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Comparison of the structure of key variants of microcystin to vasopressin

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Abstract

Microcystins (MCs) are cyclic heptapeptide compounds [where X^2 (position 2) and Z^4 (position 4) are variable L-aminoacids] produced by cyanobacteria and responsible for severe liver damage in animals ingesting acute doses of the toxic compounds. Certain variants of microcystins are more toxic than others, the differences being commonly ascribed to the hydrophobic nature of the variant. Microcystin-LR (MCLR) [X = L-leucine (L); Z = L-arginine (R); R1 = R2 = CH_3] is the most toxic of all the microcystins investigated to date. This study investigates the similarity of the structures of MCLR and selected MC variants to the liver specific hormone vasopressin. Structures were compiled in HyperChem® (professional version 5.1). Initial comparisons of the MCLR and vasopressin indicated comparable volumes, surface areas and masses. Further studies using RMS overlays show that the microcystin derivative MCLR(Dha^7) is comparably similar to vasopressin in terms of tertiary structure.

Keywords: Microcystin; Vasopressin; Polak-Ribiere gradient; RMS overlay

1. Introduction

Microcystins are recognized as potent liver toxins in humans and animals (Falconer, 2001; Kuiper-Goodman et al., 1999). They are produced by different genera of cyanobacteria in freshwater bodies, most commonly *Microcystis*, *Anabaena* and *Oscillatoria* (Sivonen, 1996). Microcystin consists of a small, cyclic heptapeptide: cyclo-(D-alanine^1-X^2-D-MeAsp^3-Z^4-ADDA^5-D-glutamate^6-Mdha^7) shown in Fig. 1. D-MeAsp, the third amino acid is D-*erythro*-β-methylaspartic acid. Mdha, the seventh amino acid is *N*-methyldehydroalanine and an unusual moiety, (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-deca-4,6-dienoic acid (ADDA), at position 5, forms the distinctive side arm of this toxin (Botes et al., 1985). The two variable L-amino acids are denoted as X^2 and Z^4 in Fig. 1. The microcystin nomenclature rests on the composition of these two variable L-amino acids with microcystin-LR, in which the two variable amino acids are leucine^2 (L) and arginine^4 (R), being the most toxic and hence most commonly studied microcystin. More than 60 different analogues of microcystin have been identified from natural blooms or laboratory cultures (Sivonen and Jones, 1999). Structural variations have been reported in all seven amino acids, but most frequently with substitition of L-amino acids at positions 2 (X^2) and 4 (Z^4) and demethylation of amino acids at position 3 and/or 7 (Ito et al., 2002, Vesterkvist and Meriluoto, 2003). Minor changes in structure may have far reaching implications in toxicity as reflected by differences in the intraperitoneal LD_{50} in mice (Sivonen and Jones, 1999).

The toxic effects of MC can be attributed to their inhibition of protein phosphatases 1 and 2A (PP1 and PP2A) (MacKintosh et al., 1990) resulting in hyperphosphorylation of the cellular cytoskeleton. The resultant sinusoidal collapse leads to severe intrahepatic heamorhaging and in severe cases, death (Falconer, 2001; Kuiper-Goodman et al., 1999). Protein phosphatase inhibition was

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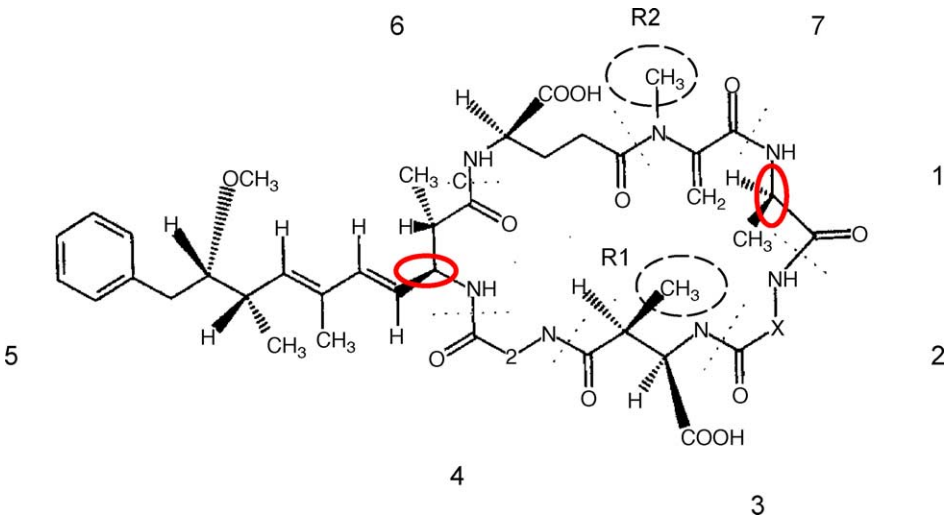

