Cyanobacterial oligopeptides isolation and structure-activity studies

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Introduction

Cyanobacteria are a rich source of secondary metabolites which show diverse structure and biological activity. Some of the metabolites play an important ecological role in interactions with aquatic biota. Other compounds have biotechnological application. Peptides belong to the most widely studied group of cyanobacterial metabolites. Many of the compounds belong to inhibitors of thrombin (e.g. aeruginosins), trypsin (e.g. cyanopeptoline), elastase and chymotrypsin (e.g. planktopeptin) and carboxypeptidase A (e.g. anabaenopeptins). Enzymes play central role in the human organism. Naturally produced inhibitors of serine proteases are considered to be potential therapeutic agents in cardiovascular disorders, thrombosis, viral infections and other health problems.

The aim of study was isolation of anabaenopeptins produced by the Baltic cyanobacteria. In this work, the biological activity of anabaenopeptins was tested in enzymatic assays.

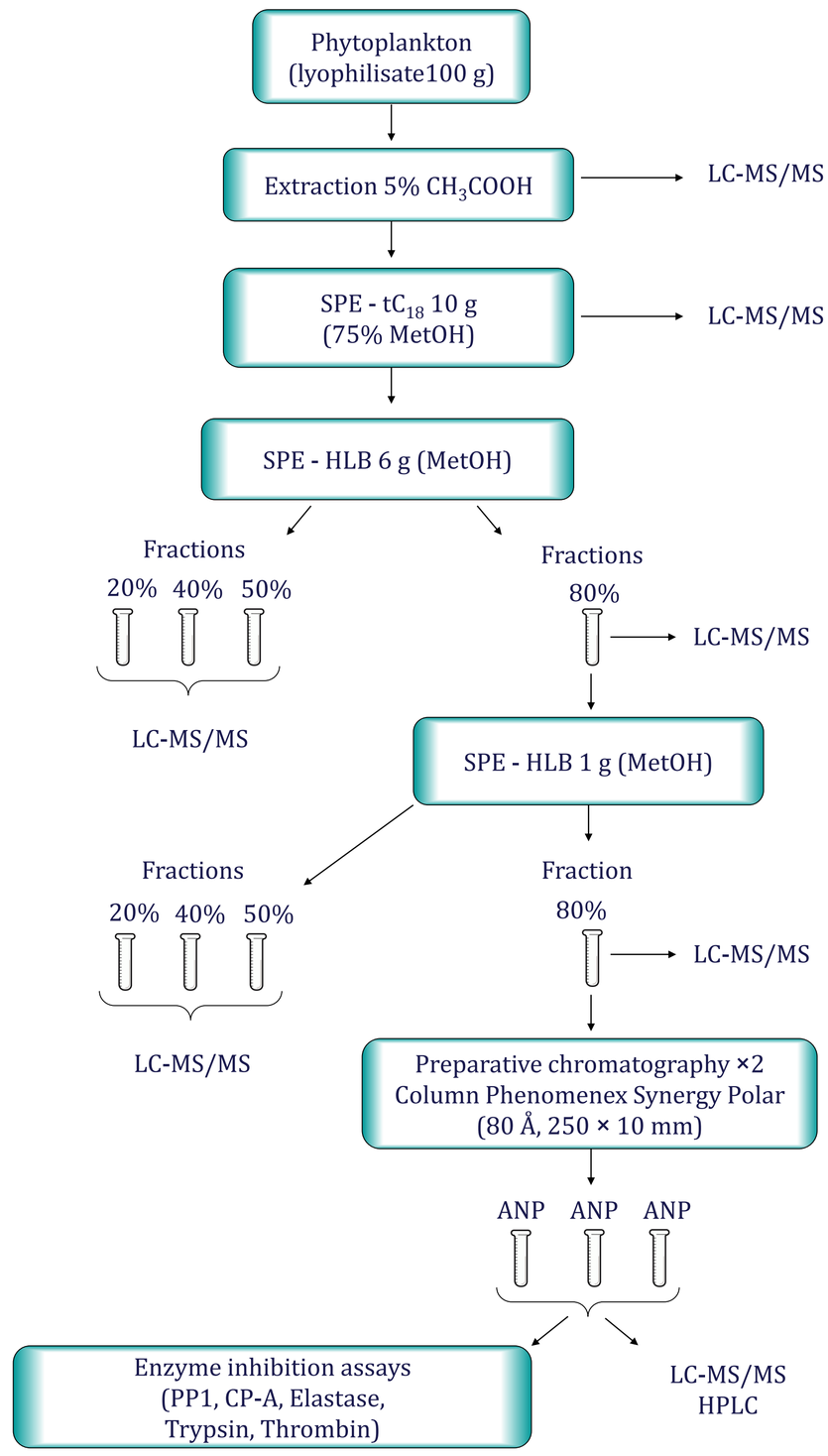
Materials and methods

After collection of cyanobacterial cells (Gulf of Gdansk, 6 July 2012) and their liophylization, extract in 5% acetic acid was prepared. The cyanobacterial extracts was purified by solid-phase extraction (tC_{18} SPE and OASIS HLB SPE) and then analyzed by LC-MS/MS system. The eluted samples containing oligopeptides were selected and subjected to further fractionation using preparative liquid chromatography HPLC. The chromatographic conditions were optimized for the best separation of peptides present in a sample. The final products were analyzed using liquid chromatography (HPLC-DAD) and different MS/MS experiments (including fragmentation of molecular ions).
