AMYLOIDOGENIC PEPTIDES: NEW CLASS OF ANTIMICROBIAL PEPTIDES WITH THE NOVEL MECHANISM OF ACTIVITY

Oxana V. Galzitskaya

agalzit@vega.protres.ru
Institute of Protein Research
The beginning of the golden age of antibiotics

The Nobel Prize in Physiology or Medicine 1945
"for the discovery of penicillin and its curative effect in various infectious diseases"

I would like to remind you that thanks to these scientists, the discovery of antibiotics has become an important event in the development of science and medicine, and not only in these areas.
The widespread use of antibiotics in medicine, animal husbandry, aquaculture, plant growing forced the microworld to adapt, which led not only to antibiotic resistance, but also the spread of this phenomenon among various taxa of microorganisms.
The problem of antibiotic resistance

Source: Infectious Diseases Society of America
Antimicrobial peptides

Biological action:
Antimicrobial
Immunomodulatory
Antibiofilm Anticancer
Antiviral

Structural classes of antimicrobial peptides

The ability of amyloidogenic peptides to exhibit antimicrobial activity

Dependence of the optical density of the cell culture of *Paracoccus denitrificans* on the concentration of peptides

Creation of new antibacterial peptides based on targeted protein aggregation

Theoretical methods

- Search for unique domains in the bacterial proteins
- Bioinformatics search for amyloidogenic regions
- Molecular dynamics modeling of the penetration of peptides through the lipid layer

Experimental methods

- Toxicity testing of peptide against fibroblasts
- Identification of protein regions in the backbone of amyloid fibrils by mass spectrometry analysis
- Co-aggregation of amyloidogenic peptide and protein-target
- Studies of the process of fibril formation by AMPs

Design and synthesis of the amyloidogenic/antibacterial peptides
A model of directed co-aggregation of an amyloidogenic peptide based on the S1 ribosomal protein and the protein itself.

Experimental data:
- S1 (0.5 mg/ml)
- V10T (0.5 mg/ml)
- S1/V10T = 1:5 (0.5 mg/ml and 2.5 mg/ml)

Dysfunction of the target protein and antimicrobial action.

Antimicrobial and Amyloidogenic Activity of Peptides Synthesized on the Basis of the Ribosomal S1 Protein from Thermus Thermophilus

by Stanislav R. Kurpe 1,2,3, Sergei Yu. Grishin 1,2,3, Alexey K. Surin 1,2,3, Olga M. Seliwanova 1,2,3, Roman S. Fadeev 4,5, Ulyana F. Dzhus 1,2,3, Elena Yu. Gorbunova 2,5, Leila G. Mustavova 2,5, Vyacheslav N. Aseyev 2,5, and Oxana V. Galitskaya 1,4,6,7,8.
Choosing the target protein

criteria

Multifunctionality

Essentialness

Uniqueness

Ribosomal S1 protein

Participates in translation Initiation and regulation

S1 knockout leads to cell death

Only present in the bacterial cell
Number of structural S1 domains in bacteria changes strictly within a limited range from one to six

(A) Number of structural S1 domains in different bacteria (according to the SMART database). (B), (C), (D) NMR structures of the fourth (2KHI), fifth (5XQ5) and sixth (2KHJ) S1 domains from E.coli.
Analysis of amyloidogenicity of the 1453 sequences of S1 proteins and its domains

Average percentage of identity (A) and amyloidogenic regions (B) predicted by the FoldAmyloid program


Thermus thermophilus as a model organism for molecular biotechnology research

**Figure 50S ribosome subunit**

*T. thermophilus*


*T. thermophilus*, *E. coli* are model organisms

*Pseudomonas aeruginosa*, *Staphylococcus aureus* are pathogenic organisms
Aggregation of S1 ribosomal proteins, problems with crystallization

EM images of S1 protein aggregates from *T. thermophilus* (bS1)


EM images of S1 protein aggregates from *P. aeruginosa* (bPaS1)

Purpose: Development of amyloidogenic and antibacterial peptides based on the S1 protein from *T. thermophilus, E. coli, P. aeruginosa, S. aureus*

Tasks:

1. Search for amyloidogenic regions of the S1 protein.
2. Synthesize peptides based on the identified amyloidogenic sites.
3. Check the amyloidogenic and antibacterial properties of the obtained peptides.
4. Determine the minimum inhibitory concentration (MIC).
5. Determine the response of the cell proteome to treatment with AMP.
6. Determine the toxicity of peptides for eukaryotic cells.
S1 from E. coli

