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BIOLOGICALLY ACTIVE PEPTIDES IN CYANOBACTERIA

Péptidos biológicamente activos en cianobacterias

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RESUMEN

Las cianobacterias se encuentran en el medio natural tanto en aguas dulces como saladas. Ellas pueden desarrollarse en grandes masas formando "blooms" (florecimientos) en aguas dulces y saladas en diferentes partes del mundo, incluyendo América del Sur. Tales florecimientos, asi como crecimientos axénicos de cianobacterias, pueden ser una rica fuente de péptidos lineales o cíclicos únicos, muchos de los cuales presentan actividad biológica. En el pasado la mayor atención ha sido puesta en las toxinas microcistina y nodularina. Estos péptidos cíclicos son hepatotoxinas que inhiben la proteína fosfatasa 1 y 2 A, después de ingresar especificamente al hepatocito mediante la captación de las sales biliares. Sin embargo, en cianobacterias se están encontrando péptidos con otras actividades biológicas. No obstante, aunque no se consideren tóxicos, estos péptidos tienen actividades biológicas tales como: una fuerte y especifica inhibición de las proteasas (tripsina, quimo-tripsina, elastasa, trombina, plasmina y la enzima procesadora angiotensina), anticianobacterias, antialgas, antihongos, inmunosupresores y promotores de diferenciación celular. Ejemplos de péptidos cianobacteriales inhibidores de proteasas son las cianopeptolinas. Las interacciones de microcistina/proteína fosfatasa y de cianopeptolina/proteasa, han sido bien estudiadas por difracción de Rayos X en cocristales y la determinación de microcistina y de otros péptidos puede ser realizada por métodos químicos y biológicos.

Ambas, microcistina y cianopeptolina han sido recientemente determinadas en blooms producidos en cuerpos de agua en Chile, utilizando cromatografía liquida de alta resolución (HPLC), espectrometria de masas (MALDI-TOF) (PSD), además de bioensayos de inhibición enzimática.

SUMMARY

Cyanobacteria are found world-wide in both fresh and brackish waters. They can develop in large masses forming "blooms" occurrence both in fresh and brackish water all over the world including South-America. Such blooms, as well as axenically grown cyanobacteria, can be a rich source of unique linear and cyclic peptides, most of them having biologically activity. In the past, attention has been paid mostly to the toxic microcystins and to nodularin. These cyclic peptides are hepatotoxins due to the inhibition of protein phosphatases 1 and 2 A after entering specifically to hepatocytes by using the bile - salt uptake system. However, other biologically active peptides are increasingly found in cyanobacteria. Although considered to be non-toxic, these peptides have biological activities, such as potent and specific inhibition

of proteases (trypsin, chymotrypsin, elastase, thrombin, plasmin, angiotensin converting enzyme), anticyanobacterial, antialgal, antifungal activities as well as they cause, immuno suppression and promotion of cell differentiation activities. Examples of protease-inhibiting cyanobacterial peptides are the cyanopeptolines. The interactions of microcystin/protein phosphatase and of cyanopeptolin/protease have been largely studied by X-ray studies of co-crystals. The detection of microcystins and other peptides can be performed through chemical and biological methods. Both, microcystins and cyanopeptolins were recently found in blooms from chilean water bodies by using high performance gas-liquid chromatography (HPLC), matrix-assisted laser desorption/ionization time-of-flight (MALI-TOF) mass spectrometry including post source decay (PSD) fragmentation as well as enzyme inhibition bioassays.

INTRODUCTION

1.- Cyanobacteria forming toxic and/or non-toxic blooms

Various cyanobacteria ("blue-green algae") can develop in large masses ("blooms") in eutrophic fresh and brackish water mainly in warm summer months all over the world (Fig. 1), including Australia, South-Africa, Brazil, Argentina, and the northern European countries (Azevedo *et al.*, 1994; Carmichael, 1994; Scarafia *et al.*, 1995). Appearance of blooms is supported by a high nitrogen- and phosphate input, such as an agricultural dung or spread fertilizer, or urbane waste including phosphate-containing detergents. Cyanobacteria prefer generally a neutral or slightly alkaline pH-value. Some cyanobacteria can accumulate on the water surface due to gas-vesicles enabling the cells to reach a suitable light intensity (Fig. 1, insert). Depending of the wind, a millimeter to decimeter thick oily and pasty green scum can be formed at the shore. This phenomenon is well known for the commonly occurring genera such as ***Microcystis***, ***Aphanizomenon***, ***Anabaena*** and ***Nodularia***. Many cyanobacteria were isolated and kept axenically as laboratory cultures *e. g.* in the well known Pasteur Collection of cyanobacteria, PCC, of the Institut Pasteur, Paris (France).

