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The search for new biological activities for selected insect peptides

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Abstract: New biological properties of selected insect peptides are presented. The subjects of the investigation included insect oostatic peptides, like *Neb*-colloostatin (I) and *Neb*-TMOF(II), and/or insect peptides with antiviral or antitumor activity, such as alloferon (III) and its analogues modified at position 1 of the peptide chain. In the study was also included the oligopeptide Any-GS (VII) and its truncated analogues. The peptides were tested for antimicrobial activity on a series of bacterial species, antiviral activity against *Human Herpes Virus* type 1 (HHV-1) *in vitro* using a Vero cell line, and the growth and development of plant pathogens *Phoma narcissi* and *Botrytis tulipae*. The results of the biological investigations indicate that among the peptides investigated, compounds VII and IX inhibit the growth of plant pathogens *P. narcissi* and *B. tulipae*, whereas compounds I and II stimulate the mycelium growth of the aforementioned pathogens. Other peptides show slow antimicrobial activity but do not inhibit the replication of HHV-1 in Vero cells.

Keywords: insect oostatic peptides, antiviral peptides, antitumor peptides, antimicrobial activity, antifungal activity, antiviral activity

INTRODUCTION

Natural products isolated from arthropods are an important source of bioactive compounds. In the last decade a number of peptides with a large variety of biological activities were isolated from insects [1-4]. These peptides show oostatic activity in insects [2] and/or antiviral or antitumor activity [3, 4]. The subject of our investigation was a search for new biological properties of a series of insect peptides (Table 1), such as oostatic peptides – *Neb*-colloostatin (**I**) and *Neb*-TMOF (**II**), antiviral or antitumor peptides – alloferon (**III**) and Any-GS (**VII**) and a series of their analogues.

Table 1. Selected insect peptides and their analogues

Peptides sequence	Organism	Biological properties	Reference
Neb-colloostatin: H-Ser-Ile-Val-Pro-Leu-Gly-Leu-Pro-Val-Pro-Ile-Gly-Pro-Ile-Val-Val-Gly-Pro-Arg-OH (**I**)	fleshfly *Neobellieria bullata*	oostatic peptides	[5]
Neb-TMOF: H-Asn-Pro-Thr-Asn-Leu-His-OH (**II**)	fleshfly *Neobellieria bullata*	oostatic peptides	[6]
alloferon 1: H-His-Gly-Val-Ser-Gly-His-Gly-Gln-His-Gly-Val-His-Gly-OH (**III**)	blow fly *Calliphora vicina*	antiviral and antitumoral capabilities	[3]
alloferon 2: H-Gly-Val-Ser-Gly-His-Gly-Gln-His-Gly-Val-His-Gly-OH (**IV**)	blow fly *Calliphora vicina*.	antiviral and antitumoral capabilities	[3]
[Lys^1]-alloferon 1: H-Lys-Gly-Val-Ser-Gly-His-Gly-Gln-His-Gly-Val-His-Gly-OH (**V**)	synthetic		
[Arg^1]-alloferon 1: H-Arg-Gly-Val-Ser-Gly-His-Gly-Gln-His-Gly-Val-His-Gly-OH (**VI**),	synthetic		
Any-GS: H-Asp-Ile-Leu-Arg-Gly-NH_2 (**VII**)	wild silkmoth *Antheraea yamamai*	antitumor activity	[4]
[2-5]-Any-GS H-Ile-Leu-Arg-Gly-NH_2 (**VIII**)	synthetic	antitumor activity	[4]
[3-5]-Any-GS H-Leu-Arg-Gly-NH_2 (**IX**),	synthetic		
[1-4]-Any-GS H-Asp-Ile-Leu-Arg-NH_2 (**X**)	synthetic		
[1-3]-Any-GS H-Asp-Ile-Leu-NH_2 (**XI**).	synthetic		

Neb-colloostatin (**I**) [5] and *Neb*-TMOF (**II**) [6] have been isolated from the fleshfly *Neobellieria bullata*. Because *Neb*-colloostatin (**I**) and *Neb*-TMOF (**II**) show a very strong inhibition of oocyte growth [7], it inspired us to search new biological properties with respect to the growth of viruses, bacteria and fungi.

Alloferon (**I**) has been isolated from the blow fly *Calliphora vicina* [3]. *In vitro*, alloferon demonstrates stimulatory activity on natural killer lymphocytes and *in vivo* this peptide has antiviral and antitumor capabilities. The pentapeptide Any–GS (**VII**) has been isolated from the wild silkmoth *Antheraea yamamai* [8]. This peptide suppresses proliferation of rat hepatoma cells [4].