<table>
<thead>
<tr>
<th>Domain</th>
<th>Peptide Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 S1 motif</td>
<td>IVRGVVVAID (23-32)</td>
</tr>
<tr>
<td>2 S1 motif</td>
<td>DEITVKLVK (239-248)</td>
</tr>
<tr>
<td>3 домен</td>
<td>DEITVKLVK (239-248)</td>
</tr>
<tr>
<td>4 домен</td>
<td>TDYGCFVEIE (288-297) и VNVGDVEV (321-330)</td>
</tr>
<tr>
<td>5 домен</td>
<td>DFGIFIGLDG (376-385), VHLSDISWNV (391-400) и EIAAVVLQVD (414-423)</td>
</tr>
</tbody>
</table>

Amyloidogenic regions
- FoldAmyloid
- Waltz
- AGGRESCAN
- PASTA 2.0
S1 from E. coli

A


1) IVRGVVVAID (23-32)  2) DEITVKVLKF (239-248)  3) TDYGCFVEIE (288-297)  4) VVNVDVVEV (321-330)  5) DFGIFIGLDG (376-385)  6) VHLSDISWNV (391-400)  7) EIAAVVLQVD (414-423)

B

E. coli

IVRGVVVAID  DEITVKVLKF  TDYGCFVEIE  VVNVDVVEV  DFGIFIGLDG  VHLSDISWNV  EIAAVVLQVD

Structure of S1 protein predicted by AlphaFold 2
S1 from *T. thermophilus*

1 MEDKATQTEQTFSMEAALQETEARKVRPQGILTGKVVLGSEGVAVDIGAKTEGI 60

61 PFNO LTTKPLSEEELRNLSPGDEVKVQVLRVDPETQOILLSRKKIEAQE KDRIQELYE 120

121 KGEPVTITKERRVKKGGVVAELDGIOQFMPSQDLRRVPNLDVFGQOVLaughAKLIEFHRK 180

181 GRVILSRPAVLEEQQKAREAFLKSLPQGVVVEGTVEVTDGFVFVNLGPDGLVHRSEI 240

241 TWGR FNHPREVIQKQKVARKVLSVPDEKERVNLISIKALIPDPWTTVAEKPVGTAVRGK 300

301 VVGLTOFGAFVEVEPGLGEHISELWTKRKPHESEVKEGDEAVEVLRDLPEEORS 360

361 LGLKQTQPDPQQLTEKYPGTVLKGKVECMTDFGVFTEIEPGIEGLVHVESELDHKRVEN 420

421 PAALFKKGDEMEVVVLNIDPEVEQVSLRKLRLPPLPQEEERPERRRSGKERARRKGAP 480

481 RREDREYEYCAVAEYNLYDASSVPTTTATVKGDLYGDLIASLGEEEAEKSRG

**Amyloidogenic regions:**

- **FoldAmyloid**
- **Waltz**
- **AGGRESCAN**
- **PASTA 2.0**

1 domain
2 domain
3 domain: 1) VVEGTVEV (211-220) and 2) DFGVFVNLG (221-229)
4 domain
5 domain: 3) VTDFGFVEI (391-400) and 4) EMEVVVLNID (430-439)
S1 from *T. thermophilus*


1) VVEGTVVEVT(211-220)  2) DFGVFVNLG(221-229)  3) VTDFGVFVEI(391-400)  4) EMEVVVLNID(430-439)

**A** *T. thermophilus*

**B**

Structure of S1 protein predicted by AlphaFold 2
Results of HPLC-MS analysis products of the hydrolysis of S1 protein from *Thermus Thermophilus*

The proportion of the number of peptides of the S1 protein (non aggregated) by domain + fragment 449-518

- Disordered (449-518): 6%
- 1 (33-104): 4%
- 2 (122-188): 17%
- 3 (209-277): 24%
- 4 (294-364): 25%

The Peptide Mapping for products of the hydrolysis of S1 protein (non aggregated)

The proportion of the number of peptides of S1 aggregates by domain + fragment 449-518

- Disordered (449-518): 12%
- 1 (33-104): 14%
- 2 (122-188): 16%
- 3 (209-277): 10%
- 4 (294-364): 24%

The Peptide Mapping for products of the hydrolysis of S1 aggregates
Peptides from products of limited proteolysis of associates S1 Thermus:

F.SMEAALQETEARRAY. L 14-25
R.VRPGQILTGKVLVSEGVAVDIGAKTEGIFP.N 30-62
F.NQLTTEKLSEELRNLLPSGDEVQVVLVDPETQILLSRKIEAQQEKWDR.I 63-114
R.RVPNLDEFVGQQLAKITEFHRKGRVILS.R 157-187
R.VKGGVVAELDGIQGFMPASQ.D 133-153
K.SLEPGQVQEGTVVEVTDGFVFNLPVVDGLVHRSERITWGRFNHPREVQKGQ.V 205-257
K.SLEPGQVQEGTVVEVTDGFVFNLPVVDGLVHRSERITWGRFNHPREVQKGQ.V 205-249
R.VRGKVGLTQFGAFVEVPGLELIHISELSGTKPKHPSEVVEVKGDEVEAV.VL.R 297-350
R.VRGKVGLTQFGAFVEVPGLELIHISEL.SW.T 297-328
K.ALPDPWTTVAEKYPGTRVRGKVGLTQFGAFVEVPGLELIHISELSW.T 278-328
K.ALPDPWTTVAEKYPGTRVRGKVGLTQFGAFVEVPGLELIHISELSW.T 278-340
F.KKGDEMEVVVLNIDPVEQRVSLRKRLLPPPLPQEEPR.R 426-465
K.RVENPAALFVKGDEMEEVVVLNIDPVEQRVSLRKRLLPPPLPQEEPR.R 417-465
K.QUITPDPWQQLTKEYPPGTVLKGKTGVTDGFVFEVEPIEGELVHSVSELDHK.R 365-416
Y.DASSVPTTATV.K 500-512
R.REDRREYEYGAVAENLYDASSVPTTATVKGDLYGDLISSLGEEEAEK.S 482-533
R.KGAPRREDREYEYGAVAEYNLYDASSVPTTATVKGDLYGDLISSLGEEEAEK.S 477-533

Amyloidogenic regions:
- **FoldAmyloid**
- Waltz
- AGGRESCAN
- PASTA 2.0
Search results for amyloidogenic sites bPaS1

Results of HPLC-MS analysis of bPaS1 aggregates

Results of HPLC-MS analysis of "unaggregated" bPaS1

Domain organization scheme bPaS1:

MSESFAEELF ESLKSLDMQP GAITGIVDD IDGDWTVHRA GLKSEGV1PV EQFYN3EQGEL

TIKVGEVHV ALADEVDFG ETKLSEKAK RAESWIVLEA AFR4ADEVVKG VINGKVKGGF

TVDVNGIRAF LGPLVDVRP VRDTHLEGK ELEFKIKLD QKRNNVVSRR SRSLEAENA

EREALLESQ EGQVKGIVK NLDTDYGVFVD LGGVDSLLHI TMAMWIRKHK PSEIVNVGDE

IDVVKLFDF DRNRSVLGLK QRGEVNWAI KARYPEGTRV MARVNLNDY GCF3AEE3EGV

EGLVHVSEM3 WTNNHIPS RKVQGEVEV QVDIDEERR RISLGKQCK SNFWEDFSSQ

FNKGDRISGT IKTITDGIF IGLDGGIDGL VHLSDISNE VGEEAVRRFK KGDELETVIS

SVDSERERIS LGIKQLEDPP FSNYASLHEK GSIVRTVKE VDAKGAVISL GDDIEGILKA

SEISRDRVED ARNVKEEGEE VEAKISIDR KSRVISLKV SKDVDEKDA MKELRKQEVE

SAPPTTIGDL IRAQMNQG

FoldAmyloid

Aggrescan

Waltz

Pasta 2.0
S1 from *P. aeruginosa*

1. AIITGIVVDI (22-31)
2. SWIVLEAAFA (94-103)
3. LHITDMAWKR (218-227)
4. ITDFGIFIGL (374-383)

**Structure of S1 protein predicted by AlphaFold 2**
**S. aureus**

Structure of S1 protein predicted by AlphaFold 2

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**D**

**S. aureus**

**VQGLVHISEI**

**VVVHINGGKF**

**GVVVRLANFG**

**QQVNVKILGI**

**Structure of S1 protein predicted by AlphaFold 2**
Development and design of peptides based on the ribosomal S1 protein *T. thermophilus*