Peptides, revealing a broad spectrum of biological activities, can be isolated from both blooms and axenic cyanobacterial cultures as well. These peptides are of general interest not only by their structure-biological activity relationship but also by a possible pharmaceutical interest. This review can cover only part of this large field of cyanobacterial secondary metabolites. Especially the large list of non-toxic peptides is constantly growing due to increasing awareness of their broad distribution within the 5th sections of cyanobacteria (Rippka *et al.*, 1979). For reviews on the intensively studied hepatotoxic peptides of the microcystin type see Carmichael (1994), Rinehart *et al.* (1994), Watanabe *et al.* (1996), and Falconer (1998), on the non-toxic peptides (Moore, 1996; Weckesser *et al.*, 1996; Namikoshi & Rinehart, 1996), and on non-ribosomal biosynthesis of peptides (Kleinkauf & von Döhren, 1995). This mini-review will focus on a general description of the hepatotoxic microcystin peptides and on the protease-inhibiting peptides of the cyanopeptolin type, and corresponding chemical and biological detection methods will be discussed. The numerous other linear and cyclic cyanobacterial peptides will be only mentioned.

2.- Hepatotoxic peptides (microcystin, nodularin) from cyanobacteria

Cyanobacterial blooms can be toxic both to animals and human beings. Death of cows after ingestion of water contaminated by cyanobacteria- was first described by Francis (1878) in South Africa, and later on death of animals including livestock was world-wide reported (Carmichael, 1994, Falconer, 1998). Roughly estimated, about 50% of cyanobacterial blooms are toxic with a predominance of hepatotoxins over neurotoxins (Sivonen *et al.*, 1990). Thus, drinking water should not contain more than 1 microgram (1 ppm) microcystin toxin (see below) per liter according to a suggestion of the World Health Organization in 1998 (WHO/EOS/98.1, Guidelines for Drinking-Water Quality, Health criteria and other supporting information, Geneva, Switzerland).

In the 70's the toxicity of water blooms could be ascribed to the cyclic peptides, called microcystins. The most studied is microcystin-LR (microcystin with Leu and Arg, Fig. 4a). It consists of cyclic ring of *D*- and *L*-amino acids, with an unusual ß-amino acid "adda" (3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4-dienoic acid) as the side chain. In brackish water, blooms can be formed by ***Nodularia spumigena***, which produces a similar peptide (nodularin, Fig. 4b). Nodularin (pentapeptide) is smaller than micro-cystin (heptapeptide).

Microcystins and nodularin are both hepatotoxic (Carmichael, 1994). In the mouse bioassay, the LD_{50} of microcystin is about 50 µg/Kg in the oral uptake. In case of a severe intoxication, death is due to necrosis of the liver and internal bleeding may cause death. Toxicity by the peptides is caused by breaking down the cytoskeleton of hepatocytes (Fig. 3 a and b). Adda is essential for hepatotoxicity. The toxin is synthesized non-ribosomally (Meißner *et al.*, 1996) and localized intracellularly (Shi *et al.*, 1995). Putative peptide synthetase genes were identified

in microcystin-producing but not in non-toxic ***Microcystis aeruginosa*** strains (Dittmann *et al.*, 1997). Recently, dialyze-patients died due acute liver failure after being treated with microcystin-LR contaminated water from a local water reservoir (Dunn, 1996; Jochimsen *et al.*, 1998). Furthermore, in sublethal doses microcystin-LR has a tumor-promoting activity even in the lower nanomolar range (Falconer, 1998), the effect was recently ascribed to the liberation of tumor-necrosis factor (TNF)-α (Fujiki & Suganuma, 1994). In China, a relative higher percentage of liver carcinomas was observed with a population, who was consuming microcystin-contaminated drinking water. Toxicity was due to the production of potent inhibitors of type 1 and type 2A protein phosphatases (MacKintosh *et al.*, 1990).

Non-toxic peptides from cyanobacteria

Many cyanobacteria form non-toxic cyclic and linear peptides which are different from microcystins. In spite of that, most of them have biological activity. Cyclic peptides like the westiellamide from ***Westiellopsis prolifica***, laxaphycins from ***Anabaena laxa*** and the hormothamnins from ***Hormothamnion enteromorphoides*** are cytotoxic and/or antimicrobial including antifungal (Prinsep *et al.*, 1992; Patterson *et al.*, 1994; Frankmölle *et al.*, 1991 and 1992; Gerwick *et al.*, 1992). The linear peptide microginin from ***Microcystis aeruginosa*** is an angiotensin-converting enzyme inhibitor (Okino *et al.*, 1993a, Neumann *et al.*, 1997). Other linear peptides inhibit specifically and potently proteases, an example is the inhibition of the serin-protease trypsin by the aeruginosins from ***Microcystis aeruginosa*** (Murakami *et al.*, 1994, 1995; Matsuda *et al.*, 1996; Shin *et al.*, 1997b; Kodani *et al.*, 1998).

