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AN ALTERNATIVE COMPUTER MODEL OF THE 3-DIMENSIONAL STRUCTURES OF MICROCYSTIN-LR AND NODULARIN RATIONALISING THEIR INTERACTIONS WITH PROTEIN PHOSPHATASES 1 AND 2A

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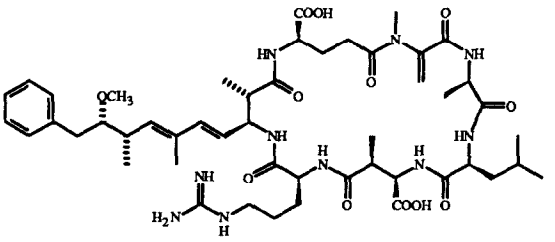
Abstract: The 3-dimensional structures of two cyclic peptides of the okadaic acid class of protein phosphatase inhibitors were found to have the same orientation of (2S,3S,8S,9S) 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(E),6(E)-dienoic acid (Adda) with respect to both peptide rings. These results are consistent with these two compounds inhibiting protein phosphatase 1 and 2A activity with almost the same specific activity.

The microcystins and nodularin are potent hepatotoxic compounds produced by certain species of cyanobacteria. These peptides produce similar gross and ultrastructural effects on hepatocytes resulting in hemorrhagic necrosis and death by hemorrhagic shock.[1] The microcystins are a class of cyclic heptapeptides while nodularin is a cyclic pentapeptide and both contain a β-amino acid, (2S,3S,8S,9S) 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4(E),6(E)-dienoic acid (Adda).[2] The microcystins have the general structure cyclo(D-Ala-L-X-D-*erythro*-β-methylisoAsp-L-Y-Adda-D-isoGlu-N-methyldehydroAla) where X and Y are variable L-amino acids while nodularin lacks the variable L-amino acid, X, and the D-Ala while the N-methyldehydroAla of the microcystins is replaced by the methyl analogue, N-methyldehydroaminobutyric acid.

While the hepatotoxicity of these compounds is similar, it has recently been demonstrated that they have similar specific molecular interactions with protein phosphatases 1 and 2A, the probable cause of the *in vivo* activity. Microcystins-YR, -LR and -RR and nodularin inhibited the activity of protein phosphatase 2A in both the cytosolic fraction of mouse skin[3] and partially purified enzyme from mouse liver[4] with ED_{50s} of 1.4 - 3.4 nM and 0.8 - 1.4 nM for the microcystins and 0.7 nM and 0.9 nM for nodularin, respectively. They inhibited the specific [^{3}H]okadaic acid binding to protein phosphatases 1 and 2A in the cytosolic fraction of mouse liver with ED_{50s} of 1.3 - 2.7 nM for the microcystins and 2.3 nM for nodularin and in the particulate fraction of mouse liver with ED_{50s} of 11 - 30 nM for the microcystins and 8 nM for nodularin.[4] These cyclic peptides belong to the okadaic acid class of compounds that bind to the okadaic acid receptors on protein phosphatases 1 and 2A.[5] These peptides are unique tools to study the process of tumour promotion and, recently, it has been shown that microcystin-LR is a tumour promoter in rat liver initiated with diethylnitrosamine.[6]

Recently, computer models of the 3-dimensional structures of microcystin-LR (**1**) and nodularin (**2**) have been published.[7] When Adda in both microcystin-LR and nodularin were fitted together, the peptide rings of microcystin-LR and nodularin formed an angle of 90° to each other.[7] Complementarity between ligand and receptor is necessary for binding and this complementarity involves steric, hydrophobic and electrostatic complementarity.[8,9] If each ligand that binds must be complementary to the receptor binding site, it follows that ligands which bind must have common features. The specific interaction of microcystin-LR and nodularin with the okadaic acid binding site on the same receptors is a convincing argument that the proposed 3-dimensional structures of microcystin-LR and nodularin do not represent the conformations of the molecules which bind to the receptors and probably do not reflect the most likely solution conformations of the molecules. A second point of some concern is that the calculated 3-dimensional structures of both molecules showed no intramolecular hydrogen bonding. As both compounds have a number of hydrogen bonding acceptors and donors it is difficult to visualize that intramolecular hydrogen bonding will not contribute to the production of stable conformations.