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>Prediction of antimicrobial activity (CAMPR3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Support vector machine (SVM) Random Tree Method (RF) Discriminant Analysis (DA)</td>
</tr>
<tr>
<td>D9G</td>
<td>DFGVFVNLG</td>
<td>0.00 0.41 0.03</td>
</tr>
<tr>
<td>E10D</td>
<td>EMEVVVLNID</td>
<td>0.10 0.45 0.01</td>
</tr>
<tr>
<td>V10I</td>
<td>VTFDFGVFVEI</td>
<td>0.80 0.44 0.03</td>
</tr>
<tr>
<td>V10T</td>
<td>VVEGTVVVEVT</td>
<td>0.08 0.50 0.00</td>
</tr>
</tbody>
</table>

### Prediction of antimicrobial activity

<table>
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<tr>
<th>Peptide</th>
<th>Sequence</th>
<th>Prediction of antimicrobial activity (CAMPR3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Support vector machine (SVM)</td>
</tr>
<tr>
<td>R23I</td>
<td>RKKRRQRRRGGAGVTDFGVFVEI</td>
<td>0.03</td>
</tr>
<tr>
<td>R23T</td>
<td>RKKRRQRRRGGAEGVVEGTVVEVT</td>
<td>0.01</td>
</tr>
</tbody>
</table>

1. Increases antimicrobial activity
2. Promotes penetration into the eukaryotic cell
3. Does not interfere with the formation of aggregates of proteins and fibrils

Structures of S1 proteins predicted by AlphaFold 2

Construction of hybrid peptides

**A. T. thermophilus**

- RKKRRQRRR + GlyGlySarGly + **VVEGTVEVT** R23T
- RKKRRQRRR + GlyGlySarGly + **VTDFGFVEI** R23I

**B. E. coli**

- RKKRRQRRR + GlyGlySarGly + **LHITDMAWKR** R23R
- RKKRRQRRR + GlyGlySarGly + **ITDFGIFIGL** R23I

**C. P. aeruginosa**

- RKKRRQRRR + GlyGlySarGly + **LHITDMAWKR** R23R
- RKKRRQRRR + GlyGlySarGly + **ITDFGIFIGL** R23I

**D. S. aureus**

- RKKRRQRRR + GlyGlySarGly + **VVEGTVEVT** R23T
- RKKRRQRRR + GlyGlySarGly + **VTDFGFVEI** R23I
Co-aggregation of amyloidogenic peptides and ribosomal S1 protein

It was similarly verified that coaggregation of the V10T peptide and the S1 protein at 5:1 ratio led to the formation of fibrils.

S1:V10T=1:5 (0.5 mg/ml and 2.5 mg/ml)

S1:R23T=1:5 (0.5 mg/ml and 2.5 mg/ml) from *T. thermophilus*
Kinetics of S1 coaggregation with peptides R23R and R23L
Fluorescence results with ThT, incubation at 37 °C

During co-aggregation of the S1 protein and R23R from P. aeruginosa, both aggregates of different sizes and fibrils of different diameters are observed. In addition, film-like polymers can sometimes be observed.
Antibacterial properties of peptides synthesized based on the predicted amyloidogenic sites of S1

\[ \mathcal{E} = 1 - \frac{A_{\text{Experiment}}}{A_{\text{Control}}} \] (1)

The evaluation of the antibacterial effect (E) was carried out according to formula (1), where A is the light absorption of the liquid culture of \(T.\ thermophilus\) after 24 hours of incubation. An E value greater than 0.5 indicates an antibacterial effect.

<table>
<thead>
<tr>
<th>Sequence peptide</th>
<th>Peptide concentration and presence (+) or absence (-) antibacterial effect</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 μg/ml</td>
</tr>
<tr>
<td>DFGVFVNLG</td>
<td>−</td>
</tr>
<tr>
<td>EMEVVVLNID</td>
<td>−</td>
</tr>
<tr>
<td>VTDFGVFVEI</td>
<td>−</td>
</tr>
<tr>
<td>VVEGTVVEVT</td>
<td>−</td>
</tr>
<tr>
<td>RKKRQRRRGSarG VTDFGVFVEI</td>
<td>−</td>
</tr>
<tr>
<td>RKKRRQRRRGSarG VVEGTVVEVT</td>
<td>−</td>
</tr>
</tbody>
</table>
Inhibitory activity of peptides against methicillin-resistant S. aureus

**A.** R23 F against MRSA (ATCC 43300 strain)

- Optical density a.u.
- Incubation time, hour
- Lines represent different concentrations of R23 F: 0.75 μM, 1.5 μM, 3 μM, 6 μM, 12 μM, and Gentamicin sulfate.