The cyanopeptolins are the largest studied among the non-toxic cyclic depsipeptides (Fig. 5). They are defined as (a) cyanobacterial 19-membered cyclic depsipeptides cyclisized by an ester-linkage of the hydroxyl-group of threonine with the carboxy terminus of the C-terminal amino acid of a proposed linear precursor, (b) an unusual 3-amino-6-hydroxy-2-piperidone unit (Ahp), and (c) a *cis*-configurated amide linkage between the amino acids in position 3 and 4 (Martin *et al.*, 1993; Weckesser *et al.*, 1996). There is a great structural variability in the composition of distinct positions within the peptide-ring as well as in the side chain. At the R1 position mainly basic amino acids (Arg, Lys, N-Me-Lys, N,N-Di-Me-Lys) or Tyr and tetrahydro tyrosine (H_4-Tyr) occur. The R2 position is occupied by strongly varying amino acids (Thr, Leu, Ile, Phe). In contrast, the R3 position with Ile or Val seems to be rather conserved. High variability is found in the side chain, where aromatic (4-hydroxyphenyllactic acid) and hydrophilic (Gln, Thr, Asp) amino acids, or non amino acid constituents (glyceric acid, aliphatic fatty acids) occur. So far, the following peptides follow the characteristics of cyanopeptolins: cyanopeptolins A to D, S and SS, the aeruginopeptins, microcystilide A and micropeptins (Martin *et al.*, 1993; Harada *et al.*, 1993, Tsukamoto *et al.*, 1993, Okino *et al.*, 1993b, Ishida *et al.*, 1995, Jakobi *et al.*, 1995, 1996, Williams *et al.*, 1996, Erhard *et al.*, 1997), oscillapeptin and oscillapeptin G from ***Oscillatoria agardhii*** (Sano & Kaya, 1995; Shin *et al.*, 1995), as well as compound A90720A from ***Microchaete loktakensis*** (Lee *et al.*, 1994) and anabaenopeptilides 90-A, 90-B, 202-A and 202-B from ***Anabaena*** sp. (Fujii *et al.*, 1995).

The cyanopeptolins are one within five classes of cyanobacterial depsipeptides: (I) Cryptophycin A and respective variants from ***Nostoc*** sp. (Trimurtulu *et al.*, 1994), (II) majusculamide C, a lipophilic heptacyclodepsipeptide from ***Lyngbya majuscula*** (Carter *et al.*, 1984), (III) hapalosin from ***Hapalosiphon welwitschii*** (Stratmann *et al.*, 1994), (IV) microviridin, a tricyclic tetradecadepsipeptide from ***Microcystis viridis*** (Ishitsuka *et al.*, 1990) and microviridins A, B and C from ***Microcystis aeruginosa*** (Okino *et al.*, 1995), (V) the cyanopeptolins, as listened in Weckesser *et al.* (1996). Another important group of peptides from cyanobacteria are the anabaenopeptins including anabaenopeptins A-G (Harada *et al.*, 1995; Fujii *et al.*, 1995; Shin *et al.*, 1997; Erhard *et al.*, 1999), Ferintoic acid A and B (Williams *et al.*, 1996), Oscillamide Y (Sano & Kaya, 1995), Nodulapeptins A and B (Fujii *et al.*, 1997), Keramamide A (Kobayashi *et al.*, 1991a) and Kombamide (Kobayashi *et al.*, 1991b). The most conspicuous property of several cyclic depsipeptides from cyanobacteria is their potent and specific protease-inhibition: Microviridins B and C inhibit the serin protease elastase (Okino *et al.*, 1995), aeruginosins 102-A and B inhibit thrombin (Matsuda *et al.*, 1996). Micropeptins A and B inhibit trypsin and plasmin among the serin proteases (Okino *et al.*, (1993b), micropeptin 90 inhibits additionally the cystein protease papain (Ishida *et al.*, 1995), compound A90720A from ***Microchaete loktakensis*** trypsin, plasmin and thrombin (Lee *et al.*, 1994), Oscillapeptin elastase and chymotrypsin (Shin *et al.*, 1995), the cyanopeptolins S and SS inhibited trypsin and plasmin more potently than thrombin (Jakobi *et al.*, 1996b).

Cyanopeptolins may have fungicidal, cytotoxic, and anti-tumor activities. Cryptophycin A is a tumor-selective cytotoxin from ***Nostoc*** sp. (Golakotiu *et al.*, 1995; Trimurtulu *et al.*, 1994; Moore, 1996; Pettit *et al.*, 1993; Kobayashi *et al.*, 1994), majuscu-lamide C inhibits growth of fungal plant pathogens (Carter *et al.*, 1984; Williams *et al.*, 1993); hapalosin has multidrug-resistance (MDR)-reversing activity (Stratmann *et al.*, 1994), microviridin strongly inhibits tyrosinase activity (Ishitsuka *et al.*, 1990; Gerwick *et al.*, 1994; Okino *et al.*, 1995), micro-cystilide A has cell-differentiation-promoting activity (Tsukamoto *et al.*, 1993), and cyanopeptolin SS was toxic to ***Daphnia***

magna, while cyanopeptolins S was not toxic in the concentrations tested (Jakobi *et al.*, 1996a, b).