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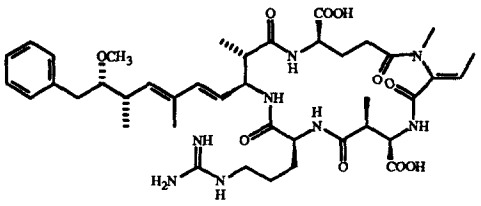

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We now present our molecular modelling results which reach a different conclusion and provide a rationalization for the receptor binding properties of the microcystins and nodularin. Calculations were performed on a VAX 11/750 using Chem-X[10] (Jan 90) molecular modelling software and on a microVAX 3600 using Chem-X (July 91). Molecules were visualized on a Macintosh IIsi terminal using the CDL GKS 3D graphics window. Microcystin-LR and nodularin were constructed using the Chem-X modification and building option. These initial structures were optimized using the Chem-X internal optimizer with the default force-field and used as starting points for the calculations of the minimum energy conformation of each molecule. A systematic conformational search which samples all "flexible" bonds about which rotation can readily occur cannot be conducted for cyclic systems such as microcystin LR and nodularin. The Chem-X interface to the distance geometry program, DGEOM[11] was thus employed. DGEOM functions by taking the explicit connectivity from the initial structure and using this to locate non-rotatable bonds and sets their distance constraints to the values found in the initial structure, therefore the amide bonds and the fact that the molecules are cyclic need not be specified This method generates random conformers one of which has the lowest calculated value but not necessarily the global minimum energy. The lowest energy conformer was subjected to both molecular mechanics energy optimization (MME) and Van der Waals energy minimization (VDWE) calculations in order to minimize the root mean square (RMS) distance between selected pairs of atoms to give the global minimum energy. The MME calculations are empirical force-field calculations which assume that the energy is dependent upon bond lengths, bond angles and non-bonding interactions thus predicting reasonable

geometries. The VDWE calculations are empirical force-field calculations which assume that all bond lengths and angles are optimized (or idealized) and that the energy is only dependent upon non-bonded interactions. Intramolecular hydrogen bonding of the Glu and D-*erythro*-β-methylisoAsp carboxylate groups was found in the optimized structures of both microcystin-LR and nodularin.

