Biophysical Characterization of the Interaction of *Limulus polyphemus* Endotoxin Neutralizing Protein (ENP) with Lipopolysaccharide

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Bacterial lipopolysaccharide exhibits a variety of biological activities in mammals which may be beneficial at low concentrations but harmful at high concentrations, leading to the severe septic shock syndrome [1]. One of the most potent antimicrobial proteins is the *Limulus* anti-LPS factor (LALF) [2]. Here we report on the interaction of bacterial lipopolysaccharide (LPS) with the recombinant form of LALF, the endotoxin neutralizing protein (ENP) using a variety of physical and biological techniques. In biological assays (*Limulus* amebocyte lysate and tumor necrosis factor-α induction in human mononuclear cells), ENP causes a strong reduction of the immunostimulatory ability of LPS in a dose-dependent manner. Concomitantly, the accessible negative surface charges of LPS and lipid A (zeta potential) are neutralized and even converted into positive values (overcompensation), due to ENP binding (Fig. 1). Gel to liquid crystalline phase transition temperatures of LPS and lipid A are shifted to significantly higher temperatures leading to a rigidification of the acyl chains. The phase transition enthalpy, however, is slightly enhanced, indicating that the hydrophobic moiety is not strongly disturbed. The aggregate structure of lipid A is converted from a cubic into a multilamellar phase upon ENP binding (Fig. 2), whereas the secondary structure of ENP does not change due to the interaction with LPS. Importantly, ENP is able to incorporate by itself into target phospholipid liposomes, and is also able to mediate the intercalation of LPS into the liposomes thus acting as a transport protein in a manner similar to lipopolysaccharide-binding protein (LBP). ENP contains a hydrophobic binding site to which the dye 1,8-anilinonaphthalenesulfonate (ANS) binds at a $K_d$ of 19 µM, which is displaced by LPS. From the fact that LBP is not able to bind to LPS when ENP and LPS are preincubated, tight binding of ENP to LPS can be deduced which is in accordance with the $K_d$ in the low nM range (220-fold lower than for the cationic polypeptide polymyxin B, PMB). These data allow the establishment of a model for LPS inactivation by ENP.

Figure 1: Zeta potential of ENP/endotoxin mixtures: ● LPS, B lipid A
Figure 2: Small-angle X-ray diffraction patterns for pure lipid A and in the presence of two ENP concentrations. [Lipid A] = 50 mM.

References