3.- Specific interaction of microcystins and cyanopeptolins with enzymes

- **Microcystins**: According to co-crystallization experiments of the cyanobacterial enzyme-inhibiting peptide and the enzyme followed by X-ray diffraction of the complex, a structural elucidation of the peptide-enzyme interaction has been achieved successfully with microcystin bound to protein phosphatase I (Fig. 2; Goldberg *et al.*, 1995). A covalent binding of microcystin to the enzyme has been observed, in that dehydro-Ala of microcystin binds to the SH-group of the cysteine 273 in the active center of the enzyme.

- **Cyanopeptolins**: In contrast to microcystins, the cyanopeptolin-like compound A90720A from *Microchaete loktakensis* interacts with the serin protease trypsin in a non-covalent, substrate-like manner through hydrogen bonds, by hydrophobic interactions and steric complementarity (Lee *et al.*, 1994). It imitates the canonical conformation of the exposed binding loop of the so-called 'small' protease inhibitors using peptidal and nonpeptidal elements, whereby the Ahp unit (3-amino-6-hydroxy-2-piperidone, Fig. 6) plays an essential role by determining the binding conformation of the inhibitor and preventing its dissociation by its transannular hydrogen bonds. For the inhibition of papain by micropeptin 90, a similar mechanism is suggested (Ishida *et al.*, 1995), and the interaction of aeruginosin 98-B with trypsin was identified at an atomic level (Sandler *et al.*, 1998).

4.- Detection and quantification of cyanobacterial peptides

There are several detection and quantification methods for microcystins and other cyanobacterial peptides, including chemical approaches and bioassays. The choice for their application depends from the desired sensitivity, specificity and the available laboratory equipment. The detection limits given below concern non-concentrated water samples. Concentration may allow the increase of sensitivity by a factor of up to about 10^3. It should be noted that detection limits of both chemical and biological assays can be given in value/L or, alternatively, in value/g cell dry weight. When the microcystin content of a water sample related to the amount of cyanobacterial cell is desired, knowledge of both sample volume (L) and cyanobacterial cell dry weight (g) is required.

a) Chemical assays

- **Detection by HPLC**: A common way for the detection of both microcystins and non-toxic peptides such as the cyanopeptolines includes the extraction of lyophilized cells by water only or in combination with acetic acid or methanol. The extracts can be analyzed directly by reversed phase high performance liquid chromatography (RP-HPLC) with trifluoroacetic acid containing water and acetonitrile as mobil phases and using a photodiode array detector (Martin *et al.*, 1990). Microcystins can be detected by their characteristic UV absorption at 238 nm, whereas other peptides by their absorption maxima at 225 and 276 nm. The RP-HPLC allows also quantification of microcystin in that standards are commercially available (Calbiochem, Bad Soden, Germany), and there exists a linear relationship between peak area (238 nm) and injected amount of 10 up to 2,000 ng toxin (ca. 10 ng toxin are detectable on a single 50 µl injection). Thus, the detection limit of HPLC-separations is about 200 µg/L for samples without any concentration procedures.

- **MALI-TOF mass spectrometry**: According to a recently published method, cyanobacterial peptides can be detected directly in whole cyanobacterial cells (without material- and time-consuming extraction) applying matrix-assisted laser desorption/ionization time-of-flight (MALI-TOF) mass spectrometry (see also below). Microcystins, micropeptins, and anabaenopeptolin have been recently detected in blooms by this method (Erhard *et al.*, 1997). In addition, this method allows typing of cyanobacterial according to their peptide pattern produced as well as differentiation between toxic and non-toxic blooms. MALDI-TOF mass spectrometry method requires only microgram amounts of cells. A safe quantification, however, is not possible so far by this method.

b) Biological assays

- **Mouse lethality bioassay**: Originally toxicity of microcystins was tested by the mouse lethality bioassay (LD_{50} is about 50 µg/kg). However, this rather insensitive and unspecific assay is replaced today by the following biological methods:

- **Immunological detection and quantification**: Both polyclonal and monoclonal antibodies against microcystin are available for ELISA-tests including a com-mercial test kit such as the "EnviroGuard™ Microcystins Plate Kit" ELISA-testsystem (Millipore Coring-System Diagnostics GmbH, Gernsheim, Germany) based on microcystin-specific polyclonal antibodies, although differentiation between the numerous variants is not possible (Chu *et al.*, 1989; Pilette *et al.*, 1995). Antisera are able to detect 1 ppb and may allow to evaluate microcystin contents in water sample rapidly. So far, no reports are available on the immunological detection of the remaining cyanobacterial peptides discussed in this review. The detection limit of

these ELISA-systems is about 10 to even 1 µg/L.