Additionally biological activitie of anabaenopeptins was tested. Enzyme inhibition assays were based on the spectrophotometric measurements. Following enzymes were used: thrombin (Ocampo Bennet 2007), trypsin (Pluotno & Carmeli 2005), elastase (Kwan et al. 2009), carboxypeptidase-a (Hass et al. 1981), protein phosphatase PP1(Rapala at al. 2002).

Table. 1. Structures of peptides detected in methanolic fractions.

P.	*m/z*	Peptide structures	Fractionation I – HLB SPE				Fractionation II – HLB SPE			
			20%	40%	50%	80%	40%	50%	60%	80%
SPU	583	Hpla-Tyr-Pro-Argal		■	■	■				
	595	Hpla-Hph-MePro-Argal		■	■					
	597	Hpla-Hty-Pro-Argal		■	■	■		■		
	599	Hpla-Hty-Pro-Argol	■	■						
	611	Hpla-Hty-MePro-Argal		■	■	■		■	■	
	613	Hpla-Hty-Pro-Arg	■	■	■					
	627	Hpla-Hty-MePro-Arg	■	■	■	■				
	639	(Hpla + 42)-Hty-Pro-Argal	■	■						
AER	561	Bu-Tyr-Choi-Argol	■	■	■	■	■			
	587	Hex-Tyr-Choi-Argal		■	■	■	■	■		
	589	Hex-Tyr-Choi-Argol		■	■	■	■			
	719	?-Tyr-Choi-?		■						
MC	995	Ala-Leu-MeAsp-Arg-Adda-Glu-MeDha			■	■			■	
NOD	811	Cyclo[Asp-Arg-Adda-Glu-Mdhb]			■	■			■	
	825	Cyclo[MeAsp-Arg-Adda-Glu-Mdhb]			■	■			■	
ANP	808	Ile-CO-[Lys-Ile-Hty-MeAla-Phe]			■	■			■	■
	824	Ile-CO[Lys-Val-Hphe-MeHty-Ser]			■	■			■	
	828	Phe-CO-[Lys-Val-Hty-MeAla-Phe]				■			■	■
	842	Phe-CO-[Lys-Ile-Hty-MeAla-Phe]				■			■	
	844	Tyr-CO-[Lys-Val-Hty-MeAla-Phe]				■			■	■
	850	Ile-CO-[Lys-Val-Hph-MeHph-AcSer]				■				■
	851	Arg-CO-[Lys-Ile-Hty-MeAla-Phe]				■			■	
	856	Ile-CO-[Lys-MetO-Hph-MeHyph-Ser]				■			■	■
	858	Tyr-CO-[Lys-Ile-Hty-MeAla-Phe]	■	■	■	■			■	■
	866	Ile-CO-[Lys-Val-Hty-MeHty-Ile]				■				■
	868	Ile-CO-[Lys-Val-Hph-MeHty-Met]				■				■
	880	Ile-CO-[Lys-Ile-Hph-MeHty-AcSer]				■				■
	884	Ile-CO-[Lys-Met-Hph-MeHph-Met]				■			■	■
	886	Ile-CO-[Lys-Met-Met-MeHty-MetO] ?				■				■
	900	Phe-CO-[Lys-Val-Hph-MeHty-AcSer]				■				■
	902	Phe-CO-[Lys-Val-Hph-MeHty-Met]				■			■	
	914	Ile-CO-[Lys-MetO-Hph-MeHty-AcSer]				■				■
	916	Ile-CO-[Lys-MetO-Hph-MeHty-Met]				■				■
	918	Phe-CO-[Lys-Val-Hph-MeHty-MetO]				■				■
	934	Phe-CO-[Lys-Val-Hty-MeHty-MetO]				■			■	■

