձև մոդելավորումը, այնպես էլ ամինաթթուների առկայությամբ ֆերմենտի ակտիվությունը որոշելը: Բացահայտվել են միացություններ, որոնք արգելակել են կոլագենազի ակտիվությունը, և դրանց համար որոշվել են IC_{50} -ի արժեքները:

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М. А. МЕЛКУМЯН, Г. Г. ОГАНЕЗОВА, Н. С. АВETИСЯН, Н. А. ОГАНЕСЯН

ВЛИЯНИЕ НЕБЕЛКОВЫХ АМИНОКИСЛОТ И ПЕПТИДОВ
НА БАКТЕРИАЛЬНУЮ КОЛЛАГЕНАЗУ

Бактериальные коллагенолитические протеазы привлекают все большее внимание из-за их существенной роли в глобальном круговороте азота и их вирулентной роли при некоторых заболеваниях. Поиск ингибиторов этих ферментов представляет интерес, поскольку они не оказывают непосредственного воздействия на патоген, а скорее блокируют колонизацию и инфильтрацию клеток хозяина клостридиями. В работе исследована способность оптически активных небелковых α -аминокислот ингибировать активность коллагеназы *Clostridium histolyticum*. Для идентификации низкомолекулярных ингибиторов коллагеназы применялся как структурно-ориентированный подход (моделирование), так и метод определения активности ферментов в присутствии аминокислот. Выявлены соединения, способные ингибировать активность коллагеназы, определены значения IC_{50} .