**B.** R23 DI against MRSA (ATCC 43300 strain)

- Optical density a.u.
- Incubation time, hour
- Lines represent different concentrations of R23 DI: 0.75 μM, 1.5 μM, 3 μM, 6 μM, 12 μM, and Gentamicin sulfate.

**C.** R23 EI against MRSA (ATCC 43300 strain)

- Optical density a.u.
- Incubation time, hour
- Lines represent different concentrations of R23 EI: 0.75 μM, 1.5 μM, 3 μM, 6 μM, 12 μM, and Gentamicin sulfate.
Inhibitory activity of peptides

A) R23F against *S. aureus* (209P strain)

B) R23DI against *S. aureus* (209P strain)

C) R23EI against *S. aureus* (209P strain)
Inhibitory activity of peptides

A. R23F against *P. aeruginosa* (ATCC 28753 strain)

B. R23DI against *P. aeruginosa* (ATCC 28753 strain)

C. R23EI against *P. aeruginosa* (ATCC 28753 strain)
## Amyloidogenic AMP peptides based on S1 ribosomal protein *P. aeruginosa*

<table>
<thead>
<tr>
<th>Sequence of hybrid peptides and folding patterns predicted by AlphaFold</th>
<th>Strain of the pathogenic microorganism</th>
<th>MIC for the tested hybrid peptide (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Based on the sequence S1 protein from <em>P. aeruginosa</em></strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| **RKKRRQRRRGGGGITDFGIFIGL** | MRSA strain ATCC 43300 (resistant to ampicillin)  
S. aureus strain 209 (resistant to aztreonam)  
P. aeruginosa (strain ATCC 28753) (resistant to sulfamethoxazole) | 12  
>12  
12 |
| **RKKRRQRRGGSarGLHITD-Nle-AWKR** | P. aeruginosa (strain ATCC 28753) (resistant to sulfamethoxazole)  
P. aeruginosa (strain PA 103) (resistant to levomycetin) | 12  
>12  
12 |
| **RKKRRQRRGGSarGITDFGIFIGL** | P. aeruginosa (strain ATCC 28753) (resistant to sulfamethoxazole)  
P. aeruginosa (strain PA 103) (resistant to levomycetin) | 12  
>12 |

## Amyloidogenic AMP peptides based on S1 ribosomal protein *S. aureus*

**Based on the sequence S1 protein from *S. aureus***

<table>
<thead>
<tr>
<th>Peptide Sequence</th>
<th>MRSA strain ATCC 43300 (resistant only to ampicillin)</th>
<th>S. aureus strain 209 (resistant to aztreonam)</th>
<th>P. aeruginosa strain ATCC 28753 (resistant to sulfamethoxazole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RKKRRQRRRGSarGVVVHI-Asi-GGKF</td>
<td>3</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>RKKRRQRRRGSarGLTQFGAFIDI</td>
<td>3</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>RKKRRQRRRGSarGVQGLVHISEI</td>
<td>6</td>
<td>12</td>
<td>&gt;12</td>
</tr>
</tbody>
</table>

Effects of peptide treatment on survival the human fibroblasts (A) and breast tumor cell line BT-474 (B). Error bars show standard errors.
Effect of peptide treatment on the survival of human fibroblasts (A) and the breast tumor cell line BT-474 (B) without preliminary incubation (18 h, 37 °C, in DMEM with 10% FBS) and after 72 h of co-incubation with peptide. Each of the experiments was carried out at least three times ($n \geq 3$)
Hypothetical mechanism of targeted co-aggregation against CoV

Conclusion

Theoretically predicted and experimentally confirmed the ability to manifest aggregation (amyloidogenic) and antibacterial properties of the studied peptides. New antibacterial peptides have been created that effectively suppress the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*, pathogenic bacteria that cause hospital infections.
Acknowledgments


The work was supported by the grants from the Russian Science Foundation 18-14-00321.
State-of-the-Art Macromolecules in Russia

Guest Editors
Prof. Dr. Ilya Nifant'ev, Prof. Dr. Oxana V. Galzitskaya
Thank you for your attention! Questions?