All peptides were synthesized by the classical solid-phase method according to previously described procedures [9].

During the biological investigations of the peptides, we performed a search for their antimicrobial activity on a series of bacterial species, antiviral activity against *Human Herpes Virus* type 1 (HHV-1) *in vitro* using a Vero cell line, and growth and development activity on plant pathogens such as *Phoma narcissi* and *Botrytis tulipae*.

MATERIALS AND METHODS

Peptides synthesis

Peptides were synthesized by the classical solid phase method according to Fmoc or Boc-procedures. Amino acids were assembled either on a Wang (peptide acids) or MBHA resin (peptide amides). All peptides were purified on a Sephadex G-15 column followed by preparative HPLC.

Biological part

Growth of *Phoma narcissi* and *Botrytis tulipae*

Five mm diameter plugs taken from a 7-day-old culture of tested fungus were placed in the middle of 90 mm Petri dishes containing PDA medium (potato-dextrose-agar) along with peptides (**I**, **II, III**, and **VII-XI**) at a concentration of 40-60 μg/cm^3. Control plates contained the culture growing on PDA without any additions. The diameter of the fungi colonies was measured within a 4-day incubation period at 25 °C in the darkness. The data were subjected to analysis of variance and a Duncans multiple range test.

Antimicrobial assay

The following strains were tested: *Bacillus subtilis*, *Enterococcus faecalis* ATCC 11420, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus aureus*

ATCC 6538, *Escherichia coli* ATCC 8739, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* ATCC 9027*, Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 16404. All microorganisms were obtained from the Polish Collection of Microorganisms (Polish Academy of Sciences, Institute of Immunology and Experimental Therapy, Wrocław, Poland).

The minimal inhibitory concentration (MIC) was determined using a microbroth dilution method with a Mueller-Hinton (MH) broth or a Sabouraud Dextrose broth (Becton Dickinson, Le Pont de Claix, France). Polypropylene 96-well plates (Nunc GmbH & Co. KG, Germany) were incubated for 18 h at 37 °C in air (for bacteria) and 72 h at 25 °C (for fungi). The MIC was taken as the lowest peptide analogue concentration at which a noticeable growth inhibition occurred. Experiments were performed in triplicate.

Antiviral activity

Cytotoxic activity of *Neb*-colloostatin (**I**) and *Neb*-TMOF (**II**) was evaluated *in vitro* by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide) assay on a Vero cell line. Antiviral activity was assessed against Human Herpes Virus type 1 McIntrie (HHV-1_{MC}) *in vitro* using a Vero cell line infected with HHV-1_{MC} 0,01$TCID_{50}$/cell.

RESULTS AND DISCUSSION

During the investigation of the influence of peptides on the growth and development of plant pathogens (*P. narcissi* and *B. tulipae*), we found that peptide **I** (44 $\mu g/cm^3$), after 24 h of incubation, stimulates the growth of mycelium *B. tulipae in vitro* by 250%, although after three and four days of incubation the stimulatory effect is considerably weaker (Tables 2 and 3). However, another insect oostatic peptide (**II**) after one day of incubation demonstrates a marked inhibitory effect on the development of both pathogens of 11% and 50%, respectively. However, after four days of incubation it shows a weak stimulatory effect on the development of *P. narcissi* and *B. tulipae.*

Table 2. Influence of selected insect peptides on the colony growth of *Phoma narcissi*

Peptides	Concentration $\mu g/cm^3$	Inhibiotory or stimulatory (+) effect on *in vitro* growth of mycelium (% of control) after incubation [days]			
		1	2	3	4
Neb-colloostatin (**I**)	44.0	+11.3	+27.3	nd	+8.1
Neb-TMOF (**II**)	44.0	11.1	+13.6	nd	+13.3
Alloferon (**III**)	40.0	5.6	+11.4	nd	+3.5
Any-GS (**VII**)	52.0	7.7	+9.1	31.0	1.7
H-Ile-Leu-Arg-Gly-NH_2 (**VIII**)	48.0	0.0	+9.1	+25.3	+1.1
H-Leu-Arg-Gly-NH_2 (**IX**)	58.0	23.1	0.0	14.1	3.5
H-Asp-Ile-Leu-Arg-NH_2 (**X**)	48.0	15.4	13.6	+26.8	14.9
H-Asp-Ile-Leu-NH_2 (**XI**)	46.0	23.1	4.5	+40.8	13.8