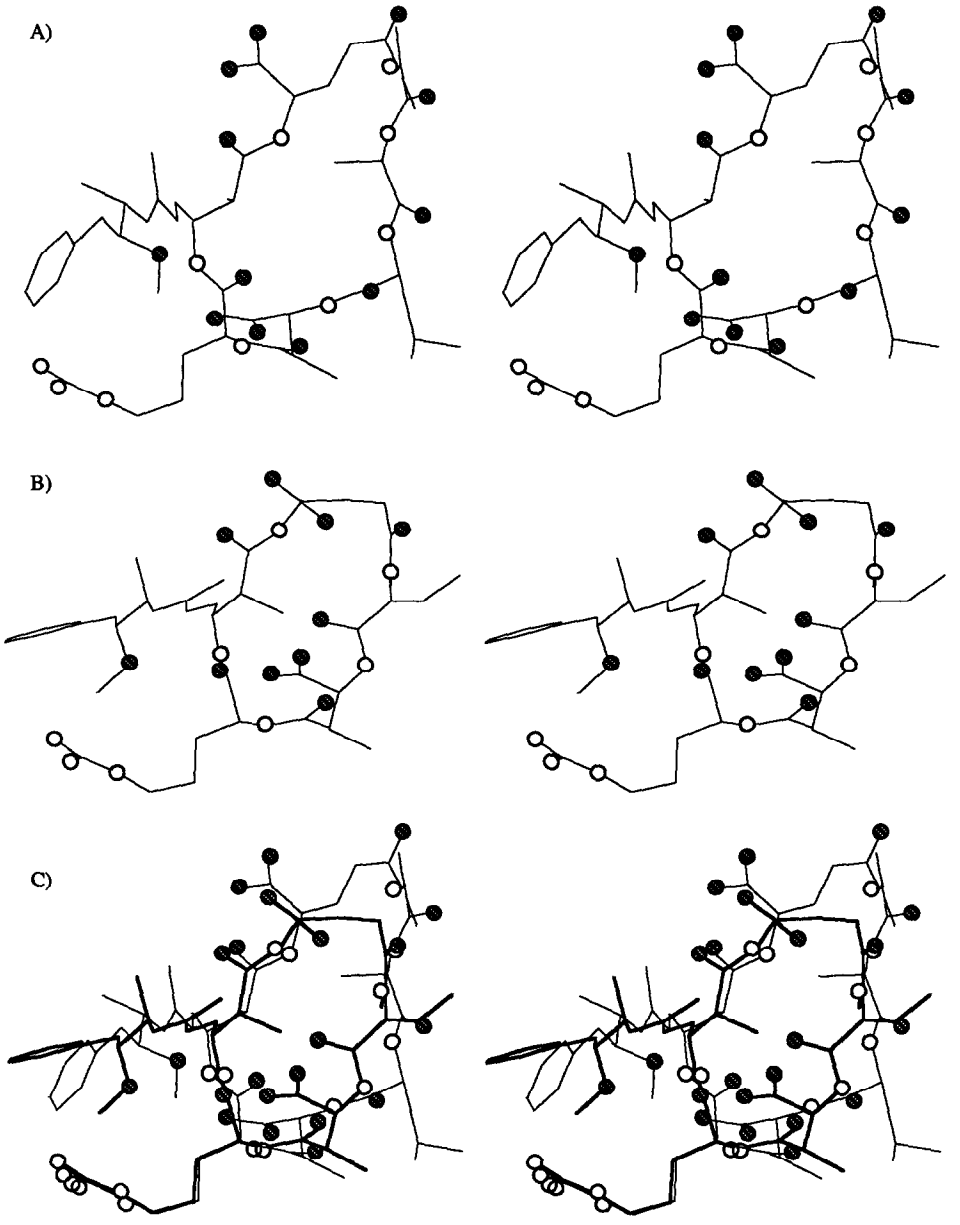

Figure 1: The three dimensional structures of microcystin-LR (A), nodularin (B) and their superimposition (C).

The energy parameters calculated for the optimized structures of microcystin-LR and nodularin are respectively: E(VDW), the total non-bonded interaction energy (net repulsion energy), 1.660, 1.396 kcals x 10^3 compared to the previously reported[7] 2.594, 2.670 kcals x 10^3; E(MME), molecular mechanics energy, 0.0952, 0.0988 kcals x 10^3 compared to the previously reported[7] 0.361, 0.443 kcals x 10^3. The lower energy values and the three-dimensional similarity of these compounds suggests they are more reasonable computer models. These structures were then used to define a microcystin-LR / nodularin model for their inhibition of protein phosphatase activity.

The optimized structures of microcystin-LR and nodularin are presented in Figure 1A and 1B in a similar orientation, showing the planarity of both peptide rings and the relative spatial alignments of the Adda and Arg side chains. Rigid superimposition of the two molecules in these orientations is shown in Figure 1C. There is close proximity of the Adda side chains, the Glu and D-*erythro*-β-methylisoAsp carboxylate groups and the peptide rings. This superimposition defines the microcystin-LR / nodularin model and coupled with the biological activity suggests the presence and the orientation with respect to the peptide ring of the Adda and the two acidic groups is necessary for activity. This result is supported by evidence that a microcystin-LR analogue having 4(E),6(Z) Adda geometry was less effective in inhibiting protein phosphatase 2A activity and [^{3}H]okadaic acid binding to protein phosphatases 1 and 2A than microcystin-LR (4(E),6(E) geometry).[12] The model is consistent with the observed biological properties of microcystin-LR, nodularin and their derivatives.

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