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Grant Title: Glycolipids as receptors for toxins of plant pathogens

Abstract

The members of the family of necrosis- and ethylene-inducing peptide 1 (NEP1)-like proteins, i.e. NLPs, elicit diverse defence reactions and cell death in eudicot plants but not monocots. NLPs are widely distributed among taxonomically nonrelated microorganisms like fungi, bacteria and oomycetes. These microorganisms are widespread, they may infect range of different crops, such as potato, tomato, soya and tobacco, and cause enormous economic loss worldwide. It was shown that NLPs function as cytolytic toxins that induce plasma membrane leakage, thus causing cytotoxicity. The mechanism by which NLP induce necrosis is poorly understood. Recently, we have identified glycosylinositol phosphorylceramides (GIPCs), a major class of plant sphingolipids, as a target molecule for NLP binding to plant plasma membranes (Lenarčič et al., Science, 2017). GIPCs consist of a polar headgroup bearing variable carbohydrate moieties and inositol phosphorylceramide core. Type and number of terminal hexose groups varies significantly between plant species and plant tissues. Binding of the GIPC terminal hexose moiety induces several conformational changes within the NLP toxin that may precede membrane attachment and host cell lysis. The main objectives of our research are (i) preparation and characterization of plant-derived GIPCs and GIPC-containing model lipid bilayers, and (ii) determination of molecular mechanism of the glycan recognition by NLPs. In the first task we prepared sufficient amounts of purified GIPCs from eudicot (tobacco and tomato) and monocot plants (leek). By using thin layer chromatography we detected several lipid species, with the majority bearing carbohydrate moieties. MALDI-MS analysis revealed that the majority of isolated eudicot (tobacco and tomato) GIPCs contained two sugar moieties, while monocot (leek) GIPC contained higher number of glycan groups. Tobacco and tomato derived GIPCs samples displayed similar TLC and MALDI-MS profiles. Preparation of purified GIPCs in sufficient amounts enabled the preparation of unique and newly developed model membrane systems, such as small unilamelar vesicles, large unilamellar vesicles, multilamellar vesicles, giant unilamellar vesicles (GUVs) and planar lipid bilayers. The size of the liposomes was confirmed by using dynamic light scattering, transmission electron microscopy and confocal microscopy (for GUVs). The properties of lipid bilayers in absence and presence of NLP toxin were further analyzed in Task 2, where molecular details of interaction between NLPs and lipid membrane were monitored by exploiting various GIPCs-containing lipid model systems and biochemical and biophysical approaches, such as sedimentation assay, surface plasmon resonance (SPR) experiments, monitoring integrity of GUVs and passage of fluorescent probes, etc. Purified lipids and newly-developed model system enabled us further insights into interaction of NLPs with lipid membranes and mechanism of lipid membrane damage.