- **Hepatotoxicity test**: An hepatotoxicity assay can be used for the determination of toxicity of microcystin including its quantification Heinze (1996), in that rat hepatocytes are exposed to different microcystin concentrations and then checked for viability by photometrical determination of dehydrogenase activity (MTT-Test). The test is performed with crude cell extracts, the detection limit is about 40 µg microcystin/g cell dry weight.

- **Protein-phosphatase inhibition assay**: This test might be the most convenient system for the detection of microcystins without the need of extensive laboratory equipment. In addition to a respective radioactive test, a colorimetric test system allows a rapid and sensitive screening (An & Carmichael, 1994). The test makes use of the specific recognition and inhibition of protein phosphatases 1 und 2A. The enzyme is commercially available (catalytic subunit of the a-isoform of protein-phosphatase 1 from rabbit muscle, Calbiochem, Bad Soden, Germany). It should be noted, however, that false negative and/or positive results cannot be excluded (Mudge & Mudge, 1994), making necessary a confirmation by the chemical methods mentioned. The detection limit of the non-radioactive protein-phosphatase inhibition assay is about 20 µg/L according to our experience, whereby a good correlation between the data resulting from this tests system and HPLC-separation was observed (Ward *et al.*, 1997; Wirsing *et al.*, 1999).

5.- Isolation and structural identification of peptides

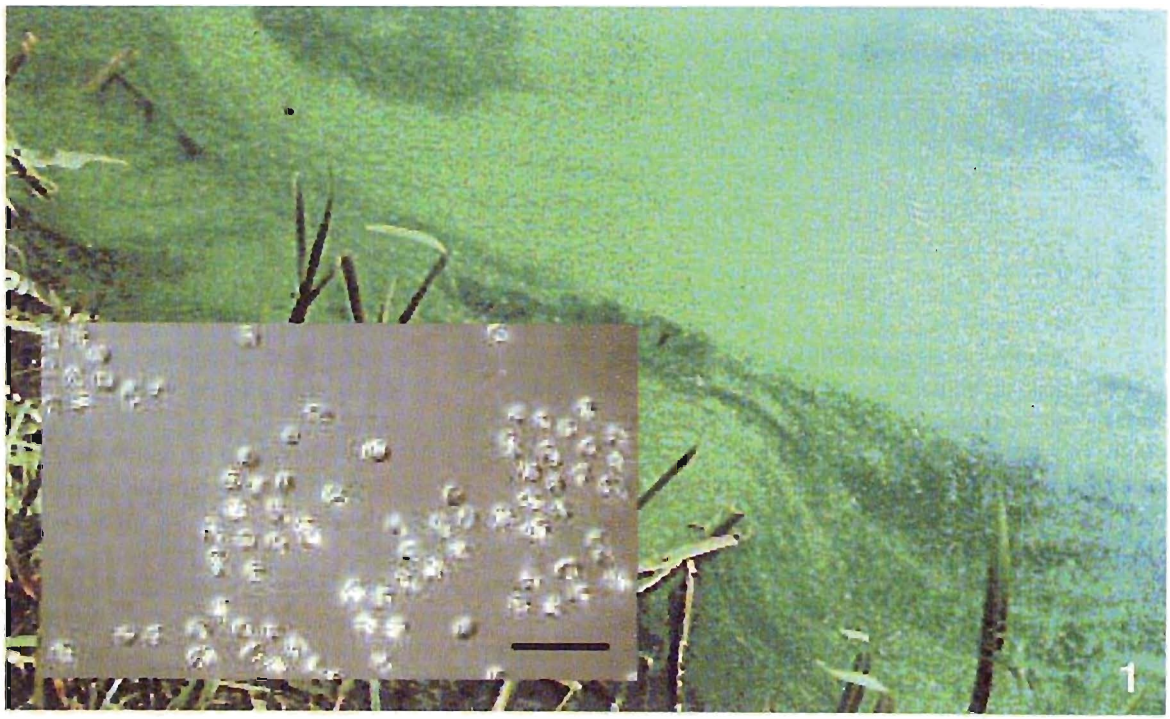
For the isolation and purification of the peptides, lyophilized cells are extracted by either water, acetic acid or methanol (Lawton *et al.*, 1994). The extracts are chromatographed on *e. g.* a LH-20 column with methanol as the eluent, whereby the separations are monitored by HPLC and UV detection at 238 nm (see above). The peptide-containing fractions are then treated by solid-phase extraction on a C-18 cartridge. The structural identification of the peptides may first include amino acid analysis and MALDI-TOF mass-spectrometry (see above). Structural details of the molecules can be obtained by a further fractionation of the molecule ion using post source decay (PSD) fragmentation (Erhardt *et al.*, 1997). Additional biophysical/chemical methods are used such as infrared spectrometry for the detection of sulphate (Jakobi *et al.*, 1996 a,b) and gas-liquid chroma-tography for the detection of fatty acids in the side chain of peptides (Martin *et al.*, 1993), respectively. ^{1}H and ^{13}C NMR spectroscopy is commonly used for the final identification. Some standards, for example the microcystin variants microcystin-LR, -RR, -YR and -LA are available commercially (Calbiochem, Bad Soden, Germany).

