

A common feature among nearly all gram-negative bacteria is the requirement for lipopolysaccharide (LPS) in the outer leaflet of the outer membrane. LPS provides structural integrity to the bacterial membrane, which aids bacteria in maintaining their shape and acts as a barrier from environmental stress and harmful substances such as detergents and antibiotics. Recent work has demonstrated that *Caulobacter crescentus* can survive without LPS due to the presence of the anionic sphingolipid ceramide-phosphoglycerate (CPG). Based on genetic evidence, we predicted that protein CpgB functions as a ceramide kinase and performs the first step in generating the phosphoglycerate head group. Here, we characterized the kinase activity of recombinantly expressed CpgB and demonstrated that it can phosphorylate ceramide to form ceramide 1-phosphate. The pH optimum for CpgB was 7.5, and the enzyme required Mg²⁺ as a cofactor. Mn²⁺, but no other divalent cations, could substitute for Mg²⁺. Under these conditions, the enzyme exhibited typical Michaelis–Menten kinetics with respect to NBD C6-ceramide ($K_{m,app} = 19.2 \pm 5.5 \mu\text{M}$; $V_{max,app} = 2590 \pm 230 \text{ pmol/min/mg enzyme}$) and ATP ($K_{m,app} = 0.29 \pm 0.07 \text{ mM}$; $V_{max,app} = 10,100 \pm 996 \text{ pmol/min/mg enzyme}$). Phylogenetic analysis of CpgB revealed that CpgB belongs to a new class of ceramide kinases, which is distinct from its eukaryotic counterpart; furthermore, the pharmacological inhibitor of human ceramide kinase (NVP-231) had no effect on CpgB. The characterization of a new bacterial ceramide kinase opens avenues for understanding the structure and function of the various microbial phosphorylated sphingolipids.