PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2014, № 3, p. 35–39

Biology

NOVEL SYNTHETIC AMINO ACIDS AND PEPTIDES AS BACTERIAL GROWTH INHIBITORS

M. A. MELKUMYAN [1], N. S. AVETISYAN [1], G. G. OGANEZOVA [1], M. B. CHITCHYAN [1], A. M. HOVHANNISYAN [2*], N. A. HOVHANNISYAN [1**]

[1] *SPC "Armbiotechnology" NAS Republic of Armenia*
[2] *Laboratory of Heterocyclic Amino Acids YSU, Armenia*

The influence of novel synthetic amino acids and peptides on the growth of bacteria included in test culture collection has been studied. The compounds were revealed to inhibit the growth of all the strains included in collection. Some compounds demonstrated antibacterial activity in relation to antibiotic-resistant *E.coli* strains, others demonstrated the specificity in relation to particular species. Thus, the novel synthetic inhibitors of bacterial growth, including antibiotic-resistant ones, were described.

***Keywords*:** non-protein amino acids, peptides, plasmid-antibiotics, alanine analog, antibiotic-resistant *E.coli* strains.

Introduction. Currently non-protein amino acids and peptides based thereon are widely used in biotechnology and pharmacology. Peptides composed of non-protein α-amino acids occupy a special place among active compounds. The biological activity of synthetic peptides is stipulated by their ability to interact with enzymes. Bacterial resistance to antibiotics is growing up day by day. Multidrug resistant microorganisms challenged the scientists to develop new antimicrobial compounds. Design of a number of modern antibacterial, antiviral, antitumor and other drugs is based on the property of non-protein amino acids and peptides either to inhibit or enhance the activity of target enzymes [1].

Short peptides synthesized from non-protein amino acids are promising alternatives to small molecule drugs. These compounds have several advantages: high activity, high specificity, targeting capabilities, minimal drug-drug interactions, low toxicity, etc. Antibacterial compounds, i.e. bacteriocins, are peptides with diverse structure and modes of action, which are produced by bacteria. The main role of bacteriocins is growth inhibition of similar or closely related bacterial strains [2]. Recently in design of novel peptides the inclusion of non-protein amino acids into the structure of natural peptides is applied [3, 4].

The biological activity of non-protein amino acids and peptides studied by our group detected some compounds with antimicrobial and mutagenic/anti-mutagenic properties [5].

* E-mail: anhovanisyan@ysu.am ** E-mail: nelliog@yahoo.fr

In this work the screening of non-protein amino acids and peptides based thereon is carried out aiming to reveal compounds that are able to inhibit the antibiotic resistant bacteria.

Materials and Methods.

Strains and Medium. The strains listed in Tab. 1 are used as test cultures.

Table 1

Strain	Genotype	Reference
Escherichia coli DH5α	Δ80d*lacZ*ΔM15, *rec*A1, *end* A1, *gyr*A96, *thi*-1, *hsdR*17(r_{K-}, m_{K+}), *supE*44, *rel*A1, *deoR*, Δ(*lacZYA-argF*)U169, *phoA*	[6]
E. coli DH5α/ pUC18	*E. coli* DH5α, Amp ^{r}	[7]
E. coli DH5α/pVOG10	*E. coli* DH5α, Km ^{r}	[8]
Citrobacter freundii 62	Prototroph	SPC "Armbiotechnology"
Erwinia sp.	Prototroph	SPC "Armbiotechnology"
Pseudomonas putida VKM-V	Prototroph	SPC "Armbiotechnology"
Corynebacterium glutamicum 191	*trp*$^-$, Sm ^{r}	SPC "Armbiotechnology"
Corynebacterium glutamicum 191/pVOG10	*trp*$^-$, Sm ^{r}, Km ^{r}	SPC "Armbiotechnology"
Corynebacterium flavum E531	*met*$^-$, *thr*, AEC $^{r-}$	SPC "Armbiotechnology"

The gram positive and gram negative bacterial strains have been included in collection used in this study. Recombinant *E. coli* DH5α and *C. glutamicum* strains with plasmid pUC18 conferring resistance to ampicillin, and plasmid pVOG10 conferring resistance to kanamycin are included in collection as well.

Bacteria have been grown on LB and synthetic medium was composed of 1% glucose, 0.5% NH_4Cl, 0.1% NH_4NO_3, 0.2% Na_2SO_4, 0.3% K_2HPO_4, 0.01% $MgSO_4$, 100 *u*/*L* thiamin, 200 *u*/*L* biotin. The concentration of amino acids in synthetic medium was 20 or 40 *u*/*mL*. The concentration of ampicillin and kanamycin in medium was 50 *u*/*mL*.

The non-protein amino acids and peptides studied in this work were synthesized in the SPC "Armbiotechnology" NAS RA and YSU.

