Abstract

Phytosulfokine-α (PSK-α), a peptidyl plant growth factor that was originally isolated from conditioned medium (CM) derived from asparagus mesophyll cell culture, stimulates cell proliferation in some plants in suspension culture. However, the physiological relationship between phytohormones and the growth factor in plants is not well understood. Using carrot (*Daucus carota* L.) non-embryogenic cell culture, I investigated the stimulatory effects of PSK-α on the cell proliferation induced by different combinations and concentrations of auxin [2,4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), or naphthaleneacetic acid (NAA)] and PSK-α. Cell proliferation was not stimulated by PSK-α at high auxin concentrations, but was promoted at low auxin concentrations. There was no proliferation in the absence of auxin. These results indicate that PSK-α stimulates cell proliferation when cell division activity is low, and that auxin is required for this process. I also evaluated PSK-α production using medium derived from non-embryogenic cell culture with high or low concentrations of auxin (2,4-D, IAA or NAA) in a competition ELISA system. More PSK-α was produced at high auxin concentrations than at low auxin concentrations. These results suggest that auxin participates in PSK-α production.

I further investigated the effects of PSK- α on cell cycle progression using carrot non-embryogenic cell culture. Analysis of cell nuclear DNA content using flow cytometry (FCM) after synchronization of the cell cycle

by aphidicolin and/or propyzamide showed that the progression of G1 cell cycle was promoted by addition of PSK- α in the presence of low concentrations of 2,4-D. Similar results were observed in positive control culture grown in the presence of a high concentration of 2,4-D without PSK- α. Using RT-PCR analysis with carrot cell cycle-related genes as marker genes, I verified the results of the FCM analyses. The increase in the transcript levels was enhanced by addition of PSK- α to low 2,4-D medium. In high 2,4-D medium, used as a positive control, levels of these transcripts were as high as those in the sample derived from cells grown in low 2,4-D medium with PSK- α. I also showed that PSK-α is involved in S-G2 phase progression outside of the G1 phase. In low 2,4-D medium with PSK- α, the percentage of cells accumulated in the G2/M phase(s) eight hours after propyzamide addition was higher than in cells growing in low 2,4-D medium without PSK- α. In high 2,4-D medium without PSK- α, the percentage was identical to that in low 2,4-D medium with PSK- α. Finally, I found that PSK- α is involved in re-entry into the cell cycle from quiescent state by RT-PCR analysis. Transcripts of cell cycle-related genes accumulated earlier or more strongly with addition of PSK- α than in low 2,4-D medium lacking PSK- α, and in high 2,4-D medium without PSK- α the transcripts were comparable to that in low 2,4-D medium with PSK- α. These results indicate that PSK- α promotes the progression of cell cycle, and suggest that PSK- α stimulates cell proliferation through regulation of

the cell cycle.

In this study, I indicated that auxin involves in the production of PSK-α and the PSK-α stimulates the cell proliferation by activating the cell cycle induced by auxin.