ELUCIDATION OF THE CONFORMATION OF FREE AND LPS-BOUND POLYMIXIN B NONAPEPTIDE IN WATER BY 2D-NMR AND RESTRAINED MOLECULAR DYNAMICS METHODS AND MOLECULAR MODELING OF POLYMIXIN B-LIPID A COMPLEX.

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The conformation of Polymixin B nonapeptide (PMN) in aqueous solution has been investigated by 400 MHz 2D-NMR methods. A crude model satisfying interresidue NOE distance constraints and hydrogen-bond criteria was subjected to restrained molecular dynamics simulation and energy minimization, yielding an ensemble of stereochemically reasonable structures compatible with NMR observations. The major features of PMN are a C2 y-turn at D-Phe Leu-Dab and an inverse C2 y-turn at Thr 38-Dab-Dab [Dab = 2,4-diaminobutyric acid]. The conformation of LPS-bound PMN was analyzed by transfer NOE methods and NOE-derived constraints were used to construct a model of Polymixin B (PMB), which was then docked on a model of lipid A and the resultant complex was energy minimized. The salient structural features of the model complex are the following: The carboxyl functions of the cyclic backbone peptide groups lie on one face of the peptide, and this polar surface interacts with the saccharide backbone of lipid A. The protonated amino functions of Dab/Db-Dab/Dab form bidentate ionic hydrogen bonds with the anionic phosphate groups on lipid A. The orientation of the acetyl portion of PMB (6-methyl octanoyl-Dab-Thr) is normal to the plane of PMB and coaxial with the acyl chains of lipid A. These findings permit the rationalization of several experimental observations concerning the binding of PMN and PMB to, and consequent neutralization of the activity of lipid A and LPS, and may be useful in the rational design of endotoxin antagonists from first principles.

Note: The numbering scheme for PMN corresponds to the positions of the amino acids in the parent PMB molecule.

AN APPROACH TO THE MECHANISM OF BINDING OF POLYMIXIN B TO RE-LPS FROM SALMONELLA MINNESOTA

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Polymyxin B (PMB) is a well-known antibiotic agent against Gram-negative bacteria. One of the main targets of PMB is the outer membrane, the defense shield of the bacterial cell. It increases extremely the permeability of the outer membrane and in moderate concentration of PMB leads to lysis of the bacteria. A similar behavior is known for PMB-nonapeptide (PMBN).

The membrane insertion of PMB/PMBN into multilayers of Re-LPS was studied. These experiments revealed that PMB/PMBN diffuse from aqueous sulphate into the monolayer. After a while, saturated Re-LPS monolayer showed an area of ~150% as compared to pure Re-LPS monolayer.

In addition X-ray diffraction was applied to mixtures of PMB/PMBN-RLPS. In contrast to the monolayer investigations, the samples were in the dry state. First series of measurements demonstrated that the stacking order in the bilayered samples almost vanished. We therefore applied a special sample treatment to improve the sample quality. It was found that there is a significant decrease of the bilayer spacing from 5.7 nm for pure Re-RLPS to 4.9 nm for a 1:1 mixture with PMB/PMBN. Both the lateral and the stacking order in the mixed samples are reduced significantly. The low resolution X-ray pictures unfortunately cannot give detailed structural information. However, the increases in the area found in the monolayer studies and decrease in bilayer thickness detected by X-ray measurements point to the following explanation.

PMB/PMBN is placed in the LPS-membrane leading to an enlarged membrane area. At the same time the state of order of the acyl chains is disturbed resulting in kinks and bends. Therefore the bilayer thickness is reduced. Since PMB/PMBN are inserted in the membrane, the decrease of membrane order seems to be related to steric misfit of molecular shape.

LIPOPOLYSACCHARIDE, LIPID A AND ANIONIC PHOSPHOLIPIDS AS VECTORS FOR PORIN RECONSTITUTION IN LIPID BILAYER

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Pore-forming protein from Yersinia pseudotuberculosis was proved to be the constituent of endotoxin. pH-Dependence of porin reconstitution into egg lecithin liposomes and model membranes from bacterial phospholipid (PL) have been studied. The protein was found to incorporate spontaneously only into negatively charged liposomes. Reconstitution vectors for porin use in the following order: dipalmitoylphosphatidylcholine (DPPC) > dipalmitoylphosphatidylethanolamine (DPEA) > lipopolysaccharide (LPS) > lipid A > p-alcohol, CL (pH 8.0); LPS > lipid A > DCP; CL (pH 5.0); DCP > PL > CL > LPS; lipid A (pH 3.0).

The ability of porin to form channels in model membrane from PL was demonstrated only at pH 5.0. But porin reconstitution occurred at pH 5.0 and 6.0 when protein was in complex with LPS. Using fluorescent probes the pH-induced conformational changes of porin have been elucidated. At pH 5.0 hydrophobicity of protein globule surface and accessibility of tryptophan residues to hydrophobic fluorescence acceptors were shown to be minimal.

Thus, porin reconstitution into negatively charged lipid membranes occurred in weakly acidic media, in addition under these condition the specific interaction between protein and endotoxin constituents (LPS and lipid A) have been observed.