Detection of Antibacterial Activity. The influence of compounds on bacterial growth was detected applying 5 or 10 *u* of 10 *mM* solution to top L-agar. The top L-agar was preliminarily mixed with 0.1 *mL* of bacterial overnight culture. The plates were incubated at an appropriate temperature for 2 days and then the transparent region (region of growth inhibition) was measured.

Results and Discussion. The influence of about 80 non-protein amino acids (aliphatic and heterocyclic substituted amino acids) and peptides based thereon the bacterial growth was studied. The compounds were divided according to their mode of inhibition of bacterial growth.

Compounds Inhibiting the Growth of All Test Cultures. The following non-protein amino acids and peptides inhibited the growth of all test cultures including antibiotic resistant strains: (*S*)-β-[4-allyl-3-(furan-2-yl)-5-thioxo-1,2,4--triazol-1-yl]-α-alanine, (*S*)-β-[4-allyl-3-(2'-chlorphenyl)-5-thioxo-1,2,4-triazol-1-yl]--α-alanine, (*S*)-β-[4-allyl-3-(pyridine-4'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, (*S*)-β-[4-allyl-3-(pyridine-3'-yl)-5-thioxo-1,2,4-triazol-1-yl]-α-alanine, N-formyl-

methionyl-(*S*)-*β*-[4-phenyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-*α*-alanine, N-formyl-methionyl-alanyl-(*S*)-*β*-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-*α*-alanine, N-formyl-methionyl-alanyl-(*S*)-*β*-[4-phenyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]--*α*-alanine N-formyl-methionyl-glycyl-(*S*)-*β*-[4-allyl-3-propyl-5-thioxo-1,2,4-triazol-1-yl]-*α*-alanine, N-formyl-methionyl-glycyl-(*S*)-*β*-[4-phenyl-3-propyl-5-thioxo-1,2,4--triazol-1-yl]-*α*-alanine. The activity of the strongest inhibitors of bacterial growth is presented in Tab. 2. Evidently these compounds have inhibited the growth of this bacterial collection not specifically.

Table 2

The influence of non-protein amino acids and peptides on the growth of test cultures

Compound, 10 *мМ*	Strains				
	C. flavum E531	*E. coli* DH5*α*	*C. freundii* 62	*P. putida* VKM-V	*Erwinia sp.*
(*S*)-*β*-[4-allyl-3-(furan-2-yl)-]-5-thioxo-1,2,4--triazol-1-yl]-*α*-alanine	8*	8	6	3	4
(*S*)-*β*-[4-allyl-3-(2'-chlorphenyl)-5-thioxo--1,2,4-triazol-1-yl]-*α*-alanine	5	7	4	5	5
N-formyl-methionyl-(*S*)-*β*-[4-phenyl-3--propyl-5-thioxo-1,2,4-triazol-1-yl]-*α*-alanine	6	6	3	5	5
N-formyl-methionyl-alanyl-*(S)*-*β*-[4-allyl-3--propyl-5-thioxo-1,2,4-triazol-1-yl]-*α*-alanine	7	7	8	5	5

* Diameter of transparent region, *mm*.

Compounds Inhibiting the Growth of Particular Bacteria. The compounds inhibiting growth of particular bacteria were detected. For example, *allo*-O-methyl-threonine inhibited *P. putida* VKM-V and tri-peptide t-BOC-alanyl-glycyl-(*S*)--imidazolylalanine inhibited *C. freundii*. Two compounds (*S*)-*β*-(N-ethanol-amino)--alanine and (*S*)-*β*-(N-methyl-amino)-alanine demonstrated inhibition specificity in relation to *Erwinia sp.* Particular growth inhibition can occur due to the difference between targets of the different species.

Table 3

Compounds inhibiting the growth of particular bacteria

Compound, 10 *мМ*	Strains				
	C. flavum E531	*E. coli* DH5*α*	*C. freundii* 62	*P. putida* VKM-V	*Erwinia sp.*
t-BOC-alanyl-glycyl-(*S*)-imidazolylalanine	+	+	7*	+	+
allo-O-methyl-threonine	+	+	+	5	+
(*S*)-*β*-(N-ethanol-amino)-alanine	+	+	+	+	7
(*S*)-*β*-(N-methyl-amino)-alanine	+	+	+	+	4
(*S*)-*β*-[4-furan-2-yl-methyl]-5-thioxo-1,2,4--triazol-1-yl]-*α*-alanine	8	+	+	+	+

\+ Bacterial growth;
* Diameter of transparent region, *mm.*

Compounds Inhibiting the Growth of Antibiotic Resistant Strains. According to obtained results, besides the compounds described above there were some

compounds that inhibited antibiotic resistant strains and didn't inhibit other strains included in the test (Tab. 4). There was no specificity of inhibition of antibiotic resistance. The growth inhibition was detected in the case of host strain as well as in the case of recombinant antibiotic resistant strain. But the compound that inhibited *E. coli* DH5α strains didn't inhibit *C. glutamicum* 191 strains. For final conclusion, more experiments involving of more bacterial species should be done.