6.- Co-occurrence of toxic and non-toxic peptides in blooms

Martin *et al.*, (1993) described the simultaneous presence of the hepatotoxic microcystin-LR and 3-demethyl-microcystin-LR with the cyanopeptolins A to D in the axenic ***Microcystis*** PCC 7806. In ***Microcystis aeruginosa*** TAC 95 the aeruginopeptins 95-A and -95-B, which are also depsipeptides of the cyanopeptolin type, were found together with microcystin-LR while ***Microcystis aeru-ginosa*** M228 produced the aeruginopeptins 228-A and -228-B together with microcystin-YR (Harada *et al.*, 1993). Interestingly, co-occurrence of microcystins and biolo-gically active cyanobacterial peptides is also found in blooms. Jakobi *et al.*, (1995, 1996 a,b) reported the presence of several microcystin variants together with cyano-peptolins S and cyanopeptolins SS in a ***Microcystis*** sp. bloom from lake Auensee/Leipzig (Germany). Co-occurrence of another, so far unidentified cyanopeptolin together with MCYST-LR, MCYST-RR, MCYST-YR was recently observed in a cyanobacterial bloom (also *Microcystis* sp.) collected near the center of Concepción (Neumann *et al.*, 1999). In the bloom from lake Walters-hofener See, the linear, ACE-inhibiting peptide microginin FR1 was found together with several variants of microcystin (Fig. 7).

7.- Cyanobacterial peptides in Chilean lakes

First reports on the occurrence of cyanobacterial blooms in Chilean lakes were published by Parra *et al.* (1981, 1986), Peñaloza (1990) and by Zuñiga & Carvajal (1990). They were dominated mainly by ***Microcystis*** species and occurred in several lakes around Concepción, in lake Aculeo located south of Santiago and in lake Peñuelas located east of Valparaíso, respectively. The bloom observed in lake Laguna Redonda, located north west of downtown Concepción, was found to be highly toxic to mice (Parra *et al.*, 1986). In case of the bloom from lake Aculeo, a soluble, purified toxin extract affected the zooplankton. In addition to a toxin dosis dependence observed, cladocerans were higher sensitive than copepods and rotifers. Within the cladocerans, the smaller-sized genera were more sensitive than the larger ones (Peñaloza, 1990). In the 1995 and 1996 blooms dominated by ***Microcystis*** sp. were observed in lake Rocuant (in the marshland near Concepción). In both years samples were tested for the presence of microcystin by RP-HPLC and for hepatotoxicity using primary rat