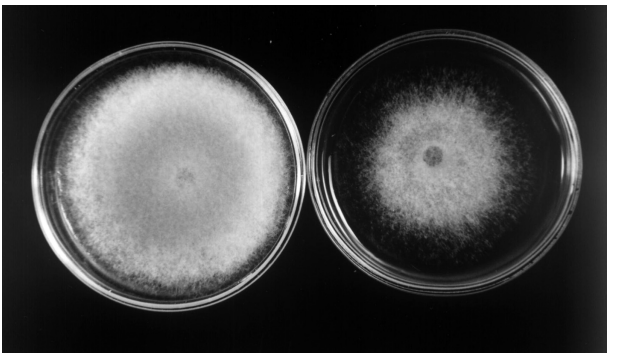
Table 3. Influence of selected insect peptides on the colony growth of *Botrytis tulipae*

Peptides	Concentration $\mu g/cm^3$	Inhibiotory or stimulatory (+) effect on *in vitro* growth of mycelium (% of control) after incubation [days]			
		1	2	3	4
Neb-colloostatin (**I**)	44.0	+250.0	+7.3	nd	+18.4
Neb-TMOF (**II**)	44.0	50.0	+54.5	nd	+10.5
Alloferon (**III**)	40.0	0.0	12.7	nd	+6.6
Any-GS (**VII**)	52.0	33.3	25.0	+17.3.0	36.2
H-Ile-Leu-Arg-Gly-NH_2 (**VIII**)	48.0	0.0	+17.9	+13.3.3	+12.6
H-Leu-Arg-Gly-NH_2 (**IX**)	58.0	33.3	21.4	1.0	25.3
H-Asp-Ile-Leu-Arg-NH_2 (**X**)	48.0	+16.7	+21.4	3.1	+12.6
H-Asp-Ile-Leu-NH_2 (**XI**)	46.0	+50.0	+28.6	5.1	+3.4

Any-GS (**VII**) inhibits the mycelium growth of *P. narcissi in vitro* at a concentration of 52 $\mu g/cm^{-3}$, by 7-31% (Table 2, Figure 1). A similar activity was observed for peptides **IX**-**X**. In the test on the growth of *B. tulipae*, alloferon (**III**) and Any-GS (**VII**) inhibit the development of this pathogenic fungi (Table 3, Figure 2), whereas peptides **VIII**, **X**, and **XI** stimulate the growth of mycelium. Further studies are required to determine structure/function relationships for the peptides studied.

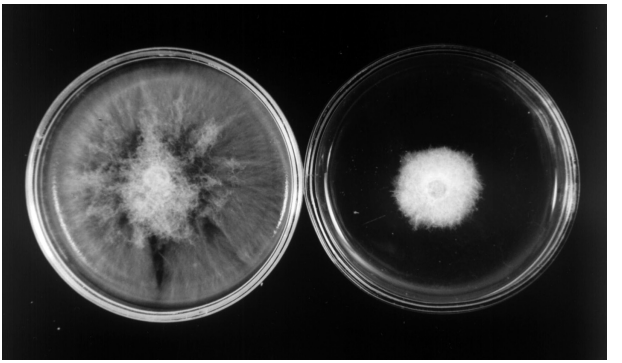
The biological investigations reported here indicate that peptides studied exert a low antimicrobial activity against all investigated strains (MIC>512 μg/ml).

In the antiviral test, the investigated peptides do not inhibit the replication of HHV-1 in Vero cells.



Control Peptide

Figure 1. Influence of Any-GS (**VII**) on the growth mycelium of *Phoma narcissi in vitro*; after 3 days of incubation.



Control Peptide

Figure 2. Influence of Any-GS (**VII**) on the growth mycelium of *Botrytis tulipae in vitro*; after 4 days of incubation.

CONCLUSION

Based on the biological results presented here we can conclude that :

- compounds **VII** and **IX** inhibit the growth of plant pathogens *P. narcissi* and

B. tulipae, whereas compounds **I**, **II**, and **VIII** stimulate the mycelium growth of *P. narcissi* or *B. tulipae*,
- all tested insect peptides demonstrate low antimicrobial activity,
- the investigated peptides do not inhibit the replication of HHV-1 in Vero cells.

The influence of the insect peptides on the plant pathogens represent the most significant results in this study, and these effects will be the subject of further investigations.

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