■ Peptide ■ Intensity e^6 ■ Intensity e^7



Scheme 1. Extraction and purification of cyanobacterial peptides.

Results

The application of this method confirmed the presence several anabaenopeptins isolated from *Nodularia spumigena*. Among the isolated compounds we found 4 new analogues of APs (*m/z* 808, 884, 916, 934). All fractions showed PP1 and CP-A inhibitory activity. Two fractions contained APs *m/z* 850 and 858 inhibited thrombin activity. In elastase and trypsin inhibitory assays no activity were detected. (Tab. 2).
The biological assays indicate which of the isolated peptides should be subjected to further studies into their potential application as pharmaceuticals.

Table. 2. Protease and protein phosphatase inhibitory activity.

m/z	Peptide structure	PP1	CP-A	Elastase	Trypsin	Thrombin
808	Ile-CO-[Lys-Ile-Hty-MeAla-Phe]	■	■			
828	Phe-CO-[Lys-Val-Hty-MeAla-Phe]	■	■			
844	Tyr-CO-[Lys-Val-Hty-MeAla-Phe]	■	■			
850	Ile-CO-[Lys-Val-Hph-MeHph-AcSer]	■	■			■
858	Tyr-CO-[Lys-Ile-Hty-MeAla-Phe]	■	■			■
866	Ile-CO-[Lys-Val-Hty-MeHty-Ile]	■	■			
868	Ile-CO-[Lys-Val-Hph-MeHty-Met]	■	■			
884	Ile-CO-[Lys-Met-Hph-MeHph-Met]	■	■			
900	Phe-CO-[Lys-Val-Hph-MeHty-AcSer]	■	■			
916	Phe-CO-[Lys-Val-Hty-MeHty-AcSer]	■	■			
918	Phe-CO-[Lys-Val-Hph-MeHty-MetO]	■	■			
934	Phe-CO-[Lys-Val-Hty-MeHty-MetO]	■	■			

■ Weak inhibition ■ Strong inhibition

References:
Hass, G. M. i Ryan, C. A. (1981). Carboxypepidase inhibitor from potatoes. Meth. Enzymol, 80, 778-791
Kwan, C.J., Taori, K., Paul, J.V. i Luesch, H. (2009). Lyngbyastatins 8–10, elastase inhibitors with cyclic depsipeptide scaffolds isolated from the marine cyanobacterium Lyngbya semiplena. Marine Drugs, 7: 528-538.
Ocampo Bennet, X. (2007). Peptide au seiner Cyanobakterien Wasserblütte (1998) aus dem Wannsee/Berlin: Strukturen and biologische Wirksamkeit. University of Freiburg, Freiburg, 28 pp.
Pluotno, A. i Carmeli, S. (2005). Banyasin A and banyasides A and B, three novel modified peptides from a water bloom of the cyanobacterium Nostoc sp. Tetrahedron, 61: 575–583.
Rapala, J., Erkomaa, K., Kukkonen, J., Sivonen, K. i Lahti, K. (2002). Detection of microcystins with protein phosphatase inhibition assay, high-performance liquid chromatography–UV-detection and enzyme-linked munosorbent assay. Comparison of methods. Analytica Chimica Acta, 466: 213-231.

This study was financially supported by the project CYANOCOST (ECOST-STSM - ES1105-070514-043323) and the National Science Centre in Poland (NCN 2012/0518/B/NZ01222).