Fig. 1. The generic structure for microcystins. Microcystin-LR has an L-leucine at position X and L-arginine at position Z (Ito et al., 2002). R1 and R2 represent methyl groups for D-*erythro*-β-methylaspartic acid and *N*-methyldehydroalanine, respectively. ⬭ = anchor molecules.

demonstrated in vitro for MCLR, MCYR (tyrosine–arginine) and MCRR (arginine–arginine) in mouse liver lysates (Yoshizawa et al., 1990) and in vivo for MCLR and MCYM (tyrosine–methionine) (Runnegar et al., 1993). [^{125}I]MCYR binds both PP1 and PP2A covalently at Cys-273 and Cys-266, respectively (MacKintosh et al., 1995; Runnegar et al., 1995a,b). Western blotting of liver homogenates of mice exposed to MCLR confirmed the covalent binding of MCLR to nuclear PPs (Guzman and Solter, 2002). There is considerable flexibility in the active site of protein phosphatase 1, which would allow a potentially wide range of MC variants to bind to Cys-273 on PP1, thereby allowing for the wide range of MC showing inhibition of protein phosphatases (Lavigne et al., 2000).

MCRR (arginine–arginine) is less toxic than MCLR with an LD_{50} in mice of 600 μg/kg (Watanabe et al., 1988) compared to the 50 μg/kg of MCLR (Botes et al., 1985). MCRR is 1/12 as toxic as MCLR when administered intratracheally to mice and yet, both toxins inhibit PP1 and PP2A to a similar degree (Ito et al., 2002). It is apparent that the lethality of a MC analogue appears to be more closely related to uptake mechanisms.

Uptake of MC into the liver occurs via a multispecific bile transport system and has been demonstrated for both MCLR and MCYM (tyrosine–methionine) (Runnegar et al., 1995b; Eriksson et al., 1990) however the identity of the receptor has not been identified. Non-hepatocyte cells are affected by exposure to MC at higher concentrations of toxin (100 μM MCLR) than required to elicit similar toxic effects in hepatocytes (0.8 μM MCLR) (McDermott et al., 1998), presumably due to the lack of an active bile transport system. The fact that non-hepatic cells are able to take up MC suggests another means of uptake, possibly diffusion through the cell membrane.

Microcystins are amphipathic molecules with polar or hydrophilic carboxylic acids in positions 3 and 6 and the often present arginine at the variable amino acid in position 2 or 4 (Kuiper-Goodman et al., 1999). Their hydrophobicity varies depending on variations to the ADDA moiety and incorporation of hydrophobic amino acids in the variable positions X and Z. MCLR, the most commonly occurring MC and the most toxic, is more hydrophobic than MCRR, which is less toxic, leading to suggestions that the hydrophobicity of microcystins may enhance their penetration capacity into lipid bilayers and hence their toxic effect in the cell (Vesterkvist and Meriluoto, 2003). Ward and Codd (1999) demonstrated increased toxicity of MCLF (leucine–phenylalanine) and MCLW (leucine–tryptophan) to MCLR in cells of the protozoa, *Tetrahymena pyriformis*. MCLW and particularly MCLF showed better membrane penetrating capacity than observed for MCLR (Vesterkvist and Meriluoto, 2003). Both of these studies suggested that toxins with greater hydrophobicity demonstrated greater membrane penetration ability and, hence increased toxicity.

Vasopressin is a nonapeptide neurohyphoseal hormone synthesised in the hypothalamus. Its major functions are regulation of readsorption of water in the kidneys and contraction of the smooth muscles in arteries, thereby regulating vasodilation (Barberis et al., 1998). Vasopressin receptor is found in the liver, smooth muscle cells in arteries and most peripheral tissues (Mouillac et al., 1995). Binding of vasopressin to its receptor is characterised by a delicate balance of hydrogen bonds as opposed to a few key molecular points of interaction (Mouillac et al., 1995). Vasopressin receptor antagonists virtually all carry bulky sidechains or hydrophobic ring structures (Barberis et al., 1998). Antagonist binding is not affected by mutations to key agonist binding sites, indicating different subsites of the receptor are involved in hormone/antagonist interactions (Mouillac et al., 1995). Binding of vasopressin to its receptor mediates cleavage of phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3