Abstract:

The study of sphingosine and sphingosine-1-phosphate is now widespread due to their immense role as intra- and extracellular messenger molecules. The balance and interplay of these ceramide metabolites is dependent on the activities of kinase and phosphatase enzymes. Sphingosine and sphingosine-1-phosphate are found in very minute quantities in cells; thus, they require highly sensitive techniques for quantitative analysis. In this study, we developed a quantitative assay for the determination of sphingosine kinase 2 (SphK2) activity both in vitro and with cell lysates, using CE-LIF. Sphingosine fluorescein was used as the substrate. The KM of SphK2 for sphingosine fluorescein was 2.8±0.8 μM with a Vmax of 2490±520 μM/min and a kcat of 1920±402/s. The inhibition of SphK2 was also investigated using four different inhibitors for which 2-(p-hydroxyanilino)-4-(p-chlorophenyl) thiazole inhibitor was the most potent for the in vitro inhibition of SphK2 while N,N-dimethylsphingosine (DMS) did not inhibit but rather increased SphK2 activity. The fluorescence-based approach for the determination of the enzymatic activity of SphK2 proves to be useful for the quantitative determination of SphK2 activity in vitro and in cell lysates, and could be extended to single-cell analysis or applied in drug screening.