



Prevention of the wortmannin-induced inhibition of phosphoinositide 3-kinase by sulfhydryl reducing agents

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Abstract:

The effects of the sulfhydryl reducing agents 2-mercaptoethanol and dithiothreitol on wortmannin-induced inhibition of phosphoinositide 3-kinase (PI3K) were studied in order to examine whether the sulfhydryl reducing agents directly affect the wortmannin inhibition of PI3K. These reducing agents are commonly used to stabilize enzyme structures by maintaining protein sulfhydryl groups in the reduced state. Preincubation of wortmannin with millimolar levels of 2-mercaptoethanol, a sulfhydryl derivative of ethanol, markedly prevented subsequent wortmannin-induced inhibition of PI3K. In contrast, ethanol, 2-mercaptoethanol lacking sulfhydryl group, and 2-(methylthio)ethanol, a methyl derivative of the sulfhydryl group of 2-mercaptoethanol, had little effect on the wortmannin-induced inhibition of PI3K, which suggests that the prevention of wortmannin-induced inhibition by 2-mercaptoethanol occurs through the sulfhydryl group of this agent. Moreover, dithiothreitol, a second sulfhydryl reducing agent, also markedly prevented wortmannin-induced inhibition of PI3K. These results indicate that the wortmannin-induced inhibition of PI3K is markedly prevented by millimolar concentrations of sulfhydryl reducing agents such as 2-mercaptoethanol and dithiothreitol in the medium, presumably by the binding of wortmannin to the agents.

Key words:

wortmannin, sulfhydryl reducing agent, phosphoinositide 3-kinase, PI3K, 2-mercaptoethanol, dithiothreitol