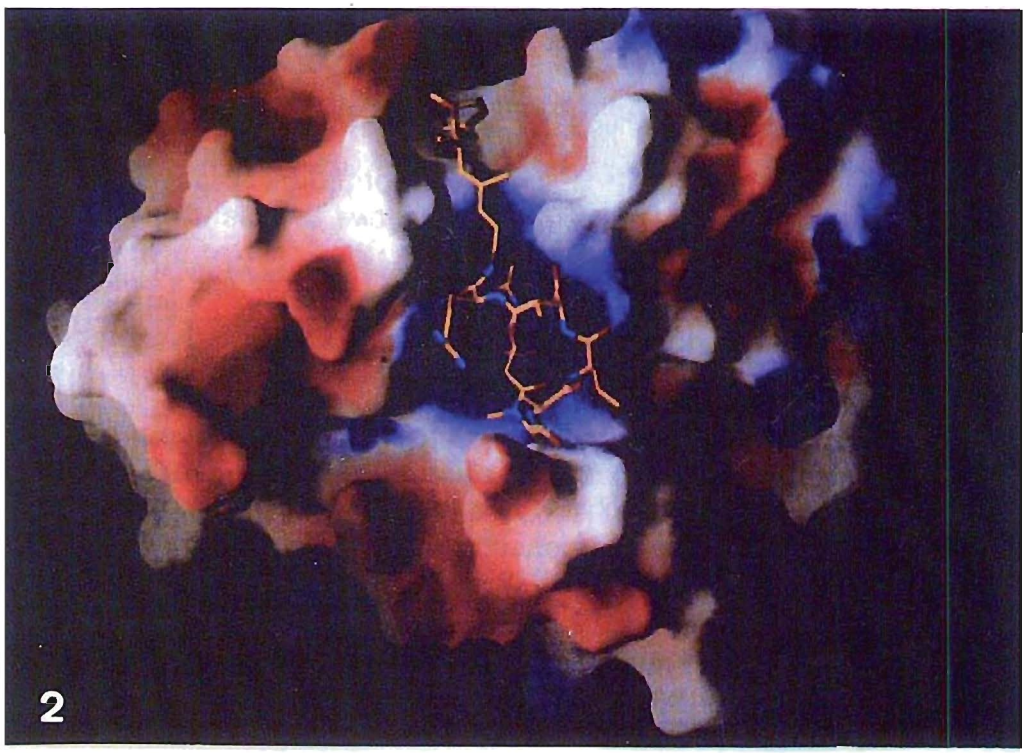
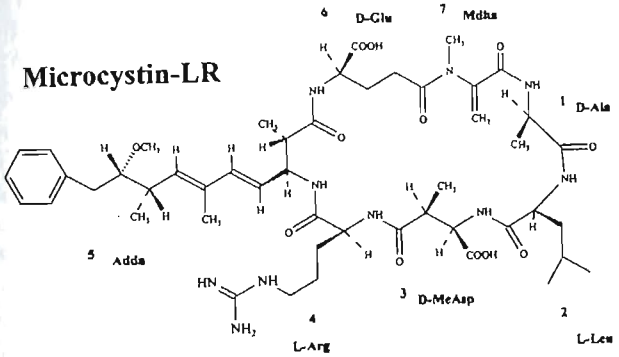
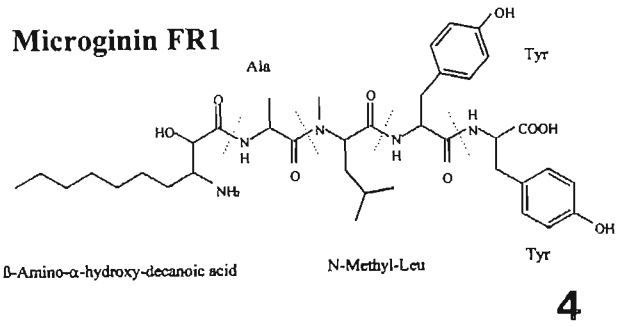
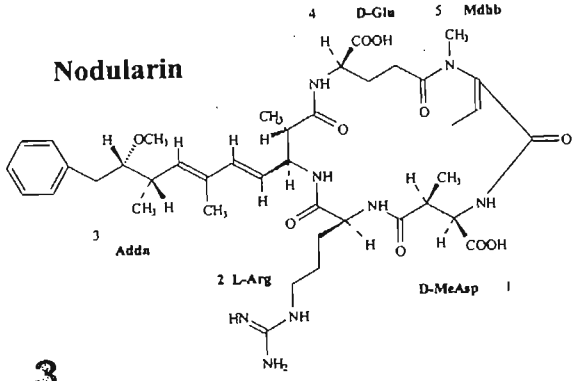
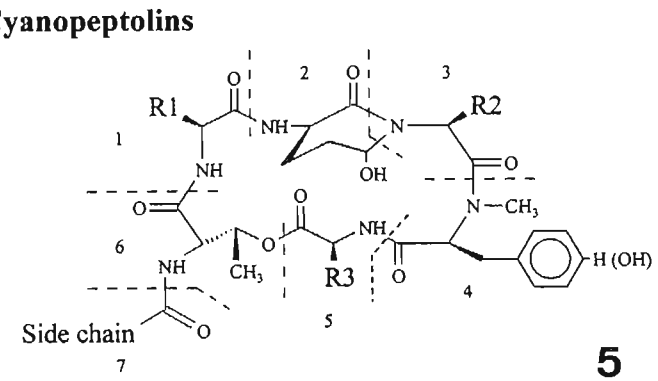


Fig. 1. Water bloom, formed by the cyanobacterium ***Microcystis*** sp., in the marshland pond ("marisma") Rocuant near Concepcion/ Chile. Insert: ***Microcystis*** sp. cells, taken by transmission light microscopy (marker: 10 µm). Note the presence of gas-vacuoles, bringing about the floating of the cells on the water surface. **Fig. 2.** Interaction of microcystin with protein phosphatase 1 at its active site. The hydrophobic Adda side chain binds non-covalently to a hydrophobic region of the active center of the enzyme, N-methl-dehydro-Ala binds covalently to a cysteine residue of the enzyme (taken with permission from Goldberg et al., 1995 (Nature 376: 745-753), slightly modified.