Table 4

The influence of non-protein amino acids and peptides on the growth of antibiotic resistant strains

Compound (10mM)	*E. coli* DH5α/ pUC18	*E.coli* DH5α/ VOG 10	*C. glutamicum* 191	*C. glutamicum* 191/pVOG10
(*S*)-β-[4-allyl-3-benzyl)-5-thixo-1,2,4--triazol-1-yl]-α-alanine	5*	+	+	+
(*S*)-β-[4-(furan-2-yl-methyl)-3-butyl)--5-thixo-1,2,4-triazol-1-yl]-α-alanine	+	+	5	5
(*S*)-β-[4-allyl-3-(2'-methoxyphenyl)-5--thixo-1,2,4-triazol-1-yl]-α-alanine	+	+	6	6
N-formyl-methionyl-(*S*)-β-[4-allyl-3--(pyridine-3'-yl)-5-thixo-1,2,4-triazol--1-yl]-α-alanine	+	+	3	3
N-formyl-methionyl-glycyl-(*S*)-β-[4--(furan-2-yl-methyl)-3-butyl)-5-thixo--1,2,4-triazol-1-yl]-α-alanine	5	3	+	+

\+ Bacterial growth;
* Diameter of transparent region, *mm.*

It is well known that a number of bacterial enzymes are involved in peptidoglycan biosynthesis, including DAla:DAla ligase, alanine racemase and D-amino acid transaminase. These enzymes are inhibited by derivatives of alanine [9]. Inhibitors of these enzymes are able to prevent construction of intact functional cell wall and thus inhibit bacterial growth. Such inhibitors may serve as new antibiotics like alanine analog, cycloserine, which is a racemase inhibitor [10]. The majority of non-protein amino acids studied in this work may be considered alanine derivatives. The results demonstrated that only few of them inhibited bacterial growth. The mode of bacterial growth inhibition by these compounds is still to be discovered. But our preliminary results of screening have shown that these inhibitors act in different ways possibly due to differences between target enzymes in different strains.

Received 30.09.2014

REFERENCES

1. **Katsuki T.** Some Recent Advances in Metallosalen Chemistry. // Synlett J., 2003, № 3, p. 281–297, DOI: 10.1055/s-2003-37101.
2. **Nishie M., Nagao J-I., Sonomoto K.** Antibacterial Peptides "Bacteriocins": An Overview of Their Diverse Characteristics and Applications. // Biocontrol Science, 2012, v. 17, p. 1–15.
3. **Derksen D.J., Stymiest J.L., Vederas J.C.** Antimicrobial Leucocin Analogues with a Disulfide Bridge Replaced by a Carbocycle or by Noncovalent Interactions of Allyl Glycine Residues. // J. Am. Chem. Soc., 2006, v. 128, p. 14252–14253.

4. **Bassetti M., Merelli M., Temperoni Ch., Astilean A.** New Antibiotics for Bad Bugs: Where are We? // Annals of Clinical Microbiology and Antimicrobials, 2013, v. 12, p. 1.
5. **Chitchyan M.B., Melkumyan M.A., Avetisyan N.S., Oganezova G.G., Hovhannisyan A.M., Hambartzumyan A.A., Hovhannisyan N.A.** Influence of Non-Protein Amino Acids and Pepides on Their Basis on Metabolizm of Microorganisms. // Biological Journal of Armenia, 2004, v. 59, № 3–4, p. 248–253.
6. **Hanahan D.** Studies on Transformation of *Escherichia Coli* with Plasmids. // J. Mol. Biol., 1983, v. 166, p. 557–560.
7. **Yanisch-Perron C., et. al.** Improved M13 Phage Cloning Vectors and Host Strains: Nucleotide Sequences of the M13mpl8 and pUC19 Vectors. // Gene, 1985, v. 33, p. 103–119.
8. **Sahakyan H.Y.** The Influence of Glutamate Dehydrogenase Gen Multiplying on Overproduction of Serine by Serine Strain-Producer *C.glutamicum* C-25. // Biological Journal of Armenia, 2005, v. 57, № 3–4, p. 225–231.
9. **Neuhaus F.C., Hammes W.P.** Inhibition of Cell Ball Biosynthesis by Analogues of Alanine. // Pharmacol. Ther., 1981, v. 14, p. 265–319.
10. **Cheung K-S., Wasserman S.A., Dudek E., Lerner S.A., Johnston M.** Chloroalanyl and Propargylglycyl Dipeptides. Suicide-Substrate-Containing Antibacterials. // J. Med. Chem., 1983, v. 26, p. 1733–1741.