Endotoxin-like properties of a rhamnolipid exotoxin from *Burkholderia (Pseudomonas) plantarii*: immune cell stimulation and biophysical characterization

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A highly purified glycolipid, \(2\text{-}\O\text{-}\alpha\text{-L-rhamnopyranosyl-\alpha\text{-L-rhamnopyranosyl-\alpha}(R)-3-hydroxytetradecanoyl-(R)-3-hydroxytetradecanoate (RL-2,2,14)}\), produced by *Burkholderia plantarii*, was analysed physicochemically and in biological test systems with respect to its pathophysiological activities as heat-stable extracellular toxin. Fourier-transform infrared spectroscopy (FTIR) was applied to study specific molecular groups as well as the gel (\(\beta\)) to liquid crystalline (\(\alpha\)) phase behaviour of the acyl chains. Synchrotron radiation X-ray diffraction was used for the elucidation of the aggregate structure. Small-angle X-ray diffraction was applied for the elucidation of the aggregate structures of RL-2,2,14 at different water contents and temperatures. The results at 95 % water content (Figure 1) at 40 and 70 °C indicate a complex structural polymorphism. At 40 °C the occurrence of the reflections at 7.89 nm, 5.60 nm, and 3.50 nm can be assigned to a cubic structure with \(a_0 = 7.89 \text{ nm}\) and \(a_0 / \sqrt{2} = 5.32 \text{ nm}\) and \(a_0 / \sqrt{5} = 3.50 \text{ nm}\), whereas those at 70 °C indicate a more complex lipid aggregation. Fluorescence resonance energy spectroscopy (FRET) allowed to test the ability of the rhamnolipid RL-2,2,14 to intercalate into phospholipid liposomes in the absence and presence of lipopolysaccharide-binding protein (LBP). Its biological activity was examined as the ability to induce cytokines in human mononuclear cells (MNC) and to activate the *Limulus* clotting cascade. Furthermore, measurements were also performed in the presence of the polycationic decapeptide polymyxin B (PMB), which effectively neutralizes bacterial endotoxins. Despite its completely different chemical structure, the rhamnolipid RL-2,2,14 exhibits a variety of endotoxin-related physicochemical characteristics such as cubic inverted aggregates and the ability to induce cytokines in MNC [1]. These data are in good agreement with our conformational concept of endotoxicity, based on the intercalation of naturally-originating virulence factors, such as LPS (endotoxin), glycolipid from *Mycoplasma fermentans* (MfGL-II) and rhamnolipids but also on synthetic lipid A-mimicking structures expressing the “endotoxic conformation” into membranes of immune cells, leading to strong mechanical stress on integral proteins eventually causing cell activation.
Figure 1: Small-angle X-ray diffraction patterns of RL-2,2,14 at 95% water content and two temperatures.

References