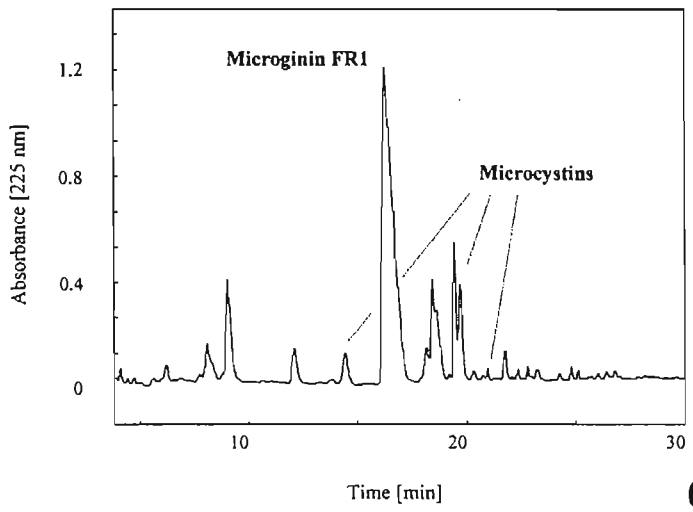


Fig. 3. Structures (a) of MCYST-LR (microcystins with Leu and Arg as variable amino acids, the numbers 1-7 refer to the position of amino acids) from ***Microcystis*** sp., and (b) of nodularin from the brackish water species ***Nodularia spumigena***. **Fig. 4.** Overall structure of depsipeptides of the cyanopeptolin type. The numbers 1-7 account for the positions of the structural units. R1, R2, and R3 represent variable amino acids. Note the 3-amino-6-hydroxy-2-piperidone (Ahp) unit in position 2 (see also Lee et al., 1994) and the ester-bond between the hydroxyl-group of threonine (position 6) with the carboxy terminus of the amino acid R3 in position 5. **Fig. 5.** Structure of microginin FR1 (Ahda-Ala-N-Me-Leu-Tyr-Tyr, whereby Ahda is ß-amino-α-hydroxy-decanoic acid). **Fig. 6.** HPLC elution profile of a methanolic extract of Microcystis sp. water bloom material obtained from the Waltershofener See/Freiburg i. Br. (Germany)

hepatocytes (Campos *et al.*, 1999). In the bloom of 1995, the microcystin content was determined to be 130 µg/g dry bloom biomass on the basis of the RP-HPLC peak area and 800 µg/g on the basis of the rat hepatotoxicity assay, respectively. In the bloom of 1996, RP-HPLC analysis revealed a microcystin content of 8.13 µg/g bloom material dry weight and no hepatotoxicity was measured using a concentration range up to 0.8 mg dry weight bloom material per ml in the rat hepatotoxicity assay. In 1998 an extensive bloom was observed in lake Tres Pascualas (near the center of Concepción). This bloom also contained microcystin. In an ELISA test applying microcystin-specific antibodies, a value of only 13 µg/g bloom material dry weight was obtained (Neumann *et al.*, 1999). Fragmentation by MALDI-TOF (see above) revealed [Asp$^{(3)}$]-MCYST-LR, MCYST-RR, [Asp$^{(3)}$]-MCYST-YR, and MCYST-FR (Neumann *et al.*, 1999). Most interestingly, however, was the finding of two additional peptides which revealed characteristic fragments of a depsipeptide of the cyanopeptolin type on MALDI-TOF (Neumann *et al.*, 1999) and, thus, were called cyanopeptolin VW-1 and cyanopeptolin VW-2 (Neumann *et al.*, 1999). A cell extract of respective bloom material was able to inhibit protein phosphatase (likely due to the microcystins found) and selectively some proteases likely due to the cyanopeptolins found (Neumann *et al.*, 1999).

CONCLUDING REMARK

Cyanobacteria produce a large number of biologically active compounds, some of them having been identified in the latest years and the list is still increasing. While the world-wide distribution of the microcystin toxin is reasonably studied, awareness of the significance of non-toxic cyanobacterial peptides is just beginning. It is also noted that cyanopeptolins can be simultaneously produced together with microcystins (Martin *et al.*, 1993; Harada *et al.*, 1993; Jakobi *et al.*, 1996 a,b). Many of the cyanobacterial peptides are able to inhibit enzymes of the central metabolism and regulation of cells such as protein phosphatases or proteases. For example microcystins, cyanopeptolins and related depsipeptides as well as other peptides from cyanobacteria may, thus, serve as tools in biochemistry and cell biology. A possible pharmacological application cannot be excluded. For the microcystins as well as for the cyanopeptolins the mode of action is known due to the co-crystallization of the peptide-enzyme complex. In should be noted, however, that the physiological *in situ* function of these peptides is essentially unknown.

Cyanobacterial peptides are even found in eukaryotes rising the question of a possible origin from cyanobacteria, as outlined in Weckesser *et al.* (1996). Tsukamoto *et al.* (1993) stated: «There has been a persisting speculation in that a number of interesting compounds - many of them being peptides - found in the sea hare ***Dolabella*** are derived from dietary algae». ***Dolabella*** is just one example of the finding of peptides in sponge, which are possibly of cyanobacterial origin.

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