

Quantitative determination of isopentenyl diphosphate in cultured mammalian cells

Isopentenyl diphosphate (IPP), an intermediate of the isoprenoid biosynthetic pathway (IBP), has several important biological functions, yet a method to determine its basal level has not been described. Here, we describe a nonradioactive and sensitive analytical method to isolate and specifically quantify IPP from cultured mammalian cells. This method applies an enzymatic coupling reaction to determine intracellular concentrations of IPP. In this reaction, geranylgeranyl diphosphate synthase catalyzes the formation of geranylgeranyl protein transferase I conjugates GGPP with a fluorescently labeled peptide. The geranylgeranylated peptide can be quantified by high-performance liquid chromatography (HPLC) with a fluorescence detector, thereby allowing for IPP quantification. The detection lower limit of the fluorescence-labeled geranylgeranyl peptide is approximately 5 pg (~0.017 pmol). This method was used to examine the effects of IBP inhibitors such as lovastatin and zoledronate on intracellular levels of IPP. Inhibition of hydroxymethylglutaryl coenzyme A reductase (HMGCR) by lovastatin (50 nM) decreases IPP levels by 78% and 53% in K562 and MCF-7 cells, respectively. Whereas zoledronic acid (10 μ M) increased IPP levels 12.6-fold when compared with untreated cells in the K562 cell line, an astonishing 960-fold increase was observed in the MCF-7 cells.