CE-MS-based Metabolomics

CE-MS analysis was performed by Human Metabolome Technologies (Tsuruoka, Japan) or by ourselves. Briefly, cells and tissues with internal standards were homogenized with ice-cold methanol and extracted by the Bligh–Dyer method using chloroform and methanol to separate and recover the water-soluble fractions. The supernatant was filtered through a 5-kDa cut-off filter (Ultrafree-MC-PLHCC, Human Metabolome Technologies) at 9,100 × *g* at 4 °C, dried in a centrifugal evaporator at 1,500 rpm at 1,000 Pa, and resuspended in 50 μl of ultrapure water before metabolome analysis using CE-MS on an Agilent 7100 capillary electrophoresis system coupled to a 6224 time-of-flight liquid chromatography mass spectrometer (CE-TOFMS) for cationic compounds and a capillary electrophoresis tandem mass spectrometer (CE-MS/MS) for anionic compounds (Agilent Technologies). Peaks detected in CE-TOFMS analysis were extracted using automatic integration software (MasterHands ver.2.16.0.15 developed at Keio University) and those in CE-MS/MS analysis were extracted using automatic integration software (MassHunter Quantitative Analysis B.06.00, Agilent Technologies). Putative metabolites were then assigned from the Human Metabolome Technologies (HMT) metabolite database on the basis of *m/z* and migration time. Concentrations of metabolites were calculated by normalizing the peak area of each metabolite with respect to the area of the internal standard and using a standard curve, obtained by